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REGULAR ARTICLE

OXIDATIVE STABILITY OF CHICKEN MEAT AFTER POLLEN EXTRACT APPLICATION IN THEIR DIET

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ABSTRACT

In the experiment were evaluated the effect of feeding the pollen extract (group I - 400 mg and group II - 800 mg) in feed mixtures for feeding Ross 308 chickens for 42 days on the oxidative stability of breast and thigh muscles stored for 6 months at -18 °C. Malondialdehyd (MDA) values were in the breast muscle in the control group from 0.065 to 0.137 in I^{st} group 0.61 to 0.111 and in II^{nd} group 0.075 to 0.96 $mg.kg^{-1}$ respectivelyfrom 1^{st}day to 6^{th}month of storage. In the thigh muscle were noted MDA values from 0.105 to 0.137 $mg.kg^{-1}$ (control group), from 0.083 to 0.111 $mg.kg^{-1}$ (I^{st} group) and 0.114 to 0.120 $mg.kg^{-1}$ (II^{nd} group). We observed lower levels of MDA $mg.kg^{-1}$ (0.095 to 0.099 - II^{nd} EG, 0.103 to 0.111 - I^{st} EG) than in the control group (0.120 to 0.137). Feeding with pollen extract had a significant effect ($P \leq 0.05$ to $P \leq 0.001$) to reduce oxidation processes in the breast muscle from 5^{th} month of storage (freezing). In the thigh muscle, were release the oxidation processes ($P \leq 0.01$) recorded after 6^{th} months of storage and freezing in the I^{st} group (MDA 0.111 $mg.kg^{-1}$) with the addition of 400 mg pollen nutrition extract in Ross 308 chickens compared to control (MDA 0.137 $mg.kg^{-1}$). The results show that pollen extract has a positive effect on shelf life and oxidative stability of the most valuable parts of the carcass Ross 308 chickens, but

statistically significant ($P \leq 0.05$ to $P \leq 0.001$) after 5^{th}, 6^{th} months of storage, freezing at -18 °C, respectively.

Keywords: broiler chicken, meat, oxidative stability, frozen storage, malondialdehyd

INTRODUCTION

Bee pollen is formed in the male reproductive organ of flowering plants. It is a fine, powdery material made of flowering plants and obtained bees workers. Workers bees (*Apis mellifera L.*) collected pollen from flowers, shape it and save them for the last few feet into the so-called cups. Pollen loads are made from pollen of higher plants, bee Bonds with their excretions and adding nectar glands. Pollen loads are formed from the pollen of one plant species, ie. monofloral pollen **(Carrión et al., 2003)**, are shaped pollen grains from different plant species called multifloral pollen, respectively **(Stanley a Linskens, 1974; Barth et al., 2009; Modro et al., 2009)**. Pollen loads or bees pollen is the basic food for the colony as a source of protein for them **(Tüylü a Sorkun, 2004)**. The protein content of pollen is 25-30% carbohydrates, 30-55% fats, including fatty acids and sterols 1-20% and also contains significant amounts of vitamins and minerals. Composition of the pollen provides valuable nutrients such as free amino acids, minerals, polyfenolytic substances and oligo-elements **(Serra Bonvehi and Escola Jordi, 1997; Villanueva et al., 2002; Bastos et al., 2004; Almeida-Muradian et al., 2005; Cocan et al., 2005; Hamamoto et al., 2006; Human and Nicolson, 2006; Yamaguchi et al., 2006)** and therefore is also used in the human diet, which provides a sense of well-being, contributes to functional and well-balanced body has antioxidant properties **(Moreira et al., 2008)**and prevents free radicals **(Hejinen et al., 2002; Villanueva et al., 2002; Bastos et al., 2004; Almeida-Muradian et al., 2005; Silva et al., 2006; Mărghitas et al. 2009; Stanciu et al., 2009)**. Pollen is also rich on carotenoids, flavonoids, phytosterols and other healthy substances **(Serra Bonvehi et al., 2001; Baltrusaityte et al., 2007; Moreira et al., 2008)**. Its use is also important in medicine in terms of its anti-inflammatory **(Wagenlehner et al., 2009)**, antiandrogenic **(Shoskes, 2002; Shoskes and Manickam, 2003**), antimicrobial **(Basim et al., 2006)** and anticancer effects **(Yang et al., 2007)** and is also used for chronic prostate and oral numbness and desensitization **(Medeiros et al., 2008)**.

