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CHEMICAL AND SENSORY CHARACTERISTICS OF CHICKEN BREAST MEAT AFTER DIETARY SUPPLEMENTATION WITH PROBIOTIC GIVEN IN COMBINATION WITH BEE POLLEN AND PROPOLIS

Lenka Trembecká[1], Peter Haščík[1], Juraj Čuboň[1], Marek Bobko[1], Petronela Cviková[1], Lukáš Hleba[2]*

Address(es): Ing. Lenka Trembecká,
[1]Slovak University of Agriculture in Nitra, Department of Animal Products Evaluation and Processing, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia.
[2]Slovak University of Agriculture in Nitra, Department of Microbiology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia.

*Corresponding author: xtrembecka@uniag.sk

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ABSTRACT

The present study evaluated the effect of probiotic in combination with bee pollen and propolis in diet of broilers on chemical and sensory characteristics of chicken breast meat. A total of 180 one-d-old chicks of mixed sex were randomly assigned to three dietary groups as follows: 1. control group (C); 2. basal diet supplemented with 400 mg bee pollen (ethanol extract) per 1 kg of feed and 3.3 g probiotic (*Lactobacillus fermentum*) per day in water (E1); 3. basal diet supplemented with 400 mg propolis (ethanol extract) per 1 kg of feed and 3.3 g probiotic (*Lactobacillus fermentum*) per day in water (E2). Chicken meat was analyzed for dry matter, crude protein, fat, ash, cholesterol, and energy value. Diet did not affect the chemical characteristics of chicken meat, except for supplementation with bee pollen and probiotic which resulted in increased fat content. As for sensory characteristics, dietary groups differ from each other in terms of aroma and overall acceptability. In addition, there was significant difference between the E1 group and groups C and E2 in terms of taste and tenderness. Significant differences were also detected between group E2 and groups C and E1 group in terms of juiciness. In conclusion, propolis + probiotic-supplemented group manifested the best results in terms of sensory characteristics. Propolis extract can be thus recommended as a potential supplement providing rich nutrients and biological active substances in chicken diet that is (together with probiotic) capable of improving the sensory quality of chicken breast meat.

Keywords: chicken breast meat, chemical composition, sensory evaluation, probiotic, bee pollen, propolis

