Fatty acid profile and cholesterol and retinol contents in different locations of Celta pig breed

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SUMMARY: The fatty acid profile (total, neutral and polar lipids) and cholesterol and retinol contents of the intramuscular - *Longissimus dorsi* (LD) and *Psoas major* (PM) -, adipose -rump, covering *Biceps femoris* muscle, ventral and dorsal-, perirenal and hepatic fat of the Celta pig breed (Galicia, northwest Spain) were evaluated. Oleic acid was the most abundant fatty acid, except in the polar lipids of the intramuscular fat, where the most abundant fatty acid was linoleic acid. The fatty acid composition of total and neutral lipids was influenced more by the location than the fatty acids of polar lipids. There were some differences only in minor fatty acids of intramuscular and subcutaneous fat between castrated females and castrated males. The fatty acids of neutral lipids were also more influenced by sex than the fatty acids of polar lipids. The cholesterol and retinol contents showed no significant differences by sex. The LD muscle showed a higher content of intramuscular fat than the PM muscle. The greatest cholesterol values were described in the liver. Subcutaneous locations showed higher cholesterol levels than muscles.

KEYWORDS: Celta pig; Cholesterol; Fatty acids; Location; Retinol; Sex

RESUMEN: Perfil de ácidos grasos y contenidos de colesterol y retinol en diferentes localizaciones del cerdo de raza Celta. Se estudió el perfil de los ácidos grasos y los contenidos en colesterol y retinol en la grasa intramuscular - *Longissimus dorsi* (LD) y *Psoas major* (PM) -, subcutánea -grupa, ventral, dorsal y la grasa que rodea al músculo *Biceps femoris* -, perirrenal y hepática de cerdos de raza Celta (Galicia, noroeste de España). El ácido oleico fue el ácido graso más importante, excepto en los lípidos polares de la grasa intramuscular, donde el ácido graso más abundante fue el ácido linoleico. La composición de ácidos grasos de los lípidos totales y neutros estuvo más influenciada por la localización grasa que los ácidos grasos de los lípidos polares. Se encontraron ligeras diferencias entre hembras y machos castrados en algunos ácidos grasos minoritarios en la grasa intramuscular y subcutánea. Los ácidos grasos de los lípidos neutros fueron más influenciados por el sexo que los ácidos grasos de los lípidos polares. El contenido en colesterol y retinol no mostró diferencias significativas entre sexos. El músculo LD presentó un mayor contenido de grasa intramuscular que el PM. Los mayores valores de colesterol fueron determinados en el hígado. En las localizaciones subcutáneas los contenidos en colesterol fueron más elevados que en la grasa intramuscular.

PALABRAS CLAVE: Ácidos grasos; Cerdo Celta; Colesterol; Localización; Retinol; Sexo


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1. INTRODUCTION

The Celta pig is an autochthonous porcine breed raised traditionally in Galicia (northwest of Spain). Because of their poor growth rate, carcass conformation and crossbreeding between local pigs and improved breeds, the Celta breed became extinct during the second half of the 20th century. At the present time, their population is part of a project of recuperation, conservation, and promotion of this breed backed by the Autonomous Regional Government of Galicia (Carril et al., 2001).

The aim of the recovery of the Celta pig breed is not only to obtain high-quality meat, but also to contribute to the environmental preservation and the recovery of traditional practices. Celta pigs can be reared in an intensive, semi-intensive or free-range system. The use of local breed and extensive or semi-extensive feeding systems based on natural feed resources (chestnuts, grass, etc.) could also help to maintain development in the rural populations of northwestern Spain. On the other hand, the recovery of the Celta pig breed also has the objective of producing high-quality dry-cured meat products such as “lacón” (Lorenzo et al., 2014), dry-cured ham (Bermúdez et al., 2012; 2014; Lorenzo et al., 2013) and “chorizo” (Gómez and Lorenzo, 2013).

The carcass quality and meat quality are determined by factors such as breed, sex, rearing system, age at slaughter or physical exercise. Among the compositional traits related to meat quality, the fat is known to be very important. Fat contributes to the texture, flavor and juiciness of the meat. The fatty acid content and its types determine the physical and nutritional characteristics of fat, and they are associated with human health which is why they are of such great interest (Václavková and Becˇková, 2007; Wood et al., 2004).

Many studies have reported that the constitutive fatty acids of the fat are influenced by factors such as genotype (Gandemer et al., 1992), types and percentages of feeding (García-Olmo et al., 2002; Rentfrow et al., 2003), age at slaughter (Bragagnolo and Rodríguez-Amaya, 2002), location in the carcass (Delgado et al., 2002; Vázquez et al., 1996), sex and castration (Cordero et al., 2010; Wood et al., 1989) or slaughter weight (Palanska et al., 1993). However, there are few studies that compare the effects of the anatomical location and sex on fatty acids, cholesterol, retinol and fat contents of the carcass of castrated females and castrated males.

The aim of this research was to study the fatty acid profile (total lipids, neutral lipids and polar lipids), cholesterol and retinol contents of the fat of the Celta pig breed of eight fat deposits in castrated females and castrated males.

2. MATERIALS AND METHODS

2.1. Pigs, samples and diet

In order to carry out this study, 12 castrated Celta pigs (6 males and 6 females) were continuously fed with commercial compound feed. The piglets, which were vaccinated and deparasitized following the usual protocols, were suckled until an age of 40 days. The males were castrated at 75 days of age and the females were castrated at 90 days of age. All pigs were reared and fattened until the age of 16 months in an intensive regime, with a livestock density of 12 animals per hectare.

