Intramuscular fat and fatty acid composition of longissimus muscle from divergent pure breeds of cattle


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ABSTRACT: The objective of this study was to compare the fatty acid (FA) composition of intramuscular fat from the LM of 3 divergent breeds of cattle: Angus (AN, n = 9), Brahman (BR, n = 7), and Romosinuano (RM, n = 11). Cattle were blocked by breed and finished 129 d before slaughter in one year and 157 d in the next year. Longissimus muscle samples were collected from each carcass between the 10th and 13th ribs, trimmed of external fat, frozen in liquid nitrogen, homogenized, and used for fat extraction, using a modified Folch procedure. Extracted fat was analyzed for FA by using a GLC system with an HP-88 capillary column. Fatty acid composition was expressed using both a normalized percentage (%) and gravimetric calculation (mg/g of fresh muscle tissue) in relation to degree of saturation, which was determined using a saturation index (ratio of total SFA to total unsaturated FA). Crude fat determination revealed that LM from AN purebred cattle had the greatest amount of intramuscular fat (7.08%; P = 0.001). Although intramuscular fat of LM from RM contained a reduced percentage of total SFA (P = 0.002) compared with AN, it had the greatest percentage of total PUFA (P < 0.001 and P = 0.020). The percentages of total MUFA were similar among the 3 breeds (P = 0.675). The gravimetric calculation, a measure of actual FA concentration, showed significantly greater concentrations of SFA (26.67 mg/g), MUFA (26.50 mg/g), and PUFA (2.37 mg/g) in LM from AN cattle, as compared with LM from BR and RM cattle (P < 0.001). Interestingly, BR purebreds had the least PUFA concentration (1.49 mg/g; P ≤ 0.001) in the LM, although their intramuscular fat content was similar to that of RM (P = 0.924). Regardless of breed, the MUFA proportion was always the greatest (47.58%; P ≤ 0.005), whereas PUFA was the least contributor to FA composition (1.49 to 2.37 mg/g and 4.36 to 8.78%; P < 0.001). Beef LM fatty acid composition was characterized by palmitic and oleic acids being the most abundant FA (P < 0.001). These results suggested a genetic variation in FA synthesis and deposition among breeds that influenced both marbling and its composition.

Key words: Angus, Brahman, cattle breed, fatty acid, intramuscular fat, Romosinuano

INTRODUCTION

The most prevalent heat-tolerant cattle in the United States are American Brahman (BR, Bos indicus) or crosses of English or Continental (Bos taurus) breeds with BR (Chase et al., 1997), providing the heat tolerance of BR and reproductive and carcass traits of B. taurus (Chase et al., 1997). Romosinuano (RM) is a tropically adapted Criollo breed that originated in the Sinu river region of Columbia (Rouse, 1977).

These cattle are noted for increased fertility, longevity, docile temperament, and crossbreeding with B. indicus (Chase et al., 1997). Riley et al. (2007) suggested that RM might be useful for crossbreeding in subtropical US environments. Some data on reproduction and carcass performance of RM are available, but little information is available on meat characteristics or composition. Beef marbling is one of the most important factors in determining beef quality and carcass value (USDA, 1997). Marbling characteristics are defined by fatty acid (FA) composition (Wood et al., 2003; Scollan et al., 2006). Different melting attributes of FA can cause variation in the firmness or appearance of intramuscular fat, and therefore in the marbling score (Wood et
Moreover, FA composition can influence oxidative susceptibility, cooked flavor, and nutritional value (Elmore et al., 1997, 1999; Elmore and Mottram, 2000; Vatansever et al., 2000; Campo et al., 2003; Wood et al., 2003). Differences in FA composition among breeds and genotypes have been related to carcass fatness; however, De Smet et al. (2004) suggested a possible genetic variation in FA metabolism among breeds that alters FA composition (Harper and Pethick, 2003; Scollan et al., 2006). Data on FA composition are lacking for BR and RM cattle, especially compared with Angus (AN), a B. taurus breed. Therefore, the objective of this study was to examine the intramuscular fat content and composition of the LM among AN, BR, and RM purebreds, focusing on FA composition and its relationship to muscle fatness.

**MATERIALS AND METHODS**

Steers were managed in accordance with the protocols approved by the Animal Care and Use Committee at the USDA, ARS, Grazinglands Research Laboratory.