INTRODUCTION

High product quality and food safety are key targets for the food industry, since they relate to customer satisfaction and ultimately to repeat purchase **(O'Sullivan, 2017)**. In addition, the aim of food researchers and producers is to increase the nutritional value of food without decreasing sensory quality or consumers' acceptability **(Miezeliene *et al.*, 2011)**. Within recent years, the poultry industry has experienced a dramatic increase in consumer consumption of chicken meat, breast meat in particular **(Owens *et al*., 2004)**. In order to produce high quality meat, it is necessary to understand the characteristics of meat quality traits and factors to control those characteristics **(Joo *et al*., 2013)**. It is generally accepted that quality of chicken meat depends strongly on chicken diet. In this view, supplementation of diet with the additives, such as probiotics, prebiotics, organic acids, enzymes, and herbal products, has been attempted to enhance the poultry feed for growth development and health **(Hassan *et al*., 2010; Wati *et al*., 2015)**.
Probiotics cover a wide range of living microorganisms with supposed positive effects on gut flora and producing many substances supporting many different effects **(Bernardeau and Vernoux, 2013)**. Prebiotics are non-digestible food ingredients that selectively stimulate the growth and activity of microorganisms in the gut. Impacts of administered probiotics and beneficial bacteria of the GIT (gastrointestinal tract) can be enhanced using prebiotics **(Gibson *et al*., 2004; Bajaj *et al*., 2015; Uyeno *et al*., 2015)**, although the biological effects of prebiotics are more limited **(Toh *et al*., 2012)**. Many feed additives currently used do not fit wholly into the strict prebiotic classification due to their differing modes of action, but they can have a similar result of a healthy GIT microbiome. These substances can be referred to as prebiotic-like substances **(Kogut and Arsenault, 2016)**.
Bee products, such as bee pollen and propolis can be used as potential supplements with prebiotic activity in animal diet, since they can provide peptides and amino acids needed for probiotic viability and bacterial growth **(Yerlikaya, 2014; Babaei *et al*., 2016)**. Bee pollen is an agglomerate of flower pollen collected from different plant sources by honeybees and mixed with plant nectar and salivary secretions **(El-Neney and El-Kholy, 2014)**. It is a source of free amino acids, polyunsaturated fatty acids **(Taha, 2015)** and most of the essential nutritional elements needed for the growth and development in animals and humans **(Farag and El-Rayes, 2016)**. In addition, bee pollen is extremely rich in carotenoids, vitamin B complex, vitamins C, D, E, lecithin (**Babińska *et al*., 2012; Babaei *et al*., 2016)**, and contains also minerals, phytosterols, phenolic compounds, and flavonoids **(Dias *et al*., 2013; Mohdaly *et al*., 2015)**. Propolis is a natural resinous material collected by honeybees from plants, particularly from flowers and leaf buds, and then transported to the hives where it is modified by their enzymes **(Mohdaly *et al*., 2015; Babaei *et al*., 2016)**. It contains amino acids, terpenoids, steroids, flavonoids, aromatic acids, diterpene acids and phenolic compounds **(Gutiérrez-Cortés and Mahecha, 2014; Eyng *et al*., 2015)**.
Both bee pollen and propolis exert a broad spectrum of positive effects on humans and animals. Besides their antibacterial, antifungal and antiviral properties, they present many beneficial biological activities such as antioxidant, anti-inflammatory, antitumor, hepatoprotective, immunostimulatory and antimutagenic **(Babińska *et al*., 2012)**.
In the past, meat quality was more closely related to the sensory perceptions, freshness, and safety aspects, whereas more recently it is associated with nutrition, well-being and functionality in relation to human health. Yet, sensory quality is crucial for consumer acceptance. Dietary supplementation is the key factor which can most easily be manipulated and has one of the most profound effects on sensory quality of meat **(Joo *et al*., 2013)**. When evaluating the sensory attributes of products, such as appearance, odor, flavor, taste, and texture, consumers respond based on their perceptions **(Chumngoen and Tan, 2015)**. By using sensory analysis, producers can identify and respond to consumer preferences more efficiently than by using the instruments, thus increasing their competitiveness and segmenting their specific market **(Sow and Grongnet, 2010)**.
This study was carried out to evaluate the chemical and sensory characteristics of chicken breast meat after dietary supplementation with probiotic given in combination with bee pollen and propolis.

MATERIAL AND METHODS

Animals and experimental design

The broiler chickens (Ross 308) were raised at the poultry station of Slovak University of Agriculture in Nitra. One-day-old chicks of mixed sex (180 pcs) were assigned to three dietary groups (C, E1, E2) using a completely randomized design. Each group consisted of 3 replicated pens with 20 broiler chickens per pen. Broilers in group C (as control) received a basal diet without any feed additive, broilers in group E1 (experimental 1) received a diet supplemented with 400 mg bee pollen (ethanol extract) per 1 kg of feed mixture and 3.3 g probiotic preparation added daily to drinking water, and broilers in group E2 (experimental 2) received a diet supplemented with 400 mg propolis (ethanol extract) per 1 kg of feed mixture and 3.3 g probiotic preparation added daily to drinking water. Besides, the groups were kept under the same conditions. Feed and water were supplied *ad libitum* for the six weeks feeding experimental period (42 days). Broilers received two phases feeding program, starter HYD-01 (1 – 21 d) and grower HYD-02 (22 – 42 d) diets. Diets were formulated according to nutrient recommendations for broilers **(Bulletin of the Ministry of Agriculture and Rural Development of the Slovak Republic, 2005)**. Ingredients and nutrient content of the basal diets are presented in Table 1. Feed mixtures, both starter and grower, were produced without any antibiotics and coccidiostats. Broilers were submitted to a continuous lighting program and were reared on the floor covered with dry wood shavings, in a temperature-controlled room; room temperature was adjusted at 33 °C in the first week and gradually decreased by 2 °C, and finally fixed at 23 °C thereafter.