After slaughtering, and after 24 hours of refrigeration, samples of intramuscular fat (Longissimus dorsi and Psoas major muscles), subcutaneous fat (rump, subcutaneous ventral, subcutaneous dorsal and covering of the Biceps femoris muscle), peripheral fat and liver fat were obtained from each carcass.

The chemical composition, retinol and cholesterol contents and fatty acid composition of the diet are shown in Table 1. The chemical composition analysis of commercial compound feed was carried out according to the methods of A.O.A.C. (2000) and fatty acids, retinol and cholesterol analysis were performed according to the methods described below.

2.2. Fat and fatty acid analyses

The intramuscular and hepatic fats were extracted following the procedure described by Folch et al. (1957) and the subcutaneous and perirenal fats were extracted following the procedure described by De Pedro et al. (1997).

In order to determine if there are significant differences between the two methods of fat extraction, fat was extracted in triplicate from two different locations with both methods, and the lipid profile was analyzed. There were no significant differences (P<0.05) in the fatty acid profile between the two extraction methods.

Intramuscular fat content (IMF) was quantified according to the ISO 1443:1979.

The neutral and polar lipids from muscles, perirenal, subcutaneous and liver samples were obtained according to the procedure developed by Kuluzny et al. (1985). Fat extracts were methylated and the fatty acid profile of both lipids were determined using the procedure described by Franco et al. (2006). Fatty acid methyl esters were analyzed by Gas Chromatography using a Thermo Finnigan Trace GC (Thermo Finnigan, Austin, TX, USA). The separation of the different fatty acids was carried out in an Innowax column: 30 m; 0.25 mm ID; 0.25 μm film thickness (Agilent Technologies, Palo Alto, CA, USA). The temperature of the detector was 250 °C and that of the injector 230 °C. The chromatographic conditions used in the determination of...
The gases used were air (350 mL·min⁻¹), hydrogen (335 mL·min⁻¹) and helium (30 mL·min⁻¹).

Each fatty acid was expressed as a percentage of the total fatty acids. The indices for the activities of Δ9, Δ6 and Δ5 desaturase and the elongase activity were determined as described by Ntawubizi et al. (2009). The thioesterase index was calculated according to Zhang et al. (2007).

2.3. Cholesterol and retinol analyses

Tissue samples and commercial compound feed were saponified according to the procedure described by Saldanha et al. (2006), the unsaponifiable material was extracted by the procedure of López-Cervantes et al. (2006) and cholesterol and retinol contents were quantified by high performance liquid chromatography (HPLC), using a Thermo Finnigan HPLC (Thermo Finnigan, Rodano, Italy) equipped with an on-line solvent degasser (model Spectrasystem SCM1000), solvent delivery system (model Spectrasystem P4000), autosampler with a column oven (model Spectrasystem AS3000), rhenodyne injector with a 10 μL loop, photodiode array detector (model Spectrasystem UV6000LP) and a system controller ChromQuest 4.1. The analytical column was Ultrasphere ODS (250 mm×4.6 mm I.D.) with a particle size 5 μm (Beckman, Fullerton, USA).

The HPLC conditions were as follows: mobile phase 68:28:4 (v/v/v) methanol:acetonitrile:water; a flow rate of 2 mL·min⁻¹; column temperature 36 °C. The detection was operated using two channels of a diode-array spectrophotometer, 325 nm for retinol and 208 nm for cholesterol. Retinol and cholesterol in the non-saponifiable fractions of the lipid fraction were identified by the comparison of retention times and UV absorption spectra with those obtained for corresponding standards (Sigma Chemical Co., Saint Louis, MO, USA). Results are expressed as mg·100 g⁻¹ of sample for cholesterol and μg·g⁻¹ of sample for retinol.

All samples and patterns of fatty acids, cholesterol and retinol were injected at least in duplicate. Repeatability tests were performed injecting a pattern and a sample consecutively six times in a day. Reproducibility tests were also carried out, injecting the pattern and the sample twice a day for 3 days, under the same experimental conditions. Significant differences were not found among the results obtained in any of the tests.

2.4. Statistical analysis

Mean and standard error of the mean values were determined for all the parameters and the results were expressed as mean and standard error of the mean for six samples in each group. Analysis of variance (ANOVA) was carried out in order to

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**TABLE 1. Chemical composition (expressed as g·100 g⁻¹) and fatty acids of commercial compound feed**

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<table>
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<tr>
<td>Retinol (μg·100 g⁻¹)</td>
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</tbody>
</table>

**Fatty acids**

| C12:0  | 5.56±0.06 |
| C14:0  | 41.70±0.19 |
| C14:1  | 0.17±0.02 |
| C15:0  | 4.87±0.00 |
| C15:1  | 0.97±0.03 |
| C16:0  | 873.46±0.37 |
| C16:1 n7 | 57.13±0.26 |
| C17:0  | 12.98±0.24 |
| C17:1  | 7.19±0.08 |
| C18:0  | 361.43±0.08 |
| C18:1 c n9 | 1048.48±0.57 |
| C18:2 n6 | 1012.74±3.37 |
| C18:3 n6 | 2.12±0.08 |
| C18:3 n3 | 97.54±0.24 |
| C20:0  | 4.98±0.04 |
| C20:1 n9 | 15.11±0.09 |
| C20:2 n6 | 5.10±0.34 |
| C20:3 n6 | 1.50±0.06 |
| C20:4 n6 | 3.73±0.20 |
| C20:3 n3 | 1.71±0.31 |
| C20:5 n3 | 1.66±0.05 |
| C22:0  | 5.89±0.07 |
| C22:1 n9 | 3.84±1.09 |
| C22:2 n6 | 306.69±4.63 |
| C23:0  | 20.65±0.27 |
| C24:0  | 1.82±1.57 |
| C24:1 n9 | 1.01±0.36 |

