

RESEARCHES REGARDING HISTOLOGICAL CHARACTERIZATION OF MUSCLES HARVESTED FROM HARES (*LEPUS EUROPAEUS PALLAS*)

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Abstract

In rabbits, the thickness of the muscle fibers of somatic muscles is less studied, and literature in this area is relatively poor. The few specific data published in the literature refers to individuals selected for growth rate and especially in some specialized medium breed hybrids for meat. In addition, very few somatic body muscle of rabbits, were addressed in such studies to date. I have not seen studies in this regard, on the meat of hares. We used the method of including in paraffin, after I preserve harvest muscle tissue in a solution of formalin. Therefore I followed: muscle fiber diameters, the cross-sectional area, and the proportion of muscle tissue and muscle connective tissue of Longissimus dorsi, Psoas muscles and Semitendinosus muscles.

Key words: hare, meat, connective tissue, muscle tissue

INTRODUCTION

Histological structure of the somatic muscles have a great theoretical and practical importance, since softness and density of muscle fibers, muscle fascicles size, the proportion of muscle and connective tissue, are factors that influence a variety of physical, chemical and technological properties of meat [3]. In turn, the internal histological structure of somatic muscles is dependent on the species, breed, individual, sex, age, etc [2].

In the following, we will briefly present some data on the histological structure of the somatic muscles of hares. Striated muscle fibers, the basic components of somatic muscles, are characterized by a certain thickness and length that evolves according to several factors, such as: species, breed, individual, somatic muscle, age and sex [1]. If muscle length is the order of centimetres, their thickness is tens of microns [5]. This thickness appears to be increasing with age and state of maintenance and fattening of the animal, being more developed in male sex than in the female, higher in some muscles and lower in others. In rabbits, the thickness of the muscle

fibers of somatic muscles is less studied, and literature in this area is relatively poor.

Generally, the histological structure of somatic muscles and of the meat, parts the tissue into two categories: skeletal muscle and respectively connective tissue [4]. The proportion that have these two tissue types and their relationship depends to a very large extent the chemical composition, caloric value, biological quality of proteins, tenderness, cut resistance, water retention capacity and other physical properties and technological properties of muscle and meat [1].

MATERIAL AND METHODS

Biological material was composed of a number of 35 hares (*Lepus europeus pallas*) (14 males and 21 females).

For all the samples collection and processing muscle histological sample was used working tools anatomical and histological composed of: knives, scalpels, forceps, scissors, spreader, wadding, gauze, beaker of different sizes and capacities (50, 100, 150, 250 and 500 ml), Petri dishes, pipettes, measuring cylinders, glass stopper bottles with different capacity, glass funnel, desiccators; battery fixing with labelled containers, battery for clarification and dehydrating, paraffin wax battery,

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thermoregulation RAYPA oven-DOD 50, bar Leuckart, histological paraffin (56-57°C), beeswax, glass substrate slides, glass slides, SARTORIUS microtome sectioning knife, colouring baths, hard wood drying slides prepared, Motic digital microscope Trinocular DMWB1-223, objective micrometer, micrometer ocular micrometer grid eyepiece, image analysis software Image Motic Plus ML, M230 digital camera and Motic Fuji Film Finepix A800 digital camera, equipped with photomicrography.

Reagents were used: 10% concentration formalin for fixation samples, absolute ethanol 85°, 90° and 96°, then absolute amyl alcohol, albumin Mayer, toluene, benzene, xylene, Harris haematoxylin, acidic fuchsin, eosin, methylene blue, Evans blue, Canada balsam and cedar oil. Experimental data acquired images were processed by computer, using software digital microscope - Motic Image Plus ML and Microsoft Excel spreadsheet application. The total number of fibers in the section was counted manually.

To test the statistical significance of differences between the characters studied, we used ANOVA Single Factor algorithm included in Microsoft Excel software package. The first stage occurred usual statistical estimators calculation - arithmetic mean (\bar{X}), variance (S^2), standard deviation (s), standard error of the mean ($S\bar{x}$) and coefficient of variation (V%) - calculated using the software algorithm.

RESULTS AND DISCUSSIONS

Were performed such measurements in terms of large and small diameter of myocytes and muscle fascicles in the order I and II and their cross-sectional area.

