Original paper

MORPHOLOGICAL CHARACTERISTICS OF BREAST AND THIGH MUSCLES OF AUTOCHTHONOUS BREEDS OF CHICKENS

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Abstract

Morphological characteristics of skeletal muscles of autochthonous breeds of chickens are very important for meat quality and comparison with current hybrids for intensive production. The autochthonous breeds used in the experiment were Sombor crested and Banat naked neck, both sexes. For the purposes of morphological examination, tissue samples were taken from the thigh muscle (*m. biceps femoris*) and muscles of the breast (*m. pectoralis profundus*) of 5 male and female animals of each breed. After a standard histological procedure for conventional light microscopy, samples were stained with hematoxylin - eosin. After the processing of the samples for the histochemical analysis, samples were stained with the enzyme succinate - dehydrogenase (SDH) with the aim of determining the presence of different muscle cell types (red, white and intermediate). Morphological parameters, in this study, were diameter of muscle cells, nucleocytoplasmic ratio of muscle cells, volume density of connective tissue within the muscle and the presence of red, white and intermediate muscle cell types. Comparison of diameters of muscle cells thigh and breast muscles between Sombor crested and Banat naked neck have showed that kind of muscle, race or gender have no significant effect on the differences in this parameter. There were no statistically significant differences in the nucleo-cytoplasmic ratio of the volume density of the connective tissue of muscles. Red muscle cells were, in both autochthonous breeds, significantly more represented in *m. biceps femoris* than *m. pectoralis profundus*. The results of this study indicate that no differences were observed between autochthonous breeds in morphological parameters for examined breast and thigh muscle.

Key words: *autochthonous breeds, morphological parameters, muscle*

Introduction

The interest of consumers in products from alternative systems (organic, free-range) is increasing mainly because these systems can be environmentally friendly, sustaining animals in good health with high welfare standards and resulting in higher quality products (Sundrum, 2001).

Alternative systems for poultry meat production could be organized by using commercial fast-growing broiler hybrids or middle and slow-growing genotypes. Bogosavljević-Bošković et al. (2007) showed that there are differences in production parameters of commercial broiler hybrids reared in poultry house or free-range. The appropriate choice of genotype for meat production in free-range system is very important because final body weights are different according to genotype (Blagojević et al., 2009). In addition, different broiler genetic potential for growth has significant influence on production results as well as on carcass quality (Škrbić et al., 2013). In the process of changing commercial rearing system with a traditional one different autochthonous breeds have very important role for poultry meat production (Pavlovski et al., 2009). Franco et al. (2013) point out that meat quality was significantly different between autochthonous breeds and commercial broiler hybrids.

The aim of this study was to evaluate the morphological parameters of breast and thigh muscles of autochthonous breeds of chickens in free-range rearing system.

Material and methods

Morphological characteristics of breast and thigh muscles of autochthonous breeds of chickens were examined on 10 chickens (5 males and 5 females) of Banat naked neck and 10 chickens (5 males and 5 females) of Sombor crested housed in free-range system. Chickens were sacrificed at the age of 12 week. Samples of tissue were removed from the *m. biceps femoris* of the leg and the *m. pectoralis profundus* of the breast. The muscle tissues were initially fixed in a 10% buffered formalin solution, followed by a sequence of dehydration and clearing. The samples were then embedded in paraffin then cut into serial 5 μm thick sections using a microtome. Histological preparations for determining diameter and nucleocytoplasmic ratio of muscle cells were stained with hematoxylin-eosin (H&E), while the Mallory method was used for showing connective tissue (Disbrey and Rack, 1970). For hystochemical analysis the samples of muscles, size of 1 cm^3, were taken from each bird, and after that, the samples were frozen by liquid nitrogen at temperature of -196˚C. In laboratory, they were cut on Cryo-cut (-20˚C, sections of 10μm). These sections were fixed on microscopic plates and stained with standard methods for succinate-dehydrogenase (SDH) (Gerebtzoff, 1970).