**Sample Collection**

Samples were collected from cattle in a 3-yr project. However, only cattle slaughtered in the last 2 consecutive years were used in the present study. Calves were born the previous year, weaned in the fall, grazed on wheat pasture, and then finished in the feedlot. Calves were gradually introduced to the final diet over a 21-d period by decreasing the proportion of alfalfa hay and increasing the proportion of corn. Diets were sampled weekly for DM content. The final ration was formulated to contain 13.6% CP, 84.1% TDN, 2.02 Mcal/kg of NE\textsubscript{AN}, 1.33 Mcal/kg of NE\textsubscript{CR}, 1.33% Ca, and 0.37% P. Calves had ad libitum access to a trace mineral block.

**Proximate Analyses**

The moisture content of LM samples was determined using AOAC official methods 950.46 and 934.01 (AOAC, 1996). The dry residue after moisture analysis was covered by a layer of nonabsorbent cotton and the pan was folded, weighed, and placed in a Soxhlet extraction apparatus with petroleum ether and extracted for 18 h to determine crude fat content by using a modification of AOAC official method 991.36 (AOAC, 1996). The sample pan was then dried for 1 h at 100 to 110°C to remove the solvent before the dried weight was recorded.

Another sample was dried as above, and the residue was analyzed for ash content by using AOAC official method 923.03 (AOAC, 1996). Protein content was analyzed by combustion as described in AOAC official method 992.15 (AOAC, 1996), using a Leco FP 2000 N analyzer (Leco Corp., St. Joseph, MI). All proximate analyses were conducted in triplicate and qualitatively controlled by the analyses of Standard Reference Material 1546 (SRM 1546) purchased from the National Institute of Standards and Technology (Gaithersburg, MD).

**Fatty Acid Analyses**

**Fat Extraction.** The procedure of Folch et al. (1957) was used for fat extraction with the modifications described by Li and Watkins (2003). One gram of muscle powder was weighed into a 50-mL test tube, where fat was extracted using a 2:1 (vol/vol) chloroform:methanol organic solvent mixture. Initially, 7 mL of methanol was added immediately into the test tube when the sample was still frozen, and the whole mixture was homog-
enized for 30 s in a Polytron PT-3100 homogenizer (Kinematica AG, Lucerne, Switzerland). An additional 14 mL of chloroform was used in combination with methanol for another 30-s homogenization. After the mixture had been filtered, 12 mL of chloroform:methanol (2:1, vol/vol) was added to rinse the test tube and homogenizing probe. The rinsing solvent was filtered and combined with the previous extract. An additional 8 mL of 0.8% potassium chloride was subsequently added to the total extract to enhance the separation of the lower organic layer containing fat from the aqueous layer. The organic solvents were completely dried under a nitrogen stream at 40°C after removal of the aqueous portion by pipette aspiration. The test tube containing the fat residue was flushed with nitrogen gas, securely capped, and stored at −80°C for subsequent FA analysis.

**Preparation of FA Methyl Esters.** Transmethylation of FA was catalyzed by sodium methoxide in methanol (Li and Watkins, 2003). The reaction was performed at 50°C in a shaking water bath for 10 min. After the reaction, 0.1 mL of glacial acetic acid and 5 mL of saturated sodium chloride solution were added, followed by extraction of the FA methyl ester (FAME) mixture using 2 × 3 mL of hexane. The FAME extract was placed in a 15-mL test tube containing a layer approximately 1 mm deep of anhydrous sodium sulfate and was completely dried under a stream of nitrogen at 40°C. The dried residue was reconstituted with 3 mL of hexane, 1 mL of which was transferred to a 2-mL vial for gas chromatographic analysis.

**Gas Chromatographic Analysis.** The FAME were quantified by a gas chromatographic system (Agilent 6890N), using an HP-88 (100 m × 0.250 mm × 0.2 µm) capillary column (Agilent Technologies Inc., Palo Alto, CA). The HP-88 column, a high cyanopropyl-containing polysiloxane gas chromatographic column, was highly polar and suitable for cis/trans FAME isomer separation (Vickers et al., 2004). One microliter of FAME mixture was injected into the gas chromatographic system with a split-splitless injector and flame-ionization detector. The inlet temperature was 260°C, and the split ratio was 100:1. The carrier gas was helium at a constant flow of 2.0 mL/min. The oven temperature was programmed at an initial temperature of 140°C and held for 5 min, followed by an increase of 4°C/min up to 240°C, and held for 10 min. The detector was kept at 260°C, with an airflow of 300 mL/min, a hydrogen flow of 60 mL/min, and a helium makeup flow of 30 mL/min. The Supelco 37 Component FAME Mix was used as an external standard, along with nonadecanoate methyl ester as an internal standard (Sigma-Aldrich, St. Louis, MO).

