The Influence of Nutrition, Sex and Slaughter Age on Characteristics of Pectoralis Major Muscle at Broiler Chickens Ross-308

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Abstract
In this paper was studied the effect of dietary energy and protein levels on characteristics of pectoralis major (P. major) muscle at broiler chickens, which were sacrificed at 35 and 42 days old. The genetic material was represented by broiler chickens that belonged to the „Ross-308" hybrid, with two groups (LC-control group and LE experimental group). During the growth periods (starter, growing and finishing) they have received compound feed ad libitum, with different energy and protein levels (LC-conforming to recommendations of Aviagen Company; LE-higher with 10%). After slaughter, from each group were sampled breast muscles (five per sex) and for P. major were determined: the weight, pH value, the thickness of myocytes (fiber diameter, cross-sectional area of fibers). At the LE group, high levels of dietary proteins and energy has significantly influenced pH value and the thickness of myocytes in the P. major muscle, as compared with LC. The sex and slaughter age has significantly influenced the fibers diameter from P. major muscle, which was thicker at female chickens, as compared with male chickens and at 42 days age vs. 35 days.

Keywords: broiler chicken, fiber diameter, pectoralis major muscle, pH value

1. Introduction

Poultry meat is food source with high biological value, has high digestibility and has superior organoleptic and technological characteristics [1]. Thus, based on these characteristics, the chicken meat is in the top of consumer preferences.
Broiler chickens are a result of selection programs for rapid growth and body conformation, especially favoring muscles development in the most valuable portions of the carcass (breast, thighs and drumstick). [2]. At broiler chickens the breast represent over 30% from whole carcass, the breast meat is appreciated by consumers, occupies a special place in human diet and is the most valuable portion of the carcass on the market [2].
The pectoral muscle characteristics (biophysical, histological and biochemical) have a decisive role on meat quality [3-5] and are influenced by several factors such as bird's age, sex, genetics strain, environment and nutrition [6-11].
Some authors have shown the presence of positive correlations between meat quality of pectoral superficial muscle and biochemical and histological properties [3-4].
Miraglia et al. [12] found that the Ross chickens have in pectoral muscle greater percentage of giant fiber ($P<0.001$) vs the Kabir chickens. The presence of many giant fibers in the pectoral muscle at the Ross broiler chickens is one of the side effects of genetic selection aimed mainly this type of muscle development for its commercial

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value, but some of the meat quality traits were particularly affected by increasing fibers diameter.
Essen-Gustavsson [13] demonstrated that muscle fibers from fast growing lines of chickens have larger fiber diameters than slow growing lines and larger fiber diameters are associated with an increased number of giant fibers.
In studies of Dransfield and Sosnicki [14] have showed that the cross-sectional area of the muscle fibers from pectoral muscle has increased proportional to chickens' age, but with negative effect on the meat quality [15-16].
In studies of Dransfield and Sosnicki [14] have showed that the cross-sectional area of the muscle fibers from pectoral muscle has increased proportional to chickens' age, but with negative effect on the meat quality [15-16]. Thus, Berri et al. [17] have reported higher values of pH and a darker meat for fibers with large diameter.
Scheuermann et al. [5] have showed that muscle fiber CSA is with about 16% greater at female than males. Nonetheless, P. major muscle weight was less than 4% lower at female than males and suggests a lower muscle fiber number in female's broiler chicken.
Most studies report that glycolytic fibres exhibit the largest CSA, suggesting that, white fibers, which have a greater glycogen quantity and a higher glycolytic potential and therefore, with a rapid drop in pH value and susceptibility to PSE (pale, soft, exudative) [18].
In their studies Tůmová and Teimouri [19] have showed the nutrition influence on the muscle fibers diameter. Thus, the quantitative and qualitative feed restriction reduces the fibers diameter [19] or in the case of diets rich in nutrients we obtain hypertrophy of the muscle fibers and growth muscle mass in breast. [20].
Nutrition is an external factor with major influence on the muscle characteristics of broiler chickens. Some authors claim that feed restriction can be used as a management tool and not significantly affect the meat characteristics [21-23]. However, the severe restriction post-hatching induces loss of muscle fiber, while moderate restriction reduces the fiber size and the protein quantity of meat [19].
Studies in this respect are necessary, in order to understand the response of the studied genotype according to sex of chickens what were fed with diets with different levels of protein and energy, and to investigate the effect of age of slaughter on meat quality of breast which is represented in the highest proportion by P. major muscle.

