EFFECTS OF DIETARY ROSEMARY EXTRACT AND $\alpha$-TOCOPHEROL ON THE PERFORMANCE OF CHICKENS, MEAT QUALITY, AND LIPID OXIDATION IN MEAT STORAGED UNDER CHILLING CONDITIONS

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Abstract

The influence of rosemary powder and $\alpha$-tocopherol, applied to broiler chickens from the 21st d to 42nd d of their feeding, on chicken performance, meat quality, and lipid oxidation in the meat was observed. The chickens were fed with a feed supplemented with rosemary powder (500 mg/kg$^{-1}$) and $\alpha$-tocopherol (40 mg/per chicken) gained higher weights (2502 and 2497 g) in comparison with the control group (2457 g) and showed an increase in $\alpha$-tocopherol concentrations in blood and breast muscles. Oxidative changes were investigated on the basis of the changes of malondialdehyde content in the breast and thigh muscles on days 0, 7, and 14 of refrigerated storage ($4^\circ$C). The results showed that the rosemary powder was effective in delaying lipid oxidation compared to the control diet at all time points, but less effective than $\alpha$-tocopherol. Thigh muscles were more susceptible to lipid oxidation compared to breast muscles. The addition of rosemary improved the sensory properties of the meat without a prolongation of feeding time and increasing the costs of feeding.

Key words: chickens, rosemary, $\alpha$-tocopherol, weight gain, poultry meat, meat quality, lipid oxidation.

Poultry meat has some advantages from the nutritional aspect e.g. the high content of proteins, essential polyunsaturated fatty acids, and minerals, and the low content of lipids (1). The increasing consumption of poultry meat leads not only to a larger extension of broiler production, but also to its effectiveness. There is an effort to breed as efficient hybrids as possible, to improve conversion of nutrients, and shorten the period of feeding (17). However, all these factors are associated with some negatives, such as problems with the cardiovascular system and frequent manifestations of heart failure, occurrence of ascites, the need of administration of antibiotics, growth stimulants, vitamins etc. (2, 12, 20). The quality of meat is partly influenced by shortening the period of feeding to 37-38 d. The meat contains excess water, and due to this, it has worse sensory properties and is not fully matured (17). Nowadays, there are some new aspects of animal feeding because of a higher attention paid to the quality of food products of the animal’s origin.

Because of this reason, experts in animal nutrition are trying to find ways on how to produce as natural and qualitative food products as possible (20). The use of extracts from herbs and spices is a very important step in animal feeding (7, 8, 19). These extracts have strong antioxidative, antibacterial, and digestive effects. They help animals to overcome stress conditions, influence the microorganisms in the alimentary tract, and improve feed utilisation (2, 8).

Rosemary ($Rosmarinus officinalis$) is a typical Mediterranean plant. It contains natural polyphenols that significantly influence the oxidative processes (10). Rosemary’s essential oils, positively affect intestinal microorganisms in poultry and monogastric animals (8). The oils $in vitro$ have antibacterial properties against pathogens such as $Salmonella$ Typhimurium, $Pseudomonas aeruginosa$, and $Bacillus subtilis$ (2). Rosemary is used in the form of powder, which is the natural extract of the plant, mildly aromatic, with typical aroma of rosemary.

The presence of $\alpha$-tocopherol in the organism of chickens and laying hens is important for maintaining optimal health conditions (3, 5). Its level in blood is an important indicator, especially with regard to prophylaxis (exudative diathesis, oedema disease, malabsorption syndrome, necrotic dermatitis). Moreover, $\alpha$-tocopherol is a highly effective natural antioxidant and its presence within muscle cell membranes reduces lipid oxidation. A raised $\alpha$-tocopherol concentration in the feeding mixture also enhances feed conversion efficiency and weight gain, and decreases feed consumption (5, 11).
The purpose of the study was to investigate the effect of rosemary and vitamin E supplementation from the 21st d of feeding on the growth, lipid oxidation, and meat quality of broiler chickens.

Material and Methods

Animals and diets. The experiment was carried out with 90 one-day-old broiler chickens, ISA 220 breed, from the hatchery Incuba a.s. Záběčice (Czech Republic). Until the 20th d of the experiment, the chickens were kept and fed together the commercial feed mixture (Table 1) and water ad libitum. A 24-h light regime was used during the first 7 d. From the 8th to 20th d of age, the light regime was changed and consisted of 20-h light and 4-h darkness period. The temperature regime for one-day-old chickens was 33°C and it was gradually decreased to 21°C at the 21st d of age. The humidity of the environment was 70%.

