Histomorphometric Features of Deltoid Muscle (Musculus Deltoideus) in the Mouflon (Ovis ammon musimon Pal.) and Domestic Sheep

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Abstract
The aim of this study was to determine histomorphometry of the skeletal muscle in rams from the Tigaie breed and mouflon males (Ovis ammon musimon Pal.) knowing that, in addition to a variety of factors, both the fiber thickness and muscle fascicles influence the meat quality. The study was conducted on the deltoid muscle fragments taken from five individuals of each species. The muscle samples were processed according to the method of embedded in paraffin, sectioned to a thickness of 5 µm and stained with Mallory's trichrome method. From the histomorphometric analysis resulted that in mouflon, the average area of muscle fascicles is larger, as compared with the ram (p < 0.05), the ratio between the fascicles muscle area and the interfascicular connective tissue being 2.939:1 for mouflon and 1.624:1 for ram. Also, in mouflon the muscle fibers are thicker, the area and perimeter registering an average value of 486.60 μm², respectively 82.83 μm while in ram, the area of the muscle fibers is 281.56 μm² and the perimeter of 62.92 μm (p <0.001). The amount of interfibrillar connective tissue is higher in ram (p<0.05) and the ratio between the muscular fibers area and interfibrillar connective tissue area was 1.649:1 in mouflon and 1.451:1 in ram (p<0.05).

Key words: muscle histomorphometry, Musculus deltoideus, Ovis ammon musimon Pal., ram

1. Introduction
The quality of meat is significantly influenced by the fatty acid composition as it affects the organoleptic characteristics, particularly the taste (flavor) [1, 2] and nutritional value of the fat for human consumption [3, 4]. Dietary fats provide support to absorb fat-soluble vitamins, being a factor that contributes to the palatability of food and, not least, these are crucial for proper development and survival during the early stages - embryonic and postnatal. A number of factors, such as breed, sex, average live weight, diet, degree of obesity, as well as the interaction of these factors proved to affect the fatty acid composition of the lamb fat [5, 6]. In the last decade, the nutritional value of meat from ruminants on human health has been reconsidered [6, 7].

The main differences in fatty acid composition in wild ruminants are the result of the effect of food [8]. A favorable content in fats and fatty acids can be found in the meat of domestic ruminants, in particular of the grazing [8, 9]. On the other hand, meat quality is determined both by the thickness of muscle bundles, the thickness of the muscle fibers but also the ratio of muscle tissue and connective tissue.
Using mouflons (Ovis ammon musimon Pal.) for new genotypes with Tigaie local sheep could be a good solution for the production of high-quality mutton [10]. Based on these aspects, the purpose of this paper was to determine the histomorphometric parameters of the deltoid muscle in rams of Tigaie breed and mouflons male.

2. Materials and methods

The study was conducted on a number of 5 mouflons male (Ovis ammon musimon Pal.) and the 5 sheep male of Tigaie breed, aged between 4 and 4.5 months. All individuals were fed ad libitum with orchard hay and a mixture of corn, wheat bran and soybean meal.

Histological technique

The histological and histomorphometric study was carried out on fragments of striated muscle tissue from deltoid muscle. The muscle samples were processed according to the method of embedded in paraffin, sectioned to a thickness of 5 µm, stained with Mallory's trichrome method [11] and the microscopic sections were examined with the research microscope Olympus CX41 (Germany), equipped with a digital camera.

Histomorphometric Analysis

For histomorphometry of the deltoid muscle of the two studied species, it was used a specific software - QuickPHOTO Micro2.2. Histomorphometric parameters determined by us were: muscle fascicles area; interfascicular connective tissue area; ratio of the muscle fascicular area and interfascicular connective tissue area (Rm.fasc.-Ic.); area and perimeter of muscle fibers; the ratio of total area of the muscle fibers and connective interfibrilar tissue - (R.m.fibbers.-Ic.). The area of muscle fascicles and interfascicular connective tissue area was determined on a total of 10 microscopic fields obtained at a power microscope resolution of 100x, the measured surface of the microscopic field being 2067987 µm². Skeletal muscle fiber area and the perimeter were determined also on a number of 10 microscopic fields obtained at a power microscope resolution of 1000x, of 10 measurements/microscopic field, the surface of each field being 20,427 µm².

