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Original Research Paper

ANTIOXIDATIVE EFFECT OF OREGANO SUPPLEMENTED TO BROILERS ON OXIDATIVE STABILITY OF POULTRY MEAT

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Summary: Effect of oil extract of oregano supplemented in diet, on growth and oxidative stability of poultry meat was studied. Broiler chickens fed with addition of oregano achieved higher weight (2563 ± 140 g) in comparison with control group (2462 ± 195 g). Oxidative processes were investigated as changes of malondialdehyde content in breast and thigh meat after 0, 3, 6, and 12 months of storage at -21 °C. Partition of defrost samples was stored at chilling conditions (4 °C) during 12 hours, ground and thermally treated at 80 °C during 15 minutes, to observe antioxidative effect of added oregano oil in poultry meat after thermal treatment. Results showed that oregano essential oil was more effective in delaying lipid oxidation compared to the control diet at all time points. Thigh meat was more susceptible to lipid oxidation compared to breast meat ($P < 0.05$). Same effects of oregano extract were observed in meat after warm heating ($P < 0.05$).

Key words: broiler performance; lipid oxidation; oregano essential oils

Introduction

Poultry meat has some advantages from nutritive aspect e.g. the high content of proteins, essential polyunsaturated fatty acids, minerals and the low content of lipids (1). At average broilers have from 3.5 to 5.0 % of fatty tissues (18). Poultry fat contain higher amount of polyunsaturated fatty acids (PUFA) than fatty tissues of other slaughtered animals. Exactly, PUFA are the most sensible fractions to oxidation processes. Lipid oxidation in meat is one of the reasons for quality degradation during storage. This process is associated with the presence of free radicals that lead to the production of aldehydes responsible for the development of rancid flavours and changes in the colour of meat (5, 11).

Antioxidants are applied to protect food and avoid oxidation processes. Antioxidants are defined as substances which occur in meat in very low concentrations in comparison with substrate sensible to oxidation and they inhibit or reduce its oxidation (2, 13, 16).

Important source of natural antioxidants are plants. Rosemary *(Rosmarinus officinalis L.)* and sage *(Salvia officinalis L.)* were recognized as plants with the highest antioxidation activity by many authors (4, 5, 10, 14). Thyme (Thymus serpyllum vulgaris L.) and marjoram *Origanum majorana L.)* have also significant antioxidation effect and they reduce autooxidation of fats (3).

Oregano (Origanum vulgare L.), is an aromatic plant with a wide distribution throughout the Mediterranean area. The essential oil from oregano obtained by steam-distillation process from leaves and flowers is well known for its antioxidative activity (7). Carvacol and thymol two main phenols that constitute about 78 – 82 % of the essential oil of oregano are principally responsible for this activity. The essential oil derived from oregano is known to possess also antimicrobial, antifungal, and insecticidal activities. Antimicrobial activity of oregano has been examined as an alternative grow promoter in broiler chicken (6), broiler turkeys (17) and pigs.

A high oxidative stability of meat is important when attempting to avoid or delay development of rancid products or warmed-over flavour. In relation to character of process of lipid oxidation, effect of antioxidants is the more significant, the sooner they are applied. Ideal situation is the fats are protected immediately after slaughtering of animals (8,

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13). This protection can be achieved due to feeding of antioxidants in live animals (12). Increased antioxidative status in the living animal and the following increased oxidative stability of the raw product is considered beneficial the consumer and processing industry.

The aim of our experiment was observation of supplementation of feedstuff with oil extract of oregano on growth and oxidation stability of frozen and thermally treated poultry meat.

Materials and methods

Animals and diets. Experiment was carried out with 60 one-day-old ROSS 308 broiler chickens. Broilers were stabled in approved animal quarters in I. Internal clinic of University of Veterinary Medicine in Košice and they were divided into two groups: First group (EO) was fed with standard diet supplemented with oil extract of oregano (Calendula Nová Ľubovňa, Slovak republic) in concentration 0.05 % per 1 kg. Essential oil of oregano, on rice substrate, was mixed to expected daily dose of standard diet every day. Control group (K) was fed with standard diet without supplementation of antioxidants. Feed and water were provided ad libitum during the 42 day on study.

