Effects of dietary n-6:n-3 fatty acid ratio on feed intake, digestibility, and fatty acid profiles of the ruminal contents, liver, and muscle of growing lambs

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ABSTRACT: This study investigated the effect of modifying the n-6:n-3 fatty acid ratio (FAR) of diets using linseed, soybean, and cottonseed oils on apparent digestibility, ruminal fermentation characteristics, growth performance, key circulating hormones, and the fatty acid profile of ruminal digesta, liver, and foreshank muscle of growing lambs fed a high concentrate diet. Forty individually housed Katahdin Dorper lambs (average of 20.0 kg of BW) were fed Bermudagrass hay in ad libitum amounts and concentrates at 3.7% of BW daily. The concentrate contained 68.9% corn, 23.8% soybean meal, 3.3% limestone, and 4.0% oil supplements (DM basis). The treatments consisted of dietary n-6:n-3 FAR of 2.3:1, 8.8:1, 12.8:1, and 15.6:1. After feeding for 35 d in metabolism crates, lambs were slaughtered 15 h after feeding, and samples of ruminal digesta, blood, liver, and foreshank muscle were collected. Increasing dietary n-6:n-3 FAR did not affect the intake of DM nor the apparent digestibility of DM, ether extract, NDF, or ADF, but did increase apparent digestibility of CP (linear, \( P < 0.05 \)). Concentrations of ruminal butyrate increased linearly (\( P < 0.05 \)) with increasing dietary n-6:n-3 FAR, whereas the valerate concentration decreased linearly (\( P < 0.001 \)). Concentrations of plasma insulin and IGF-I were not affected by dietary n-6:n-3 FAR. Concentrations of C18:3n-3 increased linearly (\( P < 0.001 \), whereas that of C18:2n-6 decreased linearly (\( P < 0.001 \)) in ruminal digesta with decreasing dietary n-6:n-3 FAR. Concentrations of trans isomers of fatty acids in ruminal digesta did not change. Proportions of C18:0 in liver and foreshank muscle were unchanged by diet. The proportion of trans11 C18:1 and cis-9 trans11 CLA decreased (\( P < 0.05 \)) in liver but increased (\( P < 0.05 \)) in foreshank muscle as dietary n-6:n-3 FAR decreased. Proportions of all measured n-3 fatty acids were greater in liver when diets contained more C18:3n-3 from linseed oil. By decreasing the dietary n-6:n-3 FAR, the proportions of n-6 fatty acids in foreshank muscle decreased dramatically; specifically, C18:2n-6 decreased linearly (\( P < 0.001 \)) from 28.0 to 16.5% and C20:4n-6 decreased linearly (\( P < 0.001 \)) from 14.7 to 8.6%. Although feeding a diet that contained more n-3 fatty acids increased the n-3 fatty acid concentration of muscle, the ratio of PUFA to SFA was decreased.

Key words: digestibility, fatty acid, lamb, liver, muscle

INTRODUCTION

Lipids in food represent a significant percentage of the daily caloric intake by the human population in the United States (>33% of total calories; Bialostosky et al., 2002). Changing the lipid content and fatty acid (FA) composition of foods can be an effective way to improve the consumer’s health. Long chain n-3 FA are important to certain tissues such as the brain and retina and may also be important in the maintenance of human health by protecting against metabolic diseases (Kris-Etherton et al., 2002). Cook (1996) reported that a n-6:n-3 FA ratio (FAR) should be between 4:1 and 8:1 to promote normal growth and development of human infants and adults. More recently, an upper daily intake limit of 6.67 g/d of linoleic acid and a minimum daily intake of 2.87 g/d of n-3 FA (linolenic, eicosapentaenoic, and docosahexaenoic acids) were proposed to be adequate for human adults (Simopoulos et al., 1999). This results in an n-6:n-3 FAR of 2.3:1. However, the typical Western-type foods that are consumed have an average n-6:n-3 FAR of 10:1 (Kris-Etherton et al., 2000).

The n-6:n-3 FAR of ruminant and pork carcasses can be influenced by the FA composition of the diet fed...
to the animals (Raes et al., 2004). Raes et al. (2004) concluded that the linoleic to linolenic acid ratios in the diet and in the intramuscular fat were linearly related for swine but that this relationship was less certain for ruminants because of the partial hydrogenation of PUFA by ruminal bacteria. The n-6:n-3 FAR in intramuscular fat can be as low as 2:1 for pasture-finished ruminants but will range between 6 and 10 for concentrate-fed ruminants (Raes et al., 2004), the latter being the most common practice of finishing ruminants in the United States.

The current study was conducted to determine the effect of modifying the n-6:n-3 FAR in a concentrate-based diet on feed intake, apparent nutrient digestibility, plasma hormones, and long chain FA composition of the ruminal contents, liver, and muscle of lambs.

**MATERIALS AND METHODS**

**Animals, Diets, and Management**

This experiment was approved by the Institutional Animal Care and Use Committee of the University of Florida. Forty crossbred Katahdin Dorper ram lambs were drenched for parasites (Ivomec, Merial Ltd., Iselin, NJ), weighed, and assigned to treatment. Lambs were allocated randomly to 4 dietary treatments: concentrates formulated to n-6:n-3 FAR of 2:1, 10:1, 16:1, and 20:1. The concentrate contained (DM basis): 68.9% ground corn, 23.8% soybean meal, 3.3% limestone, and 4.0% vegetable oils. Soybean oil (Sunlight Foods Inc., FL), cottonseed oil (Hunt-Wesson Inc., Fullerton, CA), and linseed oil (Archer Daniels Midland Northern Sun Division, Minneapolis, MN) were used as oil sources to establish the n-6:n-3 FAR. The concentrates were fed at 3.7% of BW daily (DM basis; Jurgens, 2002), with adjustments made weekly according to changing BW. Tifton 85 Bermudagrass hay [10.5% CP and 1.3% ether extract (EE), DM basis] was chopped to a 4- to 6-cm length and offered ad libitum. The concentrates were offered in 2 equal meals at 0900 and 1700. The diets were formulated to be isonitrogenous and isocaloric and to meet energy and protein requirements (Jurgens, 2002). Lambs had free access to water and a mineral block, which contained (DM basis): Cl (590 g/kg), Na (380 g/kg), P (10 g/kg), Ca (10 g/kg), Mg (5 g/kg), Mn (0.2 g/kg), I (0.15 g/kg), Fe (0.15 g/kg), vitamin A (60,000 IU/kg), and vitamin D3 (60,000 IU/kg).

Lambs were grouped according to treatment assignment and fed a common diet, consisting of 80% Tifton 85 Bermudagrass hay and 20% soybean pellet (as-fed basis) for 2 wk. After 2 wk, the lambs were housed individually in metabolism crates for a 35-d period. The first week was considered an adaptation, and data were not collected. To determine apparent nutrient digestibilities, feces were collected individually using fecal collection bags fitted on the lambs during the last 2 wk of the period. Feces were collected daily, weighed, and stored at 4°C. At the end of the 2-wk collection, 10% of each daily sample was composited into 1 sample for each lamb. The composite was dried (60°C for 48 h) and stored.

