Structure of Skeletal Muscles in Leghorn Type Chicken from Conservative and Parent Flocks

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Intensive selection conducted within closed populations has led to the creation of specialized chicken strains that differ significantly in meat yield and reproduction performance. The effect of the selection conducted on the birds is differentiation identified not only on the molecular but also on the cellular level, among other things in the skeletal muscles. The aim of this study was to compare the structure of chosen homological skeletal muscles from Leghorn chickens (LSL), originating from parent flock, intensively selected for reproductive traits and from conservative flock (G99), unselected for many generations. The structure of *musculus pectoralis superficialis* and *musculus biceps femoris* (the thickness of the muscle fibres and the share of the fibre types in the bundle) in 8 and 20 week old chickens was compared. A significant impact of the origin on all examined slaughter parameters was recorded. Body weight before slaughter, carcass weight and the weight of breast and leg muscles in 8 weeks old LSL chicken made up from 60 % to about 85 % of the respective values in the G99 Leghorn. Lack of red fibres in the breast muscles of all the individuals from the parental flock (LSL) was noted, whereas in 12 individuals (among 24) from the conservative flock (G99), red fibres were observed in this muscle from 2.75 % up to 7.09 %. White fibres in 8 week old chicks were always thicker, both in *pectoralis superficialis* and *biceps femoris* muscle in birds with higher body weight as well as higher weight of breast and legs muscles, *i.e.* in chicks from conservative flock (G99), P<0.01. However, in 20 week old birds, the diameters of the white fibres were similar in both groups. Also the diameters of the red fibres in *musculus biceps femoris* in 8 week old chickens were higher in cockerels and pullets from conservative flock (G99).

Key words: Muscle, structure, fibers, conservative and intensively selected flock.

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Intensive selection conducted within closed populations has led to the creation of specialized chicken strains that differ significantly in meat yield and reproduction performance. On one hand, intensive selection increases avian performance, on the other hand, it results in limitation of the heterozygosity in the selected population, in comparison to the unselected one (HILLEL *et al.* 2003). Forming conservative flocks to protect poultry genetic resources from extinction is one of the methods that prevent further limitation of animal biodiversity (CYWA-BENKO 1996).

The effect of selection conducted on the birds is differentiation identified not only on the molecular (BEDNARCZYK & SIWEK 1999; BEDNARCZYK *et al.* 2002), but also on the cellular level, among others in the skeletal muscles. Contemporary, highly performing laying hens have two, three, and even four times smaller body and muscle weight, including breast and legs muscles, compared to the meat type hens (KLONT *et al.* 1998; DAMME & RISTIC 2003; SCHEUERMAN *et al.* 2004; MURAWSKA & BOCHNO 2006).

Muscle size and weight are determined mainly by the total number of the muscle fibres as well as by their type and thickness. Most of the skeletal muscles have a heterogenic structure, i.e. they consist of diverse types of muscle fibres that differ morphologically, metabolically and physiologically. Furthermore, these differences determine to a large extent the meat quality. Selection for increased body weight and carcass yield involved picking individuals with muscles that include either a higher number of muscle fibres (hyperplasia – a genetically determined higher number of fibres in a given muscle) or thicker fibres (hypertrophy). A recent possibility for increasing the muscle weight is to transform the muscle structure, *i.e.* to change the proportion of particular muscle fibre types, which differ in thickness. In practice, this

means increasing the proportion of the fibres with the largest diameters, *i.e.* the white fibres in a given muscle (REMINGNON *et al.* 1995; KLONT *et al.* 1998; DRANSEFIELD & SOSNICKI 1999; KARLSSON *et al.* 1999; LE BIHAN-DUVAL 2004; SCHEUER-MANN *et al.* 2004; YANG & JIANG 2005).

Among many methods used to distinguish and classify the types of muscle fibres, one depends on determining the oxygen capacity of the muscle on the basis of oxidative enzyme activity, such as succinate dehydrogenase (SDH). This method enables distinguishing between the fibres that demonstrate oxygen metabolism, *i.e.* the red fibres (βR), and the fibres not demonstrating oxygen metabolism, called the white fibres (αW). The latter derive the energy to twitch from glycogen metabolism and have larger diameters than the three remaining types of the fibres. The third type of fibres identified with the method mentioned above are the intermediate fibres (aR), with oxidative-glycolytic metabolism (ASHMOR & DOERR 1971; KARLSSON *et al.* 1999).

