Comparative Morphological and Functional Characteristics of the Muscle Tissue of Gobies of Cattle of Three Breeds (Hereford, Kazakh White-Headed and Kalmyk)

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Abstract. Using a complex of morphological methods, the muscle tissue of 18-month-old gobies of three cattle breeds (Hereford, Kazakh white-headed and Kalmyk) was studied. The results of the study showed that in the biceps femoris muscle and the longest muscle of the back of bulls of Hereford breed, the average diameter of muscle fibers was higher than in animals of the Kazakh white-headed and Kalmyk breeds. The thickness of the endomysium in the muscles of the animals studied was in the range of 6–13 μm and had no pronounced breed differences. Among the cellular elements of endomysium, endotheliocytes prevailed. Their share was about 80%. Type 1 collagen was higher in Hereford animals. In the muscles of animals of the Kazakh white-headed and Kalmyk breeds, the content of adipose tissue was higher than in animals of the Hereford breed. The results obtained reflect typical patterns of the structural and functional characteristics of cattle muscle tissue, and also show the range of in-breed variability of the muscle tissue of these animals.

1. Introduction

Hereford, Kazakh white-headed and Kalmyk cattle breeds are among the most common breeds grown in a sharply continental climate in the south-east of Russia, in the Volga region and in the Urals. At the same time, the Hereford breed was bred in the UK, and the Kalmyk and Kazakh white-headed in Russia (Kalmyk in the XVII century, and the Kazakh white-headed in the middle of XX). The morphology of muscle tissue in cattle is reflected in a large number of publications [1–6 and others]. Despite this, many aspects of the morphofunctional organization of the muscle tissue of these animals have not been studied enough, or are debatable and need further in-depth research. The results of the quantitative parameters of various muscle structures of animals of different breeds obtained by different researchers vary (sometimes within wide limits), which can be associated with many factors (the age of the animals studied, their weight, degree of fatness, productivity, climatic features of the growing region). The results of studies may also be affected by the quality of preparation of material for histological studies.

2. Purpose of research

Clarification of the morphological and functional characteristics of the biceps femoris and the longest back muscles of three cattle breeds - Hereford, Kazakh white-headed and Kalmyk.
3. Materials and methods
The object of the study was the biceps femoris and the longest muscle of the back of gobies of cattle of Hereford, Kazakh white-headed and Kalmyk breeds (6 gobies from each breed), grown in the dry-steppe zone in the Druzhba breeding farm in the Stavropol Territory in the period 2018-2019. Cultivation of the studied animals was carried out under the same conditions of feeding and keeping. In the process of growing, the mass of calves after birth was determined at the age of 3, 8, 12, 15 and 18 months (tables 1 and 2). Muscle tissue samples for research were taken from 18-month-old animals in October 2019 during the slaughter of gobies at a meat factory in s. Marvelous of the Stavropol Territory.

| Breeds              | Age in months | 8       | 12      | 15      | 18      |
|---------------------|---------------|---------|---------|---------|---------|
| Hereford            | Newborns      | 30.4±1.72| 229.2 ±2.35| 329.8±2.67| 423.3±3.98| 488.4±1.98|
| Kazakh white-headed |               | 24.3±1.94| 216.5 ±0.77| 317.1±3.51| 406.7±3.34| 471.7±1.82|
| Kalmyk              |               | 21.9±1.69| 209.6±1.89| 309.7±3.12| 393.6±4.29| 460.5±1.28|

Table 2. Morphometric indicators of the longest muscle of the back in gobies of three breeds of cattle.

| Parameter name                  | Breeds                      |
|---------------------------------|-----------------------------|
| Muscle diameter fibers, microns | Hereford, Kazakh white-headed, Kalmyk |
| The volume of myosymplast nuclei, μm³ | 125.7±5.0, 124.7±5.2, 119.0±5.4 |
| The density of nuclei per unit length of fiber, % | ±, ± |
| The thickness of the endomysium, microns | 8.5±0.1, 8.1±0.1, 7.9±0.2 |
| The content of muscle tissue (%) | 77.1±2.1, 76.9±2.0, 76.2±1.6 |
| The content of connective tissue, % | 22.9±0.4, 23.1±0.5, 23.8±0.5 |

For histological studies, the material was fixed in a 12% aqueous formalin solution, dehydrated in ethanol of increasing concentration, and embedded in paraffin. Paraffin sections with a thickness of 5-7 micrometers, made on a rotational microtome, were stained with histological examinations (Mayer hematoxylin and eosin), histochemical (SIC reaction) and immunohistochemical methods (for the detection of type I collagen). Polyclonal antibodies to type I collagen (ABBIOTEC, USA, titer 1: 150,
incubation for 45 minutes at room temperature) and imaging system (BioGenex, USA) were used to detect collagen. On histological sections, morphometry of the structural elements of the muscles was performed. The diameter of muscle fibers was determined on transverse sections, and the volume of myosymplast nuclei was measured on longitudinal sections. At the same time, the nuclei of myosatellite cells were excluded from the calculation. The thickness of the endomysium was measured on sections. The content of muscle and connective tissue was determined on a conditional unit of the area of transverse sections of muscles (4900 μm²), and the number of cells of connective tissue was counted. The obtained digital indicators were subjected to statistical processing using the Statistica-8 program.

