EVALUATION OF MEAT QUALITY AFTER APPLICATION OF DIFFERENT
FEED ADDITIVES IN DIET OF BROILER CHICKENS

Peter Haščík, Lenka Trembecká, Marek Bobko, Juraj Čuboň, Ondřej Bučko, Jana Tkáčová

ABSTRACT
The present study was conducted to investigate the effect of natural feed additives, namely bee pollen extract, propolis extract and probiotic preparation, on technological properties of meat in order to evaluate the meat quality of Ross 308 broiler chickens. The feeding of chickens (180 pcs) lasted for a period of 42 days. The experiment was carried out without segregation between the genders. The chickens were randomly divided into 4 groups. The control group was fed a basal diet, whereas the other three groups were fed diets supplemented with natural additives, i.e. bee pollen extract at level of 400 mg.kg⁻¹ of feed mixture, propolis extract at level of 400 mg.kg⁻¹ of feed mixture, and probiotic preparation based on Lactobacillus fermentum (1.10⁹ CFU per 1 g of bearing medium) in an amount of 3.3 g added to water (for 30 pcs chickens until 21 days of age, for 20 pcs chickens from 22nd to 42nd day of age) given to group E1, group E2 and group E3, respectively. The feed mixtures were produced without any antibiotic preparations and coccidiostatics. During the whole period of experiment, the broiler chickens had ad libitum access to feed and water. The following technological properties were examined: cooling loss (after 24 h of storage at 4 °C), freezing loss (after 3 months of storage at -18 °C), roasting loss (performed on roasted meat that was stored at -18 °C for 3 months before thawing), colour parameters based on CIELab system (the L*, a*, b* values of raw breast and thigh muscle), and tenderness (as shear force of roasted breast and thigh muscle). We have made a finding, that the examined additives had only little impact on meat quality in most of the investigated parameters, except the significant increase (p ≤0.05) in redness (a*) values and the slight decrease in roasting loss and shear force determination after propolis extract supplementation. Therefore, it may be inferred that propolis extract has been shown as the most appropriate feed additive among the applied supplements.

Keywords: chicken meat; loss; colour; shear force; bee pollen extract; propolis extract; probiotic; meat quality

INTRODUCTION
From the perspective of human nutrition, poultry meat is a valuable source of proteins, vitamins and minerals. Recent studies have affirmed that the level of those compounds, as well as meat quality, is determined not only genetically, but it is also affected by the microelement and macroelement content of feeds, the way animals are housed, their breed, sex and health, slaughter procedures, and type of muscle (Debut et al., 2003; Lombardi-Boccia et al., 2005). The ever-rising trend of poultry consumption shows the importance of controlling meat quality for the poultry industry (De Genova Gaya et al., 2011). Besides, the technological quality of poultry meat is now of major importance, since poultry meat is nowadays usually consumed as cuts or as processed products rather than as whole carcasses (Nissen and Young, 2006; Le Bihan-Duval et al., 2008).

Breast and thigh meats are the most valuable muscles of the poultry carcass (Yu et al., 2005). Water holding capacity, pH, colour and tenderness, usually determined in those parts of chicken carcass are crucial for the culinary value and technological properties of chicken meat (Musa et al., 2006; Nissen and Young, 2006).

Technological indicators such as colour and tenderness are important attributes to which consumers attach a special importance (An et al., 2013), due to the close association with factors such as freshness, flavour, desirability, storage time and food safety (Girolami et al., 2013; Wu and Sun, 2013), while variation in these indicators depends on the characteristic of muscle itself. Muscle is composed of different fiber types, on the one hand, they can be affected by sex, breed, age, etc., on the other hand, muscle fiber characteristics can influence meat quality characteristics such as colour, water-holding capacity (i.e. drip loss during storage) and the texture of meat (Lyon et al., 2004; Le Bihan-Duval et al., 2008; An et al., 2013). Producers should be concerned with environmental conditions, such as feed and housing conditions that may affect these important quality attributes (Saláková et al., 2010). Development of pH, meat colour, and water-holding capacity (WHC) are closely connected and are associated with the energy status of the muscles at slaughter, which is highly influenced by the duration of transportation and the stress before and during slaughter (Nissen and Young, 2006). Variation of the meat colour is up to a certain point physiological, but the differentiation to pathological
alterations like pale, soft and exudative (PSE)-like meat is important because the latter is characterized by a paler colour, a heterogeneous appearance, a poorer texture and cohesiveness as well as a higher drip loss (Berri et al., 2007; Janisch et al., 2011).