These data were used to form a picture of the ultra structure of muscle elements, and to calculate the relationship between these two diameters (large and small), data that served to highlight the predominant form of cross-sectional profile configurations analyzed.

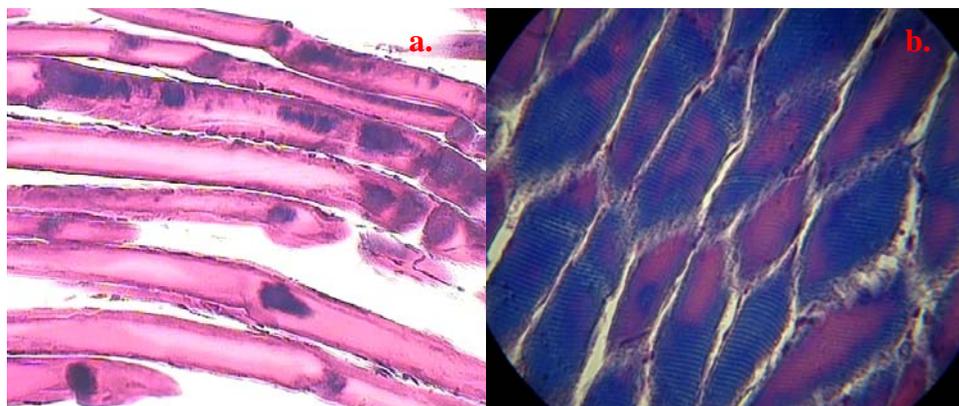


Figure 1 *Longissimus dorsi* muscle a) longitudinal section; b) transverse section

Abbreviations:

L.D. = *Longissimus dorsi* muscle

S.T. = *Semitendinosus* muscle

M.T. = muscle tissue

C.T. = connective tissue.

For ANOVA test:

n.s = insignificant difference

*=significant differences

** = very significant difference.

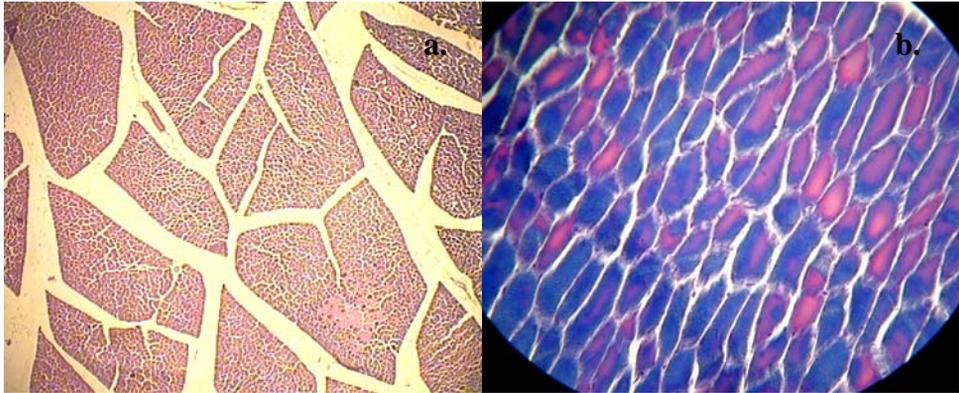


Figure 2 Transverse section a.) *psaos* muscle, b.) *semitendinosus* muscle



Figure 3 Muscle measurements: a.) area of fascicle, small diameter and big diameter of L.D. muscle; b.) area of fibres on S.T. muscle,

Table 1 Histological measurement for *Longissimus dorsi* muscles

Statistically expected	Large diameter (μ)	Small diameter (μ)	Mean diameter (μ)	Ratio BD/sD (μ)	Cross-sectional area (μ ²)
\bar{X}	67.333	41.158	54.245	1.635	1567.519
s^2	4488.20	113.713	2300.957	39.469	1016.961
s	66.994	10.663	38.828	6.282	1008.445
$\pm s_{-x}$	12.893	1.980	7.436	6.511	181.122
V%	19.148	25.908	22.528	0.739	64.333
Minimum	16.4	25.1	20.75	0.653	85.1
Maximum	284.2	64	174.1	4.440	4113.5

BD - big diameter; sD - small diameter.

Coefficient of variation for the *Longissimus dorsi* muscle has exceeded 20% symbolizing a heterogeneous population.