The commercial probiotic preparation used in the experiment contained probiotic strain *Lactobacillus fermentum*. The colony-forming unit (CFU) of *L. fermentum* in the preparation was about 10^9 CFU per 1 g of bearing medium. Bee pollen and propolis used in the experiment had origin in the Slovak Republic. Bee pollen and propolis were grounded into the powder and extracted in 80% ethanol in the 500 cm^3 flasks, according to the method described by **Krell (1996)**.

At the end of experiment, 10 broilers per group were slaughtered at the 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. Breast meat samples (*pectoralis major*) were dissected from each left half-carcass (right half-carcasses were assigned to other analysis) and stored at 4 °C until 24 h *post mortem*. The samples (boneless breast without skin) were individually packaged in labelled bags and stored at -18 °C for prior to analysis.

Table 1 Composition of feed mixtures

Ingredients (%)	Starter HYD-01 (1^{st} – 21^{st} day of age)	Grower HYD-02 (22^{nd} – 42^{nd} day of age)
Wheat	34.00	37.00
Maize	33.92	37.52
Soybean meal (48% N)	23.00	18.00
Fish meal (71% N)	5.00	3.00
Dried blood	-	1.00
Fodder lime	1.00	0.95
Monocalcium phosphate	0.80	0.70
Fodder salt	0.10	0.10
Sodium bicarbonate	0.15	0.20
Lysine	0.15	0.12
Methionine	0.18	0.21
Bergafat (palm kernel oil)	1.20	0.70
Euromix BR 0.5%[1]	0.50	0.50
Nutrient composition [$g.kg^{-1}$]		
Linoleic acid	13.53	14.05
ME_N [$MJ.kg^{-1}$]	12.07	12.16
Fibre	30.50	29.67
Crude protein	212.40	191.61
Ash	27.00	20.90
Ca	8.22	7.18
P	6.55	5.86
Na	1.77	1.70

Legend: [1]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; kobalamin 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

Chemical composition

Samples of chicken breast muscle without skin (n = 10) were analyzed for dry matter, crude protein, fat and ash, using an Infratec 1265 Meat Analyzer. The values for chemical composition were expressed as g.100 g^{-1} of breast muscle. The cholesterol content (mg.100 g^{-1}) of chicken meat was determined by spectrophotometric method according to **Horňáková *et al.* (1974)**. The energy value (kJ.100 g^{-1}) was calculated through the conversion factors for fat and protein **(Strmiska *et al*., 1988)**.

Sensory evaluation

The breast muscles were thawed and thermally processed by roasting to a core temperature of 70 °C in an electric oven (Gorenje B 3300 E), without added fat or oil, at 200 °C with regular turning of the samples. The samples were trimmed of subcutaneous fat and connective tissue, sliced into uniform sizes (about 2 cm), and cooled to room temperature before being served to the panelists. Sensory evaluation was carried out in a climate-controlled sensory analysis laboratory equipped with individual booths. Sensory profiles were determined by a 5-member semi-trained panel. Panelists included staff and PhD. students in Department of Animal Products Evaluation and Processing, Slovak University of Agriculture in Nitra; three were women and two were men, ranging from 27 to 57 years of age. They had more than 3 years of food sensory panel experience and poultry meat experience.

Before tasting, panelists were instructed on the assessment criteria. Panelists were asked to evaluate the samples of breast muscle for aroma (1 = very poor, 5 = very good), taste (1 = very poor, 5 = very good), juiciness (1 = extremely dry, 5 = extremely juicy), tenderness (1 = extremely tough, 5 = extremely tender), and overall acceptability (1 = not acceptable, 5 = extremely acceptable) on a 5-point hedonic scale. The samples were presented to the panelists monadically on plain white porcelain plates. Panelists were provided with water for neutralization of receptors before and between the samples. The panel evaluated each sample in triplicate over an 8-week period (n = 10).

Statistical analysis

A statistical analysis was computed using the ANOVA procedures of SAS software **(version 9.3, SAS Institute Inc., USA, 2008)**. Data were reported as mean ± standard deviation. Statistical significance was calculated using t-test. Differences between the groups were considered significant at $P \leq 0.05$. Spearman's correlation coefficients of XLSTAT statistical software **(Addinsoft, 2016)** were used to determine the relationships among the sensory characteristics.