The perception of colour is a very complex phenomenon that depends on the composition of the object in its illumination environment, the characteristics of perceiving eye and brain, and the angles of illumination and viewing (Wu and Sun, 2013).

The colour measurements can be conducted by visual (human) inspection, traditional instruments like colourimeter, or computer vision (Wu and Sun, 2013). Currently, meat colour is measured by colourimeter in terms of CIE \( L^*, a^*, b^* \) values, hue angle and chroma. The \( L^* \) \( a^* \) \( b^* \), or CIELab is the 3-dimensional colour expression, whereby \( L^* \) is the lightness component, which ranges from 0 to 100 (from black to white) and the parameters \( a^* \) (from green if negative to red if positive) and \( b^* \) (from blue if negative to yellow if positive) are two chromatic components which range from -120 to +120 (Leon et al., 2006; Larrain et al., 2008; Girolami et al., 2013).

Tenderness is considered the most important factor in determining the consumer-eating satisfaction of meat products (Xiong et al., 2006; Lee et al., 2009). As consumer consumption of boneless chicken meat has dramatically increased over recent years, tenderness has become increasingly important to poultry meat processors. To meet consumer expectations of tenderness, meat processors must produce tender meat products as well as understand what constitutes tender meat (Xiong et al., 2006). Meat tenderness is defined by the ease of mastication, which involves initial penetration by the teeth, the breakdown of meat into fragments and the amount of residue remaining after chewing (Kong et al., 2008). Tenderness can be determined by a trained panel (sensory analysis) or physical methods (instrumental analysis) (Cavitt et al., 2004; Li et al., 2013). Warner-Bratzler (WB) shear blade is one of the most commonly used instruments in objective estimating meat tenderness and texture quality of poultry meat, whereby the higher WB shear values are associated with less tender poultry meat (Zhuang et al., 2008). This cutting method is based on measuring the force required to shear across entire muscle fibers. The orientation of the slice needed to correspond to muscle fiber orientation so that the shearing action would be across the muscle fibers. The WB values are commonly reported in grams, kilograms, or newtons (Carranco-Jáuregui et al., 2010; Silva et al., 2015).

Water loss is directly proportional to the water holding capacity (WHC) of muscle proteins and reduced water content changes key quality parameters such as colour and texture (Ali et al., 2015). During freezing, storage and thawing, meat loses water by evaporation, sublimation and exudation, respectively. The water is also lost during the cooking. Although moisture losses make meat less attractive, they do not significantly influence its eating quality after dry-heat cooking, except in the case of very large losses, which could affect juiciness and tenderness (Pérez Chabela and Mateo-Oyague, 2006).

Chicken carcasses are chilled immediately after slaughter to reduce the temperature to 4.4 °C within 4 h (Keeton, 2001). A weight loss of 0.5% will typically occur during further processing (Sams, 2001). During the chilling, chicken carcasses usually exhibit a slight weight loss. The high relative humidity (~85%) in most coolers reduces carcass shrink and water loss (Keeton, 2001). Freezing is also responsible for weight losses of chicken meat. A slow freezing rate by the temperature zone 11.1 to 10 °C, which is the point of phase transition between intercellular crystalline ice and a combination of ice and water, results not only in large ice crystals, which generally damage the texture of meat, but also in excessive water losses when thawed (Keeton, 2001; Suzuki et al., 2006). On the contrary, a rapid freezing rate produces small ice crystals, preventing the cellular damage of meat. Furthermore, the mass transfers from cells, responsible for losses during the thawing by running water may be limited by rapid thawing (Suzuki et al., 2006). Besides the cellular and macroscopic damage, the losses also depend on the size and shape of the pieces of meat (Pérez Chabela and Mateo-Oyague, 2006).

Heating above 70 °C is often unfavourable to the meat quality due to extensive protein aggregation within the gel network, leading to water loss from the product. The gelation of the stroma protein, collagen, may also be responsible for water loss observed above 70 °C. Cooking rate can also affect the type of gel network formed and subsequent quality of heat-treated meat products. It is thought that a slower cooking rate will result in the formation of more ordered gel structures with higher water-binding abilities. Moreover, heating above 75 °C causes more fiber shrinkage, excessive moisture loss, and fat melting (Smith, 2001). Cooking loss from frozen meat depend principally on the processing of meat before freezing, especially rigor onset temperature, and on the cooking method, particularly the cooking temperature. Although cooking loss is accepted as being higher when freezing rates are slow, the effect of freezing rate on the cooking loss seem to be slight (Pérez Chabela and Mateo-Oyague, 2006).

