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Hen Performance and Egg Quality as Affected by Dietary Oregano Essential Oil and α-tocopheryl Acetate Supplementation

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Abstract: In this study, the effect of feeding oregano essential oil and α-tocopheryl acetate on hen performance and egg quality, were investigated. Ninety-six Lohmann laying hens, 32-week-old, were allocated into four groups. One of the groups was given a control diet (CONT), another group a diet supplemented with 200 mg/kg α-tocopheryl acetate (VIT-E), whereas the other two groups were given diets supplemented with oregano essential oil at levels of 50 and 100 mg/kg (OR-50 and OR-100, respectively). Following 60 days feeding, hen performance and some egg quality characteristics were determined, whereas the oxidative stability of the refrigerated stored shell eggs and liquid yolks was also examined. Results showed that there were no significant (P>0.05) differences in egg production, feed consumption, feed conversion ratio, egg weight and shape, yolk diameter, height and color, Haugh units, and shell thickness, among the dietary treatments. The extent of lipid oxidation in shell eggs differed (P<0.05) between the dietary treatments, but did not change with the storage time. In liquid yolks, lipid oxidation was higher (P<0.05) in the CONT group compared to the OR-50 group, which in turn exhibited higher (P<0.05) oxidation rate than the OR-100 group, a finding suggesting that oregano exerted a dose dependent antioxidative activity. The VIT-E group presented lower (P<0.05) lipid oxidation rate compared to all other groups.

Key words: Oregano essential oil, α-tocopherol, hen performance, yolk stability, lipid oxidation, egg quality

Introduction

Lipid composition of chicken egg has been a primary area of consumer concern due to the relationship of specific dietary lipids with the development of coronary heart disease (Simopoulos and Salem, 1992). As a result, feeding strategies are being incorporated to increase the n-3 fatty acid content of chicken eggs (Cherian *et al*., 1996). However, with increasing polyunsaturated fatty acids content of poultry diets, there is a concomitant increase in the susceptibility to oxidative deterioration of eggs, leading to losses in quality characteristics and nutritional value, lower consumer acceptability and deleterious biological effects (Addis and Park, 1989).

Supplementation of poultry diets with antioxidant substances seems to be an efficient means for improving the oxidative stability of eggs. The effect of dietary α-tocopheryl acetate supplementation on enhancing lipid stability in egg yolk has been repeatedly reported (Aymond and van Elswyk, 1995; Cherian *et al*., 1996; Lopez-Bote *et al*., 1998). Use of aromatic plant extracts including thyme (Botsoglou *et al*., 1997) and rosemary (Galobart *et al*., 2001) have also been demonstrated to delay lipid oxidation in eggs when used in hen feeding.

The essential oil of *Origanum vulgare* subsp. *hirtum*, a characteristic spice of the Mediterranean cuisine, has not been yet examined for its potential to delay lipid oxidation in eggs when used in hen feeding, although it is well known for its antimicrobial and antioxidant properties (Economou *et al*., 1991 Sivropoulou *et al*., 1996). There have been several reports on the potential of the essential oil of oregano to inhibit lipid oxidation in lard (Vekiari *et al*. 1993; Milos *et al*., 2000) and mackerel oil (Tsimidou *et al*., 1995) when added exogenously or to delay lipid oxidation of chicken, turkey and rabbit meat when supplemented into feeds (Botsoglou *et al*., 2002a,b; 2003a,b,c; Botsoglou *et al*., 2004). Major components principally responsible for these properties are carvacrol and thymol that constitute about 78-82% of the essential oil (Adam *et al*., 1998; Yanishlieva *et al*., 1999). In addition, other minor constituents such as the two monoterpene hydrocarbons, γ-terpinene and p-cymene, which often constitute about 5% and 7% of the total essential oil, respectively, also contribute to the antioxidant activity (Adam *et al*., 1998).