On the 21st d of feeding, the chickens were divided into 3 equal groups of 30 broilers in each group, and they were fed as follows: the first group (R) received the rosemary powder FlavorGuard P (Christian-Hansen A/S, Denmark) at the daily dose of 500 mg/kg -1 of feed. Rosemary was added every day, directly into estimated amount of feed mixture per day. The second group (E) was fed the commercial feed mixture and α-tocopherol (Hydrovit E forte, Pharmagal, Slovak Republic) at the daily dose of 40 mg/bird, which was administered with their drinking water. The daily dose of α-tocopherol for the whole group of chickens was added to 2 litres of water every morning. The control group (K) was fed feeding mixtures without addition of the antioxidants. The experiment was conducted up to the 42nd d of chicken feeding. The chickens were weighed individually on days 1, 20, 30, 35, and 42 of the feeding.

Processing of chickens. On the 42nd d of age, the broilers were slaughtered by decapitation and bled. The above procedure was performed by a responsible veterinarian in respect for rules established for slaughtering of animals (4). In order to determine the lipid oxidation, carcasses from 10 birds were immediately trimmed for breast and thigh muscles by removing skin, bones, and connective tissue. Next, breast and thigh muscles within each group were separately sliced, over-wrapped in transparent oxygen-permeable polyvinyl chloride film, and stored at 4°C. The rest of chicken carcasses was processed and cooled to 4°C, and within 24 h, the sensory analysis of the samples was carried out. The breast muscles were used for the analysis of the α-tocopherol content. The blood samples were taken on the 35th and 42nd d of age from the wing veins.

Determination of α-tocopherol. HPLC determination of α-tocopherol content in blood and breast muscles was performed according to the procedure described by Grau et al. (6). α-Tocopherol was analysed on a series 1050 Hewlett-Packard liquid chromatograph equipped with a C18 column (25 x 0.46 cm) packed with 5 µm - 80 A Extrasil ODS2 and a C18 precolumn packed with 5 µm - 100 A Kromasil ODS2. The compound was isocratically eluted with methanol and detected through a 1046 Hewlett-Packard spectrofluorometric detector (the excitation wavelength of 288 nm and the emission wavelength of 330 nm).

Evaluation of thiobarbituric acid. The decomposition of fats was evaluated by the measurement of thiobarbituric acid (TBA) value, expressed as amount of malondialdehyde (MDA) calculated for 1 kg of a sample. The evaluation of TBA was performed according to Marcincák et al. (15) and measured spectrophotometrically at 532 nm (Helios γ, v. 4.6, ThermoSpectronic, U.K.). The examination of the samples was carried out on days 0, 7, 9, and 14 of storage at chilling conditions (4°C).

Table 1

<table>
<thead>
<tr>
<th>Components</th>
<th>g.kg⁻¹ feed</th>
<th>chemical analysis</th>
<th>g.kg⁻¹ feed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>590</td>
<td>Dry matter</td>
<td>860</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>254</td>
<td>Crude protein</td>
<td>210</td>
</tr>
<tr>
<td>Full fat soybean</td>
<td>65</td>
<td>Crude fibre</td>
<td>35</td>
</tr>
<tr>
<td>Wheat</td>
<td>11</td>
<td>Ash</td>
<td>70</td>
</tr>
<tr>
<td>Fish meal</td>
<td>25</td>
<td>Calculated analysis</td>
<td></td>
</tr>
<tr>
<td>Yeast</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limestone</td>
<td>15</td>
<td>Linoleic acid</td>
<td>10</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>2</td>
<td>Calcium</td>
<td>8</td>
</tr>
<tr>
<td>Monocalcium phosphate</td>
<td>8</td>
<td>Phosphorus (total)</td>
<td>6</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>3</td>
<td>Lysine</td>
<td>11</td>
</tr>
<tr>
<td>Vitamin premix¹</td>
<td>1</td>
<td>Methionine + Cystine</td>
<td>7.5</td>
</tr>
<tr>
<td>Trace-mineral premix²</td>
<td>1</td>
<td>Metabolisable energy</td>
<td>12</td>
</tr>
</tbody>
</table>