Superficial chicken breast muscle consists of white fibres. According to some authors, these fibres make up as much as 100 % of all fibres that are included in this muscle (MAURYAMA & KANEMAKI 1991, see SOLOMON *et al.* 1998; LE BIHAM-DUVAL 2004). Other authors report that the breast muscle of these birds may include several per cent of the red or intermediate fibres (KŁOSOWSKA *et al.* 1979; ROSSER *et al.* 1987; UHRIN 1995; ELMINOWSKA-WENDA *et al.* 1999).

The research carried out by REMINGNON *et al.* (1995) and HALEVY *et al.* (2006), indicated that the total number of fibres included in the chicken breast muscle is determined in the embryonic period, and the subsequent increase of the muscle weight results from fiber hypertrophy. Thus, the weight of the breast muscle in the chicks selected for large body weight is the effect of an increase in the total number of muscle fibres included in this muscle, as well as in their thickness.
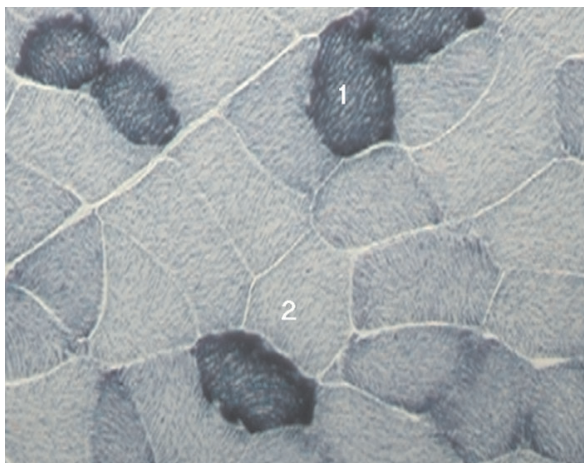
Following the changes that take place in the muscles of the birds belonging to the same breed, but subjected to different selection pressure, may contribute to explaining the complicated relations between the muscle structure and the meat quality. Therefore, the aim of this study was to compare chosen homological skeletal muscle structure from the Leghorn type chickens, originating from parent flock, intensively selected for reproductive traits and from conservative flock, unselected for many generations. The structure of *musculus pectoralis superficialis* and *musculus biceps femoris* (the thickness of the muscle fibres and the share of the fibre types in the bundle) in 8 and 20 week old chickens was compared.

Material and Methods

The research was carried out on 48 chickens belonging to the Leghorn type, at the age of 8 and 20 weeks. Twenty four birds originated from the conservative flock – strain G99 (G99), and the remaining twenty four – from the Lohmann's company parent flock, with the trade name (LSL).

Each experimental group numbered 6 cockerels and 6 pullets in both age groups. The birds were kept in closed facilities, on deep litter. The chickens were fed according to the feeding program designed for laying hens during rearing. In the 8th and 20th week of life the birds were weighed and slaughtered. Directly after slaughter, the muscle samples were taken for histological examination, and were accordingly frozen in liquid nitrogen. After cooling the carcasses, shortened carcass dissection was performed according to the method of ZIOŁECKI and DORUCHOWSKI (1989).

The muscle samples were cut in a cryostat at a temperature of -25°C into 10 μm sections. In order to distinguish the types of muscle fibres in the sec-

Fig. 1. Cross-section of *m. pectoralis superficialis* of a cockerel from conservative flock (G99), 20 weeks old. 1– red fibre; 2 – white fibre, × 200.

Fig. 2. Cross-section of *m. pectoralis superficialis* of the cockerel from parent flock (LSL), 20 weeks old. 2- white fibre, × 200.

tion, the reaction on activity of succinate dehydrogenase (SDH) was performed according to DUBOWITZ *et al.* (1973). Myofibres were classified as red (βR) and white (αW) types according to ASHMORE and DOERR (1971). The percentages of the two fibre types were defined based on the evaluation of 10 randomly selected muscle bundles for each individual. Measurements of the shortest diameters according to BROOKE (1970) were performed on ten images from each sample/individual with the use of digital picture analysis MutiscanBase v. 14.02.

The results were then subject to statistical evaluation by SAS 0.1.

Results

A significant impact of origin on all examined slaughter parameters was noted, except for carcass yield in 8 week old cockerels and 20 week chickens of both genders, as well as giblet weight in 8 and 20 week old cockerels and pullets (Table 1 and Table 2). The chickens from the parent flock (LSL), both 8 and 20 week old, were lighter and had lower breast and legs muscle weight as well as lower leg bone weight in comparison to the chickens from the conservative flock – G99 (P<0.05 and P<0.01). In relation to gender, all parameters presented in Table 1 & 2, except for carcass yield, were higher in cockerels than in pullets (l as lower leg bone weight in comparison to the chickens from the conservative flock – G99 (P<0.05 and P<0.01), which is in accordance with the results of other authors (BOCHNO & BRZOZOWSKI 1998).

