Effect of Diets with Different Energy and Protein Levels on Breast Muscle Characteristics of Broiler Chickens

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Abstract
In this paper was studied the effect of dietary energy and protein levels on breast muscle characteristics of broiler chickens, which were sacrificed at 42 days old. The genetic material was represented by broiler chickens that belonged to the „Ross-308” hybrid, with three groups (Lc-control group, L1 and L2 experimental groups). 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; L1-higher with 10%; L2-lower with 10%). After evisceration, from each group were sampled breasts from 10 carcasses (five per sex) and were determined: muscle mass, meat: bones ratio, chemical composition of meat, pH value (after evisceration up to 24 h of refrigeration) and the thickness of myocytes in the superficial pectoral muscle. For these characteristics, the highest values were obtained in the L1 group, and the lowest values in the L2 group. In the L1 group, high levels of dietary proteins and energy has significantly influenced: muscle mass, meat: bones ratio, chemical composition of meat (water, proteins and lipids), pH value and the thickness of myocytes in the superficial pectoral muscle, as compared with Lc and L2.

Keywords: breast, broiler chicken, meat, myocytes, pH value

1. Introduction
Poultry meat is food source with high biological protein value, has a relatively low fat content, has high digestibility, contains iron, some of the vitamins in the B group, and has superior organoleptic quality [1]. With these nutritional characteristics, the chicken meat is appreciated by consumers and occupies a special place in the human diet.
Broiler chickens are a result of selection programs for rapid growth and body conformation, especially favoring breast muscles development [2]. Because breast is the most valuable portion of the chicken carcass on the market, even small differences in breast yield among broiler chickens could have significant economic impact [2]. For this reason, in broiler chicken industry it is constantly necessary to run performance evaluation, considering yield breast, chemical composition and technological properties, usually determinations crucial for the culinary value in pectoral muscles [3].
Some research has demonstrated the presence of several influencing factors on poultry meat quality such as bird’s age, sex, genetics strain, environment and nutrition, with major influences on carcass and meat characteristics [4-9]. The biophysical, histological and biochemical characteristics of pectoral muscle have a decisive role on meat quality [10-12]. Some studies have revealed the presence of a positive correlation...
between meat quality and biochemical and histological properties of pectoral muscle [10, 11]. Cross-sectional area of muscle fiber had increased in size proportional to chickens’ age, but with negative effect on the meat quality [13, 14], while other authors has reported higher values of pH and a darker meat for fibers with large diameter [15]. Dransfield and Sosnicki (1999) cited by [16] reported that chickens’ fast growing had greater number of giant fibers in pectoral muscle, fibers which have a cross-section area three to five times larger than normal. The giant fibers from the breast muscle in broiler chickens are a side effect at genetic selection for increased the muscles mass in superficial pectorals [17]. Pectoral muscles have 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]. If reference is made to the feed influence, some authors argue that qualitative and quantitative restriction reduces the diameter of muscle fibers [11], or may induce an accelerated myofibres hypertrophy in the long term [19]. Roy et al. [20] claim that in the case of high density diets we obtain growth muscle mass in breast due to fibers hypertrophy. However, other authors have found that feed restrictions can be used as a management tool and not significantly affect slaughter performances and sensorial characteristics of meat [21-23]. But, the severe restriction (malnutrition) post-hatching induce loss of muscle fiber, while the moderate restriction affects the fibers hypertrophy and reduced the accumulation of protein [16].

The chemical composition of pectorals muscle is an important element of quality for this type of meat [24]. Diaz [25] relates that in breast being mostly composed of white fibers having different metabolic functions as opposed to muscles that contain red fibers. For chemical components from breast muscles, some authors reported values of over 22.50 percents for total proteins and less than 3 percents for lipid content [26-32].

The protein and lipid quantity of breast muscles is influenced by genetic and non-genetic factors [24]. Nutrition is an external factor with major influence on the chemical composition of broiler meat. Thus, diets with low protein and energy had determined reduced meat protein content, while the lipids content of the muscles had increased [24, 26-34]. However, it is unknown how a dietary energy and protein reduction will affect some meat quality characteristics (biophysical, histological and biochemical) in broilers raised in Romania. Studies in this respect are necessary, in order to understand the response the genotype and sex of chickens have to diets with different levels of protein and energy, and to investigate the effect of raw materials used in broiler nutrition on the carcasses characteristics and on the meat quality.