Concentrations of FAME were calculated using the FAME standards and correction factors obtained from nonadecanoate methyl esters (internal standard). Both gravimetric calculation (mg/g of fresh muscle tissue) and normalized percentage (%) of total FA were used to determine the differences in FA composition. The saturation index (SI) was also calculated by determining the ratio of total SFA to total unsaturated FA (MUFA and PUFA).

**Statistical Analysis**

Breed-related differences in proximate composition, individual FA, and FA categories (SFA, MUFA, and PUFA) were determined by ANOVA using a generalized randomized complete block design, with cattle breed serving as treatment and year serving as the blocking effect. The year was treated as fixed block effect because there were only 2 blocks in the experimental design. To characterize the FA composition of beef LM, differences among individual FA or FA categories were analyzed using a generalized randomized complete block design, with each FA or FA category serving as a treatment and cattle breed serving as a fixed blocking effect. The statistical analysis was accomplished using the MIXED procedure (SAS Inst. Inc., Cary, NC). Means were separated by protected t-test using the LSMEANS/PDIFF option in the MIXED procedure. The statistical significance was determined at P ≤ 0.05 unless otherwise stated. Means were reported as least squares means with SEM unless noted otherwise.

**RESULTS**

There was no interaction between cattle breed and year (P > 0.05) and no overall year effect on proximate composition of the LM, gravimetric concentrations, and normalized percentages of FA categories and individual FA (P > 0.05), except for an overall year effect on 18:3n-3 (linolenic acid, P = 0.008). Statistically, cattle breed presented an overall effect on intramuscular fat content and FA composition.

Differences in composition (crude fat, moisture, protein, and ash) of LM were evident among the 3 breeds (Table 1) because LM from AN cattle had a greater amount of intramuscular fat (7.08%) compared with LM from BR and RM (3.13 and 3.06%, respectively). The moisture, protein, and ash contents of LM from AN were less (P ≤ 0.05) than those of LM from BR and RM. A 3.95 to 4.02% (g/100 g of muscle tissue) greater intramuscular fat content of LM from AN led to 2.12 to 2.24% less moisture content compared with LM from BR and RM, respectively. Tissue protein content varied from 20.90 to 21.84% among breeds (P ≤ 0.05).

The SI and SFA percentage of total FA composition varied significantly among breeds (Figures 1 and 2). The values of LM from AN cattle were only significantly greater (P = 0.001 and P = 0.002, respectively) than the LM from RM (0.92 and 0.80, and 47.83 and 44.39%, respectively). The intramuscular fat of LM from RM contained a significantly greater (P < 0.001 and P = 0.020) percentage of PUFA as compared with intramuscular fat of LM from both AN and BR (Figure 2; 8.78, 4.36, and 6.69%, respectively). The PUFA percentage of LM from BR (6.69%) was intermediate
to and significantly different from those found in LM from RM and AN (Figure 2; \( P = 0.008 \) and \( P = 0.020 \), respectively). The percentages of MUFA were similar among all breeds evaluated (\( P = 0.675 \)).

Unlike the normalized percentage calculation, the gravimetric calculation (Figure 3), which is a measure of actual concentrations of FA in LM, revealed significantly greater concentrations of SFA (26.67 mg/g), MUFA (26.50 mg/g), and PUFA (2.37 mg/g) in LM from AN cattle as compared with LM from BR and RM cattle (\( P < 0.001 \)). Additionally, BR cattle had the least PUFA concentration (Figure 3; 1.51 mg/g) in the LM as compared with those in LM from AN and RM cattle (\( P < 0.001 \)).

There were significant differences (\( P \leq 0.05 \)) among breeds in both concentrations and percentages of specific FA (Table 2). Again, the FA profile of LM from AN was found to have significantly greater (\( P \leq 0.024 \)) concentrations not only of all SFA, consisting of myristic (14:0), palmitic (16:0), stearic (18:0), and arachidic acids (20:0), but also of most MUFA, such as myristoleic (14:1 cis-9), palmitoleic (16:1 cis-9), oleic (18:1 cis-9), and trans octadecenoic (18:1 trans). Moreover, the LM from AN had either significantly greater (\( P \leq 0.032 \)) or similar (\( P = 0.256 \) and 0.785) concentrations of PUFA as compared with those from BR and RM. Interestingly, the normalized percentages of FA showed mostly opposite results, even on some SFA. The percentages of all PUFA and the most predominant MUFA (16:1 cis-9, 18:1 cis-9, 18:1 trans) of LM from AN were either similar to (\( P = 0.129 \) and 0.439) or less than (\( P = 0.001 \) to 0.035) those from BR and RM cattle (Table 2). Surprisingly, regardless of differences in muscle fatness and unlike their concentrations, the percentages of stearic and arachidic acids were the same in all breeds (\( P = 0.490 \), \( P = 0.333 \), respectively).