At the end of the 35-d period, lambs were slaughtered at the USDA-approved Meat Processing Facility of the Animal Sciences Department, University of Florida, Gainesville, at 0800, which was approximately 15 h after the last feeding of the experimental diets. During the bleed-out process at slaughter, 20 mL of whole blood was collected from the jugular vein into tubes containing sodium heparin (Becton Dickson, Franklin Lakes, NJ) and stored immediately on ice. Blood samples were centrifuged at 1,916 × g for 20 min at 4°C to harvest the plasma. Plasma samples were stored at −20°C until analyzed. Once the stomach was removed from the carcass, approximately 100 mL of ruminal fluid was collected by sampling digesta from the ventral, caudal, and central areas of the rumen and squeezing it through cheesecloth. The pH was measured immediately (Corning Model 12, Corning Scientific Instruments, Medfield, MA). Then, 2 mL of 9 M H2SO4 was added to each sample to stop fermentation. Approximately 40 mL of ruminal fluid was centrifuged at 4°C at 2,540 × g for 20 min. The supernatant was frozen at −20°C until thawed for analysis. Samples of digesta were collected from the dorsal cranial, dorsal caudal, ventral cranial, and ventral caudal areas of the rumen, composited into 1 sample, and 100 g (wet weight) were frozen at −20°C for FA analysis. The whole liver and approximately 150 g of foreshank muscle (digital flexor) were removed from the carcass. Both were stored at −20°C until analyzed for long chain FA.

**Chemical Analyses**

Samples (500 g, as-is) of concentrates and hay were collected every 7 d, stored at 4°C, and composited across weeks. Individual lamb refusals of feed were weighed daily during the collection period and stored at 4°C until analyzed for DM (60°C for 48 h) to calculate daily DMI. Concentrates, hay samples, and feces were dried at 60°C for 48 h to determine the DM concentration, ground to pass the 1-mm screen of a Wiley Mill (A. H. Thomas, Philadelphia, PA), and analyzed for N, EE, NDF, and ADF. Crude protein was calculated as N × 6.25, using N measurements obtained with a Vario Max CN Elemental N analyzer (Elementar Americas Inc., Mt. Laurel, NJ). Ether extract was determined according to AOAC (1990) methods. The NDF and ADF (nonsequential) were determined based on the methods of Van Soest et al. (1991) using an Ankem Fiber Analyzer (Ankem Technology, Macedon, NY). Amylase and sodium sulfite were used for NDF determination, without correction for insoluble ash.

The frozen ruminal fluid was thawed and centrifuged at 5,400 × g at 4°C for 15 min, and the supernatant was saved to measure the concentrations of ammonia N and VFA. Ruminal ammonia N was determined using an adaptation for the Alpkem Auto Analyzer (Alpkem...
Corporation, Clackamas, OR) of the Noel and Hambleton (1976) procedure. The concentrations of VFA were measured using an HPLC (FL 7485, Hitachi, Tokyo, Japan) coupled to an auto sampler (L 7200, Hitachi) and a UV detector (Spectroflow 757, ABI Analytical Kratos Division, Ramsey, NJ) set at 210 nm and using the method of Muck and Dickerson (1988); 20 μL of supernatant was injected into a BioRad Aminex HPX-87H (BioRad Laboratories, Hercules, CA) column with a 0.015 M H2SO4 mobile phase and a flow rate of 0.7 mL/min at 45°C. The sample peaks were identified and calculated by the retention time and peak area of known standards (lactate, acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate; Sigma-Aldrich Co., St. Louis, MO).

For long chain FA analysis, diets (undried concentrates and hay, 2 g each), ruminal digesta (10 g), liver (5 g), and foreshank muscle (5 g) were freeze-dried (Labconco Co., Kansas City, MO), and total lipid was extracted using a 2:1 (vol/vol) chloroform:methanol solvent mixture (Folch et al., 1957). The weight of the fat was determined gravimetrically. The extracted FA methyl esters were prepared following the procedures of Sukhija and Palmquist (1988). Extracted lipid (100 mg) was transferred into an acid-washed 15-mL glass tube, 2 mL of chloroform and 3 mL of 5% methanolic HCl were added to each tube, the mixture was shaken until the lipid was dissolved completely, and the tube was incubated in a 70°C water bath for 2 h. The tubes were cooled at room temperature and, after addition of 5 mL of 0.01 M K2CO3 and 2 mL of chloroform, were centrifuged at 635 × g for 10 min at 4°C. The lower chloroform layer was collected for the analysis.

The FA methyl esters were determined using a Varian CP-3800 gas chromatograph (Varian Inc., Palo Alto, CA) equipped with an auto-sampler (Varian CP-8400), a flame ionization detector, and a Varian capillary column (CP-Sil 88, 100 m × 0.25 mm × 0.2 μm). The carrier gas was He, the split ratio was 10:1, and the injector and detector temperatures were maintained at 230 and 250°C, respectively. The sample (1 μL) was injected with an auto-sampler. The oven temperature was initially set at 120°C for 1 min, increased by 5°C/min up to 190°C, held at 190°C for 30 min, increased again by 2°C/min up to 220°C, and held at 220°C for 40 min. The peaks of samples were identified and concentrations calculated based on the retention time and peak area of known standards.

Plasma collected at the time of animal slaughter was analyzed for insulin and IGF-I. Concentrations of insulin in plasma were determined using the double-antibody RIA procedure described by Malven et al. (1987). Sensitivity of the assay was 0.2 ng/mL, and the intraassay CV was 4.2%. Concentrations of plasma IGF-I were measured using a double-antibody RIA (Selberg et al., 2005). Sensitivity of the assay was 10 ng/mL, and the intraassay CV was 2.9%. Concentrations of each hormone were determined in a single assay.

Statistics

Animal responses to dietary n-6:n-3 FAR were analyzed using the GLM procedure (SAS Inst. Inc., Cary, NC). Polynomial contrasts (linear, quadratic, and cubic effects) were used to evaluate the effects of increasing dietary n-6:n-3 FAR. The IML procedure of SAS was used to generate coefficients for testing linear, quadratic, and cubic effects of treatments with unequal spacing. Values of P ≤ 0.05 were considered significant, and those >0.05 but <0.10 were considered tendencies.

RESULTS AND DISCUSSION

Diets, Feed Intake, and Digestibility

Concentrations (DM basis) of dietary CP, EE, NDF, and ADF averaged 17.8, 7.0, 25.6, and 14.9%, respectively, across diets (Table 1). After adjusting for DMI of the concentrates and hay, the consumed dietary n-6:n-3 FAR were 2.3:1, 8.8:1, 12.8:1, and 15.6:1.

