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THE INFLUENCE OF BEE PRODUCTS IN COMBINATION WITH PROBIOTIC IN CHICKEN DIET ON OXIDATIVE STABILITY OF CHICKEN MEAT

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ABSTRACT

In the experiment, the effect of the addition bee pollen extract in combination of with probiotic and propolis extract in combination with probiotic in diet of chicken broilers Ross 308 on oxidative stability of breast and thigh muscles during 7 days storage by chilling was investigated. In the experiment were included 120 pieces of one day-old chicks, which were divided into 3 groups (control, E1 and E2). Feed mixtures and drinking water were given to chickens by *ad libitum* system until the age of 42 days. Bee pollen extract in amount of 400 mg.kg^{-1} added to feed mixtures plus 3.3 g probiotic preparation (*Lactobacillus fermentum*) added to drinking water (E1), propolis extract in amount of 400 mg.kg^{-1} added to feed mixtures plus 3.3 g probiotic preparation (*Lactobacillus fermentum*) added to drinking water (E2). During whole period of chilled storage (7 days) were higher values of MDA determined in control group (C) compared with experimental groups (E1 and E2). The higher average MDA values determined in breast muscle was in samples of control group (0.128 mg.kg^{-1}) compared with experimental groups E1 ($P>0.05$) and E2 ($P\leq0.05$) (0.127 and 0.119 mg.kg^{-1}, respectively) after 7-day of chilled storage. The higher average MDA values ($P>0.05$) were also determined in thigh muscles in control group (0.141 mg.kg^{-1}) compared with experimental groups E1 (0.139 mg.kg^{-1}) and E2 (0.128 mg.kg^{-1}) after 7-day of chilled storage. Higher amount of MDA in thigh muscle compared to breast muscle is due to by higher amount of fat occurred in thigh muscle.

Keywords: Broiler chicken, bee pollen, propolis, probiotic, meat, oxidative stability

INTRODUCTION

Lipids are an important component of meat and contribute to several desirable characteristics of meat and meat products. Lipids are important to enhance the flavour and aroma profile of meat and also increase the tenderness and juiciness of meat. However, it is generally accepted that lipid oxidation is the primary process responsible for quality deterioration of meat during storage. Quality characteristics affected in meat by lipid oxidation include flavour, colour, texture and its nutritional value. The development of rancidity in meat by lipid oxidation begins at the time of slaughter and continues during storage. Storing meat at low temperature and packaging of meat in oxygen free containers retards the rapid development of rancidity. However, oxidation of lipids may continue even during frozen storage **(Weber, 2001)**.

In the initial stages of lipid oxidation, products have a cardboard flavor. As oxidation progresses, other flavors, such as painty, rancid, and oxidized, develop **(St. Angelo *et al.*, 1990)**. Secondary compounds such as hexanal, pentanal, heptanal, and octanal produced from the oxidation of polyunsaturated fatty acids are responsible for the presence of warmed-overflavors (stale, wet cardboard, painty, grassy, rancid) associated with cooked, stored pork (**Campo *et al.*, 2006**; **Rojas and Brewer 2007**). Susceptibility of muscle tissue to lipid oxidation is related to the degree of lipid unsaturation, muscle, animal diet, additives such as salt, cooking method, manner of storage, and pH of the muscle (**Kanner 1994; Rhee and Ziprin 2001**).

Lipid oxidation can be reduced by oxygen-restrictive packaging and various additives such as antioxidants currently used in the food industry. Antioxidants are substances that at low concentrations retard the oxidation of easily oxidizable biomolecules, such as lipids and proteins in meat products, thus improving shelf life of products by protecting them against deterioration caused by oxidation (**Shahidi and Zhong, 2005).**

Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), and propyl gallate (PG) have been used as antioxidants in meat and poultry products (**Formanek *et al.*, 2001; Biswas *et al.*, 2004; Jayathilakan *et al.*, 2007**), but synthetic antioxidants have fallen under scrutiny due to potential toxicological effects **(Raghavan and Richards, 2007; Naveena *et al.*, 2008b; Nunez de Gonzalez *et al.*, 2008b)**. In response to recent demand for natural products and consumers' willingness to pay significant premiums for natural foods **(Sebranek and Bacus, 2007**), the meat and poultry industry is actively seeking natural solutions to minimize oxidative rancidity and increase products' shelf-life (**Naveena *et al.*, 2008a**). Recent investigation has focused towards identification of novel antioxidants from natural sources. Due to their high content of phenolic compounds, fruits and other plant materials are a good source of natural antioxidants and provide an alternative to currently used conventional antioxidants (**Nunez de Gonzalez *et al.*, 2008a**).

