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THE INFLUENCE OF PROPOLIS SUPPLEMENTATION ON THE TECHNOLOGICAL PROPERTIES AND MACRONUTRIENT CONTENT OF SKINLESS CHICKEN BREASTS

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Summary

The aim of this study was to determine the influence of dietary supplementation with propolis on the technological properties of skinless chicken breasts evaluated through breast muscle pH value measured 45 minutes (pH₁) and 24 hours post mortem (pH₂), water-holding capacity of breast muscle, consistency of breast muscle and its color (L*, a*, b*) and to determine its macronutrient content (protein and fat content). The study was conducted on 180 Ross 308 chickens equally distributed by sex and divided into three groups: the control group of chickens (C) fed with a basal diet and two experimental groups of chickens (E) fed with the same diet supplemented with propolis (E1 2g/kg and E2 4g/kg). There was no statistically significant difference between C and E considering pH₁ (p=0.260) but there was statistically significant difference between them considering pH₂ (p=0.037). There was statistically significant difference in L* breast muscle color (p=0.039) between C and E while there were no statistically significant differences in a* and b* breast muscle color between them (p=0.167 and p=0.637, respectively). There were no statistically significant differences between the C and E considering water-holding capacity (p=0.767) and consistency (p=0.505) of breast muscle. There were no statistically significant differences in protein and fat content between C and E (p=0.368 and p=0.244, respectively). The obtained results confirm the benefits of the tested supplementation.

Keywords: propolis, chicken breasts, chicken feeding, technological properties, macronutrient content

Introduction

Chronic non-communicable diseases are the leading cause of death globally (Dumic et al., 2017). The unbalanced or poor nutrition is the major risk factor for such diseases (Dumic et al., 2017; Dumic et al., 2018). Bearing in mind that many of chronic non-communicable diseases are directly linked to the human nutrition it is quite clear that many challenges in health care could be proactively improved by producing a healthier food supply as a preventive health care strategy (Decker and Park, 2010). Until now there has been several attempts to produce such foods but because of the complexity of this issue and many stakeholders who have interest in the subject matter the final solution has not yet been found. The one of the main challenges is to find the foodstuff that is necessary for human health and development that contains essential elements which one cannot substitute easily and to make it even more healthier and tempting for human nutrition. This is especially true for functional foods as they must be efficacious while also tasting good, being convenient and reasonably priced so that consumers will regularly purchase the products (Decker and Park, 2010). Meat continues to supply nutrients and plays a vital role in human life because of its high biological value

protein, iron, zinc, selenium and vitamin B12 contents being a crucial component of a well-balanced diet (Perreira and Vicente, 2013). Following the fact that red meat has been connected with the onset of some chronic diseases such as colon cancer and cardiovascular diseases the popularity of poultry meat is growing throughout the world including Croatia (Park et al., 2017). Within the poultry meat the chicken meat is especially popular. The popularity of chicken meat and its growing consumption is contributed by a number of factors, most notably its low prices, the long tradition of poultry farming in almost all parts of the world, the indisputable dietary and nutritional value of chicken meat, the lack of cultural and religious barriers to consumption of this type of meat, but also of the crisis in the area of food safety in the late 90s of the last century due to bovine spongiform encephalopathy (Klarić., 2014; Klarić et al., 2016). Propolis belongs to a group of natural substances of animal and vegetable origin with intense antioxidant and antimicrobial properties (Prakatur et al., 2019a). The bioactive components of propolis include polyphenolic constituents such as flavonoids, phenolic acids and their derivatives (Wang et al., 2016; Prakatur et al., 2019a). Polyphenolic constituents of propolis are responsible for its well-

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documented pharmacological activities, including antimicrobial, antioxidant, anti-inflammatory, immunomodulatory, and cardioprotective effects (Wang et al., 2016; Prakatur et al., 2019a). Just because of these properties propolis is today widely used as a health/functional food worldwide (Wang et al., 2016).

Meat has great potential for introducing important nutrients into the human diet. The nutritional composition of meat products can be altered by the direct addition of bioactive food ingredients or the inclusion of bioactive compounds in animal nutrition. This latter technique has the advantage that bioactive compounds are biologically introduced into the food and thus would not have to be declared as a food additive. This is important because food additives are often not allowed in meat products as they may violate the product identity standard (Decker and Park, 2010).