2. Materials and methods

The experiment was organized on broiler chickens of one day old belonging to the commercial hybrid „Ross-308", which were sacrificed at 35 and 42 days old. Chickens were reared in an intensive system on the permanent litter, with a density of 12 chick/ m^2. The total birds of the experimental population were 200 broilers of both sexes (2 groups × 5 replications × 20 broilers). Broilers were randomly assigned into two equal groups (control group-LC and experimental group-LE).
Broiler chickens were reared in the same house and environmental conditions, conforming to the recommendations found in the „Broiler Management Manual Ross-308" [24]. The growth technological system was in accordance with new European Union regulation on animal welfare compulsory from 2012 in all EU member states [25].
Feed and water were given ad libitum. During the growth period (1 to 42 days) chickens were fed with three recipes of compound mixtures, as follow: the starter up to 14^{th} d, the grower from 15^{th} d to 35^{th} d and the finisher from 36^{th} d to 42^{th} d. The recipes of compound feed used had different levels of energy and protein: standard at LC (SPE) [26-27] and with 10% higher at LE (HPE).
The values for different levels of crude protein (CP) and metabolizable energy (ME) are showed in Table 1.

Table 1. Features of feed compound recipes

Group		LC	LE
CP	*Starter*	24.02	26.23
	Grower	22.63	24.90
	Finisher	21.06	23.12
ME	*Starter*	3041	3270
	Grower	3144	3435
	Finisher	3190	3490

CP (g/100 g feed)-crude protein;
ME (kcal/kg feed)-metabolizable energy

After the slaughter at 35 d and 42 d, from each group were sampled 10 carcasses (five female + five males), which were cut into pieces and from

each carcass the pectorals major muscle was collected.

Breast weight was determined using gravimetric measurements, before and after deboning.

Histological samples of muscle tissue were taken from pectoral superficial muscle, after deboning. Samples for histological study were processed using the method of inclusion in paraffin. Tissue sections of 10 μm were mounted on glass slides and stained with the trichrome method Mallory's. [28]. For the study of the histological samples and data processing, an Olympus CX-41 microscope was used, calibrated at three pairs of ocular-objective. The histological slides were studied and the most successful sections were highlighted, then microphotographs and the measurements were done through a computer, using line measurement area and computation function for its software. Measurements were done in order to assess the average diameter of fibers (MD), the ratio between large diameter (LD) and small (SD) and the cross-sectional area (CSA) of the histological elements. The following mathematical relations were utilized:

$$MD\ (\mu m) = LD + SD/\ 2$$

$$CSA\ (\mu m^2) = LD\ x\ SD\ x\ \pi/\ 4$$

π = coefficient

The pH values were determined in duplicate samples using the method as described by Jeacocke [29]. The pH determinations for tissue samples collected from the pectoral muscles were made at 15 minutes, 12 and 24 h. Before recording the pH values of the solutions on a pH meter, the electrode was rinsed with distilled water and dried with soft paper.

The raw data obtained from measurements were processed using methods of biostatistics with Microsoft Excel spreadsheet application. To test the statistical significance of differences between mean values of the characters studied, an analysis of variance using tests ANOVA and MANN WHITNEY from the program MINITAB 14 was applied [30].

3. Results and discussion

By cytometric measurements were obtained dimensional elements (SD, LD, MD, LD/SD CSA), which characterizes muscles studied by us.

Data on the size of muscle fibers in the major pectoral muscle (superficial) depending on experimental factors studied are shown in Table 2.

For SD yielded was obtained values between 40.85 and 47.11 μm and for LD values from 60.97 up to 68.04 μm. higher values were obtained in the case of samples from females, at chickens slaughtered at 42 d and at LE group.