The use of antibiotics as growth promoters for poultry production has been banned in the European Union, which caused prohibition of using them as protective agents against the emergence of infectious diseases and consequently increasing the economical losses in the poultry industry (**Plail, 2006; Peric *et al.*, 2009**). Therefore, many researchers tried to find some natural feed additives such as propolis to be used in poultry farms to reduce the expected harmful effects (**Kwiecien and Winiarska-Mieczan, 2009; Hegazi *et al.*, 2012**). Antioxidative effects of bee pollen against lipid peroxidationhas been reported in some studies (**Khalil and El-Sheikh 2010; Seven *et al.*, 2011; Seven *et al.*, 2016**).

The aim of the experiment was to determine the oxidative stability in the most valuable parts of chicken carcasses (Ross 308 hybrid combination) during the cold store (7 days) after application of bee pollen extract and propolis extract added to feed mixtures in combination with probiotic added into drinking water.

MATERIAL AND METHODS

The experiment was carried out at Slovak University of Agriculture in Nitra. A total of 120 one day-old Ross 308 broiler chicks were randomly divided into 3 groups, namely, control (C) and experimental (E1, E2) of 40 pcs chickens. During the whole period of experiment (42 days), the broiler chickens had *ad libitum* access to feed and water. 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 nutrient content of basal diet is given Table 1. The feed mixtures were produced without any antibiotic preparations and coccidiostats. 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 dietary supplements as follows: 1. bee pollen extract in amount of 400 mg.kg^{-1} added to feed mixtures and 3.3 g probiotic preparation added to drinking water (E1 group), 2. propolis extract in amount of 400 mg.kg^{-1} added to feed mixtures and 3.3 g probiotic preparation added to drinking water (E2 group). The groups were kept under the same conditions. In the experiment, the probiotic preparation based on *Lactobacillus fermentum* (1.10^9 CFU per 1 g of bearing medium) was used. Bee pollen and propolis had origin in the Slovak Republic. The extracts were prepared according to **Krell (1996)**. At the end of feeding (day 42th), 20 pcs chickens were selected from each group for slaughter analysis. To determine changes in lipid degradation (determination of thiobarbiturates numbers, TBA), the samples of breast and thigh muscles were deboned, packed into polyethylene bags and stored for 7 days at 4 °C. TBA values expressed in number of malondialdehyde were measured in the process of first storage day of 1st, 3rd, 5th and 7th day. TBA number was determined by **Marcinčák *et al.* (2004)**. Absorbance of samples was measured on UV-VIS spectrophotometer T80 (PG Limeted Instruments, UK) at a wavelength of 532 nm, the translation results on the amount of malondialdehyde (MDA) in 1 kg samples. The data processing was performed by SAS software (version 9.3, SAS Institute Inc., USA. Variation-statistical values (mean and standard deviation) were calculated and significant differences between the groups were determined using F-test and t-test.

Table 1 Nutrient content of basal diet (g.kg^{-1})

Nutrient content	Starter HYD-01 (1 to 21 day)	Grower HYD-02 (22 to 42 day)
Crude protein	211.43	191.02
Fibre	30.35	29.80
Ash	25.63	20.42
Ca	8.19	7.23
P	6.66	5.79
Mg	1.59	1.53
Linoleic acid	13.52	14.13
ME_N (MJ.kg^{-1})	12.04	12.09

RESULTS AND DISCUSSION

The results of the oxidation stability measured in breast and thigh muscle of chickens Ross 308 during 7 days storage at 4 °C are shown in Table 2. Our results are in accordance with **Marcinčák *et al.* (2010)** who, after slaughtering and processing of poultry samples also show low values of MDA. During chilled storage of the breast and thigh muscles (7 days) were detected increased contents of MDA in comparison to the first day of storage. During the whole testing period of chilled storage of breast and thigh muscles were higher values of MDA measured in control group compare to experimental groups. The higher average value of MDA measured in breast muscle of broiler chickens Ross 308 was in samples of control group (0.128 mg.kg^{-1}) compared to experimental groups E1and E2 (0.127 and 0.119 mg.kg^{-1}, respectively) after 7-day of chilled storage. Significantly higher values (P≤0.05) of MDA on the end of storage were determined in control group compare to group E2.