The percentage proportion of the two fibre types in the muscle bundles of the examined muscles are presented in Table 3. Lack of red fibres in the breast muscles of all the individuals from the parent flock (LSL) was recorded. The breast muscle of the 8 and 20 week old pullets and cockerels from this flock demonstrated homogenic structure in

Table 1

Body weight and carcass parameters of 8 week old cockerels and pullets from conservative flock (G99) and parent flock (LSL) (n=6; means; SD ±)

Parameters	Conservative flock (G99)		Parent flock (LSL)	
	♂ cockerels	♀ pullets	♂ cockerels	♀ pullets
Body weight (g)	773^{Ax}± 116.6	627^{Ay}±49.72	620^{Bx}±91.65	455^{By}±78.70
Hot carcass weight (g)	530^{Ax}± 81.66	437^{Ay}±36.03	360^{Bx}±54.10	254^{By}±52.72
Breast muscle weight (g)	84.0^{Ax}± 19.04	69.6^{Ay}±12.22	52.0^{Bx}±10.60	39.6^{By}±11.16
Leg muscle weight (g)	107.0^{Ax}± 19.78	84.0^{Ay}±13.96	70.6^{Bx}±16.86	46.6^{By}±12.02
Giblet weight (g)	41.8^{cx}± 2.56	36.0^{cy}±4.33	41.6^{cx}±5.55	34.9^{cy}±4.92
Bone weight (leg) (g)	20.0^{Ax}± 3.22	17.8^{Ay}±2.22	14.0^{Bx}±1.38	9.3^{By}±1.48
Dressing percentage (%)	61.23^{ax}±2.15	61.63^{ax}±1.28	58.10^{ax}±0.95	56.60^{bx}±3.93

A, B – means (between flocks) with different letters differ significantly at P<0.01; a, b, c at P<0.05
 x, y – means (between sex) with different letters differ significantly at P<0 .05

Table 2

Body weight and carcass parameters of 20 week old cockerels and pullets from conservative flock (G99) and parent flock (LSL) (n= 6; means; SD±)

Parameters	Conservative flock (G99)		Parent flock (LSL)	
	♂ cockerels	♀ pullets	♂ cockerels	♀ pullets
Body weight (g)	2181^{Ax}±138.19	1570^{ay}±110.99	1906^{Bx}±127.54	1305^{by}±102.71
Hot carcass weight (g)	1563^{Ax}±100.72	1126^{Ay}±99.02	1201^{Bx}±80.45	823^{By}±62.71
Breast muscle weight (g)	241.0^{Ax}±22.08	206.6^{Ay}±14.78	205.2^{Bx}±24.88	167.8^{By}±17.80
Leg muscle weight (g)	345.0^{Ax}±15.56	215.0^{Ay}±20.42	279.2^{Bx}±22.92	161.6^{By}±18.06
Giblet weight (g)	69.3^{ax}±5.88	60.7^{ay}±6.65	79.7^{bx}±11.84	64.40^{ay}±6.02
Bone weight (leg) (g)	52.2^{Ax}±7.53	35.5^{Ay}±6.68	41.6^{Bx}±6.77	27.2^{By}±3.09
Dressing percentage (%)	67.14^{ax}±1.40	66.20^{ax}±3.26	63.03^{ax}±0.99	63.4^{ax}±1.27

A, B – means (between flocks) with different letters differ significantly at P<0.01; a, b at P<0.05
 x, y – means (between sex) with different letters differ significantly at P<0.05

100 %, whereas in 12 individuals from the conservative flock (G99), the red fibres were observed in this muscle in the amount of 2.7 % up to about 7.1 %.

Significant (P<0.05 and P<0.01) differences in percentage proportion of the red and white fibres in *m. biceps femoris* in 8 week old chickens were noted. It was observed that in cockerels and pullets from the conservative flock, the proportion of the red fibres was higher, and the proportion of the white fibres smaller, than in the birds of both genders from the parent flock. Such relations were not found in the muscles of 20 week old chickens.

The proportion of the white fibres in *m. biceps femoris* in both groups was higher than the proportion of the red fibres, and ranged from 65 % up to 80 % on average. In older birds the tendency towards a slightly smaller proportion of the white fibres in favour of the red fibres was evident.