The aim of the present work was to investigate the effect of diets with different energy and protein levels on breast meat quality in „Ross-308” broiler chickens.

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 to 42 days old. Chickens were reared in an intensive system on the permanent litter, with a density of 12 chick/m². The total birds of the experimental population were 300 broilers of both sexes (3 groups × 5 replications × 20 broilers). Broilers were randomly assigned into three equal groups (control group-LC and two experimental groups L 1 and L 2) which were reared in the same environmental conditions.

The environmental conditions during the rearing period were conforming to the recommendations found in the „Broiler Management Manual Ross-308” [35]. The growth technological system was in accordance with new European Union regulation on animal welfare compulsory from 2012 in all EU member states [36].

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 14th d, the grower from 15th d to 35th d and the finisher from 36th d to 42th d. The recipes of compound feed used had different levels of energy and protein: standard at LC (SPE) [37, 38]; with 10% higher at L 1 (HPE); with 10% lower at L 2 (LPE). The values for different levels of crude protein (CP) and metabolizable energy (ME) are showed in Table 1.

After the slaughter, from each group were sampled 10 carcasses, in equal number per each sex, which were weighed and were cut into pieces.
Breast weight was determined using gravimetric measurements, before and after deboning.

<table>
<thead>
<tr>
<th>Table 1. Features of feed compound recipes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
</tr>
<tr>
<td>Starter</td>
</tr>
<tr>
<td>Grower</td>
</tr>
<tr>
<td>Finisher</td>
</tr>
<tr>
<td><strong>ME</strong></td>
</tr>
<tr>
<td>Starter</td>
</tr>
<tr>
<td>Grower</td>
</tr>
<tr>
<td>Finisher</td>
</tr>
</tbody>
</table>

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

Meat: bones ratio was calculated for better highlight of meat production performances and is expressed in grams meat:1 gram bones.

\[
\text{Meat: bones ratio} = \frac{\text{meat weight}}{\text{bones weight}}
\]

*meat weight represents meat with skin

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. [39]. 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 small (SD) and large diameter (LD) and the cross-sectional area (CSA) of the histological elements. The following mathematical relations were utilized:

\[
\text{MD (μm)} = \frac{LD + SD}{2}
\]

\[
\text{CSA (μm}^2\) = \frac{LD \times SD \times \pi}{4}
\]

\[\pi = \text{coefficient}\]

The pH values were determined in duplicate samples using the method as described by Jeacocke [40]. The pH determinations for tissue samples collected from the pectoral muscles were made at 30 minutes, 2, 4, 8 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.

After deboning, using STAS methods [41], laboratory analysis for chemical composition of meat (water, dry matter, proteins, lipids and minerals) was carried out. The methods of analysis used to determine the chemical composition of the meat were: drying method for water and dry matter content (at +103°C); calcinations for total minerals content (at +550°C); Soxhlet method by modern appliances-Soxtest Raypa PG-16 E01 for total lipids; Kjeldahl method adapted on appliances FOSS TECATOR for protein substances.

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 [42].

3. Results and discussion

The pH values in pectoral muscles, at 0.5, 2, 4, 8 and respectively 24 h, of „Ross-308” broiler chickens from this study are showed in Table 2. After evisceration in maximum 30 minutes were made the first pH measurements. Thus, for pH₅ values were from 5.92 in L₂ up to 6.14 in L₁, and intermediary at L₂ (6.06), with different levels of statistical significance (P≤0.05 or P≤0.01) between studied groups. For this meat type values obtained were in the range considered normal by the literature [43]. According to the same authors, in the pectoral muscles of broiler chickens at 15 minutes post eviscerated, pH values were between 5.89 to 6.3 [43]. Ristić and Schön (1977) cited by [43] had shown that pH value under 5.8 or over 6.2 negatively influenced meat quality, because of the appearance of the typical characteristics to syndrome PSE or DFD (dark, firm and dry), respectively.
In all cases, in the first 4 h, pH values in the pectoral muscles were decreased (up to: 5.67 at L2; 5.74 at Lc and 5.82 at L1, respectively), and after 8 h was registered a slight increase (5.82 at L2; 5.89 at Lc and 5.95 at L1, respectively). While, after 24 h pH values increased up to limits of: 5.84 at L2; 5.90 at Lc, and 5.98 at L1.