4. Results and discussion

The basis of the studied muscles was muscle tissue represented by muscle fibers that form bundles of the 1st, 2nd, 3rd and higher orders, separated by layers of connective tissue, in which the blood and lymph vessels, as well as the structural elements of the nervous system, are located.

It was found that in animals of all studied breeds, the average diameter of muscle fibers in the biceps femoris significantly exceeds that in the longest muscle of the back (Tables 2 and 3).

Table 3. Morphometric indicators of the biceps femoris in gobiies of three breeds of cattle.

| Parameter name                        | Breeds           |
|--------------------------------------|------------------|
|                                      | Hereford         | Kazakh white-headed | Kalmyk     |
| Muscle diameter fibers, microns      | 49.3±2.1         | 42.8±2.5            | 41.7±2.2   |
| The volume of myosymplast nuclei, μm³ | 133.4±6.2        | 135.3±6.0           | 126.6±6.1  |
| The density of nuclei per unit length of fiber, % | ±              | ±                   | ±          |
| The thickness of the endomysium, microns | 9.2±0.4         | 8.5±0.2             | 8.1±0.2    |
| The content of muscle tissue (%)     | 76.3±1.4         | 76.9±1.1            | 77.2±2.3   |
| The content of connective tissue, %  | 23.7±0.6         | 23.1±0.6            | 22.8±0.5   |

Most muscle fibers in the studied muscles had a thickness of 20 to 50 μm, however, differences in this indicator were revealed among animals of various breeds (Tables 4 and 5). Analysis of interbreed differences in the thickness of muscle fibers in the biceps femoris and the longest muscle of the back showed that in the biceps femoris and the longest muscle of the back, the average diameter of muscle fibers in animals of Hereford breed was higher than in animals of the Kazakh white-headed and Kalmyk breeds. There were no significant differences in the thickness of muscle fibers between animals of the Kalmyk and Kazakh white-headed. There were also no differences in the average size of the nuclei of muscle fibers. Significant differences between animals of different breeds were manifested in the ratios of the proportion of muscle fibers of different diameters in the muscles (Tables 4 and 5).
Table 4. Distribution of muscle fibers by their size in the longest muscle of the back in 18-month-old Hereford gobies, Kazakh white-headed and Kalmyk cattle breeds.

| The diameter of muscle fibers (microns) | Hereford  | Kazakh white-headed | Kalmyk   |
|----------------------------------------|-----------|---------------------|----------|
| Up to 20 microns                       | 6.2±0.9   | 7.1±0.6             | 14.6±1.2 |
| 21-30 microns                          | 22.6±3.0  | 40.3±2.8            | 39.5±3.3 |
| 31-40 microns                          | 37.4±2.4  | 31.5±2.0            | 35.1±3.5 |
| 41-50 microns                          | 18.5±2.9  | 10.2±0.7            | 5.2±0.4  |
| 51-60 microns                          | 7.3±0.8   | 7.9±0.7             | 3.6±0.2  |
| Over 60 microns                        | 8.0±0.5   | 2.0±0.4             | 2.0±0.2  |

Table 5. The distribution of muscle fibers by their size in the biceps femoris in 18-month-old gobies of Hereford, Kazakh white-headed and Kalmyk cattle.

| Parameter name | Hereford | Kazakh white-headed | Kalmyk |
|----------------|----------|---------------------|--------|
| Up to 20 microns | 5.0±0.2  | 9.2±0.3             | 11.4±0.9 |
| 21-30 microns    | 21.6±2.7 | 32.3±2.1            | 353±3.6 |
| 31-40 microns    | 35.4±2.4 | 38.0±3.0            | 39.1±3.0 |
| 41-50 microns    | 15.6±1.1 | 10.0±0.7            | 6.2±0.8  |
| 51-60 microns    | 12.3±0.8 | 6.5±0.3             | 4.9±0.5  |
| Over 60 microns  | 10.1±0.8 | 4.0±0.3             | 2.1±0.3  |

It was found that the thickness of the endomysium in the muscles of the studied animals was in the range of 6–13 μm and had no pronounced differences among animals of different breeds, as well as among the two studied muscles (Tables 2 and 3, Figs. 1 and 2). Since the blood vessels of the microvasculature occupy a significant volume of endomysium, endotheliocytes prevailed among the cellular elements of endomysium, their proportion reaching 80%. The cell content of fibroblastic differential was varied in the range of 14–20%; the remaining cells accounted for a few percent.