Recent study had showed that propolis supplementation of chicken feed is a promising method to improve the quality of chicken meat since this supplementation elicited the best amino acids profile of the chicken meat (Haščik et al., 2020).

The aim of this study was to determine the influence of dietary supplementation with propolis on the technological properties of skinless chicken breasts (pH₁ and pH₂; water-holding capacity of breast muscle; consistency of breast muscle and its color) and to determine its macronutrient content (protein and fat content).

Materials and methods

Animals, diet, experimental design. The study was conducted on total 180 chickens of Ross 308 provenance, divided into 3 groups (60 chickens in each group with equally distributed sexes): one control group (C) and two experimental groups (E1 and E2). All chickens were placed on wooden sawdust under the same conditions throughout the experimental period (42 days) according to the manufacturer's recommendations for the Ross 308 hybrid (Aviagen, 2014). From day 1 to 21 of the study, chickens were fed with a starter mixture. From day 22 to 42 of the study, chickens were fed with a finisher mixture. During the whole study, feed and water were offered *ad libitum*. Throughout the study the control group (C) was fed a basal diet without additives, while the experimental groups (E1 and E2) were fed the same diet supplemented with propolis (E1 2g/kg and E2 4g/kg). The used amounts of propolis were chosen based on results of several previous studies (Klarić et al., 2018; Klarić et al., 2018a; Prakatur et al., 2019). The experimental

protocol was approved by the Committee for Animal Welfare of the Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek (Approval code: 602-04/19-01/04; 2158-94-02-19-05). Samples of raw propolis used in this study were obtained from apiaries located in naturally preserved areas of continental Croatia (around the city of Osijek, Eastern Croatia). Inclusion of propolis into the feed mixture was performed using a vertical mixer (Briketstroj Ltd., Valpovo, Croatia).

Sample collection and measurements. On day 42, after 10-hour feed withdrawal, 14 chickens from each group was slaughtered by cervical dislocation and exsanguinated for 2 minutes. The carcasses were then manually de-feathered and eviscerated. Immediately after slaughtering and de-feathering, and without cooling, the carcasses were processed. Chicken carcasses were processed according to the principle "Prepared for barbecue" (Regulation European Commission No. 543/2008).

Carcass body weight was measured by using an electronic scale Avery Berkel FX 220 (Avery Berkel, Smethwick, UK). The carcass yield was calculated as the difference between the live weight (g) and carcass body weight (g) and expressed as a percentage of live weight.

Technological characteristics of chicken meat quality were described by analyzing the average pH₁ and pH₂ of breast muscle, water-holding capacity of breast muscle, consistency of breast muscle and breast muscle color expressed as L* (lightness), a* (redness), and b* (yellowness).

Chickens' breast muscle pH values were measured in the internal section of pectoral major muscle. The pH₁ value was determined 45 minutes *post mortem* and pH₂ value was determined 24 hours *post mortem* by a contact pH meter (MP120-B, Mettler Toledo, Giessen, Germany).

Assessment of water holding capacity was determined by the method of Grau and Hamm (1953). A sample of 300 mg meat was applied to Whatman 1 paper, placed between two glass plates and subjected to an even loading of 2 kg for 5 min. From the size of the outflow area, the percentage of free water in the meat was calculated, assuming that 1 cm² of the outflow corresponded to 10 mg of water. A smaller area of the outflow (the amount of free water) indicated the greater water holding capacity of the meat. Along with the water holding capacity the consistency of breast muscle was determined.

The color of breast muscle was determined on the cooled section of muscle after 24 hours of cooling at 4 °C by using the Minolta Chroma Meter CR-410 (Minolta Camera Co. Ltd., Osaka, Japan). The

calibration of the device was done using a standard white plate (Reference No. 21633047, C Y = 94.3, x = .3135 and y = .3197; D Y = 94.3, x = .3160, y = .3232). Before the measurement, a fresh vertical incision was made in the middle of the breast muscle. The sample was left for 10 minutes at room temperature to "stabilize" the color, after which the color of the muscle was read by the Chroma meter. The color of chicken meat was expressed as CIE-L*a*b* (Commission Internationale de l'Eclairage, 1976) i.e. values of L* (lightness), a* (redness), and b* (yellowness).

