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Oregano Herb Versus Oregano Essential Oil as Feed Supplements to Increase the Oxidative Stability of Turkey Meat

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Abstract: The objective of this study was to investigate the use of oregano herb versus oregano essential oil as feed supplements to increase the oxidative stability of turkey meat stored at 4°C. Thirty 12-week-old turkeys allocated into five groups were fed a control diet and diets supplemented with 5 g oregano herb/kg, 10 g oregano herb/kg, 100 mg oregano essential oil/kg, and 200 mg oregano essential oil/kg, for 4 weeks prior to slaughter. Lipid oxidation was assessed by monitoring malondialdehyde formation in breast and thigh meat at 0, 3, 6 and 9 days of refrigerated storage. Results showed that the feed supplements increased the oxidative stability of meat without exerting any effect on feed intake and daily weight gain of turkeys. Oregano essential oil supplementation at 100 mg/kg was more effective in delaying lipid oxidation compared to the control diet at all time points, but inferior to the oregano herb at 5 g/kg. Also, oregano essential oil at 200 mg/kg was more effective than the oregano herb at 5 g/kg and equivalent to oregano herb at 10 g/kg, in delaying lipid oxidation. Thigh meat was more susceptible to lipid oxidation compared to breast meat.

Key words: Oregano, essential oil, feed supplements, oxidative stability, turkey meat, thigh, breast

Introduction

Poultry meat is relatively rich in polyunsaturated fatty acids (Igene and Pearson, 1979) and is, therefore, readily susceptible to oxidative deterioration (Kanner *et al*., 1988). The susceptibility to oxidative deterioration differs among poultry species being higher for turkey meat compared to chicken meat, although these two species present comparable fatty acid compositions (Marusich *et al*., 1975). The difference has been attributed primarily to the weaker ability of turkeys to store dietary tocopherol in their tissues compared to chickens (Sklan *et al*., 1982; Wen *et al*., 1997). Within tissues, α-tocopherol is localized in the highly unsaturated phospholipid bilayer of the cell membranes where it inhibits lipid oxidation by functioning as a free-radical scavenger. Hence, the antioxidant capacity of turkey meat depends largely on the concentration of the contained α-tocopherol, which in turn is dependent on the level of α-tocopheryl acetate added in the diet (Machlin, 1991; Wen *et al*., 1997).

Apart from tocopherol, additional dietary natural antioxidants would also have the potential to increase the antioxidant capacity of turkey meat. Several recent reports have shown that extracts of rosemary and sage (Lopez-Bote *et al*., 1998), tea catechins (Tang *et al*., 2000, 2001), oregano essential oil (Botsoglou *et al*., 2002a,b; 2003a), and a blend of several essential oils (Botsoglou *et al*., 2004) improved the oxidative stability of stored chicken meat when added in diets. However, in turkeys, only the essential oil of oregano has been yet investigated as an antioxidant feed supplement (Botsoglou *et al*., 2003b,c).

The essential oil of oregano obtained by a steam-distillation process from leaves and flowers of *Origanum vulgare* subsp. *hirtum* plants, is well known for its antioxidative activity (Economou *et al*., 1991). Carvacrol and thymol, the two main phenols that constitute about 78-82% of the essential oil of oregano, are principally responsible for this activity (Adam *et al*., 1998). In addition, other minor constituents such as α-terpinene and p-cymene, two monoterpene hydrocarbons that constitute about 5% and 7% of the total oil, respectively, also contribute to this activity (Adam *et al*., 1998; Kokkini, 1994). However, the oregano plants, apart from these volatile phenolic antioxidant compounds occurring in the essential oil (Sivropoulou *et al*., 1996; Adam *et al*., 1998), contain a variety of glycosidically bound volatile and non-volatile constituents that also exhibit biological activity after enzymatic or acid hydrolysis (Vekiari *et al*., 1993; Milos *et al*., 2000). Therefore, oregano plants might be more biologically active than their essential oil when incorporated in poultry diets. The objective of the