Figure 1. The longest muscle of the back of the goby of the Kalmyk breed of cattle. Longitudinal section. The animal is 18 months old. Coloring: Mayer hematoxylin and eosin. Magnification: x 400.
Adipose tissue is distributed in the layers of connective tissue between bundles of muscle fibers of the second and higher orders. No pronounced layers of adipose tissue were revealed between the first-order bundles, however, single fat cells were always present in the endomysium. In the muscles of the Kazakh white-headed and Kalmyk breeds, the content of adipose tissue slightly exceeds that of the animals of Hereford breed.

An analysis of the results of immunohistochemical studies for the detection of type I collagen showed a higher expression of this protein in animals of Hereford breed in comparison with the Kalmyk and Kazakh white-headed. This indicates the presence in the connective tissue of a higher proportion of thick collagen fibers. There are significant differences in the content of this protein among animals of the Kalmyk and Kazakh white-headed breeds. It was not revealed. Significant differences in glycogen content in animals of different breeds were not found.

Despite the differences identified, it should be noted that the structure of muscle tissue is a fairly stable and conservative indicator, which was formed during the evolution and domestication of cattle.

In a number of works devoted to the histological structure of muscle tissue, attempts are made to relate the shape of cross sections of muscle fibers with pedigree features [2, 4]. The authors note that first-order muscle bundles in the longest muscle of the back have various geometric shapes, among which triangular and rhomboid prevail in Kostroma bull calf, trapezoid in Kalmyk bull calf, spindle-shaped and triangular in red steppe bull, and irregular shape of muscle bundles occurs in gobies of all breeds. We believe that this statement is not sufficiently substantiated. The presence of various forms of muscle fibers on the slices is associated, first of all, not with the pedigree features, but with possible defects in the preparation of material for histological studies. The main forms of muscle fiber in sections are round, oval and polygonal (Fig. 3 and 4). It is these forms of muscle fibers that are able to adequately provide muscle contraction and relaxation.
The information obtained on the different ratio of fibers of different diameters showed that the largest proportion of fibers of a smaller diameter is contained in the muscle tissue of animals of the Kalmyk breed. Thin muscle fibers are known to belong to slow (red) muscle fibers. A higher content of thin muscle fibers indirectly indicates a higher proportion of slow (red) fibers in the studied muscles of animals of the Kalmyk breed. In a number of works [2, 4] provides data on the small size of muscle fibers, for example, indicate that the diameter of the muscle fibers of gobies of different breeds ranges from 7-80 microns. The appearance of data on such small sizes (7 μm) may be associated with the measurement of the end sections of the fibers. The presence in the muscle tissue of muscle fibers with

**Figure 4.** The biceps femoris of the goby of the Kalmyk breed of cattle. Cross section. The animal is 18 months old. Coloring: Mayer hematoxylin and eosin. Magnification: x 400.

**Figure 5.** The biceps femoris of a bullhead of Hereford cattle. Cross section. The animal is 18 months old. Coloring: Mayer hematoxylin and eosin. Magnification: x 400.
a diameter of 7 μm can only occur during their development in embryogenesis. In adult animals, such muscle fiber sizes are hardly possible.

The results showed a moderate range of interbreed variability of muscle tissue of the studied cattle breeds. We have revealed differences in a number of parameters of the studied muscles. An analysis of the morphometry of the structures of the studied muscles on histological preparations showed that significant differences were found in the diameter of muscle fibers, the ratio of muscle and connective tissue (including adipose) in the muscles.

It is known that the diameter of muscle fibers can vary significantly in animals of different breeds, so in animals of meat breeds it is usually higher than in animals of dairy breeds. It was found that the thickness of muscle fibers depends on the age (weight) of the animals, that is, with increasing age (weight) of the animals, the diameter of the muscle fibers also increases. There is no consensus on the importance of such an indicator as the thickness of muscle fibers for assessing the quality of meat products. There is an indication that the quality of meat products increases with increasing thickness of muscle fibers, and that the quality of meat increases with a reduced diameter of muscle fibers [1, 2, 4, 5]. We believe that meat products, which are characterized by a higher content of fine fibers, are also characterized by better taste.

5. Conclusion

The results obtained reflect typical patterns of the structural and functional characteristics of muscle tissue in cattle, and also show the range of in-breed variability of muscle tissue. The revealed results are morphological equivalents of high quality meat products obtained from animal breeds of domestic cattle breeding. The results of the study can be taken into account in the further study of the interior features of cattle, and also as a fundamental basis in the development of criteria for assessing the quality of meat products.

6. References

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