Deutch Federal Board of Health officially recognized bee pollen as a medicament **(Ishikawa et al., 2008)**. From this perspective, bee pollen product of great commercial interest and can also be considered as a potential source of energy and protein for human consumption **(Kroyer and Hegedűs, 2001; Campos et al., 2003)**, its nutritional composition largely depends on the species composition of pollen **(Stanley and Linskens, 1974; Modro et al., 2009).**

The most problematic technological operations is storage bee pollen, whereas there is a change of various chemical changes during storage **(Roulston, 2005)**. **Szczesna et al. (1995a,b,c)**describe various methods of preservation of pollen (freezing, drying at about 40 °C and lyofilization), which note that freezing does not cause any significant changes in the chemical composition of pollen, lyofilization significantly reduces the level of vitamin C and provitamin A and dry at 40 °C revealed the most unfavorable effects on pollen, which decreased significantly reducing sugars, total protein, vitamin C and provitamin A. Therefore, for practical use is the optimal solution pollen freezing at -20 °C in pure nitrogen, which ensures a high biological properties of bee pollen for up to 6 months. For longer storage of honey should be pollen dried by lyophilization and stored at -20 ° C in pure nitrogen.

The importance of alternative supplements, including bee pollen, which may also be used in animal nutrition gains in importance also therefore, since it can replace antibiotics and coccidiostats in European Union countries since 2006 to forbid the use of nutrition monogastric animals, including poultry and can eventually positive influence on meat utility and meat quality. For this reason, the present study was aimed to investigate the effect of 80% pollen extract Slovak multifloral addition to feed mixtures for oxidative stability of meat in the process of installation of Ross 308 chickens.

MATERIAL AND METHODS

The experiment was realized at the test station poultry of Slovak Agricultural University in Nitra on feeding of Ross 308 chicken hybrid combination. The experiment enrolled 270 pieces of one day chickens hybrid combination and were created 3 groups of animals: control (C) and two experimental (I, II) of 90 pcs of chickens. Custom fedding insisted 42 days. Chickens were fed to 21th day of age an *ad libitum* with the same starter feed mixture HYD-01 (powdery form) and from 22nd to 42nd day of age fed with the growth feed mixture HYD-02 (powdery form) in the monitored groups. The fed feed mixture HYD-01 and HYD-02 have been produced without antibiotic preparations and coccidiostats.

Tab 1 Composition of the diets

Ingredients (%)	**Starter** (1 to 21 days of age)	**Grower** (22 to 42 days of age)
Wheat	35.00	35.00
Maize	35.00	40.00
Soybean meal (48 % N)	21.30	18.70
Fish meal (71 % N)	3.80	2.00
Dried blood	1.25	1.25
Ground limestone	1.00	1.05
Monocalcium phosphate	1.00	0.70
Fodder salt	0.10	0.15
Sodium bicarbonate	0.15	0.20
Lysin	0.05	0.07
Methionin	0.15	0.22
Palm kernel oil Bergafat	0.70	0.16
Premix Euromix BR 0,5 %[1]	0.50	0.50
Analysed composition ($g.kg^{-1}$)		
Crude protein	210.76	190.42
Fibre	30.19	29.93
Ash	24.24	19.94
Ca	8.16	7.28
P	6.76	5.71
Mg	1.41	1.36
Linoleic acid	13.51	14.19
ME_N ($MJ.kg^{-1}$) by calculation	12.02	12.03

[1] active substances per kilogram of premix: vitamin A 2 500 000 IU; vitamin E 50 000 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 50 000 mg; folic acid 400 mg; biotin 40 mg; vitamin B12 10.0 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

