Histologic Characterization of Muscles Collected From Rabbits Flemish Giant Breed

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Abstract

The purpose of this paper is the histological characterization of some muscle groups from rabbits (Flemish Giant breed). The histological structure of the somatic muscles have a great theoretical and practical importance, since density of muscle fibers, the size of muscle fascicles, the proportion of muscle tissue and connective tissue are factors that influence a variety of sensory, physical, chemical and technological properties of meat. In Flemish Giant breed rabbits, the thickness of muscle fibers is less studied, and this study area is relatively poor. The few specific published data refers to some medium specialized breed hybrids for meat. For histological characterization we used the including in paraffin method. Therefore we followed: muscle fibber diameters, the cross-sectional area and the proportion of muscle tissue and muscle connective tissue of *Longissimus dorsi* muscles, *Psoas* muscles and *Semimembranosus* muscles. Following the performed determinations has been observed the highest average value for the diameter of the muscle fiber (184μ) muscle fibers area (27739μ^2) and cross-sectional area (2628290μ^2) for *Semimembranosus* muscles. In conclusion, among the analyzed muscle groups, the *Psoas* muscles are most tender, the fiber diameter being smallest.

Keywords: connective tissue, meat, muscle tissue, rabbit

1. Introduction

In meat-producing animals such as pigs and rabbits, the number of myofibers was found to be related to growth capacity and meat quality [1], properties which were taken into account in breeding programs.

The number of muscle fibers present at birth appears to determine the maximal lean meat growth capacity and meat quality of the adult animal, and seems to be dependent on the number of embryonic myocytes [1].

The heritability of meat quality and histological characteristics varies with the breed of rabbits. Meat quality can be evaluated by several important traits, including color, taste, fat content, texture, tenderness and its histochemical characteristics [2]. It has become evident that many histological characteristics of muscle such as density, diameter, cross-sectional area of the fibers, the percentage of red fibers and glycogen content are directly associated with meat quality [3]. Texture properties of meat are considered one of the most important attributes for consumers. Most of the studies on meat texture have been done on pork, beef and poultry, while information on rabbit meat is limited. There are studied only the effect of rabbit genetic line on certain carcass characteristics including textural properties [4]. The authors concluded that differences in tenderness of rabbit loin were more affected by genetic origin than by body weight [5].

A high density of small fibers also enhances the quality of the meat. The proportion of intramuscular fat and connective tissue are also

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important factors which influence the taste of rabbit meat [3].
This paper is part of a broader study aimed on comparative characterization of rabbit meat (Flemish Giant breed) and hares meat (*Lepus Europaeus Pallas).*

2. Materials and methods

Biological material comprised a number of 55 rabbits (Flemish Giant breed) (24 males and 31 females). The rabbits had an average body weight of 11.5 kg, at the age of reproductive maturity (adults: 11-12 months). They were fed ad libitum with granulated forage, grains and green fodder.
The muscle samples (*Longissimus dorsi* (L.D) muscles, *Psoas* muscles (minor and major) and *Semimembranosus* (S.M.) muscles) were collected immediately after slaughter. Immediately after collection, the samples were cut parallel to the muscle fiber direction and and were placed in 10% concentration formalin for fixation. To make histological blades were used paraffin wax battery, thermoregulation RAYPA oven-DOD 50, bar Leuckart, histological paraffin (56-57°C), beeswax, glass substrate slides, glass slides, SARTORIUS microtome sectioning knife and a coloring baths. A number of 130 samples were colored with eosin. To examine the microscopic section we used Motic digital microscope Trinocular DMWB1-223, objective micrometer, micrometer grid eyepiece, image analysis software Image Motic Plus ML, M230 digital camera and Motic Fuji Film Finepix A800 digital camera, equipped with photomicrography. 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 manually counted.
In the first stage occurred usual statistical estimators calculation-arithmetic mean ($\overline{x}$), variance (S^2), standard deviation (s), standard error of the mean ($S_{\overline{x}}$) and coefficient of variation (V%)-calculated using the software algorithm. To test the statistical significance of differences between the characters studied, we used ANOVA Single Factor algorithm included in Microsoft Excel software package.