Temperature was gradually decreased from 33 °C on day 1 to 22 °C on day 21 and then kept constant. The lighting regimen provided 24 h of continuous light per day. The humidity of environment was 70 %. All chickens were individually weighted on the 1, 7, 14, 21, 28, 35, and 42 day of age. Animal handling was according to the Guidelines established by the European Union on Animal Care (Council Directive 86/609) as required by the ethical committee of the University of Veterinary Medicine in Košice (Slovak republic).

Table 1: Composition of standard diet

Components	g.kg^{-1} feed	Chemical analysis	g.kg^{-1} feed
Corn	590	Dry matter	860
Soybean meal	254	Crude protein	210
Full fat Soybean	65	Crude fibre	35
Wheat	11	Ash	70
Fish meal	25		
Yeast	25	Calculated analysis	
Limestone	15	Linoleic acid	10.0
DL-Methionine	2	Calcium	8.0
Monocalcium phosphate	8	Phosphorus (total)	6.0
Sodium chloride	3	Lysine	11.0
Vitamin premix[1]	1	Methionine+Cystine	7.5
Trace-mineral premix[2]	1	Metabolizable energy MJ.kg^{-1}	12.0

[1] Supplying per kg diet: vit. A, min. 10000 IU; vit. D3, min. 2000 IU; vit. E, 25 mg; vit. B2, min. 4 mg; vit B12 20 μg; folic acid, 1 mg. 2Supplying per kg diet: Zn, 50 mg; Mn, 50 mg; Fe, 60 mg; Cu, 6.0 mg, Se, 0.75 mg

Processing of chickens. On the 42nd day of age broiler chickens were slaughtered. Bleeding of chickens followed after stunning in respect of rules established for slaughtering of animals and it was performed by responsible veterinary surgeon. Afterwards, all poultry carcasses were weighted, deboned, skin was removed and chilled (4 °C). Packed samples of breast and thigh muscles (in polyethylene sacks) were stored in freezer at – 21 °C during 3, 6 and 12 months. Samples were defrost at chilling conditions (4 °C) for the duration of 12 hours before analysis and homogenized in grinder.

Partition of defrost samples (10 pieces) taken from both groups was stored at chilling conditions (4 °C) during 12 hours, ground (∅ 4,5 mm) and thermally treated at 80 °C during 15 minutes, to determine antioxidative effect of added oregano oil in poultry meat after thermal treatment.

Evaluation of Thiobarbituric acid assay. Decomposition of fats is assessed by the thiobarbituric acid (TBA) assay. This test is simple, fast and it is based on the reaction of 1 molecule MDA with 2 molecules

of TBA (15). The color of the final complex is pink and the absorbance of the complex is measured spectrophotometrically. MDA is major degradation product of oxidation of polyunsaturated fatty acids. Evaluation of TBA was performed according to Marcinčák et al. (14) and measured spectrophotometrically at 532 nm (Helios ϒ, v. 4.6, Thermo spectronic, Great Britain).

Statistical analysis. Statistical processing of results was performed by Graph Pad Prism 3.0 (1999). Results are expressed as arithmetic mean (x) and standard deviation (sd). Comparison of results among groups was statistically evaluated by Student t-test. Advanced growth of malondialdehyde in both groups during storage was compared by one-way ANOVA test. Tukey comparison test was used to compare statistical differences between values and $P < 0.05$ was considering as statistically significant difference.

Results

Table 2 shows average weights during feeding period in both groups of broilers. Addition of oil extract of oregano in diet was manifested within 7 days of feeding and for the duration of feeding broilers from group EO achieved higher weight than control group (K). At the end of feeding period (42nd day), average weight of broilers supplemented with extract of oregano was 2563 g in comparison with control group 2462 g. However, weight differences between groups were not statistically significant ($P > 0.05$). No differences in feed intake and feed : gain ratio were observed in broilers fed with oregano essential oil in comparison with control (data not presented).