Table 1: Composition of basal diet

Components	g/kg	Analysis	g/kg
Maize	539.0	Chemical analysis[3]	
Soybean meal	310.0	Dry matter	893
Herring meal	25.0	Crude protein	221
Soybean oil	25.0	Fat	51
Yeast	25.0	Crude fibre	46
Corn gluten feed	40.0	Ash	63
Limestone	17.0		
Monocalcium phosphate	10.0		
Salt	3.4		
Biolysine-BASF	2.2	Calculated analysis	
DL-Methionine	1.0	Calcium	9
Vitamins premix[1]	1.0	Phosphorus	6
Trace mineral premix[2]	1.0	Lysine	13
Choline chloride 70%	0.3	Methionine+Cystine	11
Phytase-BASF	0.1	Metabolizable energy (MJ/kg)	13.1

[1]Supplying per kg diet: all-trans retinol acetate, 3.44 mg; cholecalciferol, 125 μg; α-tocopheryl acetate, 30 mg; menadione sodium bisulphite, 7 mg; thiamine hydrochloride, 5 mg; riboflavin, 10 mg; pyridoxine hydrochloride, 10 mg; cyanocobalamin, 0.02 mg; niacin, 85 mg; pantothenic acid, 25 mg; folic acid, 2 mg; biotin, 0.25 mg; ascorbic acid, 10 mg. [2]Supplying per kg diet: Zn, 100 mg; Mn, 120 mg; Fe, 20 mg; Cu, 20 mg; Co, 0.2 mg; I, 1 mg; Se, 0.3 mg. [3]According to AOAC (1990).

present study was to investigate the use of oregano herb versus oregano essential oil as feed supplements to increase the oxidative stability of turkey breast and thigh meat stored at 4°C.

Materials and Methods

Animals and diets: Thirty 10-week-old female turkeys of black strain were used in this study. These birds were separated, on the basis of body weight, into five uniform groups of 6 turkeys each, housed in individual wire floor-pens and allowed to acclimate for a period of two weeks. During this period, all birds were fed on a basal turkey diet (Table 1). Feed in form of mash and drinking water were offered to birds *ad libitum*. After the end of the acclimatization period, turkeys were weighed, and feeding of the basal diet was discontinued to all but one of the groups. The birds within this group (CON group) were given the basal diet for further 4 weeks. The experimental diets given to the remaining four groups were based on the same diet supplemented further with 5 g oregano herb/kg (ORE5 group), 10 g oregano herb/kg (ORE10 group), 100 mg oregano essential oil/kg (EO100 group), and 200 mg oregano essential oil/kg (EO200 group). Oregano essential oil was in form of a solid commercial preparation (Orego-Stim, Meriden Animal Health Ltd., Luton, UK) that contains 5% essential oil of *Origanum vulgare* subsp. *hirtum* plants and 95% natural feed grade inert carrier. The oregano herb consisted of flowered tops, leaves, stems and stalks of *Origanum vulgare* subsp. *hirtum* plants (Ecopharm Hellas, SA, Kilkis, Greece) that had been ground to pass 2 mm screen. According to the supplier, the oregano plants contained 1.22% carvacrol and 0.07% thymol. Proximate analysis showed that the herb also contained 80 g moisture/kg, 131 g crude protein/kg, 44 g fat/kg, 197 g crude fiber/kg and 88 g ash/kg.

Following 4 weeks feeding, turkeys were weighed and slaughtered under commercial conditions. On the basis of body weight and feed consumption data, the daily weight gain and feed intake per bird, were calculated. Carcass from all birds was immediately trimmed for breast (*pectoralis superficialis*) and thigh (*biceps femoris*) by removing skin, bones and connective tissue. Following trimming, breast and thigh meat samples were vacuum packaged and stored at -25 °C pending the oxidative stability study.