3. Results and discussion

Assessing the average values of calculated parameters are observed larger diameters of the area of muscle fibers and muscle fascicles belonging to male rabbits compared to females.
Further are shown some aspects of histological samples seen under a microscope (Figure 1-5) and processed using the software Motic Image Plus ML and Microsoft Excel spreadsheet application.

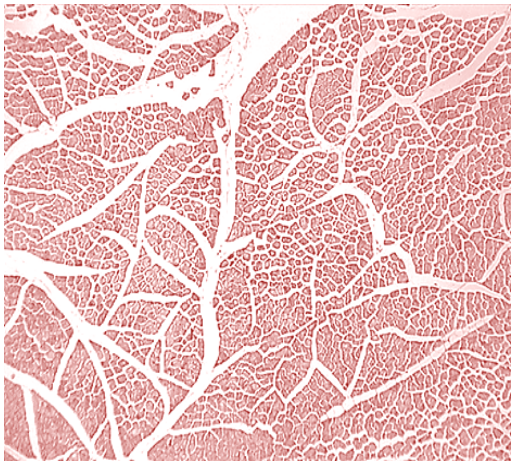


Figure 1. *Psoas* muscle staining with eosin (OC 4X OB 10X)

Figure 2. L.D. muscle staining with eosin (OC 10X OB10X)

Figure 3. L.D. muscle longitudinal section (OC10X OB 40X)

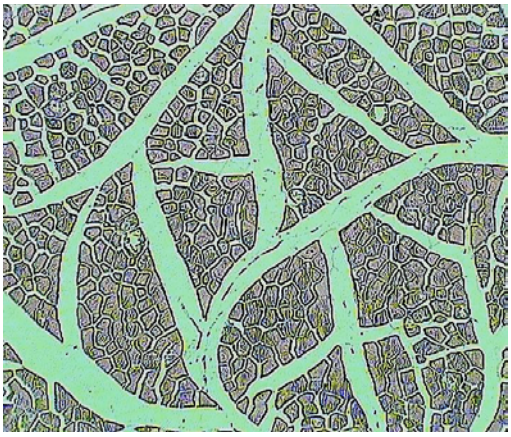
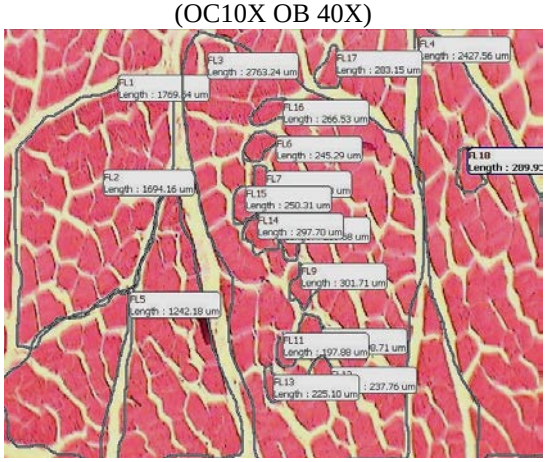


Figure 4. *Psoas* muscle (OC 10X OB10X) (with eosin)



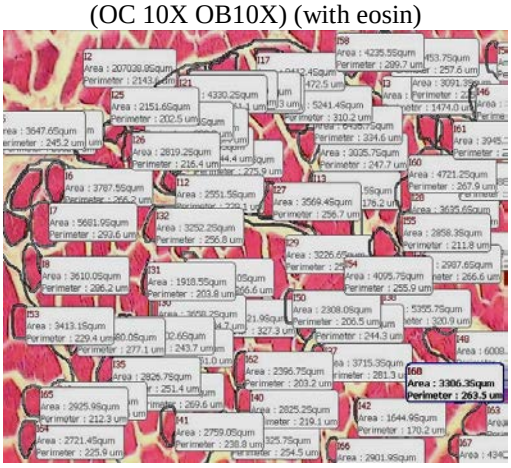


Figure 5. Measurements on rabbit *Semimembranosus* muscles fibers staining with eosin (OC 10X OB 40X)

Abbreviations: L.D.=*longissimus dorsi muscle,* S.M=*semimembranosus muscle,* M.T.=*muscle tissue,* C.T.=*connective tissue,* For ANOVA test: n.s=*insignificant difference*

The determination' results performed using this software-Motic Image Plus-were statistically processed and are presented in the following tables.