The influence of the origin of the chickens was evident in relation to the muscle fibre thickness, expressed as their smallest diameter (Table 4). The white fibres in 8 week old chickens were always thicker, both in breast muscle and *biceps femoris* muscle, in birds with higher body weight as well as

Table 3

Fibre type composition (red-βR and white-αW) in *m. pectoralis superficialis* and *m. biceps femoris* of 8 and 20 week old cockerels and pullets (within the bundle, %) from conservative flock (G99) and parent flock (LSL) (n=6; means; SD ±)

Musculus	Fibre types (%)	Sex	Age (weeks)			
			8		20	
			Conservative flock G99	Parent flock LSL	Conservative flock G99	Parent flock LSL
pectoralis superficialis	red (βR)	♂cockerels	7.09^3±1.93	–	4.80^2±1.29	–
		♀ pullets	5.28^5±1.27	–	2.75^2±1.08	–
	white (αW)	♂ cockerels	96.45^a±3.80	100.00^a±0.00	98.33^a±2.92	100.00^a±0.00
		♀ pullets	95.58^A±2.35	100.00^B±0.00	99.08^a±1.33	100.00^a±0.00
biceps femoris	red (βR)	♂ cockerels	29.12^a± 5.32	23.09^b± 3.00	34.15^c±5.41	34.37^c±4.49
		♀ pullets	27.93^A± 2.04	19.81^B± 2.86	29.93^c±2.39	27.10^c±2.26
	white (αW)	♂ cockerels	70.88^a± 5.06	76.91^a± 3.16	65.85^c±1.15	66.62^c±4.46
		♀ pullets	72.07^A± 2.05	80.19^B± 2.83	70.07^c±2.51	72.90^c±2.03

^{2} n=2; ^{3} n=3; ^{5} n=5
A, B – means (between flocks) with different letters differ significantly at P<0.01;
a, b, c at P<0.05

Table 4

Fibre diameters (red-βR and white-αW, μm) in *m. pectoralis superficialis* and *m. biceps femoris* 8 and 20 week old cockerels and pullets from conservative flock (G99) and parent flock (LSL) (n=6; means; SD ±)

Musculus	Fibre types	Sex	Age (weeks)			
			8		20	
			Conservative flock G99	Parent flock LSL	Conservative flock G99	Parent flock LSL
pectoralis superficialis	red (βR)	♂ cockerels	23.50^3±0.47	–	27.64^2±7.82	–
		♀pullets	27.98^5±4.69	–	31.21^2±5.28	–
	white (αW)	♂cockerels	36.39^A±4.67	29.38^B±2.43	45.63^c±4.79	44.76^c±5.51
		♀pullets	35.09^A±4.55	30.81^B±5.58	46.64^a±2.47	39.39^b±7.04
biceps femoris	red (βR)	♂ cockerels	23.98^A±2.55	19.41^B±1.18	36.27^c±2.88	35.98^c±5.32
		♀ pullets	28.24^A±2.94	20.29^B±4.68	32.29^c±3.91	33.55^c±2.36
	white (αW)	♂cockerels	41.49^A±4.48	33.27^B±2.67	51.87^c±4.93	53.84^c±4.26
		♀ pullets	44.70^A±5.26	33.62^B±4.35	50.94^c±3.48	53.92^c±2.51

^{2} n=2; ^{3} n=3; ^{5} n=5
A, B – means (between flocks) with different letters differ significantly at P<0.01;
a, b, c at P<0.05

higher weight of breast and leg muscles, *i.e.* in chickens from the conservative flock (G99). These differences were significant (P<0.01). However, in 20 week old birds, the diameters of the white fibres were similar in both groups, except for significantly thicker fibres *(m. pectoralis superficialis)* in 20 week old pullets G99. Also the diameters of the red fibres in *biceps femoris* were significantly (P<0.01) higher in 8 week old cockerels and pullets from the conservative flock (G99) in comparison to the birds of the same age and gender from the parent flock (LSL).

Lack of red fibres in *m. pectoralis superficialis* in chickens from the parent flock (LSL) and the presence of this type of fibre in half of the G99 population made it impossible to compare this parameter between both groups.

Discussion

As follows from the data presented in Tables 1-4, significant differences were observed in both slaughter parameters and muscle structure between the chickens originating from the flock unselected for many generations (G99) and the chickens subjected to strong selection pressure on laying traits (LSL). However, larger differences were found in slaughter parameters in 8 and 20 week old chickens, whereas the differences in the investigated parameters of the muscle structure were evident mainly in 8 weeks old birds.

Body weight before slaughter, carcass weight and the weight of breast and leg muscles in 8 week old LSL chickens made up from 60 % to about 85 % of the respective values in the G99 Leghorn.

