Histomorphometry of Deldoid Muscle (Musculus Deltoideus) in the Hybrids of Mouflon (Ovis Ammon Musimon Pal.) and Domestic Sheep

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Abstract
The aim of this study was to establish production performances upon slaughter in the hybrids obtained by crossbreeding of mouflon (Ovis ammon musimon Pal.) and domestic sheep. The morphological and histomorphometric study was carried out on fragments of deltoid muscles (Musculus deltoideus) sampled from seven MM x FT hybrids and seven MT x FM hybrids, respectively. Muscle fragments were fixed in 10% formalin solution and histological preparations were stained through Mallory trichromic method. Histomorphometric analysis reveals that MM x FT hybrids have thicker muscular fibbers and smaller amounts of inter-fibrillar connective tissue than MT x FM hybrids (p<0.001). Also, the mean area of muscular fascicles per microscopic field was larger in the MM x FT hybrids than in MT x FM hybrids (p>0.05). Results of our study show that using mouflon (Ovis ammon musimon Pal.) and local Tigaie sheep in the development of new genotypes could be a good solution for both the conservation of these threatened species and for production of high quality mutton.

Keywords: histology, histomorphometry, Musculus deltoideus, Ovis ammon musimon Pal., sheep

1. Introduction

Though meat is an important source of proteins, minerals and vitamins, it represents also a major source of fat and cholesterol that cause cardiovascular diseases, obesity, and colon cancer [1-3]. In developed countries, the highest risk for coronary diseases are saturated fatty acids from foods [4]. Therefore, lately, meat consumers are much more concerned about the quality of animal products. Of particular interest to consumers has become the content in fatty acid of meat, especially polyunsaturated fatty acids, with beneficial effects on humans’ health.

Thus, the ratio polyunsaturated fatty acids/saturated fatty acids (PUFA/SFA) seems to be much more important in human diet than total fat content [5-7]. Compared with domestic animals whose meat has a variable content in fat, between 1 and 45 g/100g, depending on species, breed, age, health state and sex, venison has a much lower fat content (<3%) and a suitable composition in fatty acids [1, 8, 9]. Mouflon meat is considered “lean” [10], with a relatively high content of proteins (22-23%) [11] with high biological value, containing vitamins such as thiamine, riboflavin and pantotenic acid [12] and a very low content of total fats (<1%) [7]. Fat content of mouflon meat is much lower than that of domestic ruminants. Thus, beef and goat have a higher content of fats (2-5%), while mutton, although has special nutritional and organoleptic features, it has a much higher content of fats (>8%) [10, 13, 14]. Nowadays, it is
increasingly accepted idea of using mouflon (*Ovis ammon musimon* Pal.) as a natural, old genetic source of meat with large amounts of high biological value protein and dietary features. Originally from countries such as Corsica and Sardinia, European mouflon was introduced in other countries from Europe in the last 10 years [15]. Because in Europe there are a few mouflon farms, the using of the new techniques for animal breeding and the modern methods of artificial insemination is useful both for genetic improvement of domesticated species and protection of threatened wild species.

In the context of the researches done at international level, the purpose of this study was to develop two different genotypes from male mouflon and female sheep and from male sheep and female mouflon, respectively, aiming at investigation of the microarchitecture and determining the histomorphometric parameters of the deltoid muscle.

### 2. Materials and methods

The study was effected on seven MM x FT hybrid males (MM—male mouflon; FT—female of Tigaie sheep breed) 4.5 months aged and seven MT x FM hybrids (MT—Male of Tigaie sheep breed; FM —female mouflon) 4 months aged. To produce such hybrids, we used European mouflon (*Ovis ammon musimon* Pal.) and Romanian Tigaie sheep breed.

**Histological technique.** The histological and histomorphometric study was done on fragments of striated muscle tissue from deltoid muscle (*Musculus deltoideus*). To do so, muscular fragments were fixed in 10% formalin solution, dehydrated in ethylic alcohol, clarified in acetone and benzene and included in histological paraffin. Paraffin blocks were sectioned into slices of 5 μ thick with microtome (Leica, Germany) and, subsequently, they were stained with Mallory trichromic method [16]. For histological studies, the microscopic sections were examined at research microscope Olympus CX41 (Germany) equipped with a digital camera.

**Histomorphometric analysis.** The histomorphometric study was done with specific software, QuickPHOTO Micro2.2. The histomorphometric parameters that we studied in the two hybrids were: area and perimeter of striated muscular fibbers, ratio between muscular fibbers and inter-fibrillar connective tissue (R.m.fibbers.-Ic.) and the ratio between muscular fascicles and inter-fascicular connective tissue (R.m.fasc.-Ic.).