**Fatty acids**

| SFA: sum of saturated fatty acids; UFA: sum of unsaturated fatty acids; PUFA: sum of polyunsaturated fatty acids; MUFA: sum of monounsaturated fatty acids. |

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The fatty acids were: initial oven temperature: 50 °C for 1 minute; ramp 1: 5 °C·min⁻¹ to 248 °C; ramp 2: 248 °C for 6 minutes.
compare the value of each parameter and the significance is given as P<0.05, P<0.01 and P<0.001. Means were compared by the least-square difference test (LSD), using the computer programme Statistica®7 for Windows (Statsoft Inc., Tulsa, OK, USA).

3. RESULTS

Table 2 shows the fatty acid composition of total, neutral and polar lipids, cholesterol and intramuscular fat content from the Longissimus dorsi–LD- and Psoas major–PM- muscles.

The intramuscular fat of Celta pigs had a high concentration of saturated (SFA) and monounsaturated (MUFA) fatty acids and a lower concentration of polyunsaturated (PUFA) fatty acids. Oleic acid (C18:1 c n-9) was the most abundant fatty acid in the total and neutral lipids and linoleic acid (C18:2 n-6) in the polar lipids. In polar lipids, the arachidonic acid (C20:4 n-6) content was higher than in total and neutral lipids.

A statistical analysis of the results showed that the total amount of SFA, unsaturated fatty acids (UFA), MUFA and PUFA of the intramuscular fat was affected by the type of muscle. LD showed higher levels of UFA and MUFA and lower levels of SFA and PUFA than the PM intramuscular fat. These differences were a result of the effect on individual fatty acids. For example, palmitoleic acid (C16:1 n-7), margaric acid (C17:0), C18:1 c n-9, C18:2 n-6, α-linolenic acid (C18:3 n-3), arachidic acid (C20:0), eicosatrienoic acid (C20:3 n-6), C20:4 n-6, behenic acid (C22:0) or lignoceric acid (C24:0) acids showed significant differences (P<0.001) in the total lipids of the intramuscular fat.

The effects of the type of muscle on the polar lipids from the intramuscular fat were minor. The highest differences between LD and PM muscles were observed for stearic acid (C18:0) and C18:1 c n-9.

Fatty acid contents were influenced more by type of muscle than by sex. In the case of total lipid content in the intramuscular fat, only C17:0 showed highly significant (P<0.001) sex effects.

The neutral lipids of the intramuscular fat showed significant differences for pentadecanoic acid (C15:0), cis-10 heptadecanoic acid (C17:1), C17:0, C18:1 c n-9, C18:3 n-3 and cis-11, 14, 17 eicosatrienoic acid (C20:3 n-3) among males and females.

The effect of sex on the polar lipids of the intramuscular fat was minor, and there were significant differences between male and female animals only concerning the nervonic acid (C24:1 n-9). An interaction between type of muscle and sex was observed for C18:0 in the polar lipids.

The fatty acid composition of total lipids, cholesterol and retinol contents from the subcutaneous fat (rump, dorsal, ventral and covering the Biceps femoris muscle) are shown in Table 3 and the fatty acid composition of neutral and polar lipids, are shown in Tables 4 and 5, respectively.

Significant differences were also observed among the fatty acid composition of the subcutaneous fat locations. The fatty acids of total and neutral lipids were affected more by location than polar lipids. The subcutaneous ventral fat showed a higher SFA content and lower UFA, MUFA and PUFA than the subcutaneous dorsal fat, subcutaneous fat from the rump and the fat covering the B. femoris muscle. In addition, the polar lipids of the subcutaneous fat showed higher levels of MUFA than in other locations.

Sex affected the fatty acids of the subcutaneous fat. However, in total lipids, there were significant differences for lauric acid (C12:0), C15:0, C17:0, C17:1 and C18:3 n-6 among males and females. In the neutral lipids of the subcutaneous fat, males had a higher content of C12:0, myristoleic acid (C14:1), palmitic acid (C16:0), C17:0, C17:1 and cis-13, 16-docosadienoic acid (C22:2 n-6) than females.

An interaction between location and sex was observed for SFA, UFA and MUFA in polar lipids. In polar lipids, the males showed higher contents of C16:1 n-7, C17:1, C18:3 n-3 and C20:3 n-3 than the females.

The fatty acid composition of total, neutral and polar lipids, cholesterol and retinol contents from perirenal and hepatic fat are presented in Table 6.

In total and neutral lipids, the hepatic fat showed higher levels of PUFA and lower levels of MUFA than the subcutaneous or intramuscular fats. These differences are a direct consequence of higher amounts of some fatty acids such as C18:2 n-6 or C20:4 n-6 in the hepatic fat and lower amounts of C18:1 c n-9, vaccenic acid (C18:1 c n-7) or cis-11-eicosanoic acid (C20:1 n-9). In polar lipids, hepatic fat showed higher levels of PUFA than the subcutaneous fat but lower than the intramuscular fat.

The sex had no significant influence on the composition of the hepatic fat.

The SFA (mainly C16:0 and C18:0) and C18:3 n-3 contents were higher in the perirenal fat compared to the other fat depots.