Regardless of cattle breed, palmitic acid was the predominant SFA (\( P < 0.001 \)), whereas oleic acid, a MUFA, was the most abundant FA in the LM (\( P < 0.001 \)). This explains why the relative proportion of MUFA,
expressed as percentage of total FA, was greater than that of SFA \((P \leq 0.005)\), even though these differences were not found to be significant when expressed as milligrams of FA per 1 g of muscle tissue \((P = 0.440)\). For all breeds, PUFA concentrations \((1.49 \text{ to } 2.37 \text{ mg/g})\) and PUFA percentages \((4.36 \text{ to } 8.78\%)\) in muscle tissue were always least \((P < 0.001)\) compared with percentages of SFA and MUFA.

Further statistical analysis indicated that most FA concentrations were positively correlated with intramuscular fat content (Table 3; \(P \leq 0.005\)), although the levels of correlation were different. However, the relationship between FA percentages and muscle fatness varied in levels of significance and correlation (Table 3). Whereas palmitic acid percentage was well correlated with intramuscular fat content \((r = 0.722; \ P < 0.001)\), the second predominant SFA, stearic acid, was not \((P = 0.902)\). As expected, the percentages of PUFA and most FA in this category were inversely correlated with muscle fatness \((P < 0.001)\).

**DISCUSSION**

The proximate analyses are important to provide an overview of muscle composition before any other in-depth analysis takes place, and they were also considered a quality control tool to ensure the accuracy of intramuscular fat content. As expected, the LM from AN had the greatest intramuscular fat associated with less moisture content. Because the fatty tissues contain little or no moisture, the greater the fat content of muscle, the less the total water content (Romans et al., 2001). Subsequently, the water-soluble components, most likely minerals in ash content, were less in LM from AN as well.