Coefficient of variation of histological determinations for *Longissimus dorsi* muscles, for Flemish Giant males, expressed a inhomogeneous population at the level of large and small diameter of myocytes (exceeding thresholds of 20%). For other indicators (mean diameter (μ), muscular fibers area (μ^2), cross-sectional area (μ^2)) was found relatively homogeneous population and are presented in table 1.

For Flemish Giant females (for *Longissimus dorsi* muscles) the coefficient of variation expressed a relatively homogeneous population (Table 1).

Applying ANOVA test for *Longissimus dorsi* muscles was observed insignificant differences between male and female.

The fiber of *Longissimus dorsi* muscles in pig , showed no significant interaction and effects of sex on all measurements of muscle fiber characteristics [1].

The coefficient of variation for *Psoas* muscles (Table 2.) showed a heterogeneous population for males and a relatively homogeneous population for females for all analyzed indicators. Applying ANOVA test for *Psoas* muscles (minor and major), we observe insignificant differences between male and female. In rabbits (Flemish Giant breed) we see a higher average size of muscle fibers (large diameter, small diameter, the average diameters, area myocytes, the cross-sectional area) compared with results obtained for hares (*Lepus Europaeus Pallas*) [2].

Table 1. The statistics estimators on the histological determinations for *Longissimus dorsi* muscles

Statistics estimators		$\overline{X} \pm S_{\bar{x}}$	V%	Minimum	Maximum
Flemish Giant Males	Large diameter (μ)	91.9±11.8 n.s.	23.2	73.4	107.2
	Small diameter (μ)	44.158±1.74	29.78	39.1	54.3
	Mean diameter (μ)	68.029±5.4	12.14	42.75	74.1
	Ratio BD/sD (μ)	2.081±5.23	14.3	0.653	4.440
	Muscular fibers area (μ^2)	2870±11.7	13.98	1793	3293
	Cross-sectional area (μ^2)	2567519±181	17.95	951213	41523135
Flemish Giant Females	Large diameter (μ)	88.7±13.5 n.s.	13.5	63.6	99.2
	Small diameter (μ)	38.18±1.3	17.42	31.4	48.3
	Mean diameter (μ)	62.39±2.4	10.19	32.9	84.1
	Ratio BD/sD (μ)	2.32±2.3	11.3	0.153	3.54
	Muscular fibres area (μ2)	2745±9.5	12.38	1293	3713
	Cross-sectional area (μ2)	2456432±74	13.45	841243	39527136

BD-big diameter; sD-small diameter

Table 2. The statistics estimators on the histological determinations for *Psoas* muscles (μ)

Statistics estimators		$\overline{X} \pm S_{\bar{x}}$	V%	Minimum	Maximum
Flemish Giant Males	Large diameter (μ)	55.8 ±13.7 n.s.	21.2	16.4	284.2
	Small diameter (μ)	35.38±1.98	31.21	25.1	64
	Mean diameter (μ)	45.59±3.436	22.93	20.75	174.1
	Ratio BD/sD (μ)	1.577±4.23	20.85	0.653	4.440
	Muscular fibers area (μ2)	1328±2.48	29.18	1135	1930
	Cross-sectional area (μ2)	1090042±181.122	22.03	626114	1250774
Flemish Giant Females	Large diameter (μ)	51.9 ±3.8 n.s.	11.2	18.4	84.2
	Small diameter (μ)	29.28±1.5	14.1	21.3	59.4
	Mean diameter (μ)	45.59±3.31	12.3	23.95	74.1
	Ratio BD/sD (μ)	1.77±5.3	10.9	0.43	3.50
	Muscular fibers area (μ2)	1285±1.7	19.12	1005	1734
	Cross-sectional area (μ2)	1126314±81.42	12.06	323564	1068912

BD-big diameter; sD-small diameter

The coefficient of variation calculated for *Semimembranosus* muscles collected from rabbits characterize a heterogeneous population for males (for all analyzed indicators) and a relatively homogeneous population for females (Table 3).

Applying ANOVA test for *Semimembranosus* muscles, we observe insignificant differences between male and female.

The mean size of muscle fibers for *Semimembranosus* muscles were much higher for rabbits (Flemish Giant breed) compared with those determined for hares (*Lepus Europaeus Pallas*) [2].

