Microstructural studies of muscle tissue of lamb of aboriginal breeds of the Volga region

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Abstract The article presents current information on the microstructure of muscle tissue of aboriginal young sheep of the Volga region. The efficiency of lifetime formation of meat productivity and consumer properties of lamb from young sheep of the Bakur breed, sold in the year of birth, is estimated by the method of microstructural analysis. The method of morphometric assessment revealed the features of individual development of animals, conditioned by hereditary patterns and environmental conditions affecting the exchange processes, that ultimately changes the individual development of animals.

1. Introduction
Currently, lamb production is considered to be the most promising and rapidly developing industry and amounts to 15 million tons per year. Slaughter and processing of sheep of regional Volga breeds can become a reserve for increase in lamb production [1, 2, 3]. Unique aboriginal coarse-haired sheep of the Bakur breed with special zoo-biological characteristics have been raised in the personal sector of the right-bank regions of the Saratov province for more than 100 years. These animals are large enough, well-adapted to local climatic and feeding conditions, and highly valued by the local population for their endurance, unpretentiousness and high efficiency. At the same time they are also characterized by high early maturity and yield high-quality lamb at an early age [4, 5, 6].

Due to the fact that sheep of this breed occupy a significant place in the meat balance of the local population, studies of microstructural indicators that determine characteristics of growth and development of muscle tissue as well as fat deposition according to the main periods of ontogenesis of these animals, their influence on the slaughter qualities and nutritional value of lamb are of undoubted interest.

2. Materials and methods
Meat samples of 30x30x30 mm were selected for histological studies, each sample was labeled with the main parameters and date. Then the samples were fixed in a 10% neutral aqueous solution of formalin at room temperature for 48 hours. After fixation the samples were washed for one day in cold running water to remove formalin. After washing, pieces of 5x10x20 mm in size were cut out of the fixed material in such a way that there was a casing of a loaf of meat product along the long side of the piece. The casing was preserved for research purposes.
Then the material was filled into gelatin. To do this, the pieces were placed in a liquid 12.5% solution for 12 hours and, for at least the same period, in a thick 25% solution. These operations were performed in a thermostat at 37°C. Then the pieces were placed in a fresh thick gelatin solution in cups and quickly cooled in the refrigerator. After 30 minutes, the resulting blocks were cut out and compacted in an aqueous solution of formalin with a concentration of 10% for 1-2 days.

Sections were made on a microtome-cryostat MK-25M, and when sectioning, orienting thin sections of ham with a preserved and tightly fitting casing perpendicular to the plane of the cutting edge of the microtome knife.

Sections were stained with Ehrlich hematoxylin and water-alcohol eosin. This coloring is designed to identify tissue and cell interrelation in the analyzed sample and allows for both qualitative research and morphometric measurements. The colored sections were placed under a cover glass in glycerine-gelatin.

Histological analysis was performed on a domestic computer image analyzer produced by “VideoTesT” company (Saint Petersburg) using the program “Morpho-4.0”.

The samples were studied and photographed using a video-computer image analysis system in several stages. The first step was to obtain an image using a light microscope. Magnification of the lens used was X25, X40. At the second stage, image readout was made using a video camera. Then, image filtration and refinement was made for quality enhancement of the entered image in accordance with the analyzer software. After that, a selection of research objects was performed interactively. The final stages were obtaining the results of measurement of the aimed parameters and data processing in accordance with the computer program used [7].

3. Results

Microstructural composition is one of the most objective indicators determining the quality of meat which allows identifying even minor changes in the structure of tissues that affect the quality of the finished product [8]. Due to the fact that the information on the microstructural features of lamb is not complete enough and does not have a systematic nature, the authors studied the structure and changes in the histological structure of m. Longissimus dorsi of Bakur rams carcasses depending on age.

The quality of meat is largely determined by the histological structure of animal muscle tissue and depends on the size of muscle fibers, the state and structure of connective and adipose tissues [9, 10].