¹ Vit. A - min. 10 000 IU; vit.D3 - min. 2 000 IU; vit. E - 25 mg; vit. B2 - min. 4 mg; vit. B₁₂ - 20 µg; folic acid - 1 mg.
² Zn - 50 mg; Mn - 50 mg; Fe - 60 mg; Cu - 6 mg; Se - 0.75 mg.
Sensory evaluation. The sensory evaluation was performed 24 h after slaughter and chilling up to 4°C. The samples were evaluated by the panel of 7 trained persons according to methods for sensory testing of poultry and poultry meat products. For testing, both boiled and grilled samples were used. The sample estimation was provided by 5-point grading system and the distinguishing triangle test (9). In each group, 14 samples were evaluated.

Statistical analysis. The statistical analysis was performed with the statistical programme GraphPad Prism version 3.0 (1999). The results were expressed as arithmetic mean with standard deviation (SD). The results obtained from each group of broilers were compared with the ANOVA test. The Turkey’s test was applied to compare statistical differences between values of each group. The average values of each group were applied to compare statistical differences between values comparing the Student’s t-test and the differences were considered significant at P<0.05.

Results

Table 2 shows the average weight gains of each group of chickens. The body weights are the same in all chickens up to the 20th d of their age, because they were fed in the same manner. The effect of addition of α-tocopherol and rosemary into commercial feed mixtures manifested already after 10 d of feeding and it became obvious at the end of the feeding period. Chickens in groups E and R achieved higher average weight than in the control group. On the 42nd d of the age, it was found that chickens with the highest body weight (2 502 g) were fed feed mixtures supplemented with rosemary powder. The addition of the rosemary increased the average weight by about 45 g and the addition of α-tocopherol to the drinking water over 40 g, compared with the control group (P>0.05). Although the increase in body weight in groups E and R was not significant, it is important that there were no evident differences in equality of body weights of chickens compared with the control group. The difference between sums of body weights of 10 the heaviest and 10 the lightest chickens was 722 g in group R, 749 g in group E, and 877 g in the control group.

The concentrations of α-tocopherol in blood and in breast muscles are shown in Table 3. The addition of rosemary powder into feed mixtures increased its level in blood. The addition of rosemary powder had the same positive impact on the content of the compound in blood. Comparing the concentrations of α-tocopherol in blood on the 42nd d, we found a moderate decrease versus the concentrations on day 35 in all tested groups. This fact is probably caused by the increase of weight of the chickens during the feeding period.

Table 3 shows that the supplementing of chicken diets with rosemary powder, or α-tocopherol elevated significantly the α-tocopherol levels also in breast muscles. Its concentrations were twofold higher after the addition of rosemary, and three times higher after the addition of α-tocopherol as compared with the control group.

The results of the determination of TBA, expressed as amount of MDA in breast and thigh muscles stored at 4°C for 14 d, is shown in Table 4. Low amount of MDA was recorded in breast muscles from all groups within 24 h after slaughter. No significant differences were found among the groups. The highest levels of MDA (0.030 mg.kg⁻¹) were determined in control samples. Statistically significant differences were found in samples of thigh muscles (P<0.05) after 24 h storage between groups fed rosemary powder and α-tocopherol (0.039 and 0.037 mg.kg⁻¹) and control group (0.055 mg.kg⁻¹).

After 7 d of storage of the samples at 4°C, the amount of MDA increased in all the samples. However, a positive impact of the antioxidants on a shortening of the oxidative processes in breast and thigh muscles was obvious. The amounts of MDA in the muscles were significantly lower compared with control (P<0.05). A positive influence of antioxidant supplementation on oxidative processes was recorded also in the muscles stored for 14 d.

The obtained results of sensory analysis according to 5-point system showed higher values of chicken meat from groups R and E in comparison with the control group (Table 5). It is important that dominant aroma and flavour of chicken meat from groups fed rosemary powder and α-tocopherol were better than those from the controls. The most significant differences were noticed at the evaluation of aroma and flavour of grilled thigh and boiled breast. Sensory analysis showed that the best sensory properties were found in meat of chickens fed rosemary. Twenty-four samples from total number of samples were recognised correctly, as a meat from chickens fed with rosemary powder (statistically 99.9%). The taste was the most expressive parameter distinguishing them from control samples.