After applying the formula for MD were obtained values from 50.91 up to 55.16 μm in the control group and from 52.83 up to 58.58 μm in the experimental group.

Applying the analysis of variance test reveal the presence of statistical differences between the mean values of the two groups of chickens (LC vs LE) between the two sexes and according with the age at slaughter.

Table 2. Sizes of the myocytes from P. major muscle

Specification	***LC***				***LE***			
	♀- 35 d *(n=100)*	♀- 42 d *(n=100)*	♂- 35 d *(n=100)*	♂- 42 d *(n=100)*	♀- 35 d *(n=100)*	♀- 42 d *(n=100)*	♂- 35 d *(n=100)*	♂- 42 d *(n=100)*
[1]SD $\bar{x}\pm S\bar{x}$ (μm)	42.90^{a} ±0.96	45.22^{b} ±0.86	40.85^{c} ±0.76	44.46^{d} ±0.70	44.10^{e} ±1.09	47.11^{f} ±0.94	43.17^{g} ±0.76	45.40^{h} ±0.85
[2]LD $\bar{x}\pm S\bar{x}$ (μm)	61,56^{a} ±0.77	65.11^{b} ±0.71	60.97^{c} ±0.77	63.22^{d} ±0.74	64.20^{e} ±0.77	68.04^{f} ±0.91	62.49^{g} ±0.71	66.33^{h} ±0.88
[3]MD $\bar{x}\pm S\bar{x}$ (μm)	52.23^{a} ±0.85	55.16^{b} ±0.82	50.91^{c} ±0.62	53.84^{d} ±0.70	54.15^{e} ±0.89	57.58^{f} ±0.86	52.83^{g} ±0.61	55.72^{h} ±0.62
LD/SD $\bar{x}\pm S\bar{x}$	1.46/1^{a} ±0.02	1.49/1^{b} ±0.01	1.48/1^{a} ±0.01	1.54/1^{c} ±0.01	1.51/1^{b} ±0.01	1.46/1^{a} ±0.02	1.54/1^{c} ±0.01	1.49/1^{d} ±0.01
[4]CSA $\bar{x}\pm S\bar{x}$ (μm^2)	2127.28^{a} ±71.78	2390.22^{b} ±95.76	1970.49^{c} ±47.13	2244.08^{d} ±59.93	2294.78^{e} ±89.50	2574.51^{f} ±86.30	2134.16^{g} ±49.89	2423.28^{h} ±91.79

Means followed by different superscript letters in the same row differ significantly to P≤0.05 by test ANOVA; n=number of measurements; $\bar{x}$=mean; ±S$\bar{x}$= Standard error; [1]SD=small diameter; [2]LD=large diameter; [3]MD=average diameter; [4]CSA=cross-sectional area

Knowing the average values of SD and LD myocyte CSA were calculated and the ratio of the two diameters. The values obtained in all cases showed superiority of females (2127.28 to 2574.51 μm^2) than males (1970.49 to 2423.28 μm^2) with lower limits at LC chickens slaughtered at 35 d and upper limits at LE chickens slaughtered at 42 d. These results are in line with studies of Scheuermann et al. [5] who reported at females CSA values for the muscle fibers from P. superficial higher than males with 16%, and Dransfield and Sosnicki [14], Aberle and Stewart [31] found that myocyte cross-sectional area increases with age.

Based on these results we can say that the level of dietary energy and protein, slaughter age and sex influenced ($p \leq 0.05$) muscle fiber thickness and CSA.

Therefore, HPE recipes accelerated muscle fiber hypertrophy and increased CSA, and the situation was reversed at SPE diets. The values obtained in this paper are consistent with the results obtained by Marcu et al. [32] which revealed differences in myocyte thickness of P. superficial muscle depending on the energy and protein levels of feed for Ross-308 broiler chickens slaughtered at 42 d. Researchers have reported the MD average values of: 51.87 μm in chickens fed with recipes LPE; 55.96 μm for HPE and intermediate values in the standard diets. Also, these results confirm the studies conducted by Roy et al. [20] which have found that diets rich in nutrients causes hypertrophy of the myocyte from pectoral superficial muscle, and Rehfeldt et al. [4] have shown that quantitative and qualitative feed restriction caused a reduction in muscle fiber diameter.