pH values measured in pectoral muscles at 0.5, 2, 4, 8 and 24 h were superior at L1, inferior at L2 and intermediate at Lc. Differences between average values of the chicken groups studied were statistically significant (P ≤ 0.05 or P ≤ 0.01) and reveal the feed influence on pH value in breast muscles. Similar results were obtained by Tang et al. [44] that studied the effect of different metabolizable energy levels and lysine on breast muscle pH in „Arbor Acres” broilers.

The pH dynamics in pectoral muscles was normal for this meat type. Depending of storage temperature and storage time in the post-slaughter meat there are normal or abnormal transformations, which more are influenced by other external factors [45].

### Table 2. pH values for pectoral muscle

<table>
<thead>
<tr>
<th>pHtime</th>
<th>LC (n=20)</th>
<th>L1 (n=20)</th>
<th>L2 (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH0.5</td>
<td>6.06b ±0.02 1.26</td>
<td>6.14a ±0.02 1.51</td>
<td>5.92c ±0.03 2.67</td>
</tr>
<tr>
<td>pH2</td>
<td>5.89b ±0.02 1.46</td>
<td>6.00a ±0.02 1.20</td>
<td>5.72c ±0.02 1.89</td>
</tr>
<tr>
<td>pH4</td>
<td>5.74b ±0.02 2.15</td>
<td>5.82a ±0.01 1.15</td>
<td>5.67c ±0.01 1.15</td>
</tr>
<tr>
<td>pH8</td>
<td>5.89b ±0.02 1.22</td>
<td>5.95a ±0.02 1.41</td>
<td>5.82c ±0.01 1.21</td>
</tr>
<tr>
<td>pH24</td>
<td>5.90b ±0.01 1.10</td>
<td>5.98a ±0.02 1.89</td>
<td>5.84c ±0.03 3.10</td>
</tr>
</tbody>
</table>

Means followed by different superscript letters in the same row differ significantly to P ≤ 0.05 by test MANN WHITNEY; n=number of determinations; \( \bar{x} \)=mean; ±S\( \bar{x} \)= Standard error; V%=coefficient of variation; \( ^* \)pHtime =pH time determination (0.5; 2; 4; 8; 24 hours, respectively)

Cross-sectional area (CSA) and thickness of the myocytes from superficial pectoral muscle are presented in Table 3.

### Table 3. Cross-sectional area and thickness of the myocytes from superficial pectoral muscle

<table>
<thead>
<tr>
<th>Specification</th>
<th>LC (n=100)</th>
<th>L1 (n=100)</th>
<th>L2 (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1SD ( \bar{x} )±S( \bar{x} ) (μm)</td>
<td>44.82b ±0.70</td>
<td>46.63a ±0.66</td>
<td>42.26b ±0.77</td>
</tr>
<tr>
<td>V%</td>
<td>15.63</td>
<td>14.23</td>
<td>18.26</td>
</tr>
<tr>
<td>2LD ( \bar{x} )±S( \bar{x} ) (μm)</td>
<td>64.24a ±0.81</td>
<td>65.28a ±0.80</td>
<td>61.48b ±0.73</td>
</tr>
<tr>
<td>V%</td>
<td>12.56</td>
<td>12.28</td>
<td>11.93</td>
</tr>
<tr>
<td>3MD ( \bar{x} )±S( \bar{x} ) (μm)</td>
<td>54.53b ±0.64</td>
<td>55.96a ±0.58</td>
<td>51.87c ±0.54</td>
</tr>
<tr>
<td>V%</td>
<td>11.66</td>
<td>10.28</td>
<td>9.66</td>
</tr>
<tr>
<td>4CSA ( \bar{x} )±S( \bar{x} ) (μm²)</td>
<td>2348.56a ±53.19</td>
<td>2465.33a ±61.68</td>
<td>2140.58b ±40.78</td>
</tr>
<tr>
<td>V%</td>
<td>22.65</td>
<td>25.02</td>
<td>19.05</td>
</tr>
</tbody>
</table>