RESULTS AND DISCUSSION

The results for chemical composition of chicken breast meat are presented in Table 2. As for dry matter and crude protein of chicken breast meat, they were not affected by dietary supplementation (P>0.05). Fat content was significantly higher in broiler chickens fed diet containing probiotic and bee pollen (1.26 ±0.278 g.100 g^{-1}) compared to control and propolis + probiotic-supplemented group (1.01 ±0.131 and 1.03 ±0.085 g.100 g^{-1}, respectively). In addition, significant differences were observed in ash content among dietary groups (P≤0.05), with the highest value in control (1.18 ± 0.03 g.100 g^{-1}) and the lowest one in propolis + probiotic-supplemented group (1.14 ±0.017 g.100 g^{-1}).
The cholesterol content and energy value in the breast chicken meat of groups E1 and E2 did not differ from those of control group (P>0.05). However, the breast meat of chickens fed with propolis and probiotic (E2) had the lowest both cholesterol content and energy value (77.94 ±5.908 mg.100 g^{-1} and 413.92 ±4.864 kJ.100 g^{-1}, respectively), whereas the breast meat of chickens fed with bee pollen and probiotic (E1) had the highest one (90.14 ±7.584 mg.100 g^{-1} and 421.69 ±10.314 kJ.100 g^{-1}, respectively). Moreover, there was significant difference (P≤0.05) between E1 and E2, in both cholesterol content and energy value. The data on chemical composition of chicken breast meat were similar to those reported in previous studies, where various supplements were used (**Ahmed *et al.*, 2014; Hossain and Yang, 2014; Swiatkiewicz *et al.*, 2014; Puvača *et al.*, 2015; Al-Yasiry *et al.*, 2017**).

Table 2 Chemical composition of chicken breast meat in relation to dietary group

Group	Parameter					
	Dry matter (g.100 g^{-1})	**Crude protein (g.100 g^{-1})**	**Fat (g.100 g^{-1})**	**Ash (g.100 g^{-1})**	**Cholesterol (mg.100 g^{-1})**	**Energy value (kJ.100 g^{-1})**
C	25.11 ±0.239	22.52 ±0.396	1.01 ±0.131^{a}	1.18 ± 0.03^{c}	87.06 ±8.861^{ab}	415.46 ±6.101^{ab}
E1	25.19 ±0.241	22.34 ±0.207	1.26 ±0.278^{b}	1.15 ±4.92×10^{-3} ^{b}	90.14 ±7.584^{b}	421.69 ±10.314^{b}
E2	24.98 ±0.279	22.40 ±0.295	1.03 ±0.085^{a}	1.14 ±0.017^{a}	77.94 ±5.908^{a}	413.92 ±4.864^{a}
P-value	0.351	0.092	0.009	0.004	0.011	0.003

Legend: Data are reported as mean ±SD (standard deviation); n = 10; C – control group; E1, E2 – experimental groups; $^{a–c}$ means with different superscripts in the same column are significantly different (P≤0.05)

The scores given by panelists for the sensory characteristics (aroma, taste, juiciness, tenderness, and overal acceptability) of breast chicken meat are presented in Table 3. There were significant effects (P≤0.05) of the supplements on the sensory attributes. It is noteworthy that the highest scores for all the sensory attributes were assigned to breast meat of chickens fed with propolis and probiotic (E2), whereas the lowest scores obtained were in breast meat of chickens fed with bee pollen and probiotic (E1). As for aroma, all the groups differ from each other, with the highest mean score found in E2 group (4.37 ±0.170) and the lowest one in E1 group (3.87 ±0.266). There was significant difference (P≤0.05) between the E1 group and groups C and E2 in terms of taste and tenderness. For both sensory attributes, panelists gave the lowest score to E1 group (3.90 ±0.149 and 3.67 ±0.182 for taste and tenderness, respectively), i.e. the breast meat from the bee pollen + probiotic-supplemented group. Statistically significant differences (P≤0.05) were detected between mean scores for E2 and those for C and E1 group in terms of juiciness, with the highest score observed in E2 (4.15 ±0.330). However, panelists were unable to differentiate both taste and tenderness of chicken breast meat from groups C and E2, as well as juiciness of meat from groups C and E1. Similar results (P≤0.05) were also detected in overall acceptability, since E2 group (4.29 ±0.229) was considered as the most acceptable for panelists and E1 group has received the lowest score (3.76 ±0.101). The present data suggest that the use of dietary supplementation of probiotic given in combination with bee pollen led to decreased sensory quality of chicken breast meat since the values in that group were seen as statistically the lowest (P≤0.05) in all the sensory attributes, except for juiciness (P>0.05). On the contrary, the sensory quality of chicken breast meat was improved significantly (P≤0.05) by the addition of propolis extract in combination with probiotic (aroma, juiciness, and overall acceptability in particular).

