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MORPHOLOGICAL CHARACTERISTICS OF BREAST AND THIGH MUSCLES OF SLOW- AND MEDIUM-GROWING STRAINS OF CHICKENS

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Abstract: Morphological characteristics of skeletal muscles of slow- and medium-growing strains of chickens are very important for meat quality and comparison with fast-growing strains. The aim of this paper was to evaluate morphological parameters of breast and thigh muscles of slow- and medium-growing strains in a free-range system. The slow-growing strains used in the experiment were autochthonous breeds Sombor crested and Banat naked neck, and the medium-growing strain was Red-bro. The tissue samples were taken from the thigh muscle and muscles of the breast of 10 chickens of each breed. Samples were stained with hematoxylin – eosin and enzyme succinate - dehydrogenase (SDH). The following morphological parameters were observed: diameter of muscle cells, nucleo-cytoplasmic ratio of muscle cells, volume density of connective tissue within the muscle and the presence of red, white and intermediate muscle cell types. Between strains, the type of muscle or genotype didn't have significant effects on diameters of muscle cells and nucleo-cytoplasmic ratio in the muscle cells. Results indicated that genotype had significant effect on volume density of the connective tissue in breast muscles. Red muscle cells were, in all strains, significantly more represented in *m. biceps femoris* than *m. pectoralis superficialis.* Genotype had significant effect on ratio between connective tissue and muscle cells and no significant effects on other morphological parameters.

Keywords: slow-growing, medium-growing, chickens, 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. *Bogosavljevic-Boskovic et al. (2007)* showed that a difference exists in production parameters of commercial broiler hybrids rearing in poultry house and free-range. The appropriate choice of genotype for meat production in free-range system is very important because final body weights are different based on genotype (*Blagojević et al., 2009*). Also, different broiler genetic potential for growth has significant influence on production results as well as on carcass quality (*Škrbić et al., 2013*). *Mobini (2015*) pointed out the correlation between intramuscular connective tissues and meat tenderness. For changing the commercial rearing system with a traditional, autochthonous breeds play a very important role for poultry meat production (*Pavlovski et al., 2009*). *Franco et al. (2013)* found out that meat quality was significantly different between slow-growing strain (autochthonous breed) and commercial (fast-growing) broiler hybrid.

The aim of this study was to evaluate morphological parameters of breast and thigh muscles of slow- and medium-growing strains of chickens in a free-range system.

Materials and methods

Morphological characteristics of breast and thigh muscles of slow-growing strains of chickens were examined on 10 chickens a both sexes of Banat naked neck and 10 chickens of Sombor crested. Ten chickens of Red-bro, as medium-growing strain of chickens were used. All chickens were rearing in free-range system. Chickens of slow-growing strains are sacrificed at age of 12 week and medium-growing strain at age of 9 weeks. Samples of tissue were removed from the *m. biceps femoris* of the thigh and the *m. pectoralis superficialis* 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 Histowax (Histolab Product Ab, Göteborg, Sweden) 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 samples of muscles were taken from each bird with a size of 1 cm^3. The samples were frozen by liquid nitrogen at a temperature of -196˚C. In the 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 succinodehydrogenase (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, nucleocytoplasmic ratio of muscle cells and identification of different muscle cell types (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 *(Weibel, 1979).*

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

The results show that the diameter of the muscle cells in the thigh ranged from 51.10μm to 53.21μm (Table 1).

Table 1. Effect of genotype, muscle and interaction GxM on the diameter of muscle cells (μm), means ± SEM.

	Banat naked neck	Sombor crested	Red-bro
Breast muscle	51.41±1.64	55.14±1.53	50.62±1.28
Thigh muscle	51.10±1.69	53.21±1.91	52.66±0.93
Source		*P* value	
Genotype (G)		n.s.	
Muscle (M)		n.s	
GxM		n.s	

Diameters of muscle cells in the breast were from 50.62μm to 55.14μm. Genotype and muscle type showed no statistically significant difference on the diameter of muscle cells.

In order to determine the dynamics of development and activity of skeletal muscle cells of different strains, the nucleo-cytoplasmic ratio of muscle cells were compared in the experiment (Table 2).

Table 2. Effect of genotype, muscle and interaction GxM on the nucleocytoplasmatic ratio of muscle cells, means ± SEM.