Desaturase and elongase activity indices are given in Table 7. Δ6 desaturase index and C18:0/C16:0 elongase index were not significantly influenced by the location or the sex in LD and PM. However, significant differences (P<0.01) were observed between males and females in the rump.

A significant effect (P<0.001) of location and sex was observed for the Δ5 desaturase index in LD and PM. PM presented a higher Δ5 desaturase index than LD in females than in males. In subcutaneous fat, differences were determined only by the location in the Δ5 desaturase index.
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<th>Longissimus dorsi</th>
<th>Psoas major</th>
<th>Significance</th>
<th>Longissimus dorsi</th>
<th>Psoas major</th>
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<td>43.28</td>
<td>0.545</td>
<td>51.95</td>
<td>52.98</td>
<td>0.492</td>
</tr>
<tr>
<td>PUFAs</td>
<td>10.67</td>
<td>10.39</td>
<td>0.314</td>
<td>17.01</td>
<td>16.72</td>
<td>0.338</td>
<td>9.40</td>
<td>8.82</td>
<td>0.308</td>
</tr>
<tr>
<td>PUFAs</td>
<td>0.28</td>
<td>0.27</td>
<td>0.003</td>
<td>0.42</td>
<td>0.42</td>
<td>0.015</td>
<td>0.23</td>
<td>0.23</td>
<td>0.008</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>39.99</td>
<td>38.10</td>
<td>1.179</td>
<td>43.10</td>
<td>44.92</td>
<td>2.446</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>IMF</td>
<td>3.22</td>
<td>3.24</td>
<td>0.190</td>
<td>2.00</td>
<td>2.20</td>
<td>0.132</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

**SFA**: sum of saturated fatty acids; **UFA**: sum of unsaturated fatty acids; **MUFA**: sum of monounsaturated fatty acids; **PUFA**: sum of polyunsaturated fatty acids; **P**: the ratio of total PUFA to total SFA; **Fem**: female; **IMF**: intramuscular fat; **S**: significantly different values as influenced by sex *(P<0.05); ***(P<0.01); ****(P<0.001); no significant difference. **L**: significantly different values as influenced by location *(P<0.05); ***(P<0.01); ****(P<0.001); SxL: interaction of sex and location *(P<0.05); ***(P<0.01); ****(P<0.001); ***(P<0.001); Means within the same row not followed by the same letter differ significantly (influence of sex) *(P<0.05); ***(P<0.01); ****(P<0.001); SEM within the same row not followed by the same letter differ significantly (influence of location) *(P<0.05).
The Δ9 desaturase index was higher in LD than in PM, showing that the conversion of palmitic acid to palmitoleic acid and stearic acid to oleic acid is higher in LD. This index was also higher in fat covering the Biceps femoris muscle than in other locations of subcutaneous fat. This index did not differ between the two sexes.

Elongase activity did not show differences between females and males in LD and PM. However, the elongase index, which provided an estimate of C16:1 n-7 to C18:1 c n-7 fatty acid elongase activity, was higher in PM than in LD. In the subcutaneous fat, significant differences were observed between sex (P<0.05) and locations (P<0.001). Finally, the thioesterase index did not differ among locations or sexes in intramuscular fat. In this case, differences between locations were observed concerning subcutaneous fat.

Table 3. Fatty acid composition (as a percentage of total fatty acids), cholesterol (mg·100 g⁻¹ of sample), retinol (μg·g⁻¹ of sample) and standard error of the mean (SEM) of total lipids from subcutaneous fat