<p>| Table 2 |
| Body weight of broiler chickens during feeding (g) |</p>
<table>
<thead>
<tr>
<th>Day of feeding</th>
<th>1</th>
<th>20</th>
<th>30</th>
<th>35</th>
<th>42</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>37 ± 4</td>
<td>659 ± 55</td>
<td>1497 ± 97</td>
<td>1888 ± 138</td>
<td>2457 ± 270</td>
</tr>
<tr>
<td>R</td>
<td>37 ± 4</td>
<td>659 ± 55</td>
<td>1509 ± 102</td>
<td>1912 ± 135</td>
<td>2502 ± 214</td>
</tr>
<tr>
<td>E</td>
<td>37 ± 4</td>
<td>659 ± 55</td>
<td>1545 ± 110</td>
<td>1935 ± 195</td>
<td>2497 ± 249</td>
</tr>
</tbody>
</table>

K – control, R – chicken fed rosemary powder, E – chicken fed α-tocopherol
Table 3

Content of α-tocopherol in blood and muscles of broiler chickens

<table>
<thead>
<tr>
<th></th>
<th>K</th>
<th>R</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood on 35th d (µmol.l⁻¹)</td>
<td>21.05 ± 3.68</td>
<td>52.36 ± 12.35</td>
<td>94.81 ± 21.06</td>
</tr>
<tr>
<td>Blood on 42nd d (µmol.l⁻¹)</td>
<td>19.26 ± 3.95</td>
<td>50.47 ± 14.41</td>
<td>93.28 ± 27.45</td>
</tr>
<tr>
<td>Breast muscles (mg.kg⁻¹)</td>
<td>1.95 ± 0.31</td>
<td>4.06 ± 0.68</td>
<td>6.30 ± 1.08</td>
</tr>
</tbody>
</table>

Explanations: as in Table 2

Table 4

TBA values, presented as MDA content (mg.kg⁻¹), in breast and thigh muscles stored at 4°C for 14 d

<table>
<thead>
<tr>
<th>Days of chilling</th>
<th>Breast</th>
<th>Thigh</th>
<th>Breast</th>
<th>Thigh</th>
<th>Breast</th>
<th>Thigh</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.030 ± 0.004</td>
<td>0.055 ± 0.013</td>
<td>0.025 ± 0.003</td>
<td>0.037 ± 0.008</td>
<td>0.024 ± 0.003</td>
<td>0.039 ± 0.007</td>
</tr>
<tr>
<td>7</td>
<td>0.337 ± 0.043</td>
<td>0.403 ± 0.058</td>
<td>0.180 ± 0.031</td>
<td>0.273 ± 0.061</td>
<td>0.209 ± 0.024</td>
<td>0.289 ± 0.032</td>
</tr>
<tr>
<td>14</td>
<td>0.456 ± 0.052</td>
<td>0.606 ± 0.059</td>
<td>0.208 ± 0.036</td>
<td>0.375 ± 0.049</td>
<td>0.253 ± 0.033</td>
<td>0.391 ± 0.039</td>
</tr>
</tbody>
</table>

Explanations: as in Table 2

Table 5

Results of sensory evaluation of meat samples 24 h after slaughter

<table>
<thead>
<tr>
<th></th>
<th>Roasted breast</th>
<th>Boiled breast</th>
<th>Roasted thigh</th>
<th>Boiled thigh</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K</td>
<td>E</td>
<td>R</td>
<td>K</td>
</tr>
<tr>
<td>Odour</td>
<td>4.2</td>
<td>4.4</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Taste</td>
<td>4.0</td>
<td>4.0</td>
<td>3.4</td>
<td>3.4</td>
</tr>
<tr>
<td>Juiciness</td>
<td>3.6</td>
<td>3.2</td>
<td>4.0</td>
<td>3.6</td>
</tr>
<tr>
<td>Tenderness</td>
<td>4.1</td>
<td>3.5</td>
<td>4.9</td>
<td>3.5</td>
</tr>
<tr>
<td>Total</td>
<td>15.9</td>
<td>15.1</td>
<td>17.2</td>
<td>14.5</td>
</tr>
</tbody>
</table>

Explanations: as in Table 2

Discussion

The obtained results indicated that the addition of rosemary powder and α-tocopherol to feed increased the slaughter weight of chickens. Our results corresponded with Hernandez et al. (8) who claimed that the addition of plant extracts to feed mixtures caused mildly higher weight of broiler chickens. Kušev et al. (12) recorded that application of vitamin E at the dose of 21 mg per capita per week increased slaughter quality of broiler chicken for about 5.68% (first quality class). It was mentioned that average weight of chickens increased by 150 g compared to control. Similar results were obtained by Kennedy et al. (11) after application of vitamin E at a dose of 180 UI/kg⁻¹ of feeding mixture, which improved feed conversion by 0.8% and increased body weight by 1.4%.