Applying ANOVA test we observe insignificant differences between male and female.

Coefficient of variation for the *Psoas* muscles has exceeded 20% symbolizing a heterogeneous population in this regard, the histological measurements.

Applying ANOVA test for *Psoas* muscles (minor and major), we observe insignificant differences between male and female.

Table 2 Histological measurements for *Psoas* muscles

Statistically expected	Large diameter (μ)	Small diameter (μ)	Mean diameter (μ)	Ratio BD/sD (μ)	Cross-sectional area (μ ²)
\bar{X}	51.825	33.979	42.902	1.525	1110.081
s^2	5096.572	142.721	2619.647	35.71	780537.4
s	71.390	11.946	41.668	5.97	883.4803
$\pm s_x$	13.739	2.218	7.978	6.19	158.6777
V%	26.510	27.164	26.837	0.97	79.58703
Minimum	22	26.9	24.45	0.81	85.1
Maximum	318.6	70.1	194.35	4.54	3319

BD-big diameter; sD-small diameter.

Coefficient of variation for muscle *Semitendinosus* has exceeded 20% for small and medium diameters, symbolizing a inhomogeneous population.

Coefficient of variation (V%) for large diameter muscle fibers exemplifies a very

homogeneous population, the amount not exceeding 10%. Applying ANOVA test for *Semitendinosus* muscles, we observe insignificant differences between male and female.

Table 3 Histological measurements for *Semitendinosus* muscles

Statistically expected	Large diameter (μ)	Small diameter (μ)	Mean diameter (μ)	Ratio BD/sD (μ)	Cross-sectional area (μ ²)
\bar{X}	147.344	34.279	90.811	4.298	2001.306
s^2	4966.838	140.845	2553.842	35.264	754507.2
s	70.475	11.867	41.171	5.938	868.623
$\pm s_x$	13.563	2.203	7.883	6.154	156.009
V%	9.205	34.620	21.912	0.265	43.402
Minimum	25.1	18.6	21.85	1.349	793.5
Maximum	293.7	64	178.85	4.589	4277.5

BD-big diameter; sD-small diameter.

The proportion of striated muscle tissue and connective tissue in the composition of the studied muscles

Connective tissue content of rabbit meat is an assessment criterion for the quality. Connective tissue is within the structure of tendons and aponeurosis of insertion and

coverage. The connective tissue itself is low in water content and high in protein, but the nutritional value of these proteins is lower because it does not contain all essential amino acids and the ratio of essential and nonessential is totally unbalanced.

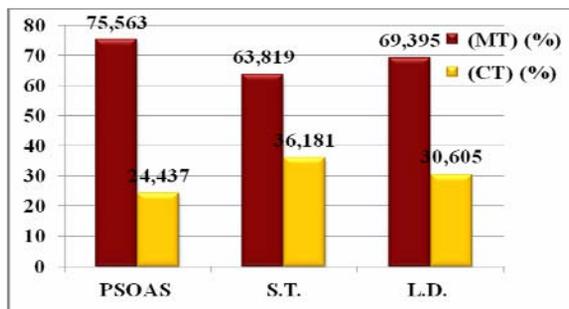


Figure 4 The proportion of muscle and connective tissue in analyzed muscles (%)

Observe the highest proportion of muscle tissue for the *psaos* muscle, with an average of 75.563%, followed by the muscles L.D. (69.395%) and then to the muscles S.T. (63.819) (fig. no. 4.). As the proportion of connective tissue, the highest values were found for S.T. muscles (36.181%), followed

by those for muscles L.D. (30.605%). The lowest value for connective tissue was recorded in *psaos* muscles (24.437%). The average density of muscle fibers (No. muscular fiber/mm²) is higher for muscles *psaos* (3423), followed by L.D. muscles (2856) and then the muscles S.T. (2789).