Since there has not been yet any report dealing with the antioxidant effect of dietary supplemented oregano on

Table 1: Composition of the basal hen diet

Components	[g/kg feed]	Chemical analysis[3]	[g/kg feed]
Maize	640	Dry matter	891
Soybean meal	125	Crude protein	150
Full-fat soybean meal	75	Ether extract	50
Herring meal	25	Crude fibre	42
Wheat bran	35	Ash	94
DL-Methionine	1	Calcium	31.9
Limestone	80	Phosphorus (total)	6.6
Dicalcium phosphate	12	Calculated analysis	
Sodium chloride	3	Lysine	8.4
Vitamin premix[1]	2	Methionine+cystine	6.7
Trace-mineral premix[2]	2	Metabolizable energy [MJ/kg]	11.6

[1]Provided per kg of diet: 12500 IU vit. A, 1250 IU vit. D_3, 30 mg vit. E, 2 mg vit. B_1, 4 mg vit. B_2, 3 mg vit. B_6, 0.02 mg vit. B_{12}, 2 mg vit. K_3, 20 mg nicotinic acid, 10 mg pantothenic acid, 1mg folic acid, 0.08 mg biotin, 50 mg vit. C, 300 mg choline.
[2]Provided per kg of diet: 80 mg Zn, 40 mg Mn, 160 mg Fe, 7 mg Cu, 0.2 mg Co, 1 mg I, 0.2 mg Se. [3]According to AOAC, 1990.

eggs, the objective of this study was to evaluate the use of the essential oil of oregano and α-tocopheryl acetate in hen feeding to promote performance and delay lipid oxidation in eggs. Possible incorporation of the antioxidant constituents of oregano into egg through feeding might help in reducing or eliminating the need for additional oxidative stabilization of processed egg yolk.

Materials and Methods

Chemicals: Analytical-grade butylated hydroxytoluene, 2-thiobarbituric acid, trichloroacetic acid, hexane, sodium azide, and 1,1,3,3-tetraethoxypropane, the precursor of malondialdehyde, were obtained from Sigma Chemical Co. (St. Louis, MO). α-Tocopheryl acetate was obtained from Roche Products Ltd. (Hertfordshire, UK), while oregano essential oil from Ecopharm Hellas S.A. (Kilkis, Greece) in form of a powder called Orego-Stim (Meriden Animal Health Ltd. (Luton, UK) that contains 5% oil of *Origanum vulgare* subsp. *hirtum* plants and 95% natural feed grade inert carrier.

Animals and diets: Ninety-six Lohmann laying hens, 32-week-old, were used in this study. The birds were assigned into four dietary treatments replicated four times with six hens per replicate. Dietary treatments included a corn-soybean-based typical layer diet (Table 1) that served as the control (CONT), two diets based on the typical diet further enriched with 100 mg/kg and 200 mg/kg oregano essential oil (OR-50, OR-100), respectively, and another diet based on the same typical diet further enriched with 200 mg/kg α-tocopheryl acetate (VIT-E). Diets were formulated to meet the requirements for nutrient and energy content for laying hens (National Research Council, 1994) and stored in airtight containers. During the feeding period that lasted 60 days, diets and water were provided *ad libitum*, whereas the lighting regimen was 15 h of continuous light per day.

All birds were weighed at the start of the experiment and at intervals of 15 days until the end of the experiment. Feed consumption, egg production and total egg weight were recorded daily. Egg quality characteristics including egg weight, egg shape index, yolk diameter, yolk height, yolk color, Haugh units and shell thickness, were measured weekly using 8 eggs from each dietary treatment.

In addition, 10 eggs from each replicate totaling 40 eggs from each dietary treatment were used for lipid oxidation studies. Egg collection for this purpose commenced 45 days after feeding the dietary treatments and lasted 2 weeks. All eggs collected during that period, were stored at 4°C, pending further handling.

Lipid oxidation studies: To investigate the effect of diet on lipid oxidation of shell eggs during refrigerated storage, 4 freshly collected eggs from each subgroup totaling 16 eggs from each dietary treatment were placed in a refrigerated cabinet at 4°C to be analyzed for malondialdehyde (MDA) levels in yolk in sets of 4 eggs at 0, 20, 40 and 60 days of storage. To investigate further the effect of diet on the oxidative stability of liquid yolk, 6 eggs from each subgroup totaling 24 eggs from each dietary treatment were broken, yolks were separated, and adhering albumen was removed by rolling on a paper towel. Yolk pools were prepared from each subgroup, and the contents were mixed with a wire whisk.