Table 3. The statistics estimators on the histological determinations for *Semimembranosus* muscles (μ)

Statistics estimators		$\overline{X} \pm S_{\bar{x}}$	V%	Minimum	Maximum
Flemish Giant Males	Large diameter (μ)	253±14.4 n.s.	23.2	241	302
	Small diameter (μ)	116.8±3.7	29.5	64.4	120
	Mean diameter (μ)	184±5.36	35.4	71.52	274.1
	Ratio BD/sD (μ)	2.011±4.315	21.4	1.233	3.340
	Muscular fibres area (μ^2)	27739±8.16	22.7	17578	39669
	Cross-sectional area (μ^2)	2628290±210.23	34.53	1710245	5228290
Flemish Giant Females	Large diameter (μ)	248±11.5 n.s.	13.1	187	292
	Small diameter (μ)	108±1.6	19.1	84.6	119
	Mean diameter (μ)	173±4.7	5.4	91.32	253.4
	Ratio BD/sD (μ)	2.29±3.15	11.7	1.53	3.40
	Muscular fibers area (μ2)	26431±5.2	6.9	19847	29233
	Cross-sectional area (μ2)	2017578±10.63	14.23	1413271	4897561

BD-big diameter; sD-small diameter.

In rabbits (Table 4), the lowest value of connective tissue was observed to *Longissimus dorsi* muscles (14.87%), followed by the *Psoas* to muscles (25.82%) and by the *Semimembranosus* muscles (31.35%).

Table 4. The proportions of the main categories of the muscles tissue Flemish Giant

Studied muscle	The number of muscle fibers	Density (nr. m.f./mm^2 muscles)	Muscle tissue (MT) (%)	Connective tissue (CT) (%)	MT/CT
PSOAS	90	2123	74.18	25.82	2.87
L.D.	78	1823	85.135	14.865	5.72
S.M.	71	1798	69.65	31.35	2.22

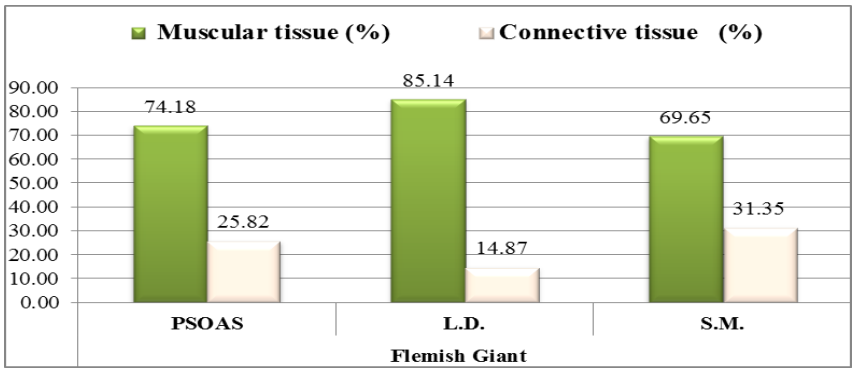


Fig. 6. The proportion of muscle tissue and connective tissue in analyzed muscles (%)

The proportion of the hare's connective tissue was observed to have higher values for *Semimembranosus* muscles (36.181%), followed by the muscles *Longissimus dorsi* (30.605%) and for the *Psoas* muscles (24.437%).

The average density of muscle fibers (no. of muscle fibers/mm^2) is highest in *Psoas* muscles (2123), followed by *Longissimus dorsi* muscles (1823), and then by the *Semimembranosus* muscles (1798); this it is related to the size of muscle fibers (Tables 1-3).

4. Conclusions

Following measurements performed on muscle fibers from rabbits we can say that the *Psoas* muscles are the finest in terms of texture (muscle fiber diameter is lowest), *Longissimus dorsi* muscle is next, followed by *Semimembranosus* muscle.

The lowest amount of connective tissue (14.865%) was found to *Longissimus dorsi* muscles so they have the highest nutritional value, knowing that a balanced level of essential amino acids is located in the muscular tissue (85.135%) and not in the connective tissue. They are followed by *Psoas* muscle (74.18%) and *Semimembranosus* muscle (69.65%). For the future, for an overview, and other muscle groups are required to be analyzed.

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