The intakes of DM, CP, EE, NDF, and ADF were not affected by increasing dietary n-6:n-3 FAR (Table 2). Mean apparent digestibility of DM (74.1%), EE (86.4%), and ADF (49.1%) were not affected, whereas CP digestibility increased linearly (P < 0.05) and NDF digestibility increased cubically (P < 0.03) with increasing dietary n-6:n-3 FAR (Table 2). Neither dietary proportions nor intakes of concentrates and Bermudagrass changed appreciably (Table 1), so differences in dietary selection by lambs cannot account for changes in apparent digestibility. Improved CP digestibility may have been due to changes in the microbial population, namely protozoa. Newbold and Chamberlain (1988) reported that dietary unsaturated 18-carbon FA could inhibit the growth of ruminal protozoa. The reduction of protozoa in the rumen often results in greater proliferation of bacteria and greater passage of bacterial N out of the rumen (Jouany, 1996). If dietary n-3 FA were more inhibitory than n-6 FA on protozoal numbers, then bacterial N production would be greater in lambs fed the 2.3:1 FAR, leading to greater excretion of N in the feces resulting in a lower apparent digestibility of CP. Although we did not measure ruminal protozoa in our study, the linear decrease in molar proportion of butyrate accompanied by the linear decrease in apparent protein digestibility (P < 0.05) with decreased dietary n-6:n-3 FAR may indicate a decreasing population of ruminal protozoa (Tables 2 and 3). The molar proportion of ruminal butyrate decreased from 11.2 to 9.4% and protozoal numbers decreased from 80.2 × 103 to 12.9 × 103/mL when dairy cows were fed a high concentrate diet and supplemented with linseed oil (Ueda et al., 2003).

Ruminal Fluid pH, Ammonia N, and VFA

Mean pH of ruminal fluid was 6.41, with lambs fed the diet of 12.8:1 n-6:n-3 FAR having the least acidic pH (cubic effect, P ≤ 0.05; Table 3). Lambs fed this treatment also had the numerically lowest concentra-
Table 1. Ingredient and chemical composition of the experimental diets consumed by growing lambs

<table>
<thead>
<tr>
<th>Item</th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>Diet 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredient, % of DM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>61.46</td>
<td>60.24</td>
<td>60.88</td>
<td>61.42</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>21.27</td>
<td>20.85</td>
<td>21.08</td>
<td>21.26</td>
</tr>
<tr>
<td>Limestone</td>
<td>2.95</td>
<td>2.89</td>
<td>2.93</td>
<td>2.95</td>
</tr>
<tr>
<td>Linseed oil</td>
<td>2.01</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cottonseed oil</td>
<td>1.54</td>
<td>—</td>
<td>1.64</td>
<td>2.24</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>—</td>
<td>3.48</td>
<td>1.87</td>
<td>1.30</td>
</tr>
<tr>
<td>Tifton 85 Bermudagrass</td>
<td>10.77</td>
<td>12.54</td>
<td>11.60</td>
<td>10.83</td>
</tr>
<tr>
<td>Chemical composition, % of DM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>86.5</td>
<td>86.7</td>
<td>86.6</td>
<td>86.4</td>
</tr>
<tr>
<td>CP</td>
<td>17.8</td>
<td>17.9</td>
<td>17.6</td>
<td>18.0</td>
</tr>
<tr>
<td>Ether extract</td>
<td>7.0</td>
<td>6.9</td>
<td>6.9</td>
<td>7.1</td>
</tr>
<tr>
<td>NDF</td>
<td>25.4</td>
<td>25.2</td>
<td>26.2</td>
<td>25.4</td>
</tr>
<tr>
<td>ADF</td>
<td>14.8</td>
<td>14.7</td>
<td>15.3</td>
<td>14.8</td>
</tr>
<tr>
<td>Total fatty acids</td>
<td>6.2</td>
<td>6.1</td>
<td>6.2</td>
<td>6.3</td>
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<tr>
<td>Fatty acid composition, % of fatty acids</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14:0, myristic</td>
<td>2.6</td>
<td>2.5</td>
<td>2.8</td>
<td>2.6</td>
</tr>
<tr>
<td>C16:0, palmitic</td>
<td>14.6</td>
<td>14.6</td>
<td>17.3</td>
<td>18.2</td>
</tr>
<tr>
<td>C16:1n-7, palmitoleic</td>
<td>0.6</td>
<td>0.4</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>C18:0, stearic</td>
<td>2.9</td>
<td>3.7</td>
<td>3.3</td>
<td>2.8</td>
</tr>
<tr>
<td>C18:1n-9, oleic</td>
<td>20.6</td>
<td>21.8</td>
<td>21.1</td>
<td>19.9</td>
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<tr>
<td>C18:2n-6, linoleic</td>
<td>40.8</td>
<td>51.2</td>
<td>50.8</td>
<td>52.4</td>
</tr>
<tr>
<td>C18:3n-3, linolenic</td>
<td>18.0</td>
<td>5.8</td>
<td>4.0</td>
<td>3.4</td>
</tr>
<tr>
<td>SFA</td>
<td>20.0</td>
<td>20.5</td>
<td>23.4</td>
<td>23.6</td>
</tr>
<tr>
<td>MUFA</td>
<td>21.2</td>
<td>22.2</td>
<td>21.8</td>
<td>20.6</td>
</tr>
<tr>
<td>PUFA</td>
<td>58.8</td>
<td>57.0</td>
<td>54.8</td>
<td>55.8</td>
</tr>
<tr>
<td>PUFA/SFA</td>
<td>2.94</td>
<td>2.74</td>
<td>2.34</td>
<td>2.37</td>
</tr>
<tr>
<td>n-6:n-3 fatty acid ratio¹</td>
<td>2.3</td>
<td>8.8</td>
<td>12.8</td>
<td>15.6</td>
</tr>
</tbody>
</table>

¹n-6:n-3 fatty acid ratio = C18:2 ÷ C18:3.

Table 2. Intake and apparent nutrient digestibilities of growing lambs fed diets differing in the ratio of n-6 to n-3 fatty acids

<table>
<thead>
<tr>
<th>Dietary ratio of n-6 to n-3 fatty acids</th>
<th>P-value²</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>L</td>
</tr>
<tr>
<td>Item</td>
<td>2.3:1</td>
</tr>
<tr>
<td>DM</td>
<td>904</td>
</tr>
<tr>
<td>Concentrate</td>
<td>808</td>
</tr>
<tr>
<td>Forage</td>
<td>96</td>
</tr>
<tr>
<td>CP</td>
<td>161</td>
</tr>
<tr>
<td>Ether extract</td>
<td>64</td>
</tr>
<tr>
<td>NDF</td>
<td>230</td>
</tr>
<tr>
<td>ADF</td>
<td>134</td>
</tr>
</tbody>
</table>

¹Intake and digestibility determined over 14 d.
²L = linear, Q = quadratic, and C = cubic effects for unequally spaced treatments.
Table 3. The pH and concentration of ammonia and VFA in ruminal fluid of growing lambs fed diets differing in the ratio of n-6 to n-3 fatty acids

<table>
<thead>
<tr>
<th>Item</th>
<th>Dietary ratio of n-6 to n-3 fatty acids</th>
<th>P-value1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.3:1</td>
<td>8.8:1</td>
</tr>
<tr>
<td>pH</td>
<td>6.23</td>
<td>6.28</td>
</tr>
<tr>
<td>Ammonia, mM</td>
<td>22.1</td>
<td>21.1</td>
</tr>
<tr>
<td>Total VFA, mM</td>
<td>119.4</td>
<td>116.5</td>
</tr>
<tr>
<td>VFA composition, molar %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>36.3</td>
<td>36.6</td>
</tr>
<tr>
<td>Propionate</td>
<td>42.0</td>
<td>42.1</td>
</tr>
<tr>
<td>Isobutyrate</td>
<td>3.6</td>
<td>5.4</td>
</tr>
<tr>
<td>Butyrate</td>
<td>9.4</td>
<td>10.5</td>
</tr>
<tr>
<td>Isovalerate + 2-methylbutyrate</td>
<td>4.4</td>
<td>4.2</td>
</tr>
<tr>
<td>Valerate</td>
<td>4.3</td>
<td>1.2</td>
</tr>
</tbody>
</table>

1L = linear, Q = quadratic, and C = cubic effects for unequally spaced treatments.