The ratio between the two muscle fiber diameters (LD/SD) ranged from 1.46/1 up to 1.51/1 in female chickens and 1.48/1 to 1.54/1 in male chickens, highlighting the section ellipsoidal profile. LC were higher values for chickens slaughtered at 42 d (1.49/1 in females and 1.54/1 in males) vs. chickens slaughtered at 35 d (1.46/1 in females and 1.48/1 in males), while at LE higher values were recorded at 35 d (1.51/1 in females and 1.54/1 in males) and lower values at 42 d (1.46/1 in females and 1.49/1 in males). Our results in this study showed a more pronounced ellipsoidal profile of LC chickens slaughtered at 42 d, while at the samples from the LE situation was reversed. In both cases, analysis of variance test showed statistical differences based on nutrition and chickens age at slaughter. Regarding the sex of offspring study, statistical differences were noted between females and males only in samples carcasses obtained from broilers slaughtered at 42 d.

The weight of the entire breast (with bone and skin) and major pectoral muscle weight were shown in Table 3.

Table 3. The weight of P. major muscle

Specification	*LC*				*LE*			
	♀- 35 d *(n=5)*	♀- 42 d *(n=5)*	♂- 35 d *(n=5)*	♂- 42 d *(n=5)*	♀- 35 d *(n=5)*	♀- 42 d *(n=5)*	♂- 35 d *(n=5)*	♂- 42 d *(n=5)*
[1]BW $\bar{x}\pm S\bar{x}$ (g)	468.23^a ±9.21	507.17^b ±8.59	546.48^c ±8.50	626.54^d ±10.13	550.40^e ±8.81	629.60^f ±6.95	648.00^g ±5.15	739.12^h ±7.09
[2]PW $\bar{x}\pm S\bar{x}$ (g)	288.40^a ±9.59	327.80^b ±7.75	379.40^c ±7.99	421.00^d ±8.79	374.20^e ±6.90	411.60^f ±4.58	422.00^g ±2.85	506.80^h ±3.99

Means followed by different superscript letters in the same row differ significantly to $P \leq 0.05$ by test MANN WHITNEY;
n=number of pieces; $\bar{x}$=mean; ±S$\bar{x}$= Standard error; [1]BW=breast weight after deboning; [2]PW=P. major weight;

In both cases (35 d and 42 d), the results showed higher values at chickens in the experimental group (550.40 g to 739.12 g) and lower values at chickens in the control group (468.23 g to 626.54 g). However, the data presented showed increased breast weight for chickens slaughtered at 42 d (females: 507.17 g at LC and 629.60 g at LE, and at males 626.54 g LC and 739.12 g LE) compared with chickens slaughtered at 35 d (females: 468.23 g LC and 550.40 g LE and males 546.48 g LC and 648 g LE). As shown in Table 3 for breast weight the highest values were in males and the lowest females.

Regarding the major pectoral muscle weight, average values were from 288.40 g to 506.80 g and had a share of more than 62% by weight of the entire breast (with bone and skin). The data presented in Table 3, shows that for P. major

muscle weight differences shown for the entire breast were maintained. In all cases, applying the test of analysis of variance indicated statistical differences and highlighted the positive impact of diets HPE level on the amount of breast meat.

The results presented in Table 2 and 3 showed statistical influence ($P \leq 0.05$) of slaughter age, nutrition and sex on the amount of meat in P. major. The results presented are consistent with the studies of Wilson et al. [33] which shown that rapid growth in the fiber size has a positive effect on muscle mass of P. major, and Mahon et al. [34] in turkeys studies have found the presence of correlations between body mass, pectoral major muscle weight and muscular fibers diameter.