Fatty acid composition can be described not only quantitatively by concentrations (gravimetric calculation) of specific FA, but also qualitatively by their relative proportions (normalized percentages to total FA; Huerta-Leidenz et al., 1991, 1993; Alfaia et al., 2006). The normalized percentage describes the interactive and comparable relationships among FA regarding lipid quality, whereas the gravimetric concentration \((\text{mg/g of fresh tissue})\) describes the actual amount of FA in tissues. It is clear that the FA concentrations are well correlated with muscle fatness (De Smet et al., 2004). Triacylglycerols depositing into adipocytes are mostly from dietary sources or de novo synthesis (Vernon, 1980; Jenkins, 1993; Gerrard and Grant, 2003). Because FA escape the rumen after being biohydrogenated (Jenkins, 1993; Demeyer and Doreau, 1999), de novo synthesis is probably more important to the occurrence of unsaturated FA because of terminal elongation and desaturation (Voet et al., 2006). Unlike nonruminant animals,
Table 2. Fatty acid concentrations, percentages, and SEM of raw LM obtained from Angus (AN, n = 9), Brahman (BR, n = 7), and Romosinuano (RM, n = 11) purebred cattle.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Concentration, mg/g</th>
<th>Percentage, %</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>AN 2.01 ± 0.14, 0.80 ± 0.05, 0.70 ± 0.07</td>
<td>3.63 ± 0.16, 3.58 ± 0.18, 2.97 ± 0.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>BR 16.11 ± 1.06, 6.16 ± 1.14, 6.30 ± 1.17</td>
<td>28.92 ± 0.27, 26.95 ± 0.31, 26.58 ± 0.49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>RM 8.50 ± 0.79, 3.52 ± 0.23, 3.52 ± 0.57</td>
<td>15.19 ± 0.55, 15.79 ± 0.60, 14.76 ± 0.61</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>16:0</td>
<td>AN 16.11 ± 1.06, 6.16 ± 1.14, 6.30 ± 1.17</td>
<td>28.92 ± 0.27, 26.95 ± 0.31, 26.58 ± 0.49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>BR 6.16 ± 1.14, 6.30 ± 1.17, 6.30 ± 1.17</td>
<td>15.79 ± 0.60, 14.76 ± 0.61, 14.76 ± 0.61</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>RM 3.52 ± 0.23, 3.52 ± 0.57, 3.52 ± 0.57</td>
<td>14.76 ± 0.61, 14.76 ± 0.61, 14.76 ± 0.61</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>18:0</td>
<td>AN 0.05 ± 0.004, 0.02 ± 0.004, 0.02 ± 0.005</td>
<td>0.89 ± 0.006, 0.88 ± 0.006, 0.88 ± 0.006</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>BR 0.19 ± 0.02, 0.15 ± 0.03, 0.15 ± 0.03</td>
<td>0.83 ± 0.008, 0.83 ± 0.008, 0.83 ± 0.008</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>RM 0.68 ± 0.14, 0.77 ± 0.14, 0.77 ± 0.14</td>
<td>0.83 ± 0.008, 0.83 ± 0.008, 0.83 ± 0.008</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>18:1 cis-9</td>
<td>AN 1.97 ± 0.13, 0.77 ± 0.14</td>
<td>3.59 ± 0.17, 2.89 ± 0.18, 3.21 ± 0.19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>BR 1.33 ± 0.13, 0.78 ± 0.15</td>
<td>2.41 ± 0.25, 2.06 ± 0.27, 3.25 ± 0.27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>RM 0.19 ± 0.02, 0.10 ± 0.01</td>
<td>0.35 ± 0.03, 0.35 ± 0.03, 0.35 ± 0.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>18:1 trans (undifferentiated)</td>
<td>AN 0.02 ± 0.004, 0.02 ± 0.005</td>
<td>0.02 ± 0.005, 0.02 ± 0.005, 0.02 ± 0.005</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>BR 0.04 ± 0.004, 0.04 ± 0.004</td>
<td>0.04 ± 0.004, 0.04 ± 0.004, 0.04 ± 0.004</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>RM 0.01 ± 0.001, 0.01 ± 0.001</td>
<td>0.01 ± 0.001, 0.01 ± 0.001, 0.01 ± 0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>18:2 (undifferentiated)</td>
<td>AN 1.87 ± 0.11, 1.53 ± 0.12</td>
<td>3.41 ± 0.43, 2.91 ± 0.47, 6.92 ± 0.48</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>BR 1.10 ± 0.12, 1.53 ± 0.12</td>
<td>4.91 ± 0.47, 6.92 ± 0.48, 6.92 ± 0.48</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>RM 0.08 ± 0.003, 0.08 ± 0.003, 0.08 ± 0.003</td>
<td>0.15 ± 0.003, 0.15 ± 0.003, 0.15 ± 0.003</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>20:0</td>
<td>AN 0.05 ± 0.006, 0.05 ± 0.006, 0.05 ± 0.006</td>
<td>0.17 ± 0.006, 0.17 ± 0.006, 0.17 ± 0.006</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>BR 0.12 ± 0.006, 0.11 ± 0.007, 0.11 ± 0.007</td>
<td>0.23 ± 0.004, 0.23 ± 0.004, 0.23 ± 0.004</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>RM 0.09 ± 0.006, 0.08 ± 0.007, 0.07 ± 0.007</td>
<td>0.17 ± 0.004, 0.17 ± 0.004, 0.17 ± 0.004</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Within each row of concentration or percentage categories, means without common letters differ (P < 0.05).

Values reported as least squares means ± SEM.

Fatty acid concentrations based on 1 g of fresh muscle tissue.

Fatty acid normalized percentages to total fatty acids.

Figure 3. Concentrations (mg/g of fresh muscle tissue) of total SFA, total MUFA, and total PUFA of raw LM obtained from Angus (AN, n = 9), Brahman (BR, n = 7), and Romosinuano (RM, n = 11) purebred cattle. The SE bars represent the variation within each breed. Within each fatty acid category, means without common letters (a-c) differ (P ≤ 0.05).
FA synthesis in beef cattle is minimal in the liver but is extensive in adipose tissue (Byers and Schelling, 1993). Despite differences in the metabolism and synthesis of FA, all FA have the possibility of occurring in adipocytes when fat continues to deposit into these cells, although their deposition rates can vary. However, SFA and MUFA always accumulate faster than does PUFA content (Scollan et al., 2006).