Growth Performance and Plasma Hormones

Intake of concentrate averaged 3.6% of BW daily, reflecting that lambs did not consume all the concentrate offered. Initial and final BW averaged 20.0 and 27.2 kg, respectively, and did not differ among treatments (Table 4). Likewise, total BW gain (7.2 kg), ADG (0.26 kg/d), DMI (0.85 kg/d), and G:F (0.30 kg/kg) were not affected by dietary n-6:n-3 FAR. Other studies reported that different dietary fat sources had no detectable effects on feed intake, growth rate, or feed conversion (Bock et al., 1991; Demirel et al., 2004).

Dietary fats can influence insulin concentrations, which in turn can affect lipolysis and lipogenesis and, therefore, tissue FA profiles. Mean plasma concentrations of IGF-I ($P = 0.15$) and insulin ($P = 0.16$) were not impacted by dietary n-6:n-3 FAR. Concentrations of plasma IGF-I were greater in lactating Holstein cows fed supplemental C18:2n-6 or C18:3n-3 compared with those fed supplemental C18:1n-9 or trans isomers of C18:1 (Amaral et al., 2006).

Fatty Acid Profiles in Ruminal Digesta

The total fat concentration in ruminal digesta ranged from 12.5 to 13.2% (DM basis; Table 5). This is more than twice the concentration of 5.5% reported for ruminal contents of steers fed a high concentrate diet containing 5% soybean oil (Beaulieu et al., 2002). It was expected that the FA concentration in the digesta would be greater than the FA concentration in the diet because the major dietary nutrient (starch) is very digestible in the rumen, whereas FA are only reduced by passage from the rumen.

Palmitic acid was the second most concentrated FA in ruminal digesta (15.5 to 18.1%) with the greatest concentration coming from lambs fed the diet with the greatest C16:0 concentration (Tables 1 and 5). As expected, the FA in greatest concentration was C18:0 (43 to 52%; Table 5). Ruminal digesta from lambs fed the 2 lowest n-6:n-3 FAR diets contained a greater proportion of C18:0 compared with those fed the 2 greatest n-6:n-3 FAR diets (quadratic effect, $P < 0.05$), indicating that C18:3n-3 underwent greater complete biohydrogenation than C18:2n-6, which agrees with other studies (Raes et al., 2004). Because diets contained greater concentrations of polyunsaturated 18-carbon FA and

Table 4. Growth performance and the concentration of IGF-I and insulin in plasma of growing lambs fed diets differing in the ratio of n-6 to n-3 fatty acids for 28 d

<table>
<thead>
<tr>
<th>Item</th>
<th>Dietary ratio of n-6 to n-3 fatty acids</th>
<th>P-value1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.3:1</td>
<td>8.8:1</td>
</tr>
<tr>
<td>Growth performance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial wt, kg</td>
<td>19.8</td>
<td>20.5</td>
</tr>
<tr>
<td>Final wt, kg</td>
<td>26.3</td>
<td>28.1</td>
</tr>
<tr>
<td>Total gain, kg</td>
<td>6.4</td>
<td>7.6</td>
</tr>
<tr>
<td>ADG, kg/d</td>
<td>0.23</td>
<td>0.27</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>0.82</td>
<td>0.90</td>
</tr>
<tr>
<td>G:F</td>
<td>0.28</td>
<td>0.30</td>
</tr>
<tr>
<td>Plasma hormones</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-I, ng/mL</td>
<td>38.4</td>
<td>38.2</td>
</tr>
<tr>
<td>Insulin, ng/mL</td>
<td>1.3</td>
<td>1.3</td>
</tr>
</tbody>
</table>

1L = linear, Q = quadratic, and C = cubic effects for unequally spaced treatments.
Table 5. Long-chain fatty acid composition in ruminal digesta of growing lambs fed diets differing in the ratio of n-6 to n-3 fatty acids

<table>
<thead>
<tr>
<th>Dietary ratio of n-6 to n-3 fatty acids</th>
<th>P-value&lt;sup&gt;2&lt;/sup&gt;</th>
<th>SEM</th>
<th>L</th>
<th>Q</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.3:1</td>
<td>8.8:1</td>
<td>12.8:1</td>
<td>15.6:1</td>
<td>0.1</td>
<td>0.01</td>
</tr>
<tr>
<td>C14:0, myristic</td>
<td>1.5</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>0.1</td>
</tr>
<tr>
<td>C16:0, palmitic</td>
<td>17.0</td>
<td>15.5</td>
<td>17.6</td>
<td>18.1</td>
<td>0.2</td>
</tr>
<tr>
<td>C16:1n-7, palmitoleic</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.02</td>
</tr>
<tr>
<td>C17:0, margaric</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.01</td>
</tr>
<tr>
<td>C18:0, stearic</td>
<td>47.7</td>
<td>51.9</td>
<td>45.2</td>
<td>43.1</td>
<td>2.0</td>
</tr>
<tr>
<td>C18:1 trans 11, vaccenic</td>
<td>11.1</td>
<td>10.5</td>
<td>11.6</td>
<td>14.9</td>
<td>2.5</td>
</tr>
<tr>
<td>C18:1n-9, oleic</td>
<td>8.0</td>
<td>6.0</td>
<td>7.6</td>
<td>6.8</td>
<td>0.5</td>
</tr>
<tr>
<td>CLA cis-9 trans 11</td>
<td>0.11</td>
<td>0.14</td>
<td>0.11</td>
<td>0.27</td>
<td>0.7</td>
</tr>
<tr>
<td>CLA trans 10 cis-12</td>
<td>0.08</td>
<td>0.10</td>
<td>0.08</td>
<td>0.09</td>
<td>0.02</td>
</tr>
<tr>
<td>C18:3n-3, linolenic</td>
<td>3.1</td>
<td>1.9</td>
<td>1.7</td>
<td>1.5</td>
<td>0.2</td>
</tr>
<tr>
<td>C20:4n-6, arachidonic</td>
<td>0.03</td>
<td>0.03</td>
<td>0.05</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>C20:5n-3, eicosapentaenoic</td>
<td>0.02</td>
<td>0.02</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>C22:5n-3, docosapentaenoic</td>
<td>0.002</td>
<td>0.005</td>
<td>0.0</td>
<td>0.003</td>
<td>0.01</td>
</tr>
<tr>
<td>C22:6n-3, docosahexaenoic</td>
<td>0.02</td>
<td>0.02</td>
<td>0.002</td>
<td>0.008</td>
<td>0.01</td>
</tr>
<tr>
<td>SFA</td>
<td>66.6</td>
<td>69.0</td>
<td>64.4</td>
<td>62.9</td>
<td>2.0</td>
</tr>
<tr>
<td>MUFA</td>
<td>19.4</td>
<td>17.1</td>
<td>18.4</td>
<td>21.9</td>
<td>2.3</td>
</tr>
<tr>
<td>PUFA</td>
<td>14.0</td>
<td>13.8</td>
<td>16.1</td>
<td>15.2</td>
<td>0.8</td>
</tr>
<tr>
<td>PUFA/SFA</td>
<td>0.21</td>
<td>0.20</td>
<td>0.25</td>
<td>0.24</td>
<td>0.01</td>
</tr>
<tr>
<td>n-6:n-3 fatty acid ratio&lt;sup&gt;3&lt;/sup&gt;</td>
<td>3.5</td>
<td>6.1</td>
<td>8.5</td>
<td>9.1</td>
<td>0.5</td>
</tr>
<tr>
<td>Total fatty acids, % of rumen digesta DM</td>
<td>13.2</td>
<td>12.5</td>
<td>12.6</td>
<td>12.8</td>
<td>1.06</td>
</tr>
</tbody>
</table>