Comparing the obtained results with the results of 10-year investigations of CYWA-BENKO (2002) concerning slaughter parameters of various indigenous breeds as well as strains from conservative flocks, including also the Leghorn breed (strain G99), it can be stated that both the body weight at the age of 8 and 20 weeks and carcass yield in examined birds were similar to the results presented in this study. However, it has to be emphasized that the laying of the hens from conservative flocks was low and ranged between 122 and 186 eggs (CYWA--BENKO 2002), whereas laying of the Leghorn-type hens from commercial flocks amounted to 255-265 eggs. Thus, the differences in the performance of hens from the same breed, expressed as the number of laid eggs, was reflected in the slaughter parameters of the chickens in the presented study.

However even larger differences in body weight between cockerels of meat and egg types were detected by MURAWSKA & BOCHNO (2006). At the age of 6 weeks, the meat type cockerels, in com-

parison to broilers of the same age, had a fourfold smaller body weight. Similarly, large differences in this characteristic were observed by GERKEN *et al.* (2003), who compared two laying strains (White Leghorn – LSL and medium heavy Lohmann Brown) with commercial broilers (Lohmann Meat).

Carcass yield of 8 week old chickens from both flocks was low, especially in the parent flock. It was even smaller in comparison to the carcass yield of 7-week Rhode Island Reds (65.51%) and Greenleg Partridges (63.74%) from conservative flock in the research of POŁTOWICZ *et al.* (2004).

Significantly larger diameter of the muscle fibres in both types (Table 4) and larger body weight, breast and legs muscle weight in 8 week old G99 chickens suggest that the increase of the muscle weight was due to the fibre hypertrophy. However, similar diameters of the red and white fibres in both flocks of the older (20 week old) birds and significantly larger body and muscle weight of the G99 chicks suggest that the increase resulted from muscle fibres hyperplasia. Finally, it can be stated that the larger values of the slaughter parameters (body weight, carcass weight, weight of breast and leg muscles) in the G99 chickens resulted from both fibre hyperplasia and hypertrophy in the muscles of birds originating from the conservative flock.

The results confirm the previous findings of REMIGNON *et al.* (1995), on chicken populations selected divergently for large and small body weight (1882 *vs.* 675 g at 77 days), which demonstrated an increase in the number of both fibre types by 20 % and a twofold increase in fiber diameters in the muscle *anterior latissimus dorsi* in chickens with larger body weight.

The presented results are also consistent with the results obtained by SCHEUERMANN *et al.* (2004), who compared the structure of the *m. pectoralis* in Leghorn chickens with commercial broilers. The broilers had a larger thickness of muscle fibres by 45 % compared to Leghorn chickens. Apart from this, the total number of fibres in *m. pectoralis* in broilers was twice as large as in Leghorn. Additionally, the number of fibres was positively correlated with body weight (r = 0.58) and breast muscle weight (r = 0.58).

The differences in the muscle structure of the chickens originating from two flocks concerned also the presence of the red fibres only in *m. pectoralis superficialis* of the birds from strain G99. The applied method of distinguishing between muscle fiber types did not detect the red fibers, either in 8 or in 20 week old chickens from the LSL parent flock. The presence of the red or intermediate fibers in the *m. pectoralis supeficialis* in chickens, in contradistinction to the deep part, is contro-

versial. Sparse literature data indicate that the proportion of these fibres varies from 0 to 9 %, and, additionally, within a given population this value demonstrates high individual variability (UHRIN 1995; ELMINOWSKA-WENDA *et al.* 1999). Perhaps the contradictory data concerning the presence of these fibres in *m. pectoralis superficialis* are due to different methods of fibre identification and different sampling sites of the muscles for the histochemical examination.

A larger proportion of the red fibres in homological muscles of the same species is caused by various factors, such as age of the animals, feeding, keeping conditions (access to the run), and also very often by the presence of genes from wild ancestors (KARLSSON *et al.* 1999; WATERS 2004; ELMINOWSKA-WENDA 2006).

Discontinuing selection on laying in the conservative flock influenced indirectly not only a decrease in laying, but also an increase in body weight, breast and leg muscle weight as well as leg bone weight. This differentiation is the result of changes that occurred in the animals' tissues on the cellular level, which was confirmed by the presented results.

The presence of the red fibres in *m. pectoralis superficialis* and larger proportion of these fibres in *m. biceps femoris* in chickens originating from the conservative flock, and thus unselected for many years, suggests that discontinuing the selection causes a return to the primary model of the skeletal muscle's structure.

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