In this study, the LM from AN was found to have a greater amount of intramuscular fat (50% greater than LM from BR and RM). Because of a significantly greater accumulation of fat, LM from AN purebreds also had greater concentrations of most FA or FA categories, as compared with LM from BR and RM. Most recent studies on FA composition have been reported using the normalized percentage of total FA (calculated by GLC-reported peak area) to determine whether a quantitative change in intramuscular fat would cause a qualitative change in FA composition (e.g., PUFA to SFA ratio or n-3 to n-6 ratio; Duckett et al., 1993; Nürnberg et al., 1999; Siebert et al., 2000; Pitchford et al., 2002; Chung et al., 2006; Indurain et al., 2006; Monteiro et al., 2006; Padre et al., 2006). Of the very few studies, the present study reported the actual concentrations of FA. At similar amounts of muscle fatness, this study was able to obtain results comparable with those from the USDA National Nutrient Database for Standard Reference (USDA, ARS, 2007) for separable lean of Choice rib-eye steak, although the information on breed and diet for cattle represented in the USDA database was unknown. The increase in FA concentrations with amounts of intramuscular fat was expected because of the positive correlation between muscle fatness and most FA concentrations, except for some PUFA. Additionally, the correlation analysis showed that the SFA and MUFA concentrations were more closely correlated with muscle fatness (r = 0.996; r = 0.988, respectively) than was the PUFA concentration (r = 0.723). This phenomenon was explained by the storing function of neutral lipids and the structural function of polar lipids (Indurain et al., 2006). An increase in intramuscular fat normally results in the continuous deposition of neutral lipids (Duckett et al., 1993; Siebert et al., 1996; Nürnberg et al., 1999; Indurain et al., 2006). Neutral lipids, predominantly triacylglycerols, are rich in SFA and MUFA (Itoh et al., 1999; Choi et al., 2000; Laborde et al., 2001), with approximately 80% of FA being 16:0, 18:0, and 18:1 cis-9 (Scollan et al., 2006; USDA, ARS, 2007) because of the mechanism of de novo FA synthesis (De Smet et al., 2004), whereas polar lipids, predominantly phospholipids, have a much greater PUFA content (Nürnberg et al., 1999; De Smet et al., 2004; Scollan et al., 2006). Therefore, the continuous accretion of intramuscular triacylglycerols expected in LM adipocytes from AN during muscle fattening could account for the dramatic aggregation of SFA and MUFA concentrations. In addition to de novo synthesis, dietary FA, which are extensively biohydrogenated in the rumen, are a great source of SFA that can be incorporated into stored triacylglyc-
erols despite limited absorption rates of long-chain SFA (Byers and Schelling, 1993).

The PUFA to SFA ratio has frequently been used to interpret FA composition and to evaluate the nutritional value of fat (Alfaia et al., 2006). However, use of the PUFA to SFA ratio unintentionally eliminates the effect of MUFA, especially oleic acid, which has been reported to be a valuable component of healthy diets (Kazala et al., 1999; Bertone et al., 2002; Baghurst, 2004; Scollan et al., 2006). In this study, the SI was used to take MUFA into consideration and to directly compare total SFA with total unsaturated FA. The SI increased with fatness, but not as much as did SFA concentrations (r = 0.555) because both SFA and MUFA concentrations became greater (r = 0.996 and r = 0.988, respectively) with increasing amounts of intramuscular fat. The increase in MUFA concentration, especially regarding the increased concentration of oleic acid (9.36 to 22.50 mg/g), might be an indicator of desaturase activity in adipose tissue (e.g., Δ⁵-desaturase system; Bauman et al., 1999). It is evident that primary lipids entering the ruminant enterocecy are predominantly free SFA, together with a small amount of 2-monoleoylglycerols (Byers and Schelling, 1993). The fact that FA desaturases may convert as much as 10% of stearic acid to oleic acid in the enterocecy (Byers and Schelling, 1993) could be a possible explanation for the significant presence of oleic acid. Alfaia et al. (2006) proposed an increase in stearoyl-CoA desaturase gene expression to explain the increased oleic acid concentration in their study. In comparison with other studies, oleic acid has long been found to be the predominant FA in beef (Kazala et al., 1999; Carnovale and Nicoli, 2000; Laborde et al., 2001; Oka et al., 2002; Silva et al., 2003; Archibeque et al., 2005; Scollan et al., 2006), although FA in these studies were reported only in normalized percentages. Again, the concentrations of oleic acids in this study were comparable with those in the USDA National Nutrient Database for Standard Reference (USDA, ARS, 2007: 32 mg of oleic acid in 1 g of separable lean only of Choice rib-eye steak with 8.3% extractable fat, as compared with 22.50 mg/g of muscle tissue with 7.08% intramuscular fat in this study). On the other hand, the relative proportions of FA did not change with muscle fatness, as was seen with FA concentrations. Saturated FA percentage still increased with fatness, which led to greater SFA percentages in LM from AN compared with LM from RM. Indeed, the correlations between fatness and SI or SFA percentage were not very high (r = 0.555 and r = 0.542, respectively). These weak correlations could explain why percentages of stearic and arachidic acids were not different among the 3 breeds. In contrast, Laborde et al. (2001) found a significant difference between Red Angus and Simmental LM only in stearic acid percentages. The palmitic and myristic acid concentrations were found to be similar between the 2 breeds, whereas arachidic acid percentage was not reported by these authors. Interestingly, they also found no difference in SFA proportion of total extractable lipid, which also did not differ between the breeds. However, Pitchford et al. (2002) observed a genetic correlation between intramuscular fat content and palmitic and oleic acids when investigating the variation in fatness and FA composition of AN, Hereford, South Devon, Limousin, Jersey, Wagyu, and Belgian Blue crossbreds. Even though SFA were not specifically reported, Nürnberg et al. (1999) revealed that an increase in intramuscular fat content of 200 bulls from 3 different breeds (Galloway, German Holstein, and White-Blue Belgian) was positively associated with SFA proportion. However, their data indicated that stearic acid did not increase with muscle fatness at 18 mo of age. Hence, these authors suggested that the positive relationship between muscle fatness and SFA proportion was probably due to the deposition of other SFA rather than stearic acid. Palmitic acid should be the most suspected FA because it is the final product of the de novo FA synthesis pathway (Voet et al., 2006). The results from the studies mentioned previously as well as those from this study suggested that the differences in proportion of total SFA and individual SFA were dependent on genetics and fatness, but these seemed to be unpredictable.