Oxidative stability study: To study the effect of the dietary treatments on lipid oxidation, breast and thigh samples were thawed overnight at 4°C, and, then, separately minced through 5 mm plates using a domestic rust-free steel meat mincer. Samples from the minced meat were wrapped in transparent oxygen-permeable polyvinyl chloride film, and placed in a non-illuminated refrigerated cabinet at 4°C for a total of nine days. Determination of malondialdehyde, the compound used as an index of lipid peroxidation, was carried out at 0, 3, 6 and 9 days by a selective third-order derivative spectrophotometric method (Botsoglou *et al.*, 1994). In brief, 1 g samples were thoroughly homogenized (Ultra-Turrax, IKA® Labortechnik, Staufen, Germany) in the presence of 8 ml of aqueous trichloroacetic acid (50 g/l) and 5 ml of butylated hydroxytoluene in hexane (8 g/l), and the mixture was centrifuged. The top layer was discarded, and a 2.5 ml aliquot from the bottom layer was mixed with 1.5 ml of aqueous 2-thiobarbituric acid (8 g/l) to be further incubated at 70°C for 30 min. Following incubation, the mixture was cooled to room temperature and submitted to conventional spectrophotometry (Shimadzu, Model UV-160A, Tokyo,

Table 2: Effect of diet supplementation with oregano herb or its essential oil on some performance parameters of turkeys

Groups of turkeys	Initial body weight[1], g (12 weeks)	Final body weight, g (16 weeks)	Daily body weight gain, g	Daily feed intake, g
CON	2969±74[a]	4540±103[a]	56,1±1[a]	194±5[a]
EO100	2974±92	4524±122	55,3±2	190±9
EO200	2980±110	4532±118	55,4±1	196±8
ORE5	2961±124	4536±112	56,2±1	191±7
ORE10	2978±81	4522±146	55,1±2	193±10

[1]Mean value of 6 birds. [a]Values within column do not differ (p> 0.05)

Table 3 : Malondialdehyde concentrations in diets and fresh breast and thigh meat of turkeys

Groups of turkeys	MDA concn, ng/g		
	Diet	Breast meat	Thigh meat
CON	162±12[a]	59±4[a]	61±2[a]
EO100	164±11[a]	45±3[b]	49±3[b]
EO200	170±7[a]	42±3[b]	43±3[b]
ORE5	158±10[a]	44±5[b]	49±2[b]
ORE10	173±8[a]	40±6[b]	44±4[b]

[a,b] Values in the same column with common superscript do not differ (P>0,05)

Japan) in the range of 400-650 nm. Third-order derivative spectra were produced by digital differentiation of the normal spectra using a derivative wavelength difference setting of 21 nm. The concentration of malondialdehyde (ng/g wet tissue) in samples was calculated on the basis of the height of the third-order derivative peak at 521.5 nm by referring to slope and intercept data of the computed least-squares fit of the standard calibration curve prepared using 1,1,3,3-tetraethoxypropane. Butylated hydroxytoluene, 2-thiobarbituric acid, and 1,1,3,3-tetraethoxypropane, the precursor of malondialdehyde, were obtained from Sigma Chemical Co. (St. Louis, MO), whereas trichloroacetic acid from Merck (Darmstadt, Germany).

Statistical analysis: Data for body weight, weight gain and feed intake were subjected to analysis of variance (ANOVA) in the general linear model using the SPSS 12.00 statistical package (SPSS Ltd., Woking, Surrey, UK). The Levene's test was applied to test the homogeneity of the variances. The development of malondialdehyde in samples during storage was analyzed by a two-way analysis of variance, fixed effects model, including main effects of dietary treatment (5 levels) and time of storage (four levels), and interaction between the two factors. When significant treatment effects were disclosed at probability level of P<0.05, the Tukey's test was applied to determine statistical differences between means.