In order to establish these relationships between muscle bundles and interfascicular connective tissue, namely the ratio of muscle fibers and interfibrilar connective tissue, there was originally determined the total area of the fascicles/muscle fibers and then, by difference made between the total area of the microscopic field and the total area of the fascicles/muscle fibers, there was calculated the total area of the interfascicular/interfibrilar connective tissue.

Statistical Analysis

The statistical analysis of the histomorphometry results was performed using the ANOVA, Student “t” tests an angular grade transformation, respectively. The results are presented as the mean±SD. Differences were considered to be statistically significant at p<0.05, distinctly significant at p<0.01 and very significant at p<0.001.

3. Results and discussion

Histomorphometric analysis performed on cross-sections of deltoid muscle (Figure 2), taken from mouflon shows that the average area of the muscle fascicles/area of the microscopic field is of 1525513 µm², mean area of the interfascicular connective tissue is 551475 µm², so that in this case, the ratio of the total area of the muscle fascicles and the total area of interfascicular connective tissue is 2.939:1 (73.76%-muscle fascicles, 26.24%-interfascicular tissue) (Table 1).

<table>
<thead>
<tr>
<th>Specification</th>
<th>MOUFLON</th>
<th>TIGAIE RAM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R. m.fasc.-Ic.</td>
<td>Muscular fascicles (%)</td>
</tr>
<tr>
<td>Mean</td>
<td>2.939&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.76&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SD</td>
<td>0.934</td>
<td>5.51</td>
</tr>
</tbody>
</table>

<sup>a,b</sup> p<0.05
The analysis of data obtained in the histomorphometry study revealed that the highest average of muscle fascicles area/the total area of the microscopic field (2067987 μm²) was recorded in mouflon (Δ=296923 μm²) (p<0.05). Interfascicular connective tissue area had higher mean values in ram, the difference between the two species being significant (p<0.05).

A statistical analysis of data from the histomorphometric study indicates that the average ratio of the mean area of muscular fascicles and the average area of the interfascicular connective tissue is 2.939:1 in mouflon and 1.624:1 in ram (Δ=1.315), the difference being, in this case, significant (p<0.05) (Figure 1).

Histomorphometric analysis performed on cross-sections of deltoid muscle with the resolution power of the microscope of 1000x, reveals that in mouflon (Figure 4a), the average area of the muscle fibers is 486.60 μm², while the average value of the perimeter of these muscle fibers is 82.83 μm. (Table 2).

In the case of ram (Figure 4b), the medium value of the muscle fibers area is 281.56 μm², while the average value of the perimeter of these muscle fibers is 62.920 μm (Table 2, Figure 3).

A statistical analysis of data from the histomorphometry study revealed that both the area and the perimeter for skeletal muscle fibers are higher in mouflon, as compared to ram, for both parameters studied, the differences were significant (p<0.001).
Table 2. Mean and statistic indices of area and perimeter of muscular fibers in *deltoid muscle* in mouflon and Tigaie ram

<table>
<thead>
<tr>
<th>Specification</th>
<th>N</th>
<th>Statistic</th>
<th>Mean</th>
<th>SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area of muscular fibers (µm²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouflon</td>
<td>100</td>
<td></td>
<td>486.60</td>
<td>188.20</td>
<td>182.00</td>
<td>924.00</td>
</tr>
<tr>
<td>Tigaie Ram</td>
<td>100</td>
<td></td>
<td>281.56</td>
<td>50.93</td>
<td>161.00</td>
<td>704.00</td>
</tr>
<tr>
<td>Perimeter of muscular fibers (µm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouflon</td>
<td>100</td>
<td></td>
<td>82.830</td>
<td>15.28</td>
<td>56.00</td>
<td>112.00</td>
</tr>
<tr>
<td>Tigaie Ram</td>
<td>100</td>
<td></td>
<td>62.920</td>
<td>5.41</td>
<td>46.00</td>
<td>97.00</td>
</tr>
</tbody>
</table>