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Rump Sub.</th>
<th>Biceps femoris Subcutaneous dorsal</th>
<th>Subcutaneous ventral</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male Fem. SEM</td>
<td>Male Fem. SEM</td>
<td>Male Fem. SEM</td>
<td>Male Fem. SEM</td>
</tr>
<tr>
<td>C10:0</td>
<td>0.01 0.02</td>
<td>0.01 0.02</td>
<td>0.01 0.01</td>
<td>0.01 0.01</td>
</tr>
<tr>
<td>C12:0</td>
<td>0.04 0.04</td>
<td>0.04 0.04</td>
<td>0.04 0.04</td>
<td>0.04 0.03</td>
</tr>
<tr>
<td>C14:0</td>
<td>1.19 1.12</td>
<td>1.16 1.11</td>
<td>1.11 1.05</td>
<td>0.03 0.001</td>
</tr>
<tr>
<td>C14:1</td>
<td>0.01 0.01</td>
<td>0.01 0.01</td>
<td>0.01 0.01</td>
<td>0.01 0.01</td>
</tr>
<tr>
<td>C15:0</td>
<td>0.04a 0.03b</td>
<td>0.03 0.03</td>
<td>0.04a 0.04b</td>
<td>0.04b 0.002b</td>
</tr>
<tr>
<td>C16:0</td>
<td>22.25 21.79</td>
<td>21.93 21.44</td>
<td>22.58 21.80</td>
<td>0.273</td>
</tr>
<tr>
<td>C16:1 n-7</td>
<td>1.72 1.60</td>
<td>1.29 1.09</td>
<td>1.73 1.57</td>
<td>0.059</td>
</tr>
<tr>
<td>C17:0</td>
<td>0.26 0.23</td>
<td>0.30 0.26</td>
<td>0.25 0.22</td>
<td>0.26 0.23</td>
</tr>
<tr>
<td>C18:0</td>
<td>11.92 11.92</td>
<td>11.89 12.24</td>
<td>11.39 12.67</td>
<td>0.312</td>
</tr>
<tr>
<td>C18:1 c n-9</td>
<td>43.98 44.68</td>
<td>43.02 43.71</td>
<td>39.45 41.76</td>
<td>0.564</td>
</tr>
<tr>
<td>C18:1 c n-7</td>
<td>2.13 2.20</td>
<td>0.26 0.23</td>
<td>1.32 1.35</td>
<td>0.018</td>
</tr>
<tr>
<td>C18:2 n-6</td>
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<td>13.16 13.06</td>
<td>11.79 11.26</td>
<td>0.351</td>
</tr>
<tr>
<td>C18:3 n-6</td>
<td>0.02a 0.01b</td>
<td>0.03 0.02</td>
<td>0.02 0.02</td>
<td>0.02 0.02</td>
</tr>
<tr>
<td>C18:3 n-3</td>
<td>0.64 0.59</td>
<td>0.66 0.64</td>
<td>0.61 0.56</td>
<td>0.024 0.024 0.024 0.024 ns ns ns</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.22 0.20</td>
<td>0.23 0.22</td>
<td>0.25 0.20</td>
<td>0.26 0.23</td>
</tr>
<tr>
<td>C20:1 n-9</td>
<td>1.31 1.36</td>
<td>1.25 1.35</td>
<td>1.02 0.99</td>
<td>0.045b</td>
</tr>
<tr>
<td>C20:2 n-6</td>
<td>0.85 0.85</td>
<td>0.83 0.81</td>
<td>0.56 0.54</td>
<td>0.033b</td>
</tr>
<tr>
<td>C20:3 n-6</td>
<td>0.07 0.06</td>
<td>0.07 0.07</td>
<td>0.07 0.07</td>
<td>0.07 0.07</td>
</tr>
<tr>
<td>C20:4 n-6</td>
<td>0.12 0.11</td>
<td>0.12 0.12</td>
<td>0.19 0.18</td>
<td>0.011b</td>
</tr>
<tr>
<td>C20:3 n-3</td>
<td>0.18 0.16</td>
<td>0.15 0.15</td>
<td>0.10 0.09</td>
<td>0.002c</td>
</tr>
<tr>
<td>C22:2 n-6</td>
<td>0.43 0.36</td>
<td>0.31 0.30</td>
<td>0.38 0.39</td>
<td>0.066 0.066 0.066 0.066 ns ns ns</td>
</tr>
<tr>
<td>C22:4 n-6</td>
<td>0.03 0.03</td>
<td>0.04 0.04</td>
<td>0.05 0.05</td>
<td>0.004b 0.004b 0.004b 0.004b ns ns ns</td>
</tr>
<tr>
<td>C24:1 n-9</td>
<td>1.16 1.17</td>
<td>1.07 1.12</td>
<td>1.25 1.44</td>
<td>0.100</td>
</tr>
</tbody>
</table>
| SFA: sum of saturated fatty acids; UFA: sum of unsaturated fatty acids; PUFA: sum of polyunsaturated fatty acids; MUFA: sum of monounsaturated fatty acids; P/S: the ratio of total PUFA to total SFA; Fem: female; S: significantly different values as influenced by sex *(P<0.05); **(P<0.01); *** (P<0.001); SxL: interaction of sex and location *(P<0.05); **(P<0.01); *** (P<0.001) ; SxL: interaction of sex and location *(P<0.05); **(P<0.01); *** (P<0.001); ns: no significant difference; L: significantly different values as influenced by location *(P<0.05); **(P<0.01); *** (P<0.001); a–b Means within the same row not followed by the same letter differ significantly (influence of sex) (P<0.05); 1–3 SEM within the same row not followed by the same letter differ significantly (influence of location) (P<0.05).
In LD muscle, significantly higher values (P<0.05) of intramuscular fat than in the PM muscle were observed (Table 2). In our study, the retinol content in muscles was not detectable.

Cholesterol contents from the subcutaneous and perirenal fat were superior to that described in intramuscular fat. The cholesterol and retinol contents of the subcutaneous locations were not strongly influenced by location. The ventral subcutaneous fat had the greatest (P<0.05) cholesterol values.

The retinol and cholesterol contents from the hepatic fat were much higher than those observed in other locations.

The percentage of intramuscular fat and cholesterol contents in the muscles and retinol and cholesterol contents in the subcutaneous and perirenal fat showed no significant differences between males and females. However, there appears to be a greater deposition (P<0.05) of retinol in the liver of males than in females.

### Table 4. Fatty acid composition (as a percentage of total fatty acids) and standard error of the mean (SEM) of neutral lipids from subcutaneous fat