The addition of α-tocopherol into the feed increased 3.5-fold its concentration in blood, compared to control. Increased content of α-tocopherol was recorded also by Young et al. (21).They found 5-fold accumulation of α-tocopherol concentration in comparison with control. Similar results were published by Coetzee and Hoffmann (5), Govaris et al. (7), and Papageorgiou et al. (19) in turkeys. It was demonstrated that the concentration of α-tocopherol in blood correlated with its addition to feed and probably responded to current nutrition status of an animal (7).

The supplementation of feed with rosemary also increased the α-tocopherol level in blood and meat. In these chickens, twofold higher content of the compound than in controls was found. Rosemary belongs to the substances with high antioxidative activity (10, 14). It is suggested that rosemary substances regenerate α-tocopherol and subsequently increase its level in the tissue (21). Botsoglou et al. (2) observed that the addition of oregano extracts at a dose of 100 mg/kg⁻¹ of feeding mixture increased significantly the levels of α-tocopherol in blood serum and in tissues of chickens. According to them, oregano extract improves antioxidative protection of α-tocopherol in the organism. α-tocopherol belongs to substances with antioxidative activity in animal tissues, and prevents unspecific oxidative reactions and helps to protect the organism against stress (3, 5). It inhibits the oxidation of polyunsaturated fatty acids in phospholipids and prevents oxidation of cell membranes. In the present time, α-tocopherol is added to feeding mixtures because it is able to decrease stress during the feeding and increase the oxidative stability of meat and meat products during storage (12, 16, 19). Higher concentration of α-tocopherol in feed caused also its higher level in animal tissues and blood (3, 7, 19). Subsequently, the meat is a very good source of α-tocopherol in human nutrition.

Poultry meat contains less amount of fat than red meat. On average, broilers contain from 3.5 to 5.0% of fat. Poultry fat has higher amount of unsaturated fatty
acids (PUFA) than fat of other food animals. Regarding higher amount of PUFA, poultry meat is more sensitive to oxidative processes (16). Feeding and conditions used for farming and slaughtering can influence oxidative stability of meat (21). The most ideal situation is when fats are protected immediately after they are obtained, i.e. just after slaughter of animals. It is possible that tissues are saturated with antioxidants as additives during the life of animals (5). Govaris et al. (7) stated that post-mortem addition of antioxidants to the mince meat also retarded lipid oxidation in the prepared patties compared to control; however, this effect was inferior to that of dietary supplementation even though the post-mortem α-tocopherol supplemented meat contained 90-fold more α-tocopherol than patties from the dietary supplemented meat.

Table 4 shows that thigh muscles were more intensively affected with lipid oxidation than breast meat samples, throughout the 14-d storage period. This is in agreement with literature report (7, 13). The greater susceptibility of thigh to lipid oxidation has been attributed to the higher content of PUFA in these muscles (7). Our results confirmed positive influence of feeding rosemary powder on sensory properties of meat. The most important improvement was obvious in feeding of rosemary powder on sensory properties of muscles (7). Our results confirmed positive influence of feeding rosemary powder on sensory properties of muscles (7).

Table 4 shows that thigh muscles were more intensively affected with lipid oxidation than breast meat samples, throughout the 14-d storage period. This is in agreement with literature report (7, 13). The greater susceptibility of thigh to lipid oxidation has been attributed to the higher content of PUFA in these muscles (7). Our results confirmed positive influence of feeding rosemary powder on sensory properties of meat. The most important improvement was obvious in feeding of rosemary powder on sensory properties of muscles (7). Additionally, sensory properties can be improved without expenses increased because of feeding. From the sensory aspect, consumer obtains good-class food products. Administration of vitamin E caused binding of α-tocopherol in muscle membranes and fats and increased oxidative stability and sensory properties (2, 6).

Acknowledgments: This study was supported by the grant VEGA No. 1/2395/05.

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