For studying the effect of diet on the oxidative stability of liquid yolk (Botsoglou *et al.*, 1997), two 9-g portions were taken from each pool and transferred into a 100-ml flask where 16 ml of water were also added. The pH of flask content was left at the value of 6.2. Water addition was indispensable for decreasing yolk viscosity and providing rapid dispersion of 50 μl of sodium azide solution (60 mg/ml) added thereafter to prevent microbial growth. All flasks were then covered with air-permeable film to retain moisture yet not to exclude air,

Table 2: Body weight of layer hens during the feeding trial with oregano essential oil and α-tocopheryl acetate for 60 days

Dietary treatments	Weight at day 0 [g]	Weight at day 15 [g]	Weight at day 30 [g]	Weight at day 45 [g]	Weight at day 60 [g]	SEM	P Value
CONT	2215	2183	2131	2118	2142	28.4	>0.05
OR-50	2126	2145	2168	2175	2193	22.3	>0.05
OR-100	2194	2176	2165	2162	2146	19.2	>0.05
VIT-E	2204	2188	2171	2184	2194	21.0	>0.05

Table 3: Effect of dietary oregano essential oil and α-tocopheryl acetate on body weight, egg production, feed consumption and feed conversion ratio

Dietary Treatments	Final Body weight [g]	Egg production [%]	Daily feed consumption [g/hen]	Feed conversion ratio [g feed/g eggs]
CONT	2142	89.8	104.8	1.77
OR-50	2193	91.9	107.0	1.80
OR-100	2146	91.0	101.8	1.74
VIT-E	2194	90.7	102.6	1.76
SEM	23.6	0.43	0.95	0.02
P value	>0.05	>0.05	>0.05	>0.05

and submitted to medium agitation on a temperature-controlled shaker bath (GFL, Gmbh, Hannover, Germany) at 20°C in artificial light. For ensuring even exposure of samples to air during the 15-days period that lasted the agitation, all flasks were inspected daily for film cracking. The first day of agitation, and at time intervals of 5 days thereafter, 2-g samples were removed from each flask and directly analyzed for MDA concentration.

Determination of MDA in yolk: Lipid oxidation was assessed on the basis of the MDA formed during refrigerated storage. MDA, the compound used as an index of lipid peroxidation, was determined by a selective third-order derivative spectrophotometric method (Botsoglou *et al.*, 1994). In brief, yolk samples were homogenized (Polytron homogenizer, PCU, Switzerland) in presence of 8 ml of 5% aqueous trichloroacetic acid and 5 ml of 0.8% butylated hydroxytoluene in hexane, 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 0.8% aqueous 2-thiobarbituric acid, to be further incubated at 70°C for 30 min. Following incubation, the mixture was cooled under tap water and submitted to conventional spectrophotometry (Shimadzu, Model UV-160A, Tokyo, 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 MDA in analyzed samples (ng/g yolk) 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 a standard calibration curve prepared using 1,1,3,3-tetraethoxypropane, the precursor of MDA.

Statistical analysis: All data were computerized using the SPSS 12.00 statistical package (SPSS Ltd., Woking, Surrey, UK). Before statistical analysis, the Levene's test was applied to test the homogeneity of the variances. Both performance and lipid oxidation data were analyzed by analysis of variance using the completely randomized design. When the effect of factors was significant, the Tukey's test was applied to test the statistical significance of data at the probability level of $P<0.05$.

Results and Discussion

Hen performance: The effect of dietary treatments on body weight values at 15-days intervals is shown in Table 2. Body weight was not significantly ($P>0.05$) changed with the dietary treatment and the feeding time. Other dietary effects on hen performance parameters including final body weight, egg production, feed consumption and feed conversion ratio values, are presented in Table 3. None of these parameters was significantly ($P>0.05$) changed with the dietary treatment. These findings come to agreement with previous reports (Jiang *et al.*, 1994; Qi and Sim, 1998) showing no significant differences in egg production and feed consumption when laying hens were given a diet supplemented with up to 200 mg α-tocopheryl acetate/kg. Jiang *et al.* (1994), in particular, reported a decreased feed consumption when hens were fed 400 mg α-tocopheryl acetate/kg, whereas Qi and Sim (1998) did not noticed such an effect even when the hens were given a diet supplemented with up to 800 mg α-tocopheryl acetate/kg. As far as the effect of dietary supplementation with oregano essential oil on layers performance is concerned, there have not been yet pertinent studies to compare with.