<sup>1</sup>The data are expressed as the percentage of identified fatty acids.
<sup>2</sup>L = linear, Q = quadratic, and C = cubic effects for unequally spaced treatments.
<sup>3</sup>n-6:n-3 fatty acid ratio = (C18:2n-6 + C20:4n-6) ÷ (C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3).

because trans<sup>11</sup> C18:1 is a mutual intermediate in the biohydrogenation of unsaturated 18-carbon FA (Harfoot and Hazelwood, 1997), more trans<sup>11</sup> C18:1 was detected in the ruminal digesta than cis-9 C18:1 (12.0 vs. 7.2%), but diet had no effect on the proportions of either. The mean concentrations of C18:0 (47%) and trans<sup>11</sup> C18:1 (12%) were similar to the data of Engle et al. (2000) who reported that the lipid in ruminal contents from steers fed a high concentrate diet supplemented with 4% soybean oil (DM basis) consisted of 48.3% C18:0 and 15.2% trans<sup>11</sup> C18:1, and to that of Bateman and Jenkins (1998) who reported that the concentrations of C18:0 and trans<sup>11</sup> C18:1 were 56.0 and 11.8% in ruminal contents collected from nonlactating cows fed a high-fiber diet supplemented with 2% soybean oil (DM basis).

Concentrations of C18:2n-6 decreased linearly ($P < 0.001$) from 13.3 to 10.6%, whereas that of C18:3n-3 increased linearly ($P < 0.001$) from 1.5 to 3.1% in the ruminal digesta of lambs fed decreasing dietary n-6:n-3 FAR (Table 5). This resulted in a linear decrease in the n-6:n-3 FAR from 9.1 to 3.5 that mimicked the dietary n-6:n-3 FAR. Conjugated linoleic acid is also an intermediate in the ruminal biohydrogenation of C18:2n-6 to C18:0 (Kepler et al., 1966). However, Beaulieu et al. (2002) reported that increased dietary intake of C18:2n-6 did not affect cis-9 trans<sup>11</sup> CLA concentrations in ruminal contents of steers fed a soybean oil supplement. This is consistent with the lack of response in cis-9 trans<sup>11</sup> CLA concentration in ruminal digesta in the current study.

Of the 8 identified FA that individually comprised \( \leq 0.4\% \) of the total FA, none were affected by diet. The mean concentrations of SFA, MUFA, and PUFA constituted 66, 19, and 15% of the total identified FA and did not differ among treatments.

**Fatty Acid Profiles in Liver**

The total fat concentration in liver ranged from 16.2 to 19.8% (DM basis; Table 6). This is similar to the 19.7% average (converted to DM basis assuming 25% DM for liver) reported for grass-fed lambs (Enser et al., 1998). As was the case for ruminal digesta, the major FA detected in liver was C18:0 with a mean proportion of 22.8% (DM basis; Table 6), with no detected differences among treatments. Other major FA detected in liver and not affected by treatment included C16:0 (14.9%) and C18:1n-9 (12.5%).

The proportions of the predominant FA in lamb liver in this study were similar to those FA proportions of the liver of bulls fed a high concentrate diet (15% C16:0, 25% C18:0, and 14% C18:1n-9) as reported by Enser et al. (1998).

Palmitic acid can be converted to C16:1n-7 through \( 9\delta \) desaturation (Cook, 1996). As in the current study, Demirel et al. (2004) reported that the concentration of C16:1n-7 in liver of lambs increased with greater intake of dietary C16:0 from palm oil, although the concentrations of C16:1 were quite low (<0.25%; Table 6).

The quadratic increase in liver concentration of trans<sup>11</sup> C18:1 with increasing dietary n-6:n-3 FAR may
be explained by the ability of ruminal bacteria to make this isomer from C18:2n-6 (Harfoot and Hazelwood, 1997). If a greater proportion of C18:3n-3 than of C18:2n-6 was completely biohydrogenated to C18:0 in the rumen, as evidenced by greater ruminal concentrations of C18:0 as dietary n-6:n-3 FAR decreased, then a greater supply of trans11 C18:1 would be expected for diets containing more C18:2n-6. Harfoot et al. (1973) reported that trans11 C18:1 was the primary end product in the rumen rather than C18:0 when C18:2n-6 was in higher concentrations. This pattern was detected in the liver but not in the ruminal digesta, possibly due to the 15-h delay between feeding and rumen sampling.

Concentrations of C18:2n-6 ranged from 16.5 to 18.2% of the identified FA (DM basis; Table 6). This is somewhat greater than the 12.3% reported for livers of bulls fed high concentrate diets (Ensor et al., 1998) but similar to the 18% reported for heifers fed high concentrate diets containing 5% soybean oil (Beaulieu et al., 2002). Liver concentration of C18:2n-6 only tended to decrease (P < 0.10) with decreasing dietary n-6:n-3 FAR, possibly because of its partial conversion to trans11 C18:1 in the rumen and to C20:4n-6 in the liver.

Concentrations of C20:4n-6 were much greater in the liver (12.0 to 16.5%) compared with the rumen (0.01 to 0.5%) due to the presence of Δ6 and Δ5 desaturase enzymes and elongase enzymes present in the liver to convert C18:2n-6 to C20:4n-6 (Cook, 1996). Concentrations of C20:4n-6 were somewhat greater in our lambs compared with the 11% reported for heifers fed high concentrate diets (Beaulieu et al., 2002). Liver concentration of C20:4n-6 only decreased when the dietary n-6:n-3 FAR decreased to 2.3:1 (quadratic effect, P < 0.001; Table 6). This decrease coincides with the major decrease in dietary concentration of C18:2n-6 (Table 1).