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Rump</th>
<th>Sub. Biceps femoris</th>
<th>Subcutaneous dorsal</th>
<th>Subcutaneous ventral</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Fem.</td>
<td>SEM</td>
<td>Male</td>
<td>Fem.</td>
</tr>
<tr>
<td>C10:0</td>
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<td>0.02</td>
<td>0.002</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>C12:0</td>
<td>0.05</td>
<td>0.04</td>
<td>0.002</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>C14:0</td>
<td>1.24</td>
<td>1.18</td>
<td>0.027</td>
<td>1.22</td>
<td>1.18</td>
</tr>
<tr>
<td>C14:1</td>
<td>0.02</td>
<td>0.01</td>
<td>0.003</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>C15:0</td>
<td>0.04</td>
<td>0.05</td>
<td>0.008</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>C16:0</td>
<td>22.32</td>
<td>21.85</td>
<td>0.281</td>
<td>22.15</td>
<td>21.82</td>
</tr>
<tr>
<td>C16:1n7</td>
<td>2.40</td>
<td>2.18</td>
<td>0.086</td>
<td>2.81</td>
<td>2.68</td>
</tr>
<tr>
<td>C18:1c n9</td>
<td>0.02</td>
<td>0.01</td>
<td>0.003</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>C18:1c n7</td>
<td>0.02</td>
<td>0.01</td>
<td>0.003</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>C18:2n6</td>
<td>0.12</td>
<td>0.08</td>
<td>0.006</td>
<td>0.12</td>
<td>0.08</td>
</tr>
<tr>
<td>C18:3n6</td>
<td>0.06</td>
<td>0.06</td>
<td>0.012</td>
<td>0.06</td>
<td>0.06</td>
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<tr>
<td>C20:0</td>
<td>0.19</td>
<td>0.20</td>
<td>0.009</td>
<td>0.15</td>
<td>0.13</td>
</tr>
<tr>
<td>C20:1n9</td>
<td>1.21</td>
<td>1.31</td>
<td>0.040</td>
<td>0.96</td>
<td>0.98</td>
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<tr>
<td>C20:2n6</td>
<td>0.82</td>
<td>0.81</td>
<td>0.022</td>
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<tr>
<td>C20:3n6</td>
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<td>0.06</td>
<td>0.006</td>
<td>0.06</td>
<td>0.06</td>
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<tr>
<td>C20:4n6</td>
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<td>0.11</td>
<td>0.009</td>
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<tr>
<td>C22:2n6</td>
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<td>0.016</td>
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<td>0.001</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>C24:1n9</td>
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<td>0.01</td>
<td>0.004</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>SFA</td>
<td>36.51</td>
<td>36.81</td>
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<td>34.77</td>
<td>34.19</td>
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<tr>
<td>MUFA</td>
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<td>63.19</td>
<td>0.454</td>
<td>65.23</td>
<td>65.81</td>
</tr>
<tr>
<td>PUFA</td>
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<td>48.94</td>
<td>0.465</td>
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<td>52.64</td>
</tr>
<tr>
<td>P/S</td>
<td>0.40</td>
<td>0.39</td>
<td>0.007</td>
<td>0.36</td>
<td>0.39</td>
</tr>
</tbody>
</table>

SFA: sum of saturated fatty acids; UFA: sum of unsaturated fatty acids; MUFA: sum of monounsaturated fatty acids; PUFA: sum of polyunsaturated fatty acids; P/S: the ratio of total PUFA to total SFA; Fem: female; S: significantly different values as influenced by sex *(P<0.05); **(P<0.01); ****(P<0.001); L: significantly different values as influenced by location *(P<0.05); **(P<0.01); ****(P<0.001); ns: no significant difference; SxL: interaction of sex and location *(P<0.05); **(P<0.01); ****(P<0.001); a–b Means within the same row not followed by the same letter differ significantly (influence of sex) (P<0.05); 1–3 SEM within the same row not followed by the same letter differ significantly (influence of location) (P<0.05).

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4. DISCUSSION

The statistical analysis showed some differences in the contents of some fatty acids of the studied locations. However, the fatty acid profile was very similar in the deposits of fat studied. These results are in agreement with previous findings by other authors in different fat locations (Cava et al., 2003; Franco et al., 2006).

The studied Celta pigs were fed with commercial compound feed (Table 1). These have high contents of carbohydrates and low contents of fat. Their fatty acid composition is characterized by high levels of C16:0 and C18:0 (Olivares et al., 2009a). On the other hand, the enzyme Δ-9 desaturase would be very active in Celta hogs, as it is favored by the carbohydrates and the MUFA, which would explain in part the high content of polar lipids in subcutaneous fat.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Rump</th>
<th>Sub. Biceps femoris</th>
<th>Subcutaneous dorsal</th>
<th>Subcutaneous ventral</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>MaleSEM</td>
<td></td>
<td>MaleSEM</td>
<td>MaleSEM</td>
<td>MaleSEM</td>
<td></td>
</tr>
<tr>
<td>C10:0</td>
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<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>ns</td>
</tr>
<tr>
<td>C12:0</td>
<td>0.04</td>
<td>0.06</td>
<td>0.06</td>
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</tr>
<tr>
<td>C14:0</td>
<td>1.29</td>
<td>1.29</td>
<td>1.29</td>
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<tr>
<td>C14:1</td>
<td>0.02</td>
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<td>0.02</td>
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<tr>
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<tr>
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<tr>
<td>C16:1 n7</td>
<td>1.83</td>
<td>1.77</td>
<td>1.51</td>
<td>1.98</td>
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</tr>
<tr>
<td>C17:0</td>
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<td>0.29</td>
<td>0.29</td>
<td>0.31</td>
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<tr>
<td>C17:1</td>
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<tr>
<td>C18:0</td>
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<td>12.66</td>
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<td>ns</td>
</tr>
<tr>
<td>C18:1 c n7</td>
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<td>2.54</td>
<td>2.61</td>
<td>2.56</td>
<td>ns</td>
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<tr>
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<td>0.19</td>
<td>0.17</td>
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<td>0.49</td>
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<td>0.09</td>
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<td>0.69</td>
<td>0.69</td>
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<td>0.08</td>
<td>ns</td>
</tr>
<tr>
<td>C24:1 n9</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>ns</td>
</tr>
<tr>
<td>SFA</td>
<td>38.55</td>
<td>41.69</td>
<td>44.30</td>
<td>44.94</td>
<td>ns</td>
</tr>
<tr>
<td>UFA</td>
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<td>58.31</td>
<td>55.70</td>
<td>55.06</td>
<td>ns</td>
</tr>
<tr>
<td>MUFA</td>
<td>45.89</td>
<td>42.62</td>
<td>41.14</td>
<td>41.45</td>
<td>ns</td>
</tr>
<tr>
<td>PUFA</td>
<td>15.56</td>
<td>15.70</td>
<td>14.56</td>
<td>13.61</td>
<td>ns</td>
</tr>
<tr>
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SFA: sum of saturated fatty acids; UFA: sum of unsaturated fatty acids; MUFA: sum of monounsaturated fatty acids; P/S: the ratio of total PUFA to total SFA; Fem: female; S: significantly different values as influenced by sex *(P<0.05); ***(P<0.001); ns: no significant difference; L: significantly different values as influenced by location *(P<0.05); ***(P<0.001); SxL: interaction of sex and location *(P<0.05); ***(P<0.001); 1–4 SEM within the same row not followed by the same letter differ significantly (influence of location) (P<0.05).
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**SFA**: sum of saturated fatty acids; **UFA**: sum of unsaturated fatty acids; **PUFA**: sum of polyunsaturated fatty acids; **MUFA**: sum of monounsaturated fatty acids; **P/S**: the ratio of total PUFA to total SFA; **Fem**: female; **S**: significantly different values as influenced by sex *(P<0.05); ***(P<0.01); ***(P<0.001); ns: no significant difference; a-b Means within the same row not followed by the same letter differ significantly (influence of sex) *(P<0.05).
TABLE 7. Desaturase and elongase activity indices