Table 2: Average weight of broilers during feeding period

	Day of feeding						
	1.	7.	14.	21.	28.	35.	42.
K	64 ± 7	192 ± 22	353 ± 29	696 ± 74	1242 ± 118	1944 ± 210	2462 ± 195
EO	68 ± 6	215 ± 17	409 ± 28	766 ± 65	1326 ± 104	1972 ± 142	2563 ± 140

In table 3 are expressed results of determination of TBA value in meat samples stored in freezer at -21 °C. Immediately after slaughter processing, concentration of MDA was slightly lower in breast muscles of broilers from group EO than in control group ($P > 0.05$). Storage of samples in freezer (- 21 °C) for 3 months caused moderate growth of MDA in control group ($P > 0.05$). MDA values in samples obtained from broilers supplemented with oil extract of oregano remained stable during this period. Significant growth of MDA concentration in samples of breast muscles became obvious in both groups within 6 months of storage at freezing conditions (- 21 °C). Growth was more significant in control group (0.184 ± 0.018 mg.kg-1) in comparison with EO group (0,138 ± mg.kg-1), what indicates higher oxidation stability of poultry meat after addition of oil extract of oregano ($P < 0.05$). The same phenomena was observed within 12 months of storage.

Immediately after slaughter processing, MDA concentrations in thigh muscles were higher than in breast muscles in both groups ($P < 0.05$) and MDA concentration in thigh muscles from control group was significantly higher than in EO group ($P < 0.05$). The same data were determined also within storage of samples for 3, 6 a 12 months in freezer at - 21 °C ($P < 0.05$).

Table 3: Oxidation changes of fats expressed as amount of malondialdehyde ($mg.kg^{-1}$) during storage of samples in freezer (- 21 °C)

		Storage (months)			
		0	3	6	12
K	Breast	0.140 ± 0.014	0.151 ± 0.015	0.184 ± 0.018	0.496 ± 0.088
	Thigh	0.235 ± 0.020	0.237 ± 0.010	0.272 ± 0.021	0,652 ± 0.102
EO	Breast	0.121 ± 0.009	0.122 ± 0.011	0.138 ± 0.013	0.311 ± 0.098
	Thigh	0.177 ± 0.013	0.181 ± 0.007	0.197 ± 0.009	0.465 ± 0.072

Table 4: Oxidation changes of fats expressed as amount of malondialdehyde (mg.kg^{-1}) after thermal treatment (80 °C, 15 min).

		Storage (months)			
		0	3	6	12
K	Breast	0.24 ± 0.06	0.38 ± 0.07	0.46 ± 0.14	1.51 ± 0.21
	Thigh	0.59 ± 0.13	0.66 ± 0.15	0.94 ± 0.21	2.35 ± 0.27
EO	Breast	0.19 ± 0.04	0.22 ± 0.05	0.31 ± 0.17	0.79 ± 0.17
	Thigh	0.34 ± 0.07	0.48 ± 0.007	0.54 ± 0.14	1.72 ± 0.14

Table 4 shows results of determination of TBA value in breast and thigh muscles from both groups after thermal treatment of samples. Thermal treatment accelerated oxidation processes in both groups in comparison with untreated samples ($P < 0.05$). Following storage and thermal treatment increased MDA concentrations as a consequence of accelerated oxidation processes. Addition of oil extract of oregano had positive effect on delaying of oxidation of fats in comparison with control samples ($P < 0.05$). Thigh meat was again more susceptible to lipid oxidation compared to breast meat.

Discussion

Obtained results indicated that addition of oregano in dose 0.05 % per kg of feed gently increased the slaughter weight of chicken. These results correspond with data (6, 14, 19), which indicate moderate higher weight of broilers after feeding of plant extracts. Plant extracts effects may be due to the greater efficiency in the utilization of feed, resulting in enhanced growth. There is evidence to suggest that herbs, spices, and various plant extracts have apetite- and digestion-stimulating properties and antimicrobial effects (9).