For LC and LE from carcasses obtained from females and males at 35 d and 42 d, within 15 minutes after sampling from P. major muscle were performed the first measurements for $pH_{0.15}$ value. Therefore, the samples was preserved for 24 h at +4°C, and two measurements were also performed at equal time intervals (12 h and 24 h), and the average values were shown in Table 4.

Table 4. pH values

$^{*}pH_{time}$	*LC*				*LE*			
	♀- 35 d *(n=10)*	♀- 42 d *(n=10)*	♂- 35 d *(n=10)*	♂- 42 d *(n=10)*	♀- 35 d *(n=10)*	♀- 42 d *(n=10)*	♂- 35 d *(n=10)*	♂- 42 d *(n=10)*
$pH_{0.15}$ $\bar{x}\pm S\bar{x}$ (UpH)	6.22 ±0.02	6.19 ±0.02	6.21 ±0.01	6.20 ±0.02	6.23 ±0.03	6.21 ±0.03	6.24 ±0.01	6.22 ±0.01
pH_{12} $\bar{x}\pm S\bar{x}$ (UpH)	5.66^{a} ±0.01	5.65^{a} ±0.02	5.68^{a} ±0.02	5.66^{a} ±0.01	5.58^{b} ±0.02	5.54^{b} ±0.02	5.59^{b} ±0.01	5.58^{b} ±0.02
pH_{24} $\bar{x}\pm S\bar{x}$ (UpH)	5.79^{a} ±0.02	5.86^{a} ±0.02	5.76^{a} ±0.02	5.83^{a} ±0.02	5.84^{b} ±0.01	5.99^{b} ±0.01	5.82^{b} ±0.01	5.89^{b} ±0.01

Means followed by different superscript letters in the same row differ significantly to P≤0.05 by test MANN WHITNEY;
10=number of determinations; $\bar{x}$=mean; ±S$\bar{x}$= Standard error; $^{*}pH_{time}$ =pH time determination (0.15 min.; 12 h; 24 h, respectively)

For $pH_{0.15}$ values were ranged between 6.19 to 6.22 UpH at LC and between 6.21 to 6.24 UpH at LE and were within the range considered normal for this type of meat [35]. After applying the test of analysis of variance the average differences compared were not statistically ($P>0.05$). In the case of P. major muscle at 15 minutes after evisceration, the literature mentions pH of 5.89 to 6.3 [35]. After Ristić and Schön (1977), cited by [35] the pH below 5.8 or above 6.2 adversely affect meat quality and are responsible for the PSE syndrome (pale, soft, exudative) and DFD (dark, firm and dry).

The pH values determined in the muscle P. major at 12 and 24 hours at both females and males were at higher at LE and lower at LC. Differences between mean values of chickens in the control group and the experimental group were statistically provided ($P \leq 0.05$) and emphasized the influence of nutrition on muscle pH of P. major. These results confirm studies of Marcu et al. [32] which have found that diets with HPE emphasizes myocyte hypertrophy and metabolism is faster, while at chickens fed with LPE recipes muscular fibers were thinner and pH dynamics was lower. At the same time, the dynamics of the pH in this study is consistent with the results reported by Tang et al. [36] who studied the influence of energy and lysine levels in the diet on the pH of the pectoral muscle in broilers „Arbor Acres".

pH dynamics of P. major muscle was normal and characteristic for white fibers, with higher glycolitic potential [37]. In all cases, at 12 h post-slaughter has been found a reduction of the pH, and after 24 h there was an upward trend. Therefore, depending on the time of slaughter and storage temperature, changes normal and abnormal occur in the muscles influenced in the same time by other external factors [38].

4. Conclusions

According to the results obtained in this study, energy and protein levels of food, sex and age at slaughter had significant effects on most studied traits. Muscle fiber size and major pectoral muscle weight increased proportionally with age at slaughter of chickens and the energy and protein level of the food and coincided with increasing of pH. However, at males, P. major muscle weight was higher and the thickness of the muscle fibers was more reduced, indicating a greater amount of

muscle in the breast with a finer texture and an accentuated tenderness of the meat.

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