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<tr>
<th></th>
<th>Longissimus dorsi</th>
<th>Psoas major</th>
<th>Rump</th>
<th>Sub</th>
<th>Sub. Dorsal</th>
<th>Sub. Ventral</th>
<th>Perirenal</th>
<th>Liver</th>
<th>Significance</th>
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<td>SEM</td>
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<td>Female</td>
<td>SEM</td>
<td>Male</td>
<td>Female</td>
<td>SEM</td>
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<td>0.002</td>
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<td>0.54</td>
<td>0.007</td>
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S: significantly different values as influenced by sex *(P<0.05); **(P<0.01); ***(P<0.001); ns: no significant differences; SxL: interaction of sex and location *(P<0.05); **(P<0.01); ***(P<0.001); SxL: interaction of sex and location *(P<0.05);

MUFA and especially of C18:1 c n-9 (Franco et al., 2006).

The SFA content was higher in hepatic and perilrenal fat than in other deposits. C18:1 c n-9 and UFA contents were higher from intramuscular and subcutaneous fat than for the hepatic and perilrenal fat. Oleic acid is synthesized by Δ9-desaturase (stearoyl-CoA-desaturase). Thompson and Allen (1969) observed that the activity of this enzyme was higher in the subcutaneous adipose tissue than in the perilrenal fat, which may explain the higher oleic acid content in the subcutaneous fat.

However, the percentage of C20:4 n-6 and PUFA content was significantly higher in hepatic fat than in other anatomical locations.

Total lipids and neutral lipids, except for the ones in the liver, showed similar fatty acid composition, as the neutral lipid represented the greatest percentage of total lipids.

The fatty acid composition of total and neutral lipids is more influenced by location than the fatty acids of polar lipids. The proportion of PUFA in the polar lipids from the intramuscular fat was significantly higher than those of the total and neutral lipids. These differences were a consequence of a higher content of some individual fatty acids in the polar lipids such as C18:2 n-6 and C20:4 n-6.

The type of fibers and their cellular metabolism determines the fatty acid composition in the muscle (Andrés et al., 2001; Leseigneur-Meynier and Gandemer, 1991). PM is a predominantly oxidative muscle, showing higher levels of PUFA than LD in both lipid classes, which has been described as a predominantly glucolitic muscle in the scientific literature.

The differences observed in the fatty acids from different locations could also be explained as a function of the amount of neutral lipids and phospholipids that they contain (Cava et al., 2003; Franco et al., 2006).

On the other hand, in monogastric animals such as the pig, the fatty acid composition of different fat deposits is affected by the diet (Viera-Alcaide et al., 2007) and some fatty acids of the diet are absorbed and deposited directly into the fat deposits. Tissue fatty acid composition is not modified to the same extent in the different locations. C18:2 n-6 and C18:3 n-3 are directly accumulated from the diet, while C20:4 n-6 is derived from dietary C18:2 n-6.

The studied Celta pigs were fed with commercial feed, which was rich in C18:2 n-6 and C20:4 n-6.

The proportion of fatty acids is also determined by endogenous synthesis. Previous studies showed that the activity of the enzymes involved in the lipogenesis is tissue specific (Buller and Enser, 1986; Narváez-Rivas et al., 2009). Our results show that desaturase and elongase activity is significantly different (P<0.001) in LD and PM muscles.
Traditionally, male and female Celta pigs are castrated to reduce the negative effect of oestrous on growth, to improve meat quality and to facilitate management. The effect of castration on lipid content and fatty acid composition has been previously described (Cai et al., 2010; Höberg et al., 2004; Ntawubizi et al., 2009; Razmaite et al., 2008). In most cases, these studies are carried out on entire males, females or castrated males. However, little is known about the effect of castration on the females.

Our results showed that the sex exerted less influence than the anatomical location on fatty acid contents. However, there were differences in some fatty acids of the intramuscular and subcutaneous fat between castrated females and castrated males. The fatty acids of neutral lipids were also more influenced by sex than the fatty acids of polar lipids.