&-b p<0.001

In mouflon, the total area of muscle fibers/total area of microscopic field (20427 µm²) has an average value of 12671 µm², while in ram, the mean area of muscle fibers/total area of microscopic field was smaller, 12041 µm² (Δ=630 µm²), the difference between the two hybrids was significant (p<0.05). The amount of interfascicular tissue is larger in the ram, where the average area of the head microscope is 8544 µm², as compared to the mouflon, where the average value of this parameter is 7756 µm² (Δ=788 µm²), the difference between the two hybrids being significant (p<0.05). A comparative analysis of these values, indicates that the ratio of muscle fibers and interfibrilar connective tissue is 1.649:1 (62.03% of muscle

![Figure 3. Average of the area (a) and the perimeter (b) in skeletal muscle fibers of the deltoid muscle structure](image)

![Figure 4. Deltoid muscle – transversal section through the muscle fascicle. Muscular fibers separated by connective tissue. a. Mouflon; b. Tigaie Ram (Mallory trichrome stain; 1000x) [original](image)
fibers; 37.97% of interfibrilar connective) in mouflon and 1451:1 (58.94% fibers, 41.06% connective) in ram ($\Delta = 0.198$), the differences between the two hybrids, and with respect to this parameter, are significant ($p <0.05$) (Table 3, Figure 5).

### Table 3. Mean and statistic indices of Rm. fibers.-Ic. and percentage of participation of muscular fibers and interfibrilar connective tissue in deltoid muscle

<table>
<thead>
<tr>
<th>Specification</th>
<th>MOUFLON</th>
<th>TIGAIE RAM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R.m.fibers-Ic</td>
<td>Muscular fibers (%)</td>
</tr>
<tr>
<td>Mean</td>
<td>1.649&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SD</td>
<td>0.1798</td>
<td>1.5006</td>
</tr>
</tbody>
</table>

<sup>a-b</sup>$P<0.05$

![Figure 5. Mean R.m.fibbers.-Ic for deltoid muscle in mouflon and Tigaie ram](image)

### 4. Discussions

Meat quality is determined by several factors such as breed, gender, diet, and the thickness of the muscular fascicles and therefore muscle fibers in their structure [5, 6]. The histomorphometry study conducted by us revealed that the mean value of the area of muscle fascicles/the total area of the microscopic field is greater in mouflon as compared to the ram ($p<0.05$), while the area of interfascicular tissue has higher values in ram ($p<0.05$). In the mouflon there were also registered higher values in terms of area and perimeter of muscle fibers ($p<0.001$). On the other hand, the amount of interfibrilar connective tissue was higher in ram ($p<0.05$). Histomorphometric studies performed on deltoid muscle originated from hybrids obtained by crossing male mouflons and female sheep of Tigaie breed (MM x TF) and Tigaie males with mouflon females (TM x MF), shows average values of muscle fibers area of 385.82 μm$^2$ and 273.03 μm$^2$. Also, the hybrids MM x TF showed lower amounts of interfascicular and interfibrilar connective tissue ($p>0.05$) [10]. Studies carried out by Winkelmayer, R., et al., [12] and Hulland, T. J, et al., [13] on sheep skeletal muscle suggest that muscular fibers thickness varies by age but also by the metabolic activity. In the same context, Peinado et al., [14] have obtained the thickness values of the muscle fibers of 150-200 μm$^2$ in sheep aged up to 60 days and 550-600 μm$^2$ in those aged over 90 days.

### 5. Conclusions

The histomorphometric study conducted on deltoid muscle reveals both a greater proportion of muscle fascicles to the detriment of interfascicular connective tissue and a higher value of muscle fascicles area in mouflon, as compared to ram. Regarding the interfascicular connective tissue area, it recorded higher average values in ram.

### References