The following parameters were observed in the analysis of histological preparations of the muscle samples: the diameter of muscle cells, the volume density of connective tissue of muscle, the nucleocytoplasmic ratio of muscle cells and identification of different muscle cells type (red, white and intermediate). Microscopy analysis was performed using a light microscope Leica DMLS with a Leica DC 300 digital camera, and the software package IM 1000 (Leica Imaging Systems Ltd, Cambridge, UK). The diameter of muscle cells was measured as the average of the longest lines drawn across the length and width of their cross-sections. For stereological analyses of the volume density of connective tissue, the nucleocytoplasmic ratio of muscle cells and percentage of certain types of muscle fibers, measurements were performed using the M42 testing system described elsewhere (Weibel, 1979; Pissinatti et al., 2003; Burity et al., 2004; Drabekova et al., 2005).

The statistical significance of differences obtained in measurements was determined using factorial ANOVA and the *post hoc* Tukey test for each of the parameters measured. The statistical significance of differences was expressed as significant at $P \leq 0.05$. Statistical

processing of data was carried out using the software package Statistica for Windows ver. 12.0 (Statsoft, 2012).

Results and discussion

The results of our trial show that the diameter of the muscle cells in the thigh was ranged from 48.83μm to 53.38 μm (Table 1). Diameters of muscle cells in the breast observed in our experiment were from 50.63 μm to 55.39 μm. Statistical analysis showed that there was no statistically significant difference in the diameter of muscle cells between genotype, muscles and sex.

Table 1. *Effect of genotype, sex and muscle on the diameter of muscle cells (μm), values are means ± SEM*

	Banat naked neck		Sombor crested	
	Male	Female	Male	Female
Breast muscle	50.63±5.04	52.32±5.80	55.39±3.63	54.88±6.29
Thigh muscle	48.83±5.76	53.38±4.25	53.37±4.85	53.04±7.67
Source	*P* value			
Genotype (G)	n.s.			
Sex (S)	n.s			
Muscle (M)	n.s			
GxSxM	n.s			

In accordance with our results of the impact of sex Mobini and Khoshoii Asadi (2013) and Mobini (2013a) suggests that sex did not significantly affect the difference in diameter of muscle fibers. But some papers show a statistically significant effect of sex on the diameter of muscle cells, *m. pectoralis profundus,* in domestic poultry (Mobini, 2013b). The results of this study indicate that the diameters of deep pectoral muscle in male individuals were significantly higher than in female ones. The comparison of our and Mobini (2013b) results show the difference in diameter and muscle cells. Comparing the results of these two studies could not be complete because in the paper of Mobini (2013b) they could not determine the age, genotype and poultry breeding system as the factors which have significant influence on muscle fiber characteristics. Branciari et al. (2009) investigated the effect of different genotypes and poultry rearing systems on characteristics of muscle cells. They observed three genotypes, slow-growing chickens (Leghorn), medium-growing (Kabir) and fast-growing chickens (ROSS) and found that genotype significantly affects the surface of the muscle fibers in *m. pectoralis superficialis*, *m. ileotibialis lateralis* and *m. semimembranosus*. Furthermore, they found that the rearing system (with respect to the conventional organic) significantly affects characteristics of muscle cells only at Leghorn genotype which is adapted for growing in organic rearing systems.

In order to determine the dynamics of development and activity of skeletal muscle cells of Sombor crested and Banat naked neck chickens, in the experiment was compared nucleo-cytoplasmic ratio of muscle cells (Table 2).

Table 2. *Effect of genotype, sex and muscle on the nucleocytoplasmic ratio of muscle cells, values are means ± SEM.*

	Banat naked neck		Sombor crested	
	Male	Female	Male	Female
Breast muscle	0.015±0.002	0.016±0.003	0.014±0.005	0.014±0.002
Thigh muscle	0.015±0.004	0.017±0.002	0.018±0.002	0.017±0.003
Source	*P* value			
Genotype (G)	n.s			
Sex (S)	n.s			
Muscle (M)	<0.05			
GxSxM	n.s			

The results of this study indicate that nucleocytoplasmic ratio ranged from 0.014 to 0.018 and that only muscle type hads significant influence on this parameter. However, Stojanović et al. (2013) show that the value of the nucleocytoplasmic ratio in ROSS broiler chicken hybrids, at the age of 42 days was 0.15. In the same study, it was shown that the age has a significant influence on this parameter. Comparing the age of individuals in our experiment we can conclude that the difference is a consequence of age, but we should not ignore the possibility that the difference is a consequence of hybrids relative to autochthonous breeds.

The trial results of the volume density of the connective tissue in the muscle directly indicate the degree and the speed of development of the skeletal muscle tissue as well as the organization and arrangement of muscle cells in muscle bundles. Stereological analysis used in our study shows the percentage between the connection tissue and muscle cells.