The quadratic increases in the concentration of omega-3 FA (C18:3n-3, C20:5n-3, and C22:5n-3) coincide with the major increase in dietary concentration of C18:3n-3 occurring with the 2.3:1 FAR diet (Table 1). These increases agree with results of Demirel et al. (2004) who supplemented lambs with linseed oil. Concentration of MUFA (P < 0.05) and n-6:n-3 FAR (P < 0.001) decreased linearly with decreasing dietary n-6:n-3 FAR. The lowest n-6:n-3 FAR in the liver was 2.4:1 which was just below the lowest n-6:n-3 FAR detected in ruminal digesta of 3.5 (Table 5), coming from lambs fed the most linseed oil. Unlike the ruminal situation, the elongation and desaturation FA products made from C18:2n-6 and C18:3n-3 in the liver played an important role in the n-6:n-3 FAR for the liver.

**Fatty Acid Profiles in Foreshank Muscle**

The total fat concentration in muscle ranged from 9.3 to 10.4% (DM basis; Table 7). This is somewhat greater than the 7.2% (converted to DM basis assuming a 25% DM for muscle) reported for longissimus thoracis of concentrate-fed lambs (Demirel et al., 2006).

Mean concentrations of C14:0 ranged from 0.5 to 2.3% and those of C16:0 ranged from 17.6 to 20.9%, both increasing quadratically as the dietary n-6:n-3 FAR
difference in the ratio of n-6 to n-3 fatty acids

### Table 6. Long-chain fatty acid composition in liver tissue of growing lambs fed diets differing in the ratio of n-6 to n-3 fatty acids

<table>
<thead>
<tr>
<th>Item</th>
<th>Dietary ratio of n-6 to n-3 fatty acids</th>
<th>P-value&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.3:1</td>
<td>8.8:1</td>
</tr>
<tr>
<td>C14:0, myristic</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>C16:0, palmitic</td>
<td>14.8</td>
<td>15.5</td>
</tr>
<tr>
<td>C16:1n-7, palmitoleic</td>
<td>0.15</td>
<td>0.18</td>
</tr>
<tr>
<td>C17:0, margaric</td>
<td>2.17</td>
<td>2.36</td>
</tr>
<tr>
<td>C18:0, stearic</td>
<td>22.6</td>
<td>23.1</td>
</tr>
<tr>
<td>C18:1 trans11, vaccenic</td>
<td>6.0</td>
<td>5.2</td>
</tr>
<tr>
<td>C18:1n-9, oleic</td>
<td>12.2</td>
<td>12.7</td>
</tr>
<tr>
<td>C18:2n-6, linoleic</td>
<td>16.9</td>
<td>16.5</td>
</tr>
<tr>
<td>CLA cis-9 trans11</td>
<td>0.31</td>
<td>0.24</td>
</tr>
<tr>
<td>CLA trans10 cis-12</td>
<td>0.06</td>
<td>0.07</td>
</tr>
<tr>
<td>C18:3n-3, linolenic</td>
<td>2.7</td>
<td>0.8</td>
</tr>
<tr>
<td>C20:4n-6, arachidonic</td>
<td>12.0</td>
<td>16.5</td>
</tr>
<tr>
<td>C20:5n-3, eicosapentaenoic</td>
<td>2.4</td>
<td>1.0</td>
</tr>
<tr>
<td>C22:5n-3, docosapentaenoic</td>
<td>4.5</td>
<td>2.9</td>
</tr>
<tr>
<td>C22:6n-3, docosahexaenoic</td>
<td>2.3</td>
<td>2.1</td>
</tr>
<tr>
<td>SFA</td>
<td>40.4</td>
<td>41.8</td>
</tr>
<tr>
<td>MUFA</td>
<td>18.4</td>
<td>18.1</td>
</tr>
<tr>
<td>PUFA</td>
<td>41.2</td>
<td>40.2</td>
</tr>
<tr>
<td>PUFA/SFA</td>
<td>1.02</td>
<td>0.96</td>
</tr>
<tr>
<td>n-6:n-3 fatty acid ratio&lt;sup&gt;3&lt;/sup&gt;</td>
<td>2.41</td>
<td>4.87</td>
</tr>
<tr>
<td>Total fatty acids, % of tissue DM</td>
<td>17.5</td>
<td>16.2</td>
</tr>
</tbody>
</table>

<sup>1</sup>The data are expressed as the percentage of identified fatty acids.

<sup>2</sup>L = linear, Q = quadratic, and C = cubic effects for unequally spaced treatments.

<sup>3</sup>n-6:n-3 fatty acid ratio = (C18:2n-6 + C20:4n-6) + (C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3).
increased (Table 7). These values were similar to the values reported for longissimus thorasis of lambs fed high concentrate diets (Bessa et al., 2005; Demirel et al., 2006). This decrease in the proportion of medium-chain FA as the dietary n-6:n-3 FAR increased may have been due to decreases in the activity and mRNA abundance of lipogenic enzymes such as acetyl CoA carboxylase and fatty acid synthase that are required for the synthesis of medium-chain FA. Others have reported that these enzymes were negatively affected in mammary tissue of cows producing milk of lowered proportions of short- and medium-chain FA during with milk fat depression (Bauman and Griinari, 2003). This response has been associated with increased concentrations of trans10, cis-12 CLA. This CLA isomer was increased linearly with increasing n-6:n-3 FAR in our study. Increased intake of C14:0 and C16:0 by men and women was reported to elevate serum concentrations of low-density lipoprotein cholesterol and total cholesterol to a greater degree than intake of oleic acid and, thus, are dietary FA to be avoided by individuals with hypercholesterolemia (Zock et al., 1994). If the feeding of additional n-3 FA to ruminants increases the concentration of these medium-chain FA in muscle, the health benefits due to increased intake of n-3 FA may be partially discounted. In partial agreement with our study, lambs supplemented with fish oil plus linseed oil had greater concentrations of C14:0 in the polar lipid fraction of lamb musculus semimembranosus than lambs supplemented with a Ca salt of palm oil, but this was not the case for the neutral lipid fraction (Demirel et al., 2004). This was likely due to a greater concentration of C14:0 in fish oil compared with palm oil. Linseed oil may be a preferred source of n-3 FA due to its low concentration of C14:0 and C16:0.

