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Effect of rearing system and gender on histological profile of chicken breast and leg muscles in hybrid (Cobb×Zk)

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Abstract: *Effect of rearing system and gender on histological profile of chicken breast and leg muscles in hybrid (Cobb×Zk).* The experiment was conducted on 930 slow-growing chickens from the crossing of a Cobb male and Greenleg Partridge female. The chicks were randomly assigned to two groups: control (BW) which did not have access to a free run and the experimental group (W) using the grassy runs from 4 weeks of age. The experiment showed a statistical effect of sex on breast ($P \leq 0.01$) and leg ($P < 0.05$) muscle fiber diameter in Cobb×Zk hybrid roosters. There were no significant gender-dependent differences in the surface area of the muscles tested. No effect was either reported of the rearing system on the histological picture of breast and leg muscles.

Key words: rearing system, broiler chickens, muscle fiber diameter

INTRODUCTION

Animal products are an essential component of a man diet. They are a source of balanced protein, they contain low levels of cholesterol and fat, which is at the same time rich in unsaturated fatty acids (Wangang et al. 2010). Poultry meat surpasses meat of slaughter animals in terms of nutritional value, because of a valuable source of balanced animal protein that is highly digestible and easy to assimilate. According to PN-65/A-82000, meat constitutes "skeletal muscles and adherent adipose, connective and bone tissues, derived from carcasses, half carcasses or quarter carcasses of particular types of animals for slaughter". The main components of skeletal muscle tissue are muscle fibers, which are highly specialized. The number, size and type of muscle fibers, their biochemical, physiological and histological characteristics may lead to changes in meat quality and sensory evaluation (Damez and Clerjon 2008). The chemical composition of skeletal muscle is 75% water, 19% protein, 0.5–8% lipids and 1% glycogen (Lefaucheur 2010). However, their main mass is formed by muscle fibers, which diameters fall within the range of 10 to 100 μm, and largely depend on the type of muscle fibers (Tumova and Teimouri 2009, Lefaucheur 2010).

Histological structure of muscles slightly varies depending on their functions (Klont et al. 1998). According to Remignon et al. (1995), the diameter of muscle fibers depends on their type and to great extent on genetic background. Other factors important in this respect include sex (Ozawa et al. 2000), age (Candek-Potokar et al. 1998), breed (Ryu et al. 2008), physical activity (Karlsson et al. 1999) and rearing system (Castellini et al. 2002a and 2002b, Branciari et al. 2009).

Nowadays consumers are becoming more aware of their needs and begin to pay attention to the welfare of the animals, making it one of the criteria when choosing products of animal origin. The research carried out on the British population clearly demonstrated that animal welfare was treated as a "very important" or "important" factor, which indirectly influences the suitability and sustainability of food (DEFERA 2011). For the most part it refers to both the health and food safety. According to Sundrum (2001), attention is increasingly drawn to the rearing system of birds, including the density per m^2 and access to free runs.

The aim of this experiment was to determine the effect of the rearing system and gender on the histological profile of breast and leg muscles in slow-growing Cobb×Zk chickens.

MATERIALS AND METHODS

The study was conducted at the Warsaw University of Life Sciences – SGGW, experimental field station in Wilanów-Obory. Experimental procedures were approved by the Ethics Commission (approval no. 27/2009 dated 16 April 2009). The study was conducted on 930 chickens from the cross of Cobb male and Greenleg Partridge female (Zk) reared for 63 days. Day-old chicks were randomly divided into two groups of 465 chicks each: BW (control) and W (experimental) in five repetitions each of 93 chicks. A differentiating factor was the possibility to use grassy free runs from 4 weeks of age by the experimental group. In each group the runs were 3×5 m in size. Half of the run area was canopied. The run area was dry, sunny, with permeable soil, covered with perennial ryegrass *Lolium perenne* L. (40%), red fescue *Festuca rubra* L. (50%) and Kentucky bluegrass *Poa pratensis* L. (10%). The free run had an area with sand where birds could use sand baths. A four-phase feeding system was applied during the rearing with the use of starter, grower 1, grower 2 and finisher feed mixtures (Table 1). Birds had unrestricted access to both feed and water.