In each individual, we determined the area and perimeter of striated muscular fibbers on 10 microscopic fields obtained at a resolution power of the microscope of 100x, of 10 measurements per microscopic field. The ratio between muscular fascicles and inter-fascicular connective tissue (R.m.fasc.-Ic.), as well as the ratio between muscular fibbers and inter-fibrillar connective tissue (R.m.fibbers.-Ic.) were determined in each individual on 10 microscopic fields obtained at a microscope resolution power of 100x and 1000x, respectively. In each parameter, the measured area of each field was identical, i.e. 2,067,987 µm² in the ratio between muscular fascicles/inter-fascicular connective tissue and 20,427 µm² in the ratio between muscular fibbers/inter-fibrillar connective tissue. To establish these ratios, we initially measured the total area of the fascicles/muscular fibbers and then we calculated the difference between the total area of the microscopic field and the total area of the fascicles/muscular fibbers and the total area of the inter-fascicular/inter-fibrillar connective tissue.

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

### 3. Results and discussion

The histological study per stained sections of deltoid muscle reveals, in both genotype hybrids, the organisation of striated muscle in muscular fascicles delimited by inner perimysium, similar to other animal species. On cross section, the muscle fibbers have a polyhedral contour and the nuclei are present at the periphery of each fibber. In endomysium there is a very developed network of capillaries with wide lumen.

The histomorphometric analysis carried out per transversal sections of deltoid muscle, at a microscopic resolution power of 100x, shows that the mean area of muscular fascicles/area of
microscopic field in MM x FT hybrid (Figure 1a) is 1,310,261.50 µm², while the mean area of interfascicular connective tissue is 757,725.50 µm². In this case, the ratio between the total area of muscular fascicles and the total area of interfascicular connective tissue is 3.174:1, i.e. 63.35% of muscular fascicles and 36.65% of interfascicular connective tissue (Table 1).

Table 1. Mean and statistical indices of Rm.fasc.-Ic. and percentage of participation of the muscular fascicles and connective tissue in the structure of deltoïd muscle

<table>
<thead>
<tr>
<th>Specification</th>
<th>MM x FT</th>
<th>MT x FM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R. m.fasc.-Ic.</td>
<td>Muscular fascicles (%)</td>
</tr>
<tr>
<td>Mean</td>
<td>1.832</td>
<td>63.35&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SD</td>
<td>0.610</td>
<td>4.406</td>
</tr>
</tbody>
</table>

<sup>a</sup> <sup>b</sup> p>0.05

Area of microscopic field (100x): 2067987 µm²

In the MT x FM hybrid (Figure 1b), the mean area of muscular fascicles/area of microscopic field is 1,266,918.00 µm², while the mean area of interfascicular connective tissue is 799,069.00 µm², the ratio between the total area of muscular fascicles and the total area of the interfascicular connective tissue is 1.606:1 (61.26% muscular fascicles; 38.74 % interfascicular connective tissue) (Table 1). Histomorphometric study data analysis shows that the highest value of the area of muscular fascicles/total area of the microscopic field (2,067,987 µm²) was in MM x FT hybrid comparative with MT x FM hybrid (Δ=43,343 µm²). The difference between the two hybrids regarding at the mean area of muscular fascicles is not significant (p>0.05).

Unlike the mean value of the area of interfascicular connective tissue, it is higher in MT x FM hybrid (Δ=126,338.6 µm²). The difference between the two genotypes regarding at the mean area of interfascicular connective tissue is not significant (p>0.05). The mean value of the ratio between the mean area of muscular fascicles and the mean area of interfascicular connective tissue is 1.832:1 in MM x FO hybrid and 1.606:1 in MB x FM hybrid (Δ=0.226 µm²), with a not significant difference, too (p>0.05) (Table 1). The histomorphometric analysis of the deltoïd muscle transversal sections (Figure 2a, b) with a microscope resolution power of 1000x reveals that in MM x FT hybrid, the mean value of muscular fibber area is 385.82 µm² and the mean value of their perimeter is 73.42 µm (Table 2). In MT x FM hybrid, the mean value of muscular fibber area is 273.03 µm², while the mean value of their perimeter is 61.06 µm (Table 2). Statistical analysis of the data from histomorphometric study shows that MM x FT hybrid has thicker muscular fibbers than MT x FM hybrid (Δ=112.79 µm²; Δ=12.36 µm), the
differences between parameters of the two hybrids being significant \(p<0.001\).

**Table 2.** Mean and statistical indices of area and perimeter of muscular fibbers in *deltoid muscle* in the two hybrid types

<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 ( (\mu m^2) )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MM x FT</td>
<td>100</td>
<td></td>
<td>385.820(^a)</td>
<td>77.202</td>
<td>233.00</td>
<td>628.00</td>
</tr>
<tr>
<td>MT x FM</td>
<td>100</td>
<td></td>
<td>273.030(^b)</td>
<td>52.859</td>
<td>125.00</td>
<td>419.00</td>
</tr>
<tr>
<td>Perimeter of muscular fibers ( (\mu m) )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MM x FT</td>
<td>100</td>
<td></td>
<td>73.42(^a)</td>
<td>6.9807</td>
<td>58.00</td>
<td>92.00</td>
</tr>
<tr>
<td>MT x FM</td>
<td>100</td>
<td></td>
<td>61.06(^b)</td>
<td>5.9115</td>
<td>44.00</td>
<td>76.00</td>
</tr>
</tbody>
</table>