Nutritional value given compound feed (Table 1) during the experiment was the same in each group, but the experimental group was in addition to feed mixtures HYD-01 and

HYD-02 added pollen extract at a dose of 400 mg.kg^{-1} (I) and 800 mg. kg^{-1} (II). Pollen extract was prepared from pulverized pollen (Slovak Republic), which was then mixed with 80% ethanol **(Krell, 1996).**

Extraction pollen solution was carried in a water bath at 80 °C under reflux for 1 hour. The mixture was cooled and centrifuged after the extraction. The resulting supernatant was evaporated on a rotary evaporator at a bath temperature 40-50 °C and then weighed. Residue in an amount of 40 g and 80 g was dissolved in 1000 cm^3 ethanol concentration of 80% and applied to 100 kg of the feed mixture. At the end of feeding (day 42th) from each group were selected for experiment 60 pieces of chicken slaughter analysis (30 pc ♀ and 30 pc ♂). To determine changes in lipid degradation (determination of thiobarbiturates numbers, TBA) of samples were boned thigh and breast muscle packed into polyethylene bags and stored for 6 months at -18 °C.

TBA value expressed in number of malondialdehyde were measured in the process of first storage day of 1st, 2nd, 3rd, 4th, 5th and 6th months. 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.

We obtained data from the statistical program Statgraphics Plus version 5.1 (AV Trading Umex, Dresden, Germany) calculated the basic variation-statistical values (arithmetic mean, standard deviation) and to determine the evidential differences between groups, were used analysis of variance followed by Scheffe´s test.

RESULTS AND DISCUSSION

The results of oxidative stability of breast and thigh muscles of Ross 308 chickens (Table 2) stored at -18 °C for 6 months expressed as TBA number is evaluated as the amount of malondialdehyde (MDA) as the main secondary breakdown products of polyunsaturated fatty acids. After slaughtering of poultry and processing of samples were MDA values in all samples low, which is in line with the statement of **Marcinčák et al. (2010)**. However, further storage of samples gradually increased MDA values in breast and thigh muscle in addition to the experimental group II in the thigh muscle where an increase in MDA levels compared to values detect after 24 hours were occurred after 6thmonths of storage. The highest average of MDA concentration in breast muscle was detected in the control group during 6 months of storage (0.095 mg.kg^{-1}) and lowest in group II (0.085 mg.kg^{-1}). Significant differences in

oxidative stability of breast muscle ($P \leq 0.05$ to $P \leq 0.001$) between control and experimental groups became evident from 5 month of storage and can be considered the most stable breast muscle group II, i.e. in the application of 800 mg pollen extract in the diet of Ross 308 chickens throughout the period of feeding. Higher average of MDA for 6 months of storage (freezing) similarly as in the breast were also seen in the thigh muscle in the control group (0.113 $mg.kg^{-1}$) compared the treatment groups (0.099 $mg.kg^{-1}$ - Group I, 0.111 $mg.kg^{-1}$ - Group II), although significant differences ($P \leq 0.01$) were observed between control and group II after 24 hours after slaughter and 6 months of storage between control and experimental group I, i.e. at a dose of pollen extract at 400 $mg.kg^{-1}$ of compound in the diet of Ross 308 chickens. The results of oxidative stability of chicken meat obtained in the experiment under review and confirm the view of other authors **(Sahin et al., 2002; Young et al., 2003; Kennedy et al., 2005, Imik et al., 2010),** that the oxidative stability decrease of the storage of chicken meat cooling, freezing except for values recorded in Group II after storage for 4 months in the thigh muscle, respectively. Various alternatives tested supplements, including pollen, pollen extract in the diet of poultry, which contain various antioxidants prevent oxidation of lipids, respectively **(Young et al., 2003; Govaris et al., 2004; Kennedy et al., 2005; Šperňáková et al., 2007; Mikulski et al., 2009; Marcinčák et al. 2010, Skřivan et al., 2010)** and increase the stability of meat during its storage thereby cooling and freezing. **Morrissey et al. (1994), Higgins et al. (1998), Jensen et al. (1998), Lawlor et al. (1999), Fellenberg and Speisky (2006)**also indicates that oxidative yellowing of the fat is one of the main causes of degradation in food for human consumption and unpleasant odors, this factor is responsible for the loss of taste, consistency, appearance, nutritional value of food increase losses of eaves, pigments, polyunsaturated fatty acids, fat soluble vitamins, reduces the right quality of meat for human consumption, and ultimately reduces its stability, shelf life and safety. **Sheehy et al. (1993)** and **Florou-Paneri et al. (2005)**also reported that a higher concentration of antioxidants in raw and boiled chicken meat has resulted in reduction of lipid oxidation, i.e. there is a reduction in TBARS values during storage and freezing, which was confirmed in the evaluation of breast muscle in chickens experiment audited Ross 308, but not confirmed in the thigh muscle in group II. Also confirmed the view **Šperňáková et al. (2007)** and **Luna et al. (2010)**, who after application of thymol and carvacrol extract, powdered rosemary were found greater stability compared to the thigh muscle breast, which in our experiment under review we have achieved just the opposite effect, respectively.