During the experiment after each week an individual body weight, food intake and death rate of chickens was checked. On the 63rd day of rearing, 24 females and 24 males were selected from each group with body weight about average for each sex in a group and specimens of breast and leg muscles were collected for histology. Samples in size of 0.5×0.5×1 cm were collected within 15 min since slaughter after appropriate exsanguination of the chickens and subsequently subjected to 24-hours fixation. The samples were then washed in ethanol to remove the fixing agent and dehydrated by a series of increasing ethyl alcohol concentrations. Dehydrated samples were saturated with paraffin. Paraffin saturation was carried out in the incubator at the melting point of paraffin. Saturation duration was adapted to muscle samples collected and amounted to a few hours. Paraffin blocks were formed after completion of the saturation process. Microtome Leica RM 2265 (Leica Microsystems, Nussloch, Germany) was used to cut paraffin sections. Muscle

TABLE 1. Feed mixture composition and nutritional value according to producer's

Specification	Starter (1–11)	Grower I (12–24)	Grower 2 (25–37)	Finisher (38–63)
Content (%)				
Corn	10.00	11.40	10	10.00
Wheat	53.00	55.00	59.60	60.80
Soybean meal	30.60	27.40	23.20	21.60
Feeding limestone	1.19	1.20	1.11	0.97
Sodium bicarbonate	0.20	0.14	0.14	0.16
NaCl	0.24	0.28	0.28	0.26
Stimulator	0.01	0.01	0.01	0.01
Dicalcium phosphate	1.18	0.78	0.70	0.64
Soybean oil	2.10	2.40	3.60	4.40
Methionine 84% calcium salt	0.48	0.42	0.36	0.28
Lysine	0.36	0.34	0.36	0.28
Threonine	0.14	0.13	0.14	0.10
Premix C196 PX05802 0.5%	0.50	0.50	0.50	0.50
Nutritional value				
ME (kcal)	2990.20	3047.19	3125.72	3217.10
Fat	3.67	4.00	5.14	5.92
Protein	21.99	20.78	19.26	18.51
Fiber	3.60	2.55	2.45	2.41
Ash	5.83	5.35	4.96	4.67
Lysine	1.38	1.28	1.19	0.97
Methionine + cystine	1.08	1.01	0.92	0.76
Available phosphorus	0.45	0.38	0.36	0.35

Provided per kilogram of diet: STARTER: vitamin A 11.00 K UL; organic phosphorus 0.59%; calcium 0.98%; phosphorus available 0.45%; calcium chloride 0.24%; sodium 0.15%; chlorine 0.27%; potassium 0.90%; magnesium 0.17%; manganese 142.32 mg; copper 31.59 mg; selenium 0.41 mg; iron 191.51 mg; sulfur 0.34%; zinc 116.80 mg; lysin1.36%; methionine 0.31%; GROWER I vitamin A 11.00 K UL; organic phosphorus 0.51%; calcium 0.87%; phosphorus available 0.38%; calcium chloride 0.28%; sodium 0.15%; chlorine 0.29%; potassium 0.85%; magnesium 0.16%; manganese 141.84 mg; copper 30.82 mg; selenium 0.41 mg; iron 174.55 mg; sulfur 0.32%; zinc 115.03 mg; lysin1.26%; methionine 0.30%; GROWER II vitamin A 11.00 K UL; organic phosphorus 0.48%; calcium 0.81%; phosphorus available 0.36%; calcium chloride 0.28%; sodium 0.15%; chlorine 0.30%; potassium 0.77%; magnesium 0.16%; manganese 141.40 mg; copper 30.21 mg; selenium 0.40 mg; iron 165.98 mg; sulfur 0.30%; zinc 113.79 mg; lysin1.17%; methionine 0.28%; FINISHER vitamin A 11.00 K UL; vitamin D3 3.00 K UL; vitamin E 40.00 mg; organic phosphorus 0.73%; calcium 0.35%; calcium chloride 0.26%; sodium 0.15%; chlorine 0.27%; potassium 0.74%; magnesium 0.15%; manganese 140.80 mg; copper 29.92 mg; selenium 0.40 mg; iron 159.92 mg; sulfur 0.28%; zinc 113.14 mg; lysin1.06%; methionine 0.27%;