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; V%=coefficient of variation; 1SD=small diameter; 2LD=large diameter; 3MD=average diameter; 4CSA=cross-sectional area.
After applying the calculation formula, for AD were obtained values of 54.53 μm in LC, 55.96 μm in L1 and 51.87 μm in L2, respectively. The coefficient of variation had values that ranged from 9.66% to 11.66% and revealed a good uniformity for average muscle fiber diameter. Analysis of variance revealed the presence of some statistical differences between average values in the studied broiler groups (P≤0.05). Thus, the muscle fiber sizes and CSA were statistically influenced by dietary energy and protein levels (P≤0.05). Therefore, diets with HPE levels determined an increased in fiber size and CSA, but situation was reversed in recipes with LPE levels. These results confirm previous studies carried out by Roy et al. [20] who found that diets with higher density accentuate the hypertrophy of the fiber from pectoral muscle. While, Rehfeldt et al. [11] found that feed restriction in quantity or quality determined a decrease in muscle fibre diameter.

For expressed the breast meat production, the meat: bones ratio for this carcass part was calculated (Table 4).

<table>
<thead>
<tr>
<th>Specification</th>
<th>L_C (n=10)</th>
<th>L_1 (n=10)</th>
<th>L_2 (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1^BW ±Sx</td>
<td>520.55±18.40</td>
<td>548.90±21.70</td>
<td>440.60±12.05</td>
</tr>
<tr>
<td>V%</td>
<td>11.18</td>
<td>12.50</td>
<td>8.65</td>
</tr>
<tr>
<td>2^BW ±Sx</td>
<td>53.98±1.65</td>
<td>54.68±1.32</td>
<td>51.70±1.67</td>
</tr>
<tr>
<td>V%</td>
<td>9.68</td>
<td>7.65</td>
<td>10.20</td>
</tr>
<tr>
<td>3^M:B</td>
<td>9.64:1±0.13</td>
<td>10.04:1±0.16</td>
<td>8.52:1±0.11</td>
</tr>
<tr>
<td>V%</td>
<td>4.22</td>
<td>5.11</td>
<td>4.05</td>
</tr>
</tbody>
</table>

Means followed by different superscript letters in the same row differ significantly to P≤0.05 by test MANN WHITNEY;

n=number of carcasses; bar=mean; Sx= Standard error; V%=coefficient of variation;

1^BW=breast weight after deboning; 2^BW=bones weight; 3^M:B=meat: bones ratio (g meat:1 g bones)

Feed energy and protein quantity statistically influenced the breast meat quantity, because data from Table 4 show different statistical significance levels between studied groups (P≤0.05 or P≤0.001). Therefore, diets with HPE level caused muscle increase in broilers from the L1 group with a difference of +0.40:1 vs. the L_C group (P≤0.05) and +1.52:1 vs. the L_2 group (P≤0.001), also and between the L_2 group (LPE) and the L_C group (SPE) there was a difference of -1.22:1 (P≤0.001).

The results presented in this paper are in accordance with other studies which have shown a significant effect of dietary energy and protein level on meat quantity from carcasses [27, 31]. The results of chemical analysis performed on samples taken from pectoral muscles are shown in Table 5.

For water content in the pectoral muscles the obtained values ranged from 72.58% in the L1 group to 74.28% in the L2 group, while for dry matter were registred quantity of 25.72% in L2, 26.46% in L_C and 27.22% in L_1, respectively.

Diets with HPE level positively influenced dry matter content in pectoral muscles, but the situation was reversed for feed LPE level. This claim is supported by the presence of some statistical differences between studied groups (P≤0.05).

Protein, lipid and mineral content in the pectoral muscles were expressed in percentages from dry matter. Samples taken from the L_1 group have the highest proteins content (92.14%) and the lowest lipids proportion (2.16%), while in L2 were registered minimum values for protein (88.57%) and maximum for lipids (6.14%). Protein and lipid contents from pectoral muscles were statistically influenced by nutrition (P≤0.05). Data presented in Table 5 show that diet HPE level increased protein quantity while the lipids decreased, but situation was reversed if recipes of compound feed had LPE level.
For the protein and lipid content of the pectoral muscles, our values can be compared with other research from the literature, who showed values of over 22.50% for protein and under 3% for lipid content, respectively [24, 26-34, 45, 46]. Normally, a decrease in both muscle protein and water is accompanied by an increase in lipids [23-34, 45, 46].