Poultry meat contains less amount of fat than red meat of slaughter animals. On average, broilers contain from 3.5 to 5.0 % of fat. Poultry fat has higher amount of unsaturated fatty acids (PUFA) than fat of other slaughter animals. Regarding higher amount of PUFA, poultry meat is more sensitive to oxidative processes (17). Feeding and conditions used for breeding and slaughtering can influence oxidative stability of meat (2, 8). The most ideal situation is when fats are protected immediately they are derived. It means before slaughtering of animals. It is possible gain this fact when tissues are saturated with antioxidants as an additives during the life of animals (6, 13). Govaris et al. (8) stated that, post-mortem addition of antioxidants to the mince meat also retarded lipid oxidation in the prepared patties compared to control; however, this effect was inferior to that of dietary supplementation even though the post-mortem α-tocopherol supplemented meat contained 90-fold more α-tocopherol than patties from the dietary supplemented meat.

Immediately after slaughter processing, MDA concentrations were low in both groups. However, following storage caused growth of MDA in samples. Obtained results indicate that addition of antioxidants had significant effect on reducing of oxidation processes in meat. Our results confirmed that thigh meat samples suffered more intensive lipid oxidation than breast meat samples, throughout the 12 months storage period. This is generally in agreement with other research studies that have investigated the effects of oregano essential oils in meat protection from oxidation through feeding (5, 6, 8, 17). Thigh meat was more susceptible to lipid oxidation compared to breast meat. The greater susceptibility of thigh to lipid oxidation has been attributed to the higher content of PUFA in this tissue (15, 17).

Process of production of meat products (cutting, grinding, and mixing) causes degradation of muscle membrane system and has a strong influence on oxidation of intracellular fat, primarily phospholipids (2). In thermally treated meat products level of oxidation depends on intensity of thermal treatment. Thermal treatment accelerated oxidation expressed in both groups as high TBA values in comparison with untreated samples ($P < 0.05$). Addition of oil extract of oregano had positive effect on delaying of oxidation of fats in comparison with control samples ($P < 0.05$). Consistently with our results, Florou-Paneri et al. (6) reported that both the oregano herb and essential oil were effective in delaying lipid oxidation of breast and thigh meat during refigerated storage, when they were dietary supplemented to turkeys. Similar results were described by Govaris et al.(8), who reported that addition of oregano oil in feed of turkeys and post mortem in ground meat increases stability of fat oxidation.

The lower MDA values found in tissues after diet supplementation with oregano, are probably the results of various antioxidants constituents that entered the circulatory system, distributed and retained in the tissues, exhibiting antioxidant activity (6)

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ANTIOKSIDATIVNI UČINEK ORIGANA KOT PREHRANSKEGA DODATKA BROJLERJEM NA OKSIDATIVNO STABILNOST PIŠČANČJEGA MESA

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Povzetek: Preiskali smo učinek oljnega ekstrakta origana na prirastek piščancev in oksidativno stabilnost njihovega mesa. Brojlerji, ki smo jih krmili z origanom kot prehranskim dodatkom so dosegali večjo telesno maso (2563 ± 140 g) v primerjavi s kontrolno skupino (2462 ± 195 g). Oksidativni proces v mesu smo spremljali z ugotavljanjem vsebnosti malondialdehida v prsnem fileju in stegenskem mesu na začetku shranjevanja pri -21°C ter po treh, šestih in dvanajstih mesecih. Da bi ugotovili antioksidativni učinek origana po termični obdelavi, smo koščke odmrznjenega mesa do 12 ur hranili pri 4 °C in jih termično obdelovali pri 80 °C petnajst minut. Rezultati kažejo, da esencialno olje origana učinkovito zadržuje oksidacijo maščob v zamrznjenem mesu v vseh preiskanih časovnih obdobjih. Stegensko meso je bolj podvrženo oksidaciji maščob, kot pa prsi ($P < 0,05$). Enak učinek ekstrakta origana je bil ugotovljen v mesu tudi po termični obdelavi ($P < 0.05$).

Ključne besede: brojlerji; prirastek; oksidacija maščob; esencialno olje - origano