cross sections had the thickness of 5 μm. Standard H&E staining was performed. The diameter and area of 200 muscle fibers was measured in each slide using a Nikon Ellipse E200 light microscope equipped with a Nikon DS-Fi2 camera and COOL view 2.7 software.

The results were analyzed statistically using the analysis of variance, calculated by least squares method with statistical software SPSS 19.0 GB (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Results of the analysis of breast muscle microstructure in chickens are shown in Tables 2 and 3. The diverse diameters of muscle fibers of breast muscles of the birds were to a greater extent correlated with gender than rearing system. The diameter of muscle fibers increases with the age of birds (Branciari et. al 2009). According to Sobolewska et al. (2011), in broiler chickens reared to the 35th day, the most intense increase in muscle fiber diameter was between the 8th and 21st day of rearing. Breast muscle fibers fully developed not before the 56th day. According to Papinaho et al. (1996) and Geyukouku et al. (2005), the average fiber thickness of *m. pectoralis major*, *m. biceps femoris*, *m. extensor hallucis longus*, and *m. gastrocnemius* is 60.0, 51.6, 59.8 and 60.45 μm, respectively. Fast-growing chickens have a much greater diameter of muscle fibers as compared to the slow-growing chickens (Khoshoii et al. 2013).

In the current experiment, the majority of muscle fibers in the cross sections had a clear shelving structure, and individual giant fibers could be distinguished, the diameter of which, according to Dransfield and Sosnicki (1999), is generally about three times greater than of the normal fibers. The hypertrophy of muscle fibers affects mainly fast-growing chickens and results from intense selection (Brocka et al. 1998, Guernec et al. 2003, Lefaucheur et al. 2010). Study by Miraglia et al. (2006) showed that the proportion of giant fibers in the *pectoralis major* in slow-growing chickens was 0.56%, in the fast-growing 3.17%, while in *ileotibialis lateralis* it was 1.70 and 3.18%, respectively. Intense muscle growth is associated with an increase in thickness and the surface of muscle fibers, rather than their number.

TABLE 2. The influence of gender on the diameter and area of chickens muscle fibers in hybrid (Cobb×Zk)

Gender	Diameter (μm)		Area (μm^2)	
	mp	mn	mp	mn
Roosters	63.02^A	50.82^A	1401.54	1142.09
Hens	48.05^B	45.69^B	955.29	808.77
SE	3.44	3.66	119.71	113.93

mp – breast muscle, mn – leg muscle; ^{A,B}Means with the different subscripts differ significantly at $P \leq 0.01$.

TABLE 3. The influence of rearing system on the diameter and area of chickens muscle in hybrid (Cobb×Zk)

Rearing system	Diameter (μm)		Area (μm^2)	
	mp	mn	mp	mn
BW	54.09	52.32	1265.32	1100.50
W	56.98	44.18	1091.51	850.35
SE	4.76	3.38	150.92	123.70

mp – breast muscle, mn – leg muscle.

Our experiment showed a statistical effect of gender on breast ($P \leq 0.01$) and leg ($P < 0.05$) muscle fiber diameter in male of Cobb×Zk hybrid (Table 2). They had a larger diameter in both breast and leg muscles (63.02 and 50.82 μm, respectively) compared to female (48.05 and 45.69 μm). Khoshoii et al. (2013) studied the effect of gender on the diameter of breast muscle fibers and demonstrated that both slow-growing and fast-growing male had a larger diameter of muscle fibers. Biesiada-Drzazga et al. (2006) demonstrated in geese that both breast and leg muscles of females had a greater diameter of muscle fibers. However Mobini (2013) showed no effect of gender on the diameter of the muscle fibers.