	Banat naked neck	Sombor crested	Red-bro
Breast muscle	0.0155±0.0008	0.0143±0.0011	0.0177±0.0008
Thigh muscle	0.0163±0.0009	0.0172±0.0007	0.0166±0.0011
Source		*P* value	
Genotype (G)		n.s.	
Muscle (M)		n.s	
GxM		n.s	

The results indicate that nucleocytoplasmatic ratio ranged from 0.014 to 0.018. No significant difference was found between the three chicken strains, muscle type and interaction GxM.

The results of 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. The genotype had significant influence of percentage of connective tissue in muscles (Table 3).

Table 3. Effect of genotype, muscle and interaction GxM on the volume density of connective tissue of muscle (%), means ± SEM.

	Banat naked neck	Sombor crested	Red-bro
Breast muscle	23.72±1.62^{a}	26.59±2.34^{a}	16.19±1.80^{b}
Thigh muscle	24.14±1.78	21.19±1.81	20.40±3.03
Source		*P* value	
Genotype (G)		p<0.05.	
Muscle (M)		n.s	
GxM		n.s	

A significant difference was observed in breast muscle, where Red-bro stains had significantly smaller volume density of connective tissue compared with other strains. A significant difference in thigh muscle was not found.

The examination of the percentage of the different types of muscle cells being determined based on the activity of the enzyme succinate dehydrogenase (SDH), suggest that the type of muscle significantly affects the observed parameter (Table 4).

Table 4. Effect of genotype, muscle and interaction GxM on the muscle fiber type percentage (%), means ± SEM.

Fiber type	Banat naked neck		Sombor crested		Red-bro	
	Breast muscle	Thigh muscle	Breast muscle	Thigh muscle	Breast muscle	Thigh muscle
Red	0.99±0.29[a]	22.29±1.02[b]	0.88±0.23[a]	24.43±1.00[b]	1.04±0.14[a]	23.32±0.39[b]
White	27.99±1.04[a]	16.60±2.23[b]	29.82±0.87[a]	16.04±1.35[b]	28.82±0.28[a]	16.07±0.74[b]
Intermed.	71.01±1.14[a]	61.11±2.41[b]	69.29±0.89[a]	59.53±1.20[b]	70.14±0.32[a]	60.61±0.72[b]
Source	*P* value					
Genotype (G)	n.s					
Muscle (M)	<0.01					
GxM	n.s					

Means in a row without a common superscript letter differ significantly, P<0.01.

The results show that in the thigh muscle of both genotypes intermediate muscle cells are the dominant type, followed by red and white muscle cells, whereas in the breast muscle all groups the intermediate are dominant, followed by white muscle cells, whereas the red- muscle cells are extremely minor.

Discussion

Our results indicated no significant effects of genotype and muscle on diameter of muscle cells. According to our results, *Khoshoii et al. (2013)* also reported that a statistically significant difference in diameter of muscle fibers in pectoral muscle between native chickens and broiler strains does not exist. Results are presented as the mean value for both sexes because our previous paper (*Žikić et al., 2014)* as papers of other authors (*Mobini and Khoshoii, 2013; Mobini, 2013a*) suggest that sex did not significantly affect the difference in diameter of muscle fibers. The comparison of our and *Mobini (2013b)* results show the difference in diameter of muscle cells. Comparing the results of these two studies could not be fully done because in the paper of *Mobini (2013b)* it could not be determined 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 in the Leghorn strain which is adapted for growing in organic rearing systems.

Our results suggest no significant effects of genotype and muscle on the nucleo-cytoplasmic ratio where values were between 0.015 and 0.017. But *Stojanović et al. (2013)* shows that the value of the nucleo-cytoplasmic ratio in

broiler chickens Ross hybrids, at the age of 42 days was 0.15. In the same study, it was shown that age has significant influence on this parameter. Comparing age of birds 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 strains used in our study.