As diet is one of the most important factors affecting meat quality (Tateo et al., 2013), various benefits in regard to meat quality characteristics can be gained by supplementing broiler diets, particularly using probiotics as feed additives (Karaoğlu et al., 2004).

In the present study, probiotics, bee pollen extract and propolis extract were used in Ross 308 broiler chickens diet to investigate effects on selected technological properties of chicken meat (cooling loss, freezing loss and roasting loss) and breast and thigh muscle (colour, shear force), as the major high-value cuts of chicken meat.

**MATERIAL AND METHODOLOGY**

**Chicks and diets**

The experiment was carried out in test poultry station of Slovak University of Agriculture in Nitra. A total of 180 one day-old Ross 308 broiler chicks were randomly divided into 4 groups, namely, control (C) and experimental (E1, E2, E3) of 45 pcs chickens. The experiment lasted for 42 days and was carried out without segregation between the genders. The broiler chickens
were bred on breed litter (wood shavings), in a temperature-controlled room; the temperature began at 33 °C and was decreased gradually to 19 °C until the end of experiment. The lighting regime was steady during the feeding period. During the whole period of experiment, the broiler chickens had *ad libitum* access to feed and water.

The feeding lasted 42 days. During that period, experimental broiler chickens were fed with a starter complete feed mixture HYD-01 (until 21 days of age) and a grower feed mixture HYD-02 (from 22nd to 42nd day of age). The composition of feed mixtures is given Table 1. The feed mixtures both starter and grower were produced without any antibiotic preparations and coccidiostatics. Nutrients content and metabolizable energy in feed mixtures were balanced, in terms of broiler chickens needs (Vestník MP SR, 2005).

All the groups were fed with the same feed mixtures. However, chickens in the control group were fed with basal diet containing no special supplement, while the diet of chickens in experimental groups contained the diet supplements as follows: bee pollen extract in amount of 400 mg.kg⁻¹ added to feed mixtures given to the group E1, propolis extract in amount of 400 mg.kg⁻¹ added to feed mixtures given to the group E2, probiotics in an amount 3.3 g added daily to the water given the group E3 (for 30 pcs chickens until 21 days of age, for 20 pcs chickens from 22nd to 42nd day of age). Besides, the groups were kept under the same conditions.

In the experiment, the probiotic preparation "Propoul" based on *Lactobacillus fermentum* (1.10⁹ CFU per 1 g of bearing medium) was used.

Bee pollen and propolis had origin in the Slovak Republic. The extracts were prepared from minced bee pollen and propolis in the conditions of the 80% ethanol in

### Table 1 Composition of feed mixtures.

<table>
<thead>
<tr>
<th>Ingredients (%)</th>
<th>Starter HYD-01 (1. – 21. day of age)</th>
<th>Grower HYD-02 (22. – 42. day of age)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>35.00</td>
<td>35.00</td>
</tr>
<tr>
<td>Maize</td>
<td>35.00</td>
<td>40.00</td>
</tr>
<tr>
<td>Soybean meal (48% N)</td>
<td>21.30</td>
<td>18.70</td>
</tr>
<tr>
<td>Fish meal (71% N)</td>
<td>3.80</td>
<td>2.00</td>
</tr>
<tr>
<td>Dried blood</td>
<td>1.25</td>
<td>1.25</td>
</tr>
<tr>
<td>Ground limestone</td>
<td>1.00</td>
<td>1.05</td>
</tr>
<tr>
<td>Monocalcium phosphate</td>
<td>1.00</td>
<td>0.70</td>
</tr>
<tr>
<td>Fodder salt</td>
<td>0.10</td>
<td>0.15</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>0.15</td>
<td>0.20</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.05</td>
<td>0.07</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.15</td>
<td>0.22</td>
</tr>
<tr>
<td>Palm kernel oil Bergafat</td>
<td>0.70</td>
<td>0.16</td>
</tr>
<tr>
<td>Premix Euromix BR 0.5%*</td>
<td>0.50</td>
<td>0.50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nutrient composition [g.kg⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
</tr>
<tr>
<td>Fibre</td>
</tr>
<tr>
<td>Ash</td>
</tr>
<tr>
<td>Ca</td>
</tr>
<tr>
<td>P</td>
</tr>
<tr>
<td>Mg</td>
</tr>
<tr>
<td>Linoleic acid</td>
</tr>
<tr>
<td>MEₜ [MJ.kg⁻¹]</td>
</tr>
</tbody>
</table>