Table 4: Effect of dietary oregano essential oil and α-tocopheryl acetate on some egg quality characteristics

Dietary treatments	Egg weight, g	Egg shape index	Yolk diameter, mm	Yolk height, mm	Yolk color	Haugh units	Shell thickness, mm
CONT	66.0	77.3	42.6	20.1	10.0	87.0	0.36
OR-50	65.3	77.8	43.1	19.8	10.2	85.7	0.36
OR-100	65.2	77.8	42.4	19.7	10.0	85.7	0.37
VIT-E	65.3	76.5	42.8	19.9	10.2	86.4	0.37
SEM	0.20	0.15	0.18	0.10	0.06	2.2	0.24
P value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05

Egg quality: The effect of dietary treatments on some egg quality characteristics is shown in Table 4. There were no significant (P>0.05) differences in egg weight and shape, yolk diameter, height and color, Haugh units, and shell thickness, among the dietary treatments. Previous reports on the use of α-tocopheryl acetate in hen diets at the level of 200 mg/kg also showed no significant effect on egg weight, yolk weight and Haugh units (Jiang *et al.*, 1994; Qi and Sim, 1998). Pertinent reports on the effect of dietary supplementation with oregano essential oil on egg quality characteristics there have not been yet in literature to compare with.

Lipid oxidation of shell eggs: The effect of dietary treatments on lipid oxidation of shell eggs refrigerated stored for 60 days is shown in Fig. 1. The extent of lipid oxidation, as measured by MDA formation, differed (P<0.05) between the dietary treatments but did not change with storage time. The VIT-E treatment exhibited the lower (P<0.05) MDA values among treatments. The OR-100 treatment exhibited MDA values lower (P<0.05) than the OR-50 treatment, whereas the CONT treatment presented the higher (P<0.05) MDA values among treatments.

The MDA found in yolks of the fresh eggs might be attributed to either the consumption and subsequent deposition of MDA that was already present in the diets or to *in vivo* production of MDA by the hens during the feeding trial. The former possibility appears unlikely because in that case the levels of MDA should have been equal among treatments. However, MDA analysis revealed not differing (P>0.05) values among diets. Therefore, the latter possibility seems to reasonably explain the lower values of MDA found in eggs from the hens fed oregano essential oil or α-tocopherol as compared to controls (Fig. 1). Possible transfer of the antioxidant constituents of the essential oil of oregano or α-tocopherol into hen organism through feeding might inhibit the chain reaction involved in oxidation of the consumed lipids, thus decreasing the oxidation products transferred into the yolk.

Consistently with these results, other workers (Marshall *et al.*, 1994; Aymond and Van Elswyk, 1995) also found MDA levels that could not be attributed to lipid oxidation during shell egg storage. The higher MDA

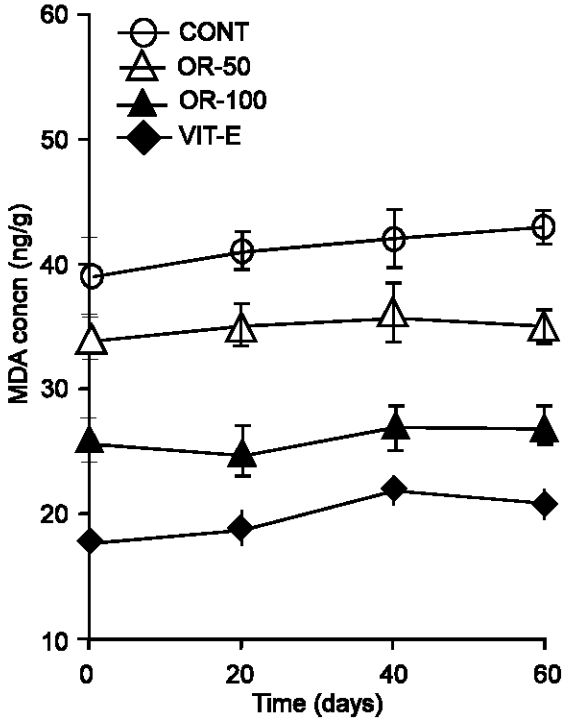

Fig. 1: Effect of refrigerated storage on lipid oxidation of yolks of shell eggs as a function of dietary supplementation with oregano essential oil at 50 mg/kg (OR-50) and 100 mg/kg (OR-100), and α-tocopheryl acetate at 200 mg/kg (VIT-E), compared to control diet (CONT). All data points represent mean MDA values of four samples and their standard deviations, some of which however lie within the data points.

concentrations (180 ng/g) found by these workers most likely reflect differences in the methods applied to determine lipid oxidation in eggs, as the widely used distillation method (Tarladgis *et al.*, 1964) may cause oxidation of lipids during the distillation stage even in presence of added antioxidants (Raharjo *et al.*, 1993). As far as the antioxidant effect of the essential oil of oregano on egg yolk is concerned, direct comparison with other studies cannot be made due to lack of pertinent reports. Nevertheless, other workers have