The proportion of muscle FA having 18 carbons was quite consistent across the 4 treatment groups, averaging between 62.4 and 63.9% of the identified FA (Table 7). Mean foreshank muscle concentrations of C18:0, C18:1n-9, C18:2n-6, and C18:3n-3 were 13, 24, 21, and 1.2% of FA, respectively. Bessa et al. (2005) reported similar concentrations of C18:0 (15%), C18:1n-9 (27%), and C18:3n-3 (0.6%) but lower C18:2n-6 (10%) concentrations in the FA of longissimus thoracis of Merino Branco lambs fed high concentrate diets (90% of dietary DM) containing 10% soybean oil. On the other hand, Demirel et al. (2006) reported concentrations of 20, 37, 12, and 0.8% of identified FA for these same 4 FA, respectively, in the longissimus thoracis of lambs fed 75% concentrate diets without oil supplementation. The lower proportions of C18:0 (13 vs. 20%) and C18:1n-9 (24 vs. 37%) and greater proportions of C18:2n-6 (21 vs. 12%) reported by Demirel et al. (2004) compared with our study may be due to differences in the extent of ruminal biohydrogenation of C18:2, with less biohydrogenation in our study due to an acidic ruminal pH resulting from the 89% concentrate diet. The proportion of cellulolytic bacteria, the bacteria primarily responsible for biohydrogenation, were greatly reduced when an 80% concentrate:20% forage diet was fed to cows (Latham et al., 1972). The extent of ruminal biohydrogenation is thought to be decreased by increasing rumi-

Table 7. Long-chain fatty acid composition in foreshank tissue of growing lambs fed diets differing in the ratio of n-6 to n-3 fatty acids

<table>
<thead>
<tr>
<th>Measure</th>
<th>Dietary ratio of n-6 to n-3 fatty acids</th>
<th>P-value2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.3:1</td>
<td>8.8:1</td>
</tr>
<tr>
<td>C14:0, myristic</td>
<td>2.3</td>
<td>2.5</td>
</tr>
<tr>
<td>C16:0, palmitic</td>
<td>20.9</td>
<td>20.5</td>
</tr>
<tr>
<td>C16:1n-7, palmitoleic</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>C17:0, margaric</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>C18:0, stearic</td>
<td>12.4</td>
<td>12.7</td>
</tr>
<tr>
<td>C18:1 trans11, vaccenic</td>
<td>3.5</td>
<td>3.8</td>
</tr>
<tr>
<td>C18:1n-9, oleic</td>
<td>28.9</td>
<td>25.0</td>
</tr>
<tr>
<td>C18:2n-6, linoleic</td>
<td>16.5</td>
<td>19.5</td>
</tr>
<tr>
<td>CLA cis-9 trans11</td>
<td>0.46</td>
<td>0.48</td>
</tr>
<tr>
<td>CLA trans10 cis-12</td>
<td>0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>C18:3n-3, linolenic</td>
<td>2.1</td>
<td>0.9</td>
</tr>
<tr>
<td>C20:4n-6, arachidonic</td>
<td>8.6</td>
<td>10.3</td>
</tr>
<tr>
<td>C20:5n-3, eicosapentaenoic</td>
<td>1.3</td>
<td>0.8</td>
</tr>
<tr>
<td>C22:5n-3, docosapentaenoic</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>C22:6n-3, docosahexaenoic</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>SFA</td>
<td>36.9</td>
<td>37.4</td>
</tr>
<tr>
<td>MUFA</td>
<td>32.9</td>
<td>29.2</td>
</tr>
<tr>
<td>PUFA</td>
<td>30.2</td>
<td>33.4</td>
</tr>
<tr>
<td>PUFA/SFA</td>
<td>0.83</td>
<td>0.91</td>
</tr>
<tr>
<td>n-6:n-3 fatty acid ratio3</td>
<td>5.5</td>
<td>9.6</td>
</tr>
<tr>
<td>Total fatty acids, % of tissue DM</td>
<td>10.4</td>
<td>9.8</td>
</tr>
</tbody>
</table>

1 The data are expressed as the percentage of identified fatty acids.
2 L = linear, Q = quadratic, and C = cubic effects for unequally spaced treatments.
3 n-6:n-3 fatty acid ratio = (C18:2n-6 + C20:4n-6) - (C18:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3).
nal acidity (Harfoot and Hazelwood, 1997). As a result, the escape of C18:2n-6 from the rumen was likely greater in our study, and this may account for the greater accumulation of C18:2n-6 in the muscle tissue of our lambs compared with other reports. Demirel et al. (2006) reported that the concentration of C18:2n-6 in longissimus thorasis of lambs could be increased dramatically from 5.0 to 11.7% of identified FA when the dietary ratio of grass hay to concentrate was changed from 75:25 to 25:75 (DM basis). This increase in C18:2n-6 was likely due to a combination of greater intakes of C18:2n-6 and a lower extent of ruminal biohydrogenation of C18:2n-6 associated with a more acidic pH in the rumen of lambs fed the high concentrate diet. Cooper et al. (2004) reported concentrations of 5.6 and 16.0% C18:2n-6 (expressed as a percentage of identified FA and corrected for proportions of neutral lipids and phospholipids) in longissimus muscle of lambs fed supplemental linseed oil or oilseeds encapsulated in formaldehyde-treated protein, respectively. Their work demonstrates that muscle tissue of lambs can accumulate C18:2n-6 when C18:2n-6 that is partially protected from ruminal biohydrogenation is fed. An additional reason for elevated C18:2n-6 may be due to the breed used in this study. Demirel et al. (2004) reported that Suffolk × Lleyn crossbreed sheep had 11% greater concentration of C18:2n-6 in the polar lipid fraction of musculus semimembranosus compared with the Scottish Blackface breed (11.2 vs. 10.0 g/100 g of total FA). The Katahdin Dorper breed used in our study may store greater C18:2n-6 concentrations in its muscle than other breeds. Lastly, muscle tissues can differ in FA profile. Popova (2006) reported that the phospholipids of the musculus semimembranosus of lambs had 16% greater concentrations of C18:2n-6 than the longissimus lumborum (19.0 vs. 16.3% of total FA). The shank muscles used in the current study are usually leaner than longissimus muscles and therefore likely contain a greater proportion of the FA as phospholipids. Because polyunsaturated FA are stored preferentially in phospholipids, the concentrations of C18:2n-6 and C20:4n-6 would be greater in shank than in muscles having greater internal fat. Arachidonic acid was detected in much greater concentrations in our study than others (11.5 vs. 2% as reported by Bessa et al., 2005; Demirel et al., 2006). This may have been due to a greater delivery and uptake of C18:2n-6 under our experimental conditions as discussed earlier because C18:2n-6 is the precursor for C20:4n-6 in muscle.

A greater escape of C18:2n-6 than of C18:3n-3 from the rumen in our study would result in lower concentrations of C18:0 in muscle. Lower concentrations of C18:0 in muscle would likely result in lower concentrations of C18:1n-9 because C18:0 in muscle serves as a substrate for the synthesis of C18:1n-9, a reaction requiring the Δ9 desaturase enzyme (Cook, 1996). An additional reason for low concentrations of C18:1n-9 in our study may be the repressive action that elevated concentrations of polyunsaturated FA in tissue can have on the activity of Δ9 desaturase (Ntambi, 1999).