Table 5. Chemical composition of pectoral muscles

<table>
<thead>
<tr>
<th>Specification</th>
<th>$L_c$ (n=10)</th>
<th>$L_1$ (n=10)</th>
<th>$L_2$ (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water $\bar{X}\pm S\bar{X}$ (%)</td>
<td>73.54$^a\pm0.16$</td>
<td>72.78$^a\pm0.08$</td>
<td>74.28$^a\pm0.19$</td>
</tr>
<tr>
<td>V%</td>
<td>0.68</td>
<td>0.33</td>
<td>0.79</td>
</tr>
<tr>
<td>Dry matter $\bar{X}\pm S\bar{X}$ (%)</td>
<td>26.46$^b\pm0.14$</td>
<td>27.22$^a\pm0.11$</td>
<td>25.72$^c\pm0.16$</td>
</tr>
<tr>
<td>V%</td>
<td>1.68</td>
<td>1.25</td>
<td>1.98</td>
</tr>
<tr>
<td>*Proteins $\bar{X}\pm S\bar{X}$ (%DM)</td>
<td>92.52$^a\pm0.42$</td>
<td>92.14$^a\pm0.38$</td>
<td>88.57$^b\pm0.35$</td>
</tr>
<tr>
<td>V%</td>
<td>1.42</td>
<td>1.31</td>
<td>1.26</td>
</tr>
<tr>
<td>*Lipids $\bar{X}\pm S\bar{X}$ (%DM)</td>
<td>3.59$^b\pm0.06$</td>
<td>2.61$^c\pm0.07$</td>
<td>6.14$^a\pm0.08$</td>
</tr>
<tr>
<td>V%</td>
<td>5.66</td>
<td>8.95</td>
<td>4.22</td>
</tr>
<tr>
<td>*Minerals (%DM)</td>
<td>4.20$\pm0.04$</td>
<td>4.19$\pm0.04$</td>
<td>4.08$\pm0.04$</td>
</tr>
<tr>
<td>V%</td>
<td>6.24</td>
<td>9.68</td>
<td>8.12</td>
</tr>
</tbody>
</table>

Means followed by different superscript letters in the same row differ significantly at $P\leq0.05$ by test MANN WHITNEY; $n=$number of samples; $\bar{X}=$mean; $\pm S\bar{X}=$ Standard error; V%=$coefficient of variation; *Proteins, *lipids and *minerals expressed in percent from dry matter.

If we refer to the mineral content of pectoral muscles similar values, were obtained for the three groups of studied broilers. The significance level was over 0.05 ($P>0.05$) and does not show statistical differences between compared mean values. Therefore, as shown in Table 5, we can say there was no significant effect of diet on mineral content of the pectoral muscles.

The results obtained in this paper are consistent with other studies that showed some differences in meat quality, depending on the feeding energy and protein levels [24, 26-34, 46].

4. Conclusions

The increase of dietary protein and energy levels had significant effects on most traits studied. Consequently, the muscle fiber diameter and cross-sectional area increased and coincides with increasing pH values.

Increasing muscle fiber CSA determined the growth of pectoral muscle weight and higher values for the meat: bones ratio.

According to the results obtained in the present study, dietary energy and protein level had significant effects on the chemical composition of pectoral muscles. Thus, the meat with a higher protein content and low quantity of lipid was obtained by feeding with higher level of energy and protein, while lowering energy and protein from diet had produced less protein and more fat in pectoral muscles.

References

9. Young L. L., J. K. Northcutt, R. J. Buhr, C. E. Lyon, Ware G. O., Effects of age, sex, and duration of post-mortem aging on percentage yield of parts from broiler chicken carcasses, Poultry Science, 2001, 80, 376–379.
17. Miraglia D., Mammoli R., Branciari R., Ranucci D., Cenci Goga B. T., Characterization of muscle fibre type and evaluation of the presence of giant fibres in two meat chicken hybrids, Veterinary Research Communications, 2006, 30 (Suppl. 1), 357–360.