* active substances per kilogram of premix: vitamin A 2 500 000 IU; vitamin E 20 000 mg; vitamin D3 800 000 IU; niacin 12 000 mg; d-pantothenic acid 3 000 mg; riboflavin 1 800 mg; pyridoxine 1 200 mg; thiamine 600 mg; menadione 800 mg; ascorbic acid 20 000 mg; folic acid 400 mg; biotin 40 mg; cobalamine 8.0 mg; choline 100 000 mg; betaine 50 000 mg; Mn 20 000 mg; Zn 16 000 mg; Fe 14 000 mg; Cu 2 400 mg; Co 80 mg; I 200 mg; Se 50 mg.
the 500 cm³ flasks, according to Krell (1996). The extraction was accomplished in a water bath at 80 °C for one hour. After that, the extracts were cooled and centrifuged. The obtained supernatants were evaporated in a rotary vacuum evaporator at bath temperature 40–50 °C and weighed. Residues in an amount of 40 g were dissolved in 1000 cm³ of 80% ethanol and used for 100 kg of feed mixture.

**Slaughter and measurements**

The chickens were slaughtered at 42 days of age at the experimental slaughterhouse of Slovak University of Agriculture in Nitra. After evisceration, the carcasses were kept at approximately 18 °C for 1 h post mortem and thereafter longitudinally divided into two parts. After that, the half-carcasses were weight and stored at 4 °C until 24 h post mortem, when the first measurements were done. The left half-carcass was used in order to determine the technological properties as described below, whereas the right one was assigned to different analysis.

After 24 h, the color of breast (Musculus pectoralis major) and tight muscle from the left half-carcass (n=10) was assessed using a Minolta CM 2600D spectrophotometer (Konica Minolta, Japan) and reported in the CIE system values of lightness (L*), redness (a*) and yellowness (b*). All the color readings were taken on meat without skin, in an area free of obvious color defects (over scald, bruises, and blood accumulation).

The cooling loss was determined in whole left half-carcass as the percentage of weight loss over a 24 h period, by calculating the weight differences before and after cooling.

Afterwards, the same half-carcasses were stored at -18 °C for 3 months prior to next analysis. Thereafter, the samples were thawed. After thawing was completed, the weight of the samples was obtained. To determine the freezing loss (%), the weight differences before and after freezing process were calculated (n=10). All the weight measurements were performed using the precision balance Kern 440 (Kern&Sohn, Germany) with accuracy of 0.01 g.

The heat treatment of samples was carried out in oven (Gorenje B 3300 E) at 200 °C for 60 minutes. After allowing the samples to cool at room temperature, the samples were weighed so as to calculate the percentage of roasting losses. The roasting loss was expressed as the percent weight reduction of the heat-treated sample compared to the raw sample (n=10).

The samples used to determine the roasting loss were the same used to evaluate the shear force. For this reason, tenderness of breast (Musculus pectoralis major) and thigh muscle was subsequently evaluated. Results have been expressed as shear force (kg·cm⁻²) (five measurements were performed on each sample to obtain an average value). First, the five cores with the same size (2.0 cm wide, 5.0 cm long and 1.5 cm high) were removed from each heat-treated sample (n=10). Then, the cores were sheared perpendicular to the muscle fibres orientation using a Warner-Bratzler shear device (Chatillon, USA), in accordance with Goodson et al. (2002).

**Statistical analysis**

The data processing for technological attributes of raw and heat-treated samples of meat was performed using a statistical program Statgraphics Plus Version 5.1. For the determination of significant difference between the tested groups, analysis of variance (ANOVA) with Scheffé’s method was used.

**RESULTS AND DISCUSSION**

The results of experiment with Ross 308 broiler chickens, which was aimed at selected technological properties, are presented as follows: the results of cooling loss, freezing loss and roasting loss of meat are given Table 2, the results of colour and shear force of breast and thigh muscle are given Table 3.