The thickness of muscle fibers is considered to be one of the important indicators responsible for the tenderness of meat. Objective measurements have shown that the diameter of the muscle fibers of the lamb under study increases with age and live weight of animals. Experimental values are shown in table 1.

| Animal age, months | Live weight, kg | Bakur breed arithmetic average | Variance |
|-------------------|----------------|-------------------------------|----------|
| 2                 | 17.6±0.3       | 22.7                          | 21.5 – 23.2 |
| 4                 | 27.5±0.5       | 36.1                          | 35.0 – 37.7 |
| 6                 | 32.9±0.4       | 38.7                          | 36.8 – 39.7 |
| 12                | 45.7±0.6       | 45.8                          | 43.9 – 46.9 |

The obtained regularity gives reason to believe that the age-related growth of muscle tissue and, consequently, the increase in live weight, occurs due to the thickening of individual fibers. The intensity of linear increase in the muscle tissue is the highest in rams when they reach the live weight of 27.5; 32.9 kg, that is 4-6 months, then it sharply decreases.

The thickness of the muscle fiber of the m. Longissimus dorsi in the 4 month-old Bakur rams with an increase in live weight from 17.6 to 27.5 kg increased by 13.5 μm; from 27.5 to 32.9 kg - by 1.8 μm; from 32.9 to 45.7 kg - by 7.1 μm. All this suggests that the experimental animals after they reached the
Age of 4 months and weight of 27.5 kg grew not only due to the increase in the tissue bulk, but also due to the development of other tissues and, above all, adipose tissue.

When conducting microstructural examination of the muscle tissue of the \textit{m. Longissimus dorsi} of 2-month-old Bakur rams, having a live weight of 17.6 kg, it was found that an hour after slaughter, the muscle fibers are undulated, patchily corrugated and lie freely relatively to each other. Cross-striation is weakened, however, as a result of the myofibrils contraction in the bulk of the tissues, the intensity of longitudinal striation increases. In the deep muscle layers, small, single over-contraction nodes of oval form are noted. The fiber nuclei are round or oval, with a clear chromatin structure. There were no destructive changes in the structure of muscle tissue (figure 1). The muscle fibers are of polygonal shape on cross sections, the boundaries between them are clearly marked.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure1.jpg}
\caption{\textit{M. Longissimus dorsi}. 2 months. Longitudinal section. Cross-striation of muscle fibers is weakened (H.E. * 200).}
\end{figure}

The average diameter of muscle fibers in bundles is 19.4 μm. The diameter of the muscle fibers ranges from 15 μm to 28 μm, while small tissues up to 20 μm make up 65.9%.

Connective tissue sheets lie freely relatively to bundles of muscle fibers, of undulatred or convoluted shape, 30-100 μm thick, include fat cells about 35 μm in size. There are sheets of adipose tissue 100-180 μm thick between the secondary bundles of the muscle fibers (figure 2).

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure2.jpg}
\caption{\textit{M. Longissimus dorsi}. 2 months. Cross section. Muscle fibers are polygonal (H.E. * 200).}
\end{figure}

The microstructure of the muscle tissue of the \textit{m. Longissimus dorsi} of 4-month-old Bakur rams having live weight of 27.8 kg was characterized by undulated or convoluted, patchily corrugated muscle fibers. Cross-striation is insignificant. The boundaries between the fibers are well-marked. The nuclei of fibers are round or oval with a clear chromatin structure. Destructive changes are not detected (figure 3). The muscle fibers are polygonal in shape on the cross-sections, the boundaries between them are clearly marked. The connective tissue sheets are loose, consisting of individual bands of bundles of
collagen fibers and individual fat cells. There are sheets of adipose tissue formed from fat cells with a diameter of $14 \pm 1.3 \, \mu m$ between the secondary bundles of muscle fibers.

The microstructure of the muscle tissue of the *m. Longissimus dorsi* of 4-month-old Bakur rams after slaughter was characterized by undulated or convoluted, patchily corrugated muscle fibers. Cross-striation is weakened, shallow. The boundaries between the fibers are well-marked. The nuclei of fibers are round or oval with a clear chromatin structure. Destructive changes are not detected (figure 4).