Trend of oxidation stability in thigh muscle of chickens Ross 308 was during 7 days of chilled storage similar to that in breast muscle. The higher average values of MDA measured in thigh muscle were in samples of control group (0.141 mg.kg^{-1}) compared to experimental groups E1 (0.139 mg.kg^{-1}) and E2 (0.128 mg.kg^{-1}). We have not found statistically significant differences (P>0.05) between testing groups after 7-day of chilled storage. However, during the whole testing period of chilled storage was the trend with lower MDA values in experimental groups, where significant differences (P≤0.05) were between control and E1 group (0.042 and 0.028 mg.kg^{-1}, respectively) in 1st day of storage. Higher concentration of MDA in thigh muscle compare to breast muscle is due to by higher amount of fat passes into thigh muscle.

Achieved results of oxidation stability determined in chicken meat of hybrid combination Ross 308 after addition antioxidants in their diet are in accordance with **Betti *et al.* (2009)** and **Yasin *et al.* (2012).**

The possibilities of using alternative feed supplements containing various antioxidant active substances for poultry which increase the oxidation stability of the meat during its period of chilling storage are presented in studies of **Ahadi *et al.* (2010), Karaalp and Genc (2013).**

Degradation pathways of fatty substances play one of the main causes of foods deterioration and unpleasant odours. This factor is also responsible for the loss of sensory properties such as flavour, texture, appearance, nutritional value of food, increases the drop losses, pigment, polyunsaturated fatty acids, fat-soluble vitamins, reduces the quality of meat intended for human consumption and ultimately reduces its stability, shelf life and safety **(Ramos Avila *et al.*, 2013)**.

Higher concentration of antioxidants in poultry meat has the effect on reducing lipid oxidation reported by **Rojas and Brewer (2007)**, **Smet *et al.* (2008)** and **Doolaege *et al.* (2012)**, i.e. there is a reduction in MDA values during chilling storage, which was confirmed by our results.

Additionally, similar values were in study of **Bobko *et al.* (2015a, b)** who found higher oxidative stability of chicken meat in breast and thigh muscles after application of bee products (bee pollen and propolis) as well as other feed supplements when compared with control group. Significantly higher oxidative stability (P≤0.05) of chicken meat after storage by cooling and freezing found also **Haščík *et al.* (2014)** after propolis extract application in an amount 600 and 800 mg.kg^{-1} feed mixture that is in accordance with results in the present study, where we also found the highest stability in the group with propolis extract addition (E2) in the most valuable parts of chicken carcass.

Table 2 Effect of storage (4 ºC) on the concentration of malondialdehyde (mg.kg^{-1}) in breast and thigh muscle (mean±SD)

Time of storage	Groups		
	Control	E1	E2
Breast muscle			
Day - 1	0.035 ±0.009^a	0.030 ±0.002^a	0.020 ±0.009^a
Day - 3	0.048 ±0.005^a	0.043 ±0.003^a	0.040 ±0.002^a
Day - 5	0.083 ±0.005^a	0.081 ±0.010^a	0.077 ±0.009^a
Day - 7	0.128 ±0.004^a	0.127 ±0.009^{ab}	0.119 ±0.010^b
Thigh muscle			
Day - 1	0.042 ±0.002^a	0.028 ±0.003^b	0.029 ±0.003^a
Day - 3	0.058 ±0.005^a	0.052 ±0.012^a	0.048 ±0.012^a
Day - 5	0.096 ±0.011^a	0.094 ±0.011^a	0.090 ±0.015^a
Day - 7	0.141 ±0.018^a	0.139 ±0.022^a	0.128 ±0.016^a

Legend: Mean values in the same lines with different superscripts (a, b) are significantly different at P≤0.05 level

CONCLUSION

Results achieved in the experiment showed that the addition of bee pollen and propolis extracts in feed mixtures in combination with probiotic added into drinking water for broiler chickens Ross 308 had positive effect on the reduction of oxidative processes in the breast and thigh muscles during 7-days of chilling storage.

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