In the current study, the losses during the storage ranged from 3.79 to 4.04% for cooling, from 3.53 to 4.85% for freezing, from 28.50 to 30.03% for roasting. There were very similar values of cooling losses, at which 4.04% of loss, as the highest value among the tested groups, was observed in group E3. The lowest value was observed in group E2 (3.79%). The cooling losses, however, did not differ significantly (P≥0.05). It is, thus, likely, that the extracts of bee products (propolis, bee pollen), as well as probiotics, do not affect losses during the cooling of chicken meat. Nevertheless, different results were obtained for freezing and roasting losses, where significant differences (p ≤0.05) were found between the groups. Among the groups, group E2 and E3 showed the highest freezing value (4.85%), whereas control group showed the lowest one (3.53%). These differences, although significant, are of little relevance as the losses in control group were lower than losses in experimental groups.

As far as roasting loss is concerned, group E3 showed the highest losses (30.03%) also in that parameter, while the lowest losses were obtained in group E2 (28.50%), i.e. the propolis extract, which was included in the feed mixture, has been shown to have the most favourable influence on losses during the roasting of chicken meat, among all the tested natural additives. These findings indicate that the probiotics supplementation (group E3) results in higher losses during the cooling, freezing and roasting than those in the other groups.

Similarly, insignificant differences between the tested groups were found in study of Haščík et al. (2008), in which cooling and freezing losses of chicken meat, after probiotic supplementation (Lactobacillus fermentum) were investigated. Yet, both cooling (2.74±0.34%) and freezing losses (2.00±1.15%) were lower than those in the control (3.14±0.57% and 3.10±1.44%, respectively). Bobko et al. (2009) investigated the weight losses of chicken meat by cooling and roasting after the probiotic supplementation (Enterococcus faecium) besides the other feed supplements. They found out higher losses in experimental group than those in control group not only in regard to cooling (2.49±0.57% and 1.88±0.42%, respectively), but also to roasting (32.27±1.75% and 32.01±2.45%, respectively).
In terms of probiotics, various effects on meat quality of chickens were found in other studies. However, it was difficult to directly assess different studies using probiotics because the efficacy of a probiotic application depended on many factors (Patterson and Burkholder, 2003), such as species composition and viability, administration level, application method, frequency of application, overall diet, bird age, overall farm hygiene, and environmental stress factors (Zhou et al., 2010). Moreover, Mihok et al. (2010) suggest to examine the technological properties of other livestock species, since introducing new trends in animal nutrition can result not only in the positive effect, but also in the negative. It was clear from this study that the administration of probiotic, Lactobacillus fermentum via the drinking water, had not quite the effects we had expected, as regards not only the determined losses, but also the shear force.

From the data obtained by shear force measurement follows that there were significant differences between the groups. As shown in Table 3, higher value was observed in group E3 (probiotic-supplemented group), in both breast and thigh muscle (2.28 ±0.48 and 1.67 ±0.25 kg.cm⁻², respectively), as compared with the other groups. On the contrary, as for group E2 (propolis-supplemented group), there was the lowest values observed, in both breast and thigh muscle (1.89 ±0.33 and 1.25 ±0.19 kg.cm⁻², respectively). These findings were not in agreement with the results determined by Zhang et al. (2005), who investigated the effects of Saccharomyces cerevisiae cell components on meat quality of male broilers. The shear forces determined in cooked breast and thigh muscle in experimental groups decreased as compared with the control. It might be explained by the different probiotic strains and culture days. In the study of Zhou et al. (2010), beneficial effects on shear force of chicken meat was observed, using different concentrations of Bacillus coagulans as diet supplement. In the present study, the water losses (determined as weight losses), as the important indicator of meat juiciness, have been coincided with trend of the shear force results. Thus, it may be deduced unfavourable effect of Lactobacillus fermentum on tenderness and juiciness of chicken meat.

According to Volpato et al. (2008), meat tenderness as a quality attribute can be negatively affected by heat-treating due to a decrease in the water content of meat during the process. Consequently, it might be appropriate the

Table 2 Cooling loss, freezing loss and roasting loss of chicken meat (mean ±SD).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>E1</td>
</tr>
<tr>
<td>Cooling loss [%]</td>
<td>3.97 ±0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.79 ±0.36&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Freezing loss [%]</td>
<td>3.53 ±1.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.81 ±0.84&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Roasting loss [%]</td>
<td>29.54 ±1.16&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>29.82 ±1.12&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Legend: C – control group; E1, E2, E3 – experimental groups; mean – average, SD – standard deviation; a, b – means with different superscripts within row differ significantly; S – significance; **p ≤0.05; NS = not significant.