On cross-sections, the muscle fibers are polygonal in shape, the boundaries between them are clearly marked. The diameter of the fibers ranges from 18 to 30 \, \mu m. The average fiber diameter is 23.7 \, \mu m, while small fibers up to 20 \, \mu m account for 52.9% of the total fiber number in the bundle.

The connective tissue sheets 30-170 \, \mu m thick are loose, consisting of individual bands of collagen fiber bundles, including individual fat cells. There are sheets of adipose tissue 120 to 300 \, \mu m thick, formed from cells of medium size about 35 \, \mu m, between the secondary bundles of muscle fibers.

A microstructural study of the muscle tissue of the *m. Longissimus dorsi* of 6-month-old Bakur rams having live weight of 33.0 kg showed that the muscle fibers had a clear boundary and well-expressed tinctorial properties. The direction of the bundles of muscle fibers is slightly-undulated. Cross-striation is well-expressed. The nuclei of myofibrils have a thickened, fusiform shape and fuzzy boundaries (figure 5). Muscle fibers have a slightly-convoluted shape on cross-sections. In individual fibers, more or less extended areas with the presence of longitudinal striation are detected, indicating the presence of contraction zones. The sheets of endomysium are very thin and tender, consisting mainly of cellular elements. The functional state of muscle tissue in this muscle is of the same type.
Connective tissue sheets are located freely relatively to the bundles of muscle fibers, between which there is adipose tissue with a certain amount of fat deposition (figure 6).

A histological examination of the structure of muscle tissue of the m. Longissimus dorsi of 12-month-old Bakur rams having live weight of 45.8 kg revealed that the muscle fibers are predominantly straight. The boundaries of the fibers are clearly defined due to their non-dense fit to each other. The degree of contraction of sarcomeres in the fibers is different.

There are fibers with a shallow cross-striation, down to its absence, which indicates their contracted state, in the latter case, their longitudinal striation, formed by individual myofibrils, is expressed. The nuclei of muscle fibers are located directly under the sarcolemma and have an elongated, often oval, shape with a well-defined chromatin structure of a granular-lumpy type. Destructive changes are presented in the form of microcracks, while the sarcolemma remains intact (figure 7).

On cross-sections, muscle fibers are polygonal in shape. The average fiber diameter is 45.8 ± 1.2 μm. Between bundles of muscle fibers there are poorly developed sheets of connective tissue: thinner - endomysium and thicker – perimizium (figure 8). The nuclei of cellular elements of connective tissue are also detected.
Figure 7. *M. Longissimus dorsi*. 12 months. The shape of the nuclei of muscle fibers is oval (H.E. * 200).

There are well-developed adipose tissue sheets formed by fat cells between the bundles of muscle fibers and inside them (figure 9).

Figure 8. *M. Longissimus dorsi*. 12 months. Dense bundles of connective tissue (H.E. * 200).

Figure 9. *M. Longissimus dorsi*. 12 months. Large fat cells are between the fibers * Sudan black “B” * 200.

4. Conclusion
The structure and changes in the histological structure of *m. Longissimus dorsi* of Bakur rams’ carcasses were studied depending on their age. The conducted research allows concluding that generally, the dynamics of changes in the structure of the muscle tissue of *m. Longissimus dorsi* of the Bakur rams has some features. The location of myofibrils in this muscle is rectilinear-undulated. *M. Longissimus dorsi* has a high rate of fat deposition. Fat drops appear in young animals at the age of 4 months having live weight of 27.5 kg. The cross-striation of muscle tissue at all values of age and live weight of the studied...
animals is expressed rather weakly. The volume of connective tissue development in 4-month-old Bakur rams having live weight of 27.5 kg is insignificant. Based on these facts, the muscle tissue of *m. Longissimus dorsi* has a high saleable quality due to the thinnest muscle fibers with small fat inclusions and is considered the most tender. Research has confirmed the practicability of widespread use of Bakur sheep in the production of young lamb. For the purpose of its rational processing, it is recommended to slaughter rams for meat at the age of 4-6 months.

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