There were no significant gender-dependent differences in the surface area of the muscles tested. However males had a greater surface area of muscle fibers in both breast and leg muscles (1,401.54 and 1,142.09 μm^2, respectively) compared to females (955.29 and 808.77 μm^2). The largest area was observed in breast muscles of males and the smallest in females leg muscles. The study by Scheuermann et al. (2004) clearly showed that gender had an influence on the surface area of the breast muscle. This trend continued on 7^{th}, 21^{st} and 35^{th} day of rearing at a significance level of $P < 0.05$. According to Choi and Kim (2008), differences in fiber number and size are primarily under the control of sex hormones. Differences in fibers of males and females can arise from hormonal action if differences in androgen hormones are at a sufficiently high level during periods of prenatal fiber formation.

There was no significant effect observed of the rearing system on the histological picture of breast and leg muscles (Table 3). However birds that could use free runs had a larger diameter of breast muscles (56.98 μm) and a lesser diameter of leg muscles (44.18 μm) compared to birds that did not benefit from grassy free runs (54.09 and 52.32 μm, respectively). Sowińska (2002) either did not identify the influence of the rearing system on skeletal muscle morphology. Polak et al. (2010) compared *m. pectoralis* and *m. gastroceneminus* in 215 Anak Titan and Isa chickens and showed that fiber size was affected by the rearing system. In general, in birds of both lines, the fiber diameter of *m. pectoralis* was smaller in groups with outdoor access ($P < 0.01$).

Birds from the control group had a greater surface area of muscle fibers in both breast and leg muscles (1,265.32 and 1,100.50 μm^2, respectively) compared to chickens from the experimental group (955.29 and 808.77 μm^2). The opposite trend was shown in the study by Branciari et al. (2009) where they compared an impact of the rearing system and genotype on muscle fiber area. These authors showed that the fast-growing chickens (Ross) reared under conventional conditions had the largest surface area of muscle fibers. The opposite trend was observed in the case of slow-growing chickens where the fiber surface area was larger than in the conducted experiment. In the conventional rearing it ranged from 955 to 1,610 μm^2 and from 1,272 to 2,396 μm^2, for breast and leg muscles, respectively. Whereas in the organic rearing system, the fiber surface area ranged from 908 to 1,699 μm^2 and from 1,758 to 3,006 μm^2 for breast and leg muscles, respectively.

CONCLUSIONS

The experiment showed a statistical effect of gender on breast ($P \leq 0.01$) and leg ($P < 0.05$) muscle fiber diameter in Cobb×Zk hybrid roosters. There were no significant gender-dependent differences in the surface area of the muscles tested. No effect was either confirmed of the rearing system on the histological picture of breast and leg muscles. The increase in thickness of the muscle fibers could have influence on tenderness and juiciness.

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Streszczenie: *Wpływ systemu utrzymania oraz płci na profil histologiczny mięśni piersiowych i nóg kurcząt mieszańca Cobb×Zk.* Doświadczenie przeprowadzono na 930 kurczętach wolno rosnących pochodzących z krzyżowania koguta Cobb oraz kury zielononóżki kuropatwianej. Pisklęta losowo przydzielono do dwóch grup: kontrolnej (BW) niemającej dostępu do wybiegu oraz grupy doświadczalnej (W), korzystającej z trawiastych wybiegów począwszy od 4. tygodnia życia. W przeprowadzonym doświadczeniu wykazano statystyczny wpływ płci na średnicę włókien mięśni piersiowych ($P \leq 0{,}01$) oraz nóg ($P < 0{,}05$)

kogutów mieszańca Cobb×Zk. Nie stwierdzono istotnych różnic dla wartości pola powierzchni badanych mięśni w zależności od płci. Nie wykazano wpływu systemu utrzymania na wyniki obrazu histologicznego mięśni piersiowych i nóg.

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