Table 3 Sensory evaluation of chicken breast meat in relation to dietary group

Group	Sensory attribute				
	Aroma	**Taste**	**Juiciness**	**Tenderness**	**Overall acceptability**
C	4.22 ±0.122^{b}	4.18 ±0.225^{b}	3.81 ±0.360^{a}	4.06 ±0.365^{b}	4.07 ±0.221^{b}
E1	3.87 ±0.266^{a}	3.90 ±0.149^{a}	3.60 ±0.132^{a}	3.67 ±0.182^{a}	3.76 ±0.101^{a}
E2	4.37 ±0.170^{c}	4.33 ±0.221^{b}	4.15 ±0.330^{b}	4.31 ±0.347^{b}	4.29 ±0.229^{c}
P-value	<0.001	<0.001	<0.001	<0.001	<0.001

Legend: Data are reported as mean ±SD (standard deviation); C – control group; E1, E2 – experimental groups; $^{a–c}$ means with different superscripts in the same column are significantly different (P≤0.05)

There were significant correlations (P≤0.05) among sensory characteristics of chicken breast meat, as seen it Tables 4, 5, and 6. As for C group (control), there was significant association (P≤0.05) between the tenderness and taste (r = 0.659), tenderness and juiciness (r = 0.823), overall acceptability and taste (r = 0.764), overall acceptability and juiciness (r = 0.832), overall acceptability and tenderness (r = 0.954). As for E1 group (probiotic plus bee pollen extract), overall acceptability correlated with taste (r = 0.802). In addition, tenderness was correlated negatively with aroma and juiciness (r = -0.047; r = -0.027, respectively). Regarding the E2 group (probiotic plus propolis extract), tenderness correlated positively (P≤0.05) with both taste and juiciness (r = 0.672; r = 0.825, respectively). Also, overall acceptability correlated positively (P≤0.05) with taste (0.798), juiciness (r = 0.867), and tenderness (r = 0.875), which was similar to control group.

There are few studies that have been done on sensory evaluation of chicken meat after dietary supplementation of probiotics, bee pollen, and propolis. The results of present study are consistent with those of **Haščík *et al.* (2012)**, where propolis extract supplementation (200 mg.kg^{-1} of feed mixture) led to an improvement in sensory quality of chicken breast meat. The results are, however, not in agreement with findings by **Haščík *et al.* (2013)**, who demonstrated positive effect of bee pollen extract supplemented separately in various doses (1000, 1500, 2500, 3500, and 4500 mg.kg^{-1} of feed mixture) on sensory profile of chicken breast meat.
There is considerable variation in published studies that evaluate the effect of probiotic strains on sensory quality of chicken meat.

Table 4 Spearman's correlation coefficients among sensory attributes of chicken breast meat – C group

Variables	Aroma	Taste	Juiciness	Tenderness	Overall acceptability
Aroma	1	-	-	-	-
Taste	0.242	1	-	-	-
Juiciness	0.142	0.521	1	-	-
Tenderness	0.247	0.659*	0.823*	1	-
Overall acceptability	0.436	0.764*	0.832*	0.954*	1

Legend: *significant correlation (P≤0.05)

In the study of **Pelicano *et al*. (2005)**, sensory evaluation of breast meat was not affected by the use of different probiotics, prebiotics and symbiotics in Cobb male broilers. The study evaluated probiotics based on *Bacillus subtilis*, *Lactobacillus acidophilus* and *casei*, *Streptococcus lactis* and *faecium*, *Bifidobacterium bifidum* and *Aspergillus oryzae*, prebiotics based on mannan oligosaccharide and organic acidifier, and their combinations. Similarly, **Brzoska *et al.* (2010)** found no significant improvement in sensory parameters of breast muscles from broiler chickens with dietary addition of probiotics and prebiotics (*L. acidophilus*, *Pediococcus* together with mannan oligosaccharide and fumaric acid). On the contrary**, Khan *et al.* (2017)** demonstrated that diet supplemented with probiotics based on *Lactobacillus acidophilus* and *Streptococcus cerevisiae*, and selenium-enriched probiotics significantly increased the sensory characteristics of chicken breast meat.