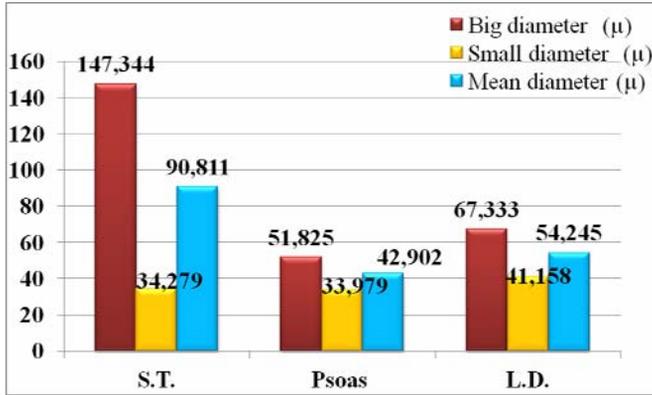


Figure 5 Average value of muscles fibers diameters

From measurements made on muscle fibers from hares it can be said that the *psaos* muscles are the fine in terms of texture, muscle fiber diameter (fig. no. 5) and collagen content was the lowest, *Longissimus dorsi* muscle is next, followed closely by *Semimembranosus* muscle.

The results obtained for average value of muscles fibres diameters of male and female of hare, applying ANOVA test, are presented in table no. 4.

Table 4 Statistical significance on ANOVA test \hat{F}

Parameters	Psoas	L.D.	S.T.
Muscle fibre diameter	$\hat{F} = 0,826$, $F_{5\%}(1;32)=4,149$; $\hat{F} < F_{5\%}=n.s.$	$\hat{F} = 0,895$, $F_{5\%}(1;32)=4,149$; $\hat{F} < F_{5\%}=n.s.$	$\hat{F} = 3,688$, $F_{5\%}(1;48)=4,042$; $\hat{F} < F_{1\%}=n.s.$
Large diameter (μ)	$\hat{F} = 1,126$, $F_{5\%}(1;32)=4,149$; $\hat{F} < F_{5\%}=n.s.$	$\hat{F} = 0,395$, $F_{5\%}(1;32)=4,149$; $\hat{F} < F_{5\%}=n.s.$	$\hat{F} = 3,688$, $F_{5\%}(1;48)=4,042$; $\hat{F} < F_{1\%}=n.s.$
Small diameter (μ)	$\hat{F} = 2,075$, $F_{5\%}(1;31)=4,159$; $\hat{F} < F_{5\%} n.s.$	$\hat{F} = 2,038$, $F_{5\%}(1;32)=4,149$; $\hat{F} < F_{5\%} n.s.$	$\hat{F} = 1,305$, $F_{5\%}(1;32)=4,149$; $\hat{F} < F_{5\%} n.s.$
Mean diameter (μ)	$\hat{F} = 2,263$, $F_{5\%}(1;32)=4,149$; $\hat{F} < F_{5\%}=n.s.$	$\hat{F} = 1,775$, $F_{5\%}(1;32)=4,149$; $\hat{F} < F_{5\%}=n.s.$	$\hat{F} = 1,113$, $F_{5\%}(1;48)=4,042$; $\hat{F} < F_{1\%}=n.s.$
Ratio BD/sD (μ)	$\hat{F} = 0,956$, $F_{5\%}(1;32)=4,149$; $\hat{F} < F_{5\%}=n.s.$	$\hat{F} = 0,793$, $F_{5\%}(1;32)=4,149$; $\hat{F} < F_{5\%}=n.s.$	$\hat{F} = 1,418$, $F_{5\%}(1;48)=4,042$; $\hat{F} < F_{1\%}=n.s.$
Cross-sectional area (μ ²)	$\hat{F} = 2,715$, $F_{5\%}(1;32)=4,149$; $\hat{F} < F_{5\%}=n.s.$	$\hat{F} = 3,435$, $F_{5\%}(1;32)=4,149$; $\hat{F} < F_{5\%}=n.s.$	$\hat{F} = 1,384$, $F_{5\%}(1;48)=4,042$; $\hat{F} < F_{1\%}=n.s.$

CONCLUSIONS

Following histological determinations, it can be observed the highest proportion of muscle tissue for the *psoas* muscles, with an average of 75.563%, followed by the muscles L.D. (69.395%) and then to the muscles S.T. (63.819%). As far the proportion of connective tissue, the highest values were found for the S.T. muscles (36.181%), followed by those for the muscles L.D. (30.605%). The lowest value for connective tissue was recorded in the *psoas* muscles (24.437%). With the measurements taken it can be said that the *psoas* muscles are the finest in terms of texture and muscle's fiber diameter and collagen content was lowest.

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