Table 3. *Effect of genotype, sex and muscle on the volume density of connective tissue of muscle (%), values are means ± SEM*

	Banat naked neck		Sombor crested	
	Male	Female	Male	Female
Breast muscle	21.62±6.82	25.82±1.19	23.52±8.05	29.66±5.94
Thigh muscle	27.67±4.84	20.62±4.13	19.80±6.18	22.57±5.53
Source	*P* value			
Genotype (G)	n.s			
Sex (S)	n.s			
Muscle (M)	n.s			
GxSxM	n.s			

The results of our study indicate that neither the types of muscles or sex, nor genotype can affect the volume density of the connective tissue within muscle. Mobini (2013c), comparing the amount of intramuscular connective tissue of *m. pectoralis profundus,* found no differences between domestic poultry and ROSS broilers hybrids. The same author also points out that sex did not affect the percentage of intramuscular connective tissue. However, Mobini (2013c) observed significant histological differences in epimysium. The number of collagen connective fibers was higher in epimysium in broiler chickens compared to domestic poultry. The softness of the meat depends on the amount of collagen in the connective fibers epimysium and perimysium and in accordance with the results of the authors we can conclude

that the autochthonous breeds have better meat quality than commercial hybrids. In order to compare the volume density of connective tissue within the muscle of broiler and autochthonous breeds, we compared the results of our studies and the results from earlier research done on broiler chickens (Stojanović et al., 2013). The results of the study on autochthonous breeds show that the volume density of connective tissue was from 19.80 to 29% while in broiler chickens average value was 17.97 % in the pectoral muscle of ROSS 308 broiler chickens at 42 days (Stojanović et al., 2013). The same paper points out that with the increase of age of broilers there occurs reduction in volume density of the connective tissue. Due to the volume density in autochthonous breeds in our study it was measured in the 12th week of life, while the dynamics changes depending on the age were not determined, therefore a complete comparison is not possible.

Examination of the percentage of different types of muscle cells which were determined on the basis of the activity of the succinate - dehydrogenase (SDH) enzyme, in our study, suggests that the type of muscle significantly affects the observed parameter. The results show that in the thigh muscle of both genotype the intermediate muscle cells are dominant type, followed by red and white muscle cells, whereas in the breast muscle in all groups dominant are the intermediate muscle cells, followed by white muscle cells, whereas the red-muscle cells are extremely minor.

Table 4. *Effect of genotype, sex and muscle on the muscle fiber type percentage (%), values are means ± SEM*

		Banat naked neck		Sombor crested	
		Male	Female	Male	Female
Breast muscle	Red	0.82±0.27	1.19±1.09	0.49±0.26	1.36±0.73
	White	27.05±4.46	28.35±1.42	29.88±3.25	29.75±1.37
	Intermediate	71.67±4.67	70.58±1.87	69.62±3.35	68.88±1.30
Thigh muscle	Red	23.93±2.70	20.65±2.24	24.29±1.21	24.56±4.15
	White	15.78±8.75	17.36±3.82	14.97±4.32	17.12±3.53
	Intermediate	60.24±10.11	61.98±2.11	60.74±4.27	58.32±2.20
Source		*P* value			
Genotype (G)		n.s			
Sex (S)		n.s			
Muscle (M)		<0.01			
GxSxM		n.s			

Our results of the activities of the succinate - dehydrogenase enzyme are consistent with the results obtained in the experiments of other authors. Šijački et al. (1986) studied the percentage of red, white and intermediate muscle cells in two breeds of poultry in the post-natal period and also concluded that the skeletal muscles have all three types of cells, and that the red muscle cells are much more present in the leg muscle than in the breast. Uščebrka et al. (1999) examined the percentage in the red, white and intermediate muscle cells in skeletal muscle of the partridges. It has been shown the existence of three types of muscle cells in the muscles of the breast and the legs. It is noted the dominance of intermediate muscle cells and in the breast and leg muscles, while red muscle cells were significantly more presented in the leg muscles compared with breast muscle.

Conclusion

The results from this study indicate that there are no significant differences in morphometric parameters of breast and thigh muscles of autochthonous breeds reared under the same environmental conditions. Because the morphometric characteristics significantly affect the quality of meat, studies like this are important to provide answers about usage of autochthonous breeds in alternative rearing systems.

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