Chemical composition of meat. Fat content of meat was determined by Soxhlet extraction method and Protein content by AOAC official method 928.08 (Kjeldahl method) (AOAC, 2000). All analyses were performed in duplicates. Energy content of samples was calculated using the Atwater general energy conversion factors where 4.0 kcal/g of protein and 9.0 kcal/g of fats (FAO, 2003).

Statistical analysis. The statistical analysis was carried out using statistical package Statistica for Windows 2010 (version 10.0, Stat Soft Inc., Tulsa, OK). Normality of data distribution was tested with the Kolmogorov-Smirnov test. The numerical variables were described as mean \pm standard deviation (SD). ANOVA was used for the comparison of numerical variables among the groups. On all statistical analyses, two-sided P-values of 0.05 and lesser ones were considered significant.

Results and discussion

This study showed that there was no statistically significant difference between C and E considering pH1 ($p=0.260$) but there was statistically significant difference between them considering pH2 ($p=0.037$) (Table 1). The results of this study are opposite to the results of the study done by Šulcerová et al. (2011) who showed how pH2 values of experimental groups were lower than those from control group while in this study those values were higher than in control group. When observing all measured chickens' breast muscle pH values, it can be said that they indicate good quality of chicken meat of all groups since the pH values were not below 5.4 and not above 7.0 when autolysis of meat appears (Haščík et al., 2012). The results of this study clearly indicate that pH value drops after slaughter and therefore the meat pH2 values are lower than the pH1 values. The lowering of the chickens' breast muscle pH values is due to the fact that glycogen from the slaughtered animals is degraded in glucose. Glucose then passes the glycolysis process, but due to lack of oxygen, the formation of lactic acid leads to decrease of muscle

tissue pH (Šulcerová et al., 2011). The described drop in pH value helps to convert muscle to meat.

Table 1. pH values (pH₁, pH₂) of the chickens' breast muscle

Parameters	Group of chickens $\bar{x} \pm s$			p*
	C	E1	E2	
pH ₁	5.76 \pm 0.13	5.82 \pm 0.10	5.82 \pm 0.10	0.260
pH ₂	5.63 \pm 0.10	5.70 \pm 0.08	5.71 \pm 0.06	0.037

*ANOVA; \bar{x} = mean; s = standard deviation; C = control group; E1 = feed mixture + 2.00 g of propolis/kg of feed mixture; E2 = feed mixture + 4.00 g of propolis/kg of feed mixture; pH₁ - pH value measured 45 minutes post mortem; pH₂ - pH value measured 24 hours post mortem

The study revealed that there was statistically significant difference in L* breast muscle color ($p=0.039$) between C and E while there were no statistically significant differences in a* and b* breast muscle color between them ($p=0.167$ and $p=0.637$, respectively) (Table 2). These results are slightly opposite to the results of study by Haščík et al. (2012) who did not find statistically significant differences in breast muscle color between control and experimental groups of chickens. However, our results are in concordance with the results of the study done by Šulcerová et al. (2011) who also showed how L* breast muscle color was statistically significant higher in experimental groups of chicken in comparison to control group. Meat color is a characteristic that significantly determines meat quality, as it is the first visual criterion by which consumers judge the appearance and appeal of a meat. Following that, fresh chicken breast muscle should be pink in color, and any deviation from this shade is considered unacceptable to the consumers (Garcia et al., 2010; Kralik G. et al., 2011). The results of our study clearly confirm that the type of chicken feeding significantly influences the color of meat, as has been shown previously in other studies (Karaoglu et al., 2006; Saláková et al., 2009).