Tab 2 Effect of storage in freeze (-18 ºC) on the concentration of malondialdehyde ($mg.kg^{-1}$) in breast and thigh muscle (mean±SE)

Time of storage	Group			S
	Control (no bee pollen added)	I. (bee pollen 400 mg. kg^{-1})	II. (bee pollen 800 mg. kg^{-1})	
Breast muscle				
Day – 1	0.065a±0.037	0.061a±0.018	0.075a±0.026	NS
Month – 1	0.077a±0.007	0.077a±0.004	0.078a±0.006	NS
Month – 2	0.079a±0.009	0.077a±0.007	0.080a±0.006	NS
Month – 3	0.086a±0.004	0.083a±0.006	0.081a±0.009	NS
Month – 4	0.098a±0.016	0.098a±0.009	0.093a±0.009	NS
Month – 5	0.120a±0.016	0.103b±0.015	0.095b±0.010	**
Month – 6	0.137a±0.016	0.111b±0.024	0.099b±0.007	***
Thigh muscle				
Day - 1	0.105a±0,019	0.083b±0,004	0.114a±0,015	**
Month – 1	0.102a±0.023	0.093a±0.023	0.111a±0.020	NS
Month – 2	0.103a±0.011	0.093a±0.023	0.108a±0.015	NS
Month – 3	0.108a±0.012	0.097a±0.015	0.106a±0.011	NS
Month – 4	0.115a±0.015	0.105a±0.016	0.103a±0.013	NS
Month – 5	0.123a±0.021	0.108a±0.013	0.113a±0.016	NS
Month – 6	0.137a±0.016	0.111b±0.016	0.120ab±0.018	**

Legend: a,b– means with different superscripts differ significantly, determined by Scheffe's test S – siginificance; ***$P \leq 0.001$;**$P \leq 0.01$; NS – not significant

CONCLUSION

In theexperiment,was evaluated theeffectof feeding with 400 mg and 800mg ofpollenextractin feedmixturesfor feedingRoss 308chickensfor oxidativestability ofbreastandthigh musclesstored for6 monthsat -18 °C.The results obtainedshow thatfeedingpollenextracthada significant impactfor degreaseoxidation processesin thebreastmuscle,particularlyfrom 5thmonth of storage (freezing),whereinexperimental groupsreportedlower levelsMDAmg.kg^{-1} ($P \leq 0.05$ to$P \leq 0.001$)thanin the control group. Inthe thighmuscle, were significantlyreduced theoxidation processesobserveduntilafter 6months of

storage (freezing) andin the groupwith the addition of400 mg ofpollenextractin the diet ofRoss 308chickensversus control($P \leq 0.01$).

Based on the resultsof the experimentshows thatpollenextract hasa positive effect onshelf life andoxidativestability,particularlybreastmuscleRoss 308chickensinstorage byfreezing,butthe future isto becheckedfor stabilitythe most valuableparts of the carcassof chickensandat higher dosesof nativepollen, pollenextractin their diets, respectively.

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