Results and Discussion

Feeding the experimental diets to turkeys, performance parameters were not affected. There were no differences (P>0.05) in final body weights, daily weight gain or feed intake among treatments (Table 2). This indicated that neither the oregano herb itself nor the essential oil exerted any growth-promoting effect when incorporated in turkey diets. These results give support to a previous study where dietary supplementation of oregano essential oil at levels of 100 and 200 mg/kg in turkeys had no beneficial effect on growth performance (Papageorgiou *et al.*, 2003). Consistently with these results, Botsoglou *et al.* (2002a) reported that dietary oregano essential oil at levels of 50 and 100 mg/kg in chickens did not influence growth performance. Also, Basmacioglu *et al.* (2004) found that dietary oregano essential oil at levels of 150 and 300 mg/kg did not improve performance in chickens. However, Hertrampf (2001) noted that oregano essential oil supplemented through the drinking water at 300 ml/1000 l improved chicken performance.

The observed lack of growth-promoting effect, although inconsistent with the high antimicrobial activity of oregano essential oil (Sivropoulou *et al.*, 1996), may relate to the digestibility of the basal diet and/or the environmental conditions. Growth promoting agents may have more impact when the diet used is less digestible. In addition, well-nourished healthy birds do not respond to growth promoting supplements when they are housed under clean, disinfected conditions and moderate stocking density. Consequently, little or no response can be expected at high performance standards, but with a substandard performance the response to dietary herbal essential oils might increase. In the present study, the birds were kept in clean environment, possibly leading to diminished efficacy, if any, of the dietary additives.

Although neither the dietary oregano herb nor the essential oil of oregano could improve turkey performance, both additives were effective in increasing the oxidative stability of the fresh meat. Both breast and thigh fresh meat of the control group presented mean MDA values higher (P<0.05) than all other groups, which did not differ among each other (Table 3). The higher levels of MDA found in the control meat, indicated higher

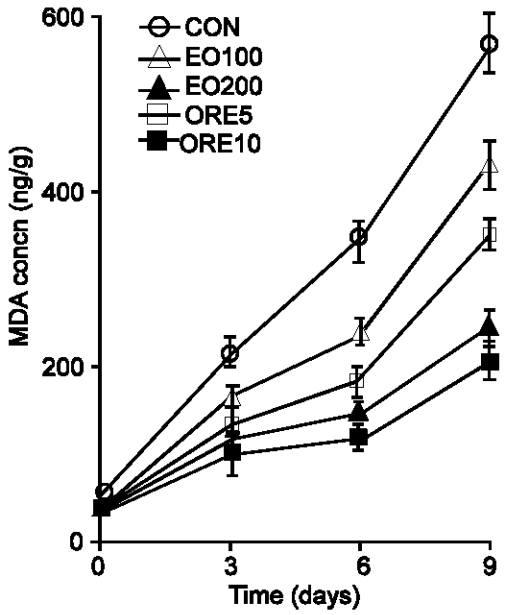

Fig. 1: Effect of refrigerated storage on lipid oxidation of raw turkey breast meat as a function of diet supplementation with ground oregano at 5 g/kg (ORE5) and 10 g/kg (ORE10), and essential oil of oregano at 100 mg/kg (EO100) and 200 mg/kg (EO200), compared to control diet (CON). All data points represent mean MDA values of six samples and their standard deviations.

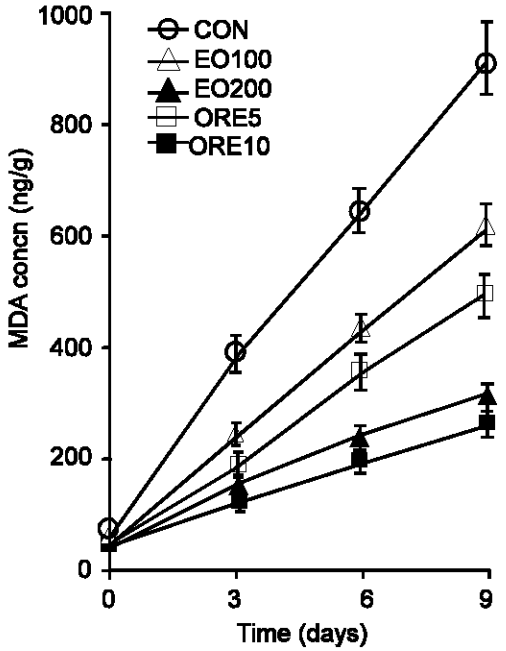

Fig. 2: Effect of refrigerated storage on lipid oxidation of raw turkey thigh meat as a function of diet supplementation with ground oregano at 5 g/kg (ORE5) and 10 g/kg (ORE10), and essential oil of oregano at 100 mg/kg (EO100) and 200 mg/kg (EO200), compared to control diet (CON). All data points represent mean MDA values of six samples and their standard deviations.