The muscle appeared to accumulate more of the dietary C18:2n-6 than did the liver (mean of 21.5 vs. 17.4%). The proportion of phospholipids as a percentage of total lipids between muscle and liver will influence the relative concentrations of polyunsaturated FA, such as C18:2n-6, because these are used to a greater degree for phospholipid than for triglyceride synthesis. The fat in the muscle may have contained a greater proportion of phospholipids than did the fat in the liver of these lambs, thus accounting for greater concentrations of C18:2n-6. Concentrations of C18:2n-6 were greater in the phospholipid fraction of muscle than in liver (10.6 vs. 4.9%), but the neutral lipid fraction of muscle had a lower concentration of C18:2n-6 (1.7 vs. 5.2%; Demirel et al., 2004). Unlike the liver, which contained increasing concentrations of trans11 C18:1 as the dietary n-6:n-3 FAR increased, the foreshank muscle tended to contain decreasing concentrations of trans11 C18:1. Generally, a positive relationship has been reported between the concentrations of dietary C18:2n-6 and tissue cis-9 trans11 CLA in ruminants (Kepler et al., 1966). The trans11 C18:1 isomer is a common intermediate in the microbial biohydrogenation of dietary C18:1n-9, C18:2n-6, and C18:3n-3 (Harfoot and Hazelwood, 1997). Studies have reported a positive relationship between trans11 C18:1 and cis-9 trans11 CLA in mammary cells (Grinari and Bauman, 1999) and in intramuscular lipid (Santos-Silva et al., 2004). Certainly in our study, the pattern of response of trans11 C18:1 and cis-9 trans11 CLA to changing dietary n-6:n-3 FAR, whether increasing in the case of liver or decreasing in the case of muscle, agreed. In our study, dietary concentrations of C18:2n-6 were similar among the 3 higher n-6:n-3 FAR diets (51 to 52% of FA, Table 1). However, trans11 C18:1 concentration in foreshank muscle, which could be converted to cis-9 trans11 CLA by the muscle, tended to decrease quadratically with increasing dietary n-6:n-3 FAR. Likewise, the concentrations of cis-9 trans11 CLA in foreshank tissue decreased quadratically as lambs consumed diets of increasing n-6:n-3 FAR, with the concentration decreasing dramatically in lambs fed the 15.6:1 n-6:n-3 FAR diet (Table 7). This result may be explained by the quadratic increase in C18:2n-6 in lambs fed the 15.6:1 FAR diet, indicating that less C18:2n-6 was isomerized to cis-9 trans11 CLA and hydrogenated to trans11 C18:1 in the rumen of lambs fed the highest n-6:n-3 FAR leading to less deposition in muscle. Zheng et al. (2005) reported that when cows were fed cottonseed oil, soybean oil, or corn oil as the fat supplement (oils of similar C18:2n-6 concentrations but soybean oil having a greater C18:3n-3 concentration), the concentrations of trans11 C18:1 as well as cis-9 trans11 CLA in milk fat were greater in cows fed soybean oil than in cows fed the other treatments. Therefore, the synthesis of these isomers from C18:3n-3 may have been more efficient than that from C18:2n-6.
With decreasing dietary n-6:n-3 FAR, the foreshank muscle concentrations of the following FA and ratios decreased: C18:2n-6 (linear, P < 0.001), trans10 cis-12 CLA (linear, P < 0.05), C20:4n-6 (linear, P < 0.001), C22:5n-3 (quadratic, P < 0.05), C22:6n-3 (linear, P < 0.001), PUFA (quadratic, P < 0.05), PUFA/SFA ratio (quadratic, P < 0.01), and n-6:n-3 FAR (quadratic, P < 0.01). Conversely, concentrations of the following FA increased as the dietary n-6:n-3 FAR decreased: C14:0 (quadratic, P < 0.05), C16:0 (quadratic, P < 0.05), C16:1n-7 (linear, P < 0.05), C18:1n-9 (linear, P < 0.001), cis-9 trans11 CLA (quadratic, P < 0.05), C18:3n-3 (quadratic, P < 0.001), C20:5n-3 (quadratic, P < 0.05), SFA (quadratic, P < 0.01), and MUFA (linear, P < 0.001).

Concentrations of the n-6 FA (C18:2n-6 and C20:4n-6) decreased linearly (P < 0.001) with decreasing dietary n-6:n-3 FAR (Table 7). Concentration of C18:3n-3 was greatest in foreshank muscle (2.1%) of lambs fed the most C18:3n-3 but was similar (0.7 to 0.9%; quadratic effect, P < 0.001) in muscles of lambs fed the other n-6:n-3 FAR diets. These longer chain n-3 FA (C20:5n-3, C22:5n-3, and C22:6n-3) in foreshank muscle are the metabolic products of C18:3n-3. However, only concentrations of C20:5n-3 followed a similar quadratic response (P < 0.05) to C18:3n-3 concentrations. Raes et al. (2004) documented that concentrations of C22:5n-3 and C22:6n-3 were increased in ruminant muscle only when they were fed; for example, in ruminants fed marine products. Muscles do not appear to synthesize these longer-chain FA from C18:3n-3 in appreciable amounts, possibly due to a lack of sufficient enzyme activity.

Decreasing the dietary n-6:n-3 FAR by 6.8 fold (from 15.6 to 2.3; Table 1) via oil supplementation changed that same ratio by 2 fold in the foreshank muscle from 10.6 to 5.5 (Table 7). When high concentrate diets are fed, replacing an oil supplement having a high proportion of C18:2n-6 with one having a high proportion of C18:3n-3, such as linseed oil, can reduce in half the n-6:n-3 FAR in muscle, thus making it a more attractive meat for individuals with hypercholesterolemia. In our study, changes in n-6 and n-3 fatty acids in liver and foreshank tissue were detected after 28 d of feeding. If the trial had been extended, the differences may have been greater. Others have shown an increase in the n-6:n-3 FAR of tissue when feeding a fat source having a high concentration of C18:2n-6, namely soybean oil. When soybean oil (0 or 8% of dietary DM) was fed to lambs consuming a basal diet of conditioned lucerne hay or pelleted lucerne hay, the n-6:n-3 FAR in longissimus thoracis increased from 2.1 to 5.0 (Santos-Silva et al., 2004). Bessa et al. (2005) reported a similar response using lucerne-based diets. The n-6:n-3 FAR of lamb muscle increased from 2.0 to 6.1 when soybean oil was fed at 0 or 10% of dietary DM. When these same authors fed a concentrate instead of lucerne-based diet, adding soybean oil to the diet did not affect the n-6:n-3 FAR, 7.7 vs. 7.5 for 0 and 10% soybean oil, respectively. Therefore, the FA profile of the basal diet is an important factor in selecting a fat supplement. The n-6:n-3 FAR is often 2 or less for ruminants finished on pasture, whereas it is often between 6 and 10 for ruminants finished with concentrate-based diets (Raes et al., 2004). In order to increase the n-3 FA concentration of lamb muscle and therefore intake of n-3 by the human consumer, the dietary FA had to contain >5.8% C18:3n-3 in these high concentrate diets.

**IMPLICATIONS**

Increasing the omega-3 fatty acids in the diet with select oil sources decreased the omega-6 to omega-3 ratio in ruminal digesta, liver, and foreshank muscle of growing lambs fed high concentrate diets. This change would likely improve the suitability of lamb meat as a healthful food. Foreshank muscle was less responsive to increases in dietary omega-3 fatty acids than was liver. The conjugated linoleic acid concentration in tissue seems to be affected not only by the dietary concentration of linoleic acid but also by the dietary concentration of linoleic acid.

**LITERATURE CITED**


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