Further analysis of the relationship between SI and PUFA percentages and intramuscular fat suggested that an increase in SI by greater fatness in LM from AN was also caused by a decrease in its PUFA proportion (r = −0.822), not only by an increase in the SFA proportion itself. Numerous studies have confirmed that the PUFA proportion is decreased with an increased abundance of intramuscular fat. Warren et al. (2003) reported that Aberdeen-Angus purebreds exhibited a greater muscle fat content compared with Holstein-Friesians, which resulted in a reduced PUFA proportion for the Aberdeen-Angus. A clear inverse relationship between the PUFA to SFA ratio and total intramuscular fat was graphically summarized in the study by De Smet et al. (2004), with values for the PUFA to SFA ratio and intramuscular fat content being collected from many other studies (Marmer et al., 1984; Duckett et al., 1993; Enser et al., 1996; Itoh et al., 1999; Choi et al., 2000; French et al., 2000; Raes et al., 2001; Nürnberg et al., 2002). Despite the different results in SFA, the previously mentioned studies (Nürnberg et al., 1999; Laborde et al., 2001; Pitchford et al., 2002) all showed a decrease in PUFA proportion during muscle fattening. Therefore, it was not surprising that the LM from RM with reduced intramuscular fat content had a greater PUFA percentage as compared with the LM from AN. As mentioned, the decreased muscle fat content led to a decreased abundance of triacylglycerols deposited, which increased the proportion of PUFA-rich polar lipids in LM from RM (De Smet et al., 2004; Scollan et al., 2006), to contribute more to total muscle lipids. However, it was interesting and important to realize that even though BR and RM had the same amounts of LM intramuscular
fat, the LM from RM cattle was significantly greater than the LM from BR cattle in both PUFA percentage and concentration.

The de novo synthesis in adipocytes of muscle as well as elongase and desaturase activities might play an important role because so far, no study has suggested breed-related differences in biohydrogenation in the rumen. Rumen biohydrogenation results in more SFA and very little PUFA (Byers and Schelling, 1993; Jenkins, 1993). Because of the decreased absorption rate of FA by an increase in FA saturation (Byers and Schelling, 1993; Voet et al., 2006), a significant part of SFA and long-chain PUFA probably came from de novo synthesis catalyzed by the FA synthase complex, followed by elongation and desaturation (Voet et al., 2006). Hence, it is reasonable to suggest from this study that breed differences could quantitatively influence not only fat deposition but also FA synthesis, which was also highlighted in the review by De Smet et al. (2004). This hypothesis was supported by the greater percentage of palmitic acid in LM from AN as compared with the LM from BR and RM and by the fact that the final product of FA synthesis is, as mentioned, mostly palmitate, the esterified form of palmitic acid (Voet et al., 2006).