Table 3 Instrumental colour values and shear force value of chicken breast and thigh muscle (mean ±SD).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>S</th>
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<tbody>
<tr>
<td></td>
<td>C</td>
<td>E1</td>
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<tr>
<td>Colour parameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIE L*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>breast</td>
<td>52.24 ±2.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.12 ±1.81&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>thigh</td>
<td>51.64 ±1.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.17 ±2.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CIE a*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>breast</td>
<td>0.07 ±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.59 ±0.55&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>thigh</td>
<td>1.94 ±0.64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.33 ±0.46&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CIE b*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>breast</td>
<td>10.08 ±1.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.14 ±0.98&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>thigh</td>
<td>9.60 ±1.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.56 ±1.33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Shear force value [kg.cm⁻²]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>breast</td>
<td>1.97 ±0.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.18 ±0.60&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>thigh</td>
<td>1.33 ±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.66 ±0.36&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Legend: C – control group; E1, E2, E3 – experimental groups; mean – average, SD – standard deviation; a, b – means with different superscripts within row differ significantly; S – significance; **p ≤0.05; NS = not significant.
optimising of heat-treating conditions to get better results of meat tenderness. Since the lower shear force values indicate tenderer meat, the present study suggests that the dietary supplementation of propolis could improve meat tenderness of broilers, although the underlying mechanism is not readily understood. Anyway, propolis has been shown as the most favourable diet supplement in order to get good meat tenderness as well as getting the lowest roasting losses (mentioned above). Overall, the shear force values obtained by measurement have been appropriate owing to the fact demonstrated in study of Lyon and Lyon (2001), that if the shear force value is below 3.61 kg.cm\(^{-2}\), chicken meat can be considered as very tender. In our study, all the shear force values were below this level. The values were similar to those in study of Alfaig et al. (2014), in which the shear force value of breast muscle in the probiotic-supplemented group was obtained at a level of 2.63 ±0.28 kg.cm\(^{-2}\). What is more, the value in experimental group was higher than that in control group.

The shear force values in our study also resembled the values observed by Rababah et al. (2005), who investigate chicken breast meat infused with various plant extracts. The values ranged from 1.64 to 2.28 kg.cm\(^{-2}\). Pelicano et al. (2005) evaluated effects of different probiotics (Bacillus subtilis, Lactobacillus acidophilus and casei, Streptococcus lactis and faecium, Bifidobacterium bifidum and Aspergillus oryzae) on quality attributes of chicken meat, including the meat tenderness. They concluded that the probiotics used as diet supplements did not affect the meat quality, because of slight changes in shear force values in experimental groups as compared with the control. Moreover, the values in the experimental groups (3.84 – 4.08 kg.cm\(^{-2}\)) were slightly higher than those in our study.

As far as colour parameters are concerned, only colour parameter redness (a* value) has been shown to express the significant differences between the tested groups (Table 3). The highest a* value of thigh muscle was observed in control group (1.94 ±0.64). The a* value 1.33 was, on the one hand, observed in breast muscle as the highest (E2 group), on the other hand it was observed in thigh muscle as the lowest (E1 group). The lowest a* value in breast muscle was found in control group (0.07 ±0.06). The redness (a* value) of breast muscle was increased significantly (p ≤0.05) after the addition of propolis in the diet, whereas the redness (a* value) of thigh muscle was not significantly (p ≥0.05) affected by addition of natural supplements. In the colour parameters lightness (L* value) and yellowness (b* value), the groups did not differ significantly from each other. The colour parameters ranged from 52.24 to 53.48 for L* value, from 0.07 to 1.94 for a* value, and from 9.60 to 10.88 for b* value. The addition of natural supplements imparted neither darker nor lighter colour of chicken meat, since the L* values were not as significant as the a* component. Furthermore, the supplements did not cause changes in the yellowness (b*) values.