Table 5 Spearman's correlation coefficients among sensory attributes chicken breast meat – E1 group

Variables	Aroma	Taste	Juiciness	Tenderness	Overall acceptability
Aroma	1	-	-	-	-
Taste	0.280	1	-	-	-
Juiciness	0.059	0.177	1	-	-
Tenderness	-0.047	0.126	-0.027	1	
Overall acceptability	0.615	0.802*	0.424	0.341	1

Legend: *significant correlation (P≤0.05)

Table 6 Spearman's correlation coefficients among sensory attributes chicken breast meat – E2 group

Variables	Aroma	Taste	Juiciness	Tenderness	Overall acceptability
Aroma	1	-	-	-	-
Taste	0.558	1	-	-	-
Juiciness	0.392	0.483	1	-	-
Tenderness	0.450	0.672*	0.825*	1	-
Overall acceptability	0.553	0.798*	0.867*	0.875*	1

Legend: *significant correlation (P≤0.05)

In the study of **Liu *et al.* (2012)**, improvement in sensory attributes of breast muscle was observed in broilers fed with the probiotic based on *Bacillus licheniformis*. **Alfaig *et al*. (2014)** observed improved overall acceptability of chicken breast meat after dietary supplementation of probiotics (*Bacillus subtilis*) given in combination with thyme essential oil, whereas probiotic supplementation was perceived by panelists as the least acceptable.

The findings of another study **(Pelicano *et al*., 2003)** evidenced that various probiotic strains (*Bacillus subtilis*; *Bacillus subtilis* and *Bacillus licheniformis*; and *Saccharomyces cerevisiae*) added to both feed and water of male Cobb chickens improved the sensory traits of breast samples. Also, improvement in sensory attributes of breast meat in broilers fed with bacterium *Rhodopseudomonas palustris* was observed in the study of **Xu *et al.* (2014)**.

Many natural dietary supplements have little aroma in meat until cooking. However, complex processes such as lipid oxidation, thermal degradation of thiamine, and Maillard reactions result in enhancing the chicken flavour (combination of aroma and taste) **(Jayasena *et al*., 2013)**. In the study of **Chulayo *et al.* (2011)**, addition of medicinal plants (*Aloe ferox* and *Agave sisalana*) in chicken diet improved juiciness, tenderness, and overall flavour of meat.

Teye *et al*. (2015) reported that administration of palm kernel oil residue inclusion up to 17.5% in chickens appeared to have no effect on sensory quality of meat.

Another study **(Adeyemo and Sani, 2013)** demonstrated the improvement of sensory properties of meat from chickens fed 50% cassava based meal, especially the overall acceptability, compared to that from the other groups.

The study of **Bartlett and Beckford (2015)** has shown that the inclusion of sweet potato in the diet of broilers enhanced the juiciness, tenderness, flavor, and overall acceptability of meat.

CONCLUSION

The present results suggest that dietary supplementation with probiotic given in combination with propolis in chickens was effective in improving the sensory quality of chicken breast meat since panellists preferred meat from that group more than any other meat as indicated by the sensory evaluation. On the contrary, breast meat of chickens fed with bee pollen and probiotic obtained the lowest scores for all the sensory attributes. Dietary supplements did not influence markedly the chemical characteristics of chicken breast meat, except for fat content which was significantly the highest in chickens fed with bee pollen and probiotic. As a potential natural supplement with prebiotic activity in chicken diet, propolis extract can be recommended to be used for improving the sensory quality of chicken meat. In further studies, propolis supplementation combined to probiotics should be investigated to clarify the underlying mechanisms that contribute to those effects.

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