Results from this study confirmed the characteristics of FA composition of beef LM, with palmitic, oleic, and linoleic (undifferentiated) acids being the predominant SFA, MUFA, and PUFA, respectively (De Smet et al., 2004; Padre et al., 2006; Scollan et al., 2006; USDA, ARS, 2007). The fact that the percentages of 18:1 cis-9 and total MUFA were not different among these 3 breeds might indicate similar Δ⁹-desaturase activity in LM. There was no difference in Δ⁹-desaturase indices (data not shown; determined by (16:1 cis-9)/(16:1 cis-9 + 16:0) for C16 and (18:1 cis-9)/(18:1 cis-9 + 18:0) for C18; Malau-Aduli et al., 1997, 1998) among the 3 breeds. The correlation analysis also indicated that Δ⁹-desaturase indices did not correlate with muscle fatness (r = 0.144 for C16; r = 0.089 for C18). Cameron et al. (1994) also found no difference in stearyl Co-A desaturase activity and messenger RNA among subcutaneous adipose tissues from AN and Wagyu steers, even though adipose tissue was found to possess the greatest stearyl Co-A desaturase activity (St. John et al., 1991; Chang et al., 1992). In addition, Archibeque et al. (2005) suggested that FA indices did not reflect the actual stearyl Co-A desaturase activity in adipose tissue after they studied FA composition of adipose tissues from 45 AN steers that had been fed an oil-supplemented finishing diet. Therefore, the effect of Δ⁹-desaturase activity in this study was inconclusive because MUFA from dietary sources, another important factor that should be accounted for, were not monitored.

Genetic differences among AN, BR, and RM purebreds affected FA composition of LM by differentiating muscle fattening and FA synthesis. To confirm this hypothesis, the whole FA composition values of LM from the 3 breeds were recalculated and standardized to 1 g of intramuscular fat. The concentrations of FA were expressed as milligrams per gram of intramuscular fat (data not shown). Because the muscle fatness was the same (1 g), any statistical variation among the 3 breeds should be an indicator of genetic effects on FA synthesis. As expected, differences in FA concentration (mg/g of intramuscular fat) were found for myristic acid (14:0), palmitic acid (16:0), and total SFA; for myristoleic acid (14:1) and palmitoleic acid (16:1); and for total PUFA and most FA in this category. In fact, the FA mentioned previously were mainly from de novo synthesis. Interestingly, genetic variation was again found to cause no effect on stearic and oleic acids, as well as total MUFA (data not shown), which agreed with the statistical analyses of FA percentage previously reported. The results suggested that stearic and oleic acids might be more affected by dietary sources and the elongation and desaturation processes, rather than by FA synthesis itself. The analyses of Δ⁹-desaturase indices after intramuscular standardization (data not shown) confirmed these findings because no difference was found in C16 and C18 indices among the breeds. These important findings suggest that FA composition needs to be approached both quantitatively (concentration) and qualitatively (percentage or relative proportion).

Some previous studies determined that a few genes such as GDF8 (myostatin; Wegner et al., 1998; Harper and Pethick, 2004) and the TG5 SNP (the thyroglobulin 5’ leader sequence; Barendse et al., 2004) may affect marbling deposition and FA composition. However, the differences in these genes among breeds are not well elucidated. Leptin, a hormone in the fat metabolism pathway produced by the obese gene, which has been shown to affect the amount of fat deposition in beef (Geary et al., 2003; Nkrumah et al., 2005), could be another genetic variant. Leptin is a 16-kDa protein hormone secreted from white adipocytes (Houseknecht et al., 1998). A DNA variant altering a critical AA will affect the folding and function of this hormone. A cytosine-to-thymine transition will encode an AA change of an arginine to a cysteine (Buchanan et al., 2002). The cytosine allele has been associated with leaner carcasses, whereas the thymine allele has been associated with fatter carcasses (Buchanan et al., 2002; Nkrumah et al., 2005). These authors found that animals with the fatter genotype had a 39% increase in serum leptin concentration and a 9 to 13% increase in marbling score. In Buchanan et al. (2002), AN cattle were found to have greatest frequencies of the fatter allele of 58% as compared with Hereford, Charolais, and Simmental cattle, which might explain the increased marbled characteristics of AN. Hence, although genetic information was not determined in the present study, the differences in marbling deposition and FA composition of LM from AN, BR, and RM purebreds in this study were surely affected by genetic diversity.

Carcass fatness is very important in determining carcass value. In addition to the amount of marbling fat, which is scored visually, the FA composition of marbling
can be a determinant of beef flavor, quality, and shelf life. This study revealed significant differences among AN, BR, and RM purebred cattle in both muscle fatness and intramuscular fat composition. These traits have been found to be relatively heritable and could be used in developing breeding management strategies. These results will also help elucidate the effects of crossingbreeding strategies on FA composition, beef flavor, fat oxidation, and ultimately retail shelf life.

**LITERATURE CITED**