Results reported in the literature about the effect of sex on fatty acids show that the most important differences between sexes in fatty acid composition are caused by a difference in metabolism between females and males or between castrated and uncastrated pigs. However, most of these sex differences are eliminated by the castration of males and females because the castration causes a removal of hormones that affects the fatty acid metabolism (Högberg, 2002).

In addition, the age of castration has an influence on the effect of sex on meat quality. In the case of castrated animals at a young age, the sexual hormones have little influence on growth (Mayoral et al., 1999). The differences observed in the literature could also, at least in part, be due to the different ages of castration.

Our results showed differences among some fatty acids in castrated females and castrated males. For example, C17:0 in the total lipid contents from the intramuscular fat, C15:0 in total lipids and C17:1 in polar lipids from the subcutaneous fat showed significant effects of the sex. In contrast to our results, Ramírez and Cava (2008) did not find differences in fatty acid compositions in subcutaneous and intramuscular fat from LD and Biceps femoris muscles between castrated females and castrated males. Serrano et al. (2008) did neither observe any difference in the fatty acid contents of the subcutaneous fat of castrated females and castrated males. Cordero et al. (2010) did not find any difference in the fatty acid composition of the intramuscular fat from LD between castrated females and castrated males in the Iberian×Duroc pig. However, these authors found differences in C18:1 c n-9, SFA and the MUFA of the subcutaneous fat between sexes.

Peinado et al. (2008) did not observe differences between the fatty acids of subcutaneous fat of intact females, castrated females and castrated males, except for the C18:2 n-6 content, which was higher for intact females than for castrated females and castrated males.

On the contrary, a significant effect (P<0.001) of location and sex was observed for the Δ5 desaturase index. PM presented a higher Δ5 desaturase index than LD in females than in males. Δ-5 desaturase activity, which is an indicator of the stearoyl-CoA desaturase influence on the conversion of C20:3 n-6 to C20:4 n-6, was higher in castrated females than in castrated males.

Hasty et al. (2002) showed that fatty acid metabolism follows a different course according to sex. Cordero et al. (2010) observed that the subcutaneous fat from castrated males had lower MUFA/SFA and C18:1 c n-9/C18:0 ratios than those from castrated females. However, Zhang et al. (2007) did not observe any differences in the indices of Δ9-desaturase, thioesterase, or elongase between males and females.

The cholesterol contents of muscles coincided with those described by Bragagnolo and Rodríguez-Amaya (2002) and Salvatori et al. (2008). The fact that the PM muscle presented a higher cholesterol content (P>0.05) than the LD muscle could be due to that oxidative muscles are richer in phospholipids than glycolytic muscles, and as a result, they are richer in cholesterol (Alasnier et al., 1996; Chizzolini et al., 1999).

Intramuscular fat content also affects the amount of cholesterol, since the muscles with high content of intramuscular fat present proportionately less membrane polar lipids and therefore lower amounts of the cholesterol associated with these membranes (Alasnier et al., 1996). This would explain, in part, the fact that the LD muscle has a lower cholesterol content than the PM muscle.

Bragagnolo and Rodriguez-Amaya (2002) found that cholesterol contents decreased significantly with increasing age at slaughter. In turn, Salvatori et al. (2008) found a significant relationship between carcass weight and cholesterol content, the last one decreased as the weight of the carcass increased. This may explain, in part, the low-cholesterol in Celta pig muscles, as these pigs were slaughtered at an older age (16 months), presenting a high carcass weight and high percentage of intramuscular fat. So, despite not being significant, we found that there was an inverse correlation between carcass weight and cholesterol content.

Several authors suggest that castration causes increased intramuscular fat deposition (Latorre et al., 2003; Mersmann, 1984; Mourot et al., 1999; Peinado et al., 2008), because the pigs have greater energy, resulting in an increased fat storage (Gandemer et al., 1979; Kolstad et al., 1996). These differences were not noted in our study, as both males and females were castrated.

There are few studies that describe the content of retinol in pig muscles. Höberg et al. (2002) and Olij avere et al. (2009a) described a retinol content of approximately 0.03 and 0.04 μg·g⁻¹ of pork loin.
samples, respectively, showing that the retinol content from muscles is very low.

The retinol and cholesterol contents from hepatic fat were much higher than those observed in other locations. This is mainly due to the fact that liver synthesized and accumulated these substances, which explains their high contents in relation with the other locations. The retinol content present in the Celta pork liver was above the one described by Olivares et al. (2009a, 2009b) possibly due to a higher content of vitamin A in the diet of the Celta pig (Table 1).

The percentage of intramuscular fat and cholesterol contents in muscles and the retinol and cholesterol contents in the subcutaneous and perirrenal fat showed no significant differences between males and females. However, there appears to be a greater deposition (P<0.05) of retinol in the liver of males compared to females.

The absence of significant differences in the content of cholesterol and retinol due to sex can be due to the fact that castration removed hormones that affect cholesterol and retinol metabolism and, as noted above, differentiate males from females.

5. CONCLUSIONS

The effects of gender and location on the fatty acid profile were less intense in polar lipids than in total and neutral lipids. The differences determined by the location could be due to the different activity of the elongase and desaturase enzymes. In turn, the cholesterol content decreased with increasing age of slaughter, weight, and amount of intramuscular fat.

Castration removed hormones that differentiate sexes, so as both male and female pigs were castrated, only significant differences could be seen in some minor fatty acids. Cholesterol, retinol and intramuscular fat contents showed no significant differences between sexes.

ACKNOWLEDGEMENT

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REFERENCES