\(^a\) \(p<0.001\)

**Figure 2.** *Deltoid muscle* – transversal section through the muscle fascicle. Muscular fibbers separated by connective tissue. a. MM x FT hybrid; b. MT x FM hybrid (Mallory trichrome stain; 1000x) [original]

In the MM x FT hybrid, the mean value of muscular fibbers area in a microscopic field of \(20,427 \mu m^2\) was \(12,632.80 \mu m^2\), while in the MT x FM hybrid it was lower, i.e. \(12,588.50 \mu m^2\) \(\Delta=44.4 \mu m^2\) \(p>0.05\). The amount of interfibrillar connective tissue is higher in the MT x FM hybrid, where its mean area/area of microscopic field is \(7,794.20 \mu m^2\), compared to the MM x FT hybrid, whose mean value of the parameter is \(7,838.50 \mu m^2\) \(\Delta=44.3 \mu m^2\) \(p>0.05\). The comparative analysis of the values shows that the ratio between muscular fibbers and interfibrillar connective tissue is 1.620:1 (61.84% muscular fibbers; 38.16% interfibrillar connective tissue) in the MM x FT hybrid (Table 3) and 1.605:1 (61.63% muscular fibbers; 38.37% interfibrillar connective tissue) in the MT x FM hybrid \(\Delta=0.015\) \(p>0.05\) (Table 3).

**Table 3.** Mean and statistical indices of R.m.fibbers-Ic. and percentage of participation of muscular fibbers and interfibrillar connective tissue in *deltoid muscle*

<table>
<thead>
<tr>
<th>Specification</th>
<th>MM x FT</th>
<th>MT x FM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>R. m.fibbers-Ic</td>
<td>Muscular fibbers (%)</td>
</tr>
<tr>
<td>Mean</td>
<td>1.620</td>
<td>61.84</td>
</tr>
<tr>
<td>SD</td>
<td>0.310</td>
<td>2.587</td>
</tr>
</tbody>
</table>

\(^a\) \(p<0.05\)

Area of microscopic field (1000x): \(20427 \mu m^2\)

The histological study of *deltoid muscle* reveals the organisation of striated muscle fascicles delimited by inner perimysium, in the hybrids of both genotypes, similar to other animal species. In both genotypes, the muscular tissue is intensely
vascularised, with a well developed capillary network in endomysium. Information obtained by histomorphometry is a major element in determining of morphological features of the muscle tissue and, implicitly, on some aspects of meat quality [17].

Our histomorphometric analysis reveals that in deltoid muscle, the mean area of muscle fascicles/microscopic field was higher in the MM x FT hybrids than MT x FM hybrids (p>0.05). In MM x FT hybrid, the amount of inter-fascicular connective tissue/area of microscopic field is lower in MT x FM hybrid, with non-significant differences between the two genotypes (p>0.05). Also, muscular fibbers are thicker in MM x FT hybrid, their area and perimeter, on cross sections, having higher values compared to those of the MT x FM hybrid (p<0.001).

Histochemical and histomorphometric studies made on skeleton muscles in sheep reported values ranging between 1,112 and 2,208 µm² for the area of muscle fibbers in longissimus dorsi, with variations depending on age and metabolic activity [18, 19]. Peinado et al. [20] reported mean values of the area of muscular fibbers from the structure of skeleton muscles ranging between 150 and 200 µm² in young sheep aged up to 60 days and about 550-600 µm² in individuals aged over 90 days. In large ruminants, i.e. in young cattle of Brown breed, Teuşan et al. [21] report mean values of the diameter of skeleton muscle fibbers from the structure of longissimus dorsi ranging between 41.60 and 44.81 µ and, as a result, mean values of these fibbers ranging between 1,358.5 µm² and 1,575 µm².

The amount of inter-fibrillar connective tissue is lower in the hybrid MM x FT. Thus, the ratio between the total area of muscular fibbers and the inter-fibrillar connective tissue was 1.620:1 in MM x FT hybrid and 1.605:1 in MT x FM hybrid (p>0.05). Histomorphometric data from our study regarding the quality of skeleton muscle tissue in two different genotypes are similar to those reported in literature by other authors. Thus, studies carried out by Vacca et al. [22] on productive performances and meat quality in the hybrids from mouflon x Sarda and Sarda x Sarda point out to the presence of a larger amount of muscular tissue in the hybrids from mouflon x Sarda compared to the hybrids from Sarda x Sarda (p<0.01) where the amount of connective tissue and, implicitly, of fat tissue, was higher (p<0.05).

4. Conclusions

Histomorphometric analysis of deltoid muscle sections reveals differences between the two genotypes regarding both the percentage of muscular fascicles and inter-fascicular connective tissue, area and perimeter of muscular fibbers, and ratio between total area of muscular fibber and total area of inter-fibrillar connective tissue. Our results indicated many good qualities in both genotypes and suggest that using mouflon (Ovis ammon musimon Pal.) to develop new genotypes with local Tigaie sheep could be a good solution for the production of high quality mutton.

References


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