in vivo production and deposition of MDA in these tissues. These levels of the control group could not be attributed to consumption and subsequent deposition of the MDA already present in the diets since they all contained not differing (P<0.05) MDA levels (Table 3). Possible transfer of antioxidant constituents of oregano herb and essential oil of oregano into turkey organism through feeding might inhibit the chain reaction involved in lipid oxidation, thus decreasing MDA in meat.

The susceptibility of breast meat to lipid oxidation as a function of the dietary supplementation with oregano herb or the essential oil of oregano and the time of refrigerated storage is presented in Fig. 1. At all time points, the Control group presented MDA values higher (P<0.05) than all other groups. At day 3, all groups exhibited higher (P<0.05) MDA values compared to day 0. At this day, the EO100 group showed a mean MDA value lower (P<0.05) than the control group but higher (P<0.05) than the ORE5, EO200 and ORE 10 groups, which in turn did not differ (P>0.05) among each other. At day 6, the EO100 group continued to present an MDA value higher (P<0.05) than the ORE5 group; at this day, however, the mean MDA value of the ORE5 group was higher (P<0.05) than those of the EO200 and ORE10 groups, which did not differ (P>0.05) each other. The lipid oxidation profile observed at day 6 did not change at day 9, however, MDA values were much higher (P<0.05), indicating more intense lipid oxidation.

The susceptibility of thigh meat to lipid oxidation as a function of the dietary supplementation with oregano herb or the essential oil of oregano and the time of refrigerated storage is presented in Fig. 2. The observed lipid oxidation profile was generally comparable to that of breast meat, however, lipid oxidation was more intense. All groups exhibited progressively higher (P<0.05) MDA values on days 3, 6 and 9 of storage. Again, the EO100 group showed a mean MDA value lower (P<0.05) than the control group but higher (P<0.05) than the ORE5 group, which in turn was higher (P<0.05) than the EO200 and ORE10 groups that did not differ (P>0.05) among each other.

The higher susceptibility of thigh meat to oxidation has been attributed to its higher absolute content of polyunsaturated fatty acids with more than two double bonds (Jensen *et al.*, 1997). Although breast meat has a higher percentage of these acids in fat, the absolute amount in thigh meat is three times higher than in breast meat regardless of the dietary treatment because the total fat content in thigh meat is approximately five times that of breast meat (Jensen *et al.*, 1997). In

addition, the large amount of pro-oxidative agents originating from tissue myoglobin and other iron containing proteins found in thigh meat may also reduce the oxidative stability in this tissue (Rhee and Ziprin, 1987).

The results presented in this study show that both the oregano herb and the essential oil were effective in delaying lipid oxidation of breast and thigh meat during refrigerated storage, when they were dietary supplemented to turkeys. Oregano herb at the supplementation level of 10 g/kg was more effective than the level of 5 g/kg, but equivalent to oregano essential oil supplemented at the level of 200 mg/kg. Oregano essential oil at the supplementation level of 100 mg/kg diet was less effective than oregano herb at the level of 5 g/kg. The better oxidative stability offered by dietary oregano herb and its essential oil are probably the result of antioxidant components that entered the circulatory system and distributed in tissues, exhibiting antioxidant activity. However, there has not been yet developed analytical method capable for identification and quantification of any of the oregano oil constituents at trace levels in tissues. Therefore, the bioavailability of any of these compounds cannot be yet directly demonstrated.

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