In the present study, the colour of raw meat was not altered after addition of natural supplements so that was unacceptable for consumers. As mentioned Pelicano et al. (2005), the different additives might be used since they did not affect meat colour, which is an extremely important parameter that is related to the choice made by the consumer. As mentioned Mancini and Hunt (2005), the instrumental measures of L* and a* can easily be applied to muscle colour, whereas the colours represented by b* (blue and yellow) are not typical related to meat. Generally, as reported Karaoglu et al. (2004), when a* and b* values increase, L* value declines and the colour gradually darkened. With reference to study of Lindahl et al. (2001), variation in a* values is affected by pigment content and redox state in muscle, while b* values are influenced only by redox state. In addition, L* values are slightly correlated with haem pigment and metmyoglobin contents. In the present study, a* values in breast muscle were rather lower due to lower pigment in the breast as compared with that in thigh.

A study of Bianchi and Fletcher (2002), comparison of absolute colour values between the different studies is difficult, because of colour difference measurements as well as differences in measurement conditions.

In the study of Pelicano et al. (2005), the L*, a*, b* measurements from CIELab system were evaluated, besides the above-mentioned meat tenderness. The L* values were in the range 45.25 – 46.37, the a* values were in the range 3.80 – 3.88, and the b* values ranged from 2.87 to 3.36. These results were similar to findings reported by Bianchi and Fletcher (2002), who investigated the effect of chicken meat thickness on colour measurement.

In another study, Rababah et al. (2005) observed colour parameters in the raw chicken meat, after the plant extracts supplementation, as follows: the lightness component (L*) in the range 51.07 – 62.15, the a* component in the range 0.95 – 3.32, and the b* component in the range 5.21 – 7.16. In a similar manner, Janisch et al. (2011), who analyzed the colour of breast muscle depending on the broiler genetic line, observed in Ross 308 line the averaged values, as follows: 51.18 ±0.47, 3.44 ±0.19 and 8.73 ±0.25 for L* component, a* component and b* component, respectively. Kilic et al. (2014) determined the colour parameters in raw chicken meat quite similar to those above-mentioned (L* 51.15 ±0.13, a* 3.56 ±0.19) except for b* value, which was slightly lower as compared with those in other studies (1.50 ±0.07).

In another study, Ali et al. (2015) determined the influence of multiple freeze-thaw cycles on colour of chicken meat. They obtained the lightness (L*) value in the range 43.6 – 46.57, the redness (a*) values in range 2.72 – 3.92, the yellowness (b*) values in the range 4.17 – 5.62. As the L* component ranges from black to white and the a* component ranges from green to red, it can be inferred that meat in the study of Ali et al. (2015) was observed as darker and redder as compared with that in our study.

Karaoglu et al. (2004) investigated the effect of slaughtering at different ages and the use of probiotic preparation contained Saccharomyces cerevisiae in chicken diet on the colour properties. In probiotic-supplemented groups, the L* values were in the range 63.69 – 65.21, the a* values were in the range 2.38 – 2.59, and the b* values were in the ranged...
10.49 – 10.64. In addition, they demonstrated that darkness of colour has increased as time progressed.

Colour parameters in breast and thigh muscle of chickens were also evaluated in study of Haščík et al. (2014), in which the bee pollen extract was included into diet of broilers. The measurement was conducted after 45 minutes post mortem. For this reason, some values were completely different from those in our study. The $L^*$ values were found in the range 49.38 – 52.5 and 52.31 – 53.96 for breast and thigh muscle, respectively. The $a^*$ values were found in the range -0.98 – 2.05 and 4.53 – 7.38 for breast and thigh muscle, respectively. The $b^*$ values were found in the range 7.14 – 9.52 and 5.17 – 13.56 for breast and thigh muscle, respectively.

**CONCLUSION**

Based on the results of present study, it may be concluded that any of applied natural additives in feed mixtures has not notable impact on losses caused by cooling, freezing and roasting, since the lowest losses were not found in experimental groups as has been expected. The results of shear force measurement, however, indicate favourable effect of propolis addition on meat tenderness, in both breast and thigh muscle. Besides, the other applied supplements did not influence the tenderness significantly. When considering the colour parameters, it can be inferred that the propolis extract addition increase the redness ($a^*$) values in breast muscle significantly, whereas the other supplements induce rather decrease in the redness ($a^*$), in both breast and thigh muscle. On the contrary, the lightness ($L^*$) and the yellowness ($b^*$) were not changed after addition of natural supplements. In addition, the results showed that probiotic administration via drinking water did not improve the technological properties of chicken meat, since the most of them were observed as the least convenient. On the whole, the addition of natural supplements in chicken diet requires further research to clearly understand their influence in chicken organism and the effects on technological properties of meat.

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