Table 2. Average color values of chickens' breast muscle expressed as CIE-L*a*b* according to the groups of chickens

Parameters	Group of chickens $\bar{x} \pm s$			p*
	C	E1	E2	
L*	64.46 \pm 2.71	66.26 \pm 1.60	66.19 \pm 1.55	0.039
a*	11.32 \pm 1.27	10.86 \pm 1.26	11.83 \pm 1.46	0.167
b*	12.08 \pm 2.09	11.65 \pm 2.81	11.27 \pm 1.68	0.637

ANOVA; \bar{x} = mean; s = standard deviation; C = control group; E1 = feed mixture + 2.00 g of propolis/kg of feed mixture; E2 = feed mixture + 4.00 g of propolis/kg of feed mixture; L - lightness; a* - redness; b* - yellowness

The study further showed that there were no statistically significant differences between the C and E considering water-holding capacity ($p=0.767$) and consistency ($p=0.505$) of breast muscle (Table 3 and Table 4). These results are in concordance with the results of the study done by Klarić (2014) who also did not find statistically significant differences in mentioned parameters between control and experimental groups of chicken. The water-holding capacity is a very important parameter of meat quality since the color, juiciness and tenderness of the meat depend partially on the ability of the meat to retain moisture during normal storage conditions and during its heat treatment, making this parameter important for both fresh meat quality and for the quality of meat products (Mehaffey et al., 2006; Wang et al., 2009).

Table 3. Water-holding capacity of chickens' breast muscle (%) according to the groups of chickens

Parameter	Group of chickens $\bar{x} \pm s$			p*
	C	E1	E2	
Water-holding capacity	2.62±0.45	2.53±0.58	2.66±0.34	0.767

*ANOVA; \bar{x} = mean; s = standard deviation; C = control group; E1 = feed mixture + 2.00 g of propolis/kg of feed mixture; E2 = feed mixture + 4.00 g of propolis/kg of feed mixture

Table 4. Consistency of chickens' breast muscle according to the groups of chickens

Parameter	Group of chickens $\bar{x} \pm s$			p*
	C	E1	E2	
Consistency	2.15±0.25	2.15±0.25	2.07±0.15	0.505

*ANOVA; \bar{x} = mean; s = standard deviation; C = control group; E1 = feed mixture + 2.00 g of propolis/kg of feed mixture; E2 = feed mixture + 4.00 g of propolis/kg of feed mixture

Both, protein and fat content were lower in both experimental groups 20.26±1.61 g of proteins/100 g in E1 and 20.50±1.00 g of proteins/100 g in E2 in comparison to average of 21.16±2.28 g of proteins/100 g in control group; 2.03±0.58 g of fat/100 g in E1 and 1.78±0.50 g of fat/100 g in E2 in comparison to average of 2.12±0.56 g of fat/100 g in control group. Obtained values result in lower caloric value of skinless chicken breast (99.3±7.1 kcal/100g in E1 and 98.0±6.1 kcal/100g in E2 in comparison to 103.7±10.0 kcal/100g in C). Reduction in protein, fat content and energy value was not statistically significant ($p=0.368$; $p=0.244$; $p=0.149$, respectively)

(Table 5) which confirms the plausibility of selected feeding profile from the aspect of macronutrient content.

Table 5. Protein and fat content of chickens' breast muscle according to the groups of chickens

Parameter	Group of chickens $\bar{x} \pm s$			p*
	C	E1	E2	
Proteins (%)	21.16±2.28	20.26±1.61	20.50±1.00	0.368
Total fat (%)	2.12±0.56	2.03±0.58	1.78±0.50	0.244
Energy (kcal/100 g)	103.7±10.0	99.3±7.1	98.0±6.1	0.149

*ANOVA; \bar{x} = mean; s = standard deviation; C = control group; E1 = feed mixture + 2.00 g of propolis/kg of feed mixture; E2 = feed mixture + 4.00 g of propolis/kg of feed mixture; protein content determined by Kjeldahl method; Fat content determined by Soxhlet extraction

Conclusions

The results of this study had justified the usage of propolis as a feed supplement in chickens feeding. This type of feeding opens up the possibility of the production of enriched chicken meat, which is of utmost importance in the context of the prevention of chronic non-communicable diseases, especially cardiovascular diseases, and the general improvement of the health of the population. Further studies are needed to determine the most optimal amounts of propolis for chickens feeding.

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