The results of our study indicate that the genotype affect the volume density of the connective tissue within the muscle. Precisely, volume density of connective tissue in breast muscle was significantly smaller in medium-growing strain then in slow-growing strains. *Mobini (2013c)* comparing the amount of intramuscular connective tissue of *m. pectoralis profundus* did not find the differences between domestic poultry and Ross hybrid broilers. Also, the same author 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 found in the epimysium and perimysium. These results are in accordance with authors who conclude that the autochthonous breeds have better meat quality then commercial hybrids (*Pavlovski et al., 2012*). In order to compare the volume density of connective tissue within the muscle of broiler (fast growing strains), Red-bro as medium growing strain and autochthonous breeds as slow-growing strains, we compared the results of our study and the results from earlier research done with broiler chickens (*Stojanović et al., 2013*). The results of our study showed that the volume density of connective tissue in pectoral muscle was from 16.19% at Red-bro to 26.59% at Sombor crested, while in broiler chickens the average value was 17.97 % in the pectoral muscle broiler chickens Ross 308 at 42 days (*Stojanović et al., 2013*). The same paper points out that the increase of age of broilers causes a reduction in volume density of the connective tissue. Due to the facts that we measured the volume density in slow- and medium-growing strains at the 12th week of life and we could not determine dynamics changes depending on the age, a complete comparison is not possible.

Our results of the activities of enzymes succinate dehydrogenase are consistent with the results obtained in the experiments of other authors. *Šijački et al. (1986)* examined the percentage of red, white and intermediate muscle cells in two breeds of poultry in the post-natal period. He 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. *Uśćebrka et al. (1999)* examined the percentage in the red, white and intermediate muscle cells in the skeletal muscle of partridges. It has been shown that there exists of three types of muscle cells in the muscles of the breast and the legs. It is noted that intermediate muscle cells dominate both in the breast and leg muscles, while red muscle cells were significantly more present in the leg muscles compared with breast muscle. *Dahmane Gosnak et al. (2010)* investigated the effect of selection (slow-growing

line and fast-growing line) on the metabolic activity of muscle fiber in the muscles of chickens and found that genotype significantly affects the metabolic activity of muscle fiber. Our results indicate that the only muscle (thigh or breast) has significant influence on ratio between muscle fiber types, but it has be noticed that in contrary to *Dahmane Gosnak et al. (2010)*, in our study slow-growing and medium-growing strains were compared.

Conclusion

The results from this study indicate that genotype have a significant effect on volume density of connective tissue in the breast muscle. Within the other morphological parameters there are no significant differences between strains and muscles. Because the morphometric characteristics significantly affect the quality of meat, researches like this are important to provide answers about usage of different strains in alternative rearing systems.

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Morfološke karakteristike mišića grudi i bataka sporo-rastućih i srednje-rastućih pilića

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Rezime

Izučavanje morfoloških karakteristika skeletnih mišića sporo- i srednje-rastućih pilića je veoma važno za kvalitet mesa kao i mogućnost poređenja sa brzo-rastućim hibridima. Cilj ovog rada je bio da se ispitaju morfološke karakteristike mišića grudi i bataka sporo- i srednje-rastućih pilića gajenih u *"free-range"* sistemu. Sporo-rastući pilići korišćeni u ovom ispitivanju su bile dve autohtone rase Somborska kaporka i Banatski gološijan, dok su srednje-rastući pilići bili hibrida Red-bro. Uzorci tkiva su uzimani od mišića grudi i bataka od po 10 pilića iz svake grupe u ispitivanju. Uzorci su bojeni hematoksilin-eozinom i metodom za detekciju sukcinat-dehidrogenaze (SDH). Od parametara su određivani: dijametar mišićnih vlakana, nukleo-citoplazmatični odnos, volumenska gustina vezivnog tkiva i prisustvo crvenih, belih i intermedijarnih mišićnih vlakana. Između grupa

pilića, korišćenih u ovom ispitivanju, nije uočena statistički značajna razlika u pogledu dijametra mišićnih vlakana i nukleo-citplazmatičnog odnosa. Rezultati ovog ispitivanja ukazuju da je genotip značajno uticao na volumensku gustinu vezivnog tkiva u mišićima grudi. Crvene mišićne ćelije su, kod svih grupa pilića, bile značajno prisutnije u *m. biceps femoris* u odnosu na *m. pectoralis superficialis*. Genotip je imao značajan uticaj na odnos između vezivnog tkiva i mišićnih ćelija i nije imao statistički značajan efekat na druge ispitivane parametre.

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