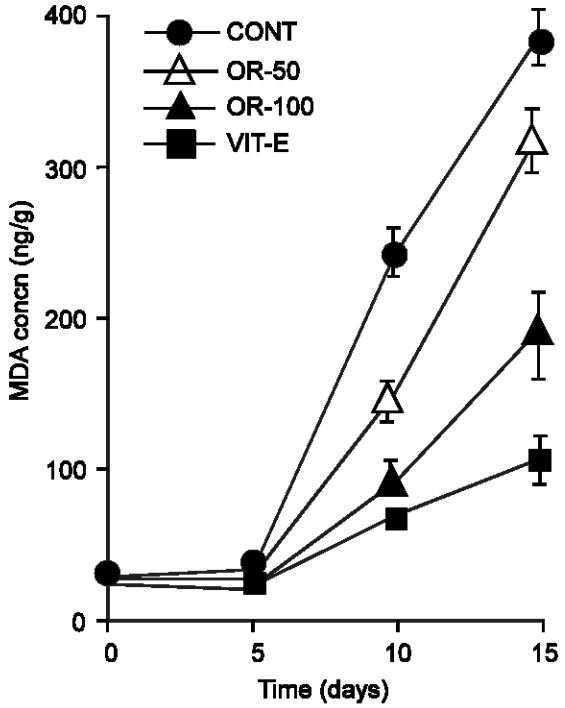

Fig. 2: Effect of refrigerated storage on lipid oxidation of liquid yolks as a function of dietary supplementation with oregano essential oil at 50 mg/kg (OR-50) and 100 mg/kg (OR-100), and α-tocopheryl acetate at 200 mg/kg (VIT-E), compared to control diet (CONT). All data points represent mean MDA values of four samples and their standard deviations, some of which however lie within the data points.

found increased oxidative stability of egg yolks when a thyme extract was fed to laying hens (Botsoglou *et al.*, 1997).

Lipid oxidation of liquid yolk: Since shell eggs were inherently resistant to oxidative deterioration upon refrigerated storage, additional experiments were carried out in order to evaluate yolk lipid stability under conditions that could promote lipid oxidation. Thus, the effect of dietary treatments on the oxidative stability of yolks agitated for 15 days in presence of light was investigated. Figure 2 shows the susceptibility of yolks to lipid oxidation during agitation as a function of the dietary supplementation oregano essential oil or α-tocopheryl acetate. The extent of lipid oxidation, as measured by MDA formation, differed ($P<0.05$) between the dietary treatments at all time points. Yolks from the CONT treatment presented mean MDA values that were higher ($P<0.05$) than those of the OR-50 treatment, which in turn were higher ($P<0.05$) than those of the OR-100 treatment, a finding suggesting that dietary oregano essential oil exerted a dose dependent antioxidative activity. The VIT-E treatment presented MDA values that were lower ($P<0.05$) compared to all other treatments at all time points.

In pertinent studies with extracts of other aromatic plants, Galobart *et al.* (2001) found that the dietary supplementation of a rosemary extract to laying hens had no effect on lipid oxidation of eggs. In contrast, Krause and Ternes (2000) and Botsoglou *et al.* (1997) observed an improvement of the oxidative stability of egg yolk when carnosic acid, the main antioxidant constituent of rosemary, and a thyme extract, respectively, were used as dietary supplement in laying hens.

The relatively low MDA values presented in Fig. 2 indicated a moderate rate of lipid oxidation. Considering this lipid oxidation profile, it appears that antioxidant constituents of the essential oil of oregano had passed through feeding into the developing yolk, thus providing egg with antioxidant properties. By now, no method is available for the determination of all of the antioxidant constituents of oregano essential oil passed into egg yolk. Additional research is needed towards developing such a method, which could identify and quantify each of the main antioxidant constituents of oregano deposited into egg yolk.

References

Adam, K., A. Sivropoulou, S. Kokkini, T. Lanaras and M. Arsenakis, 1998. Antifungal activities of *Origanum vulgare* ssp. *hirtum*, *Mentha spicata*, *Lavandula angustifolia*, and *Salvia fruticosa* essential oils against human pathogenic fungi. J. Agri. Food Chem., 46: 1739-1745.

Addis, P.B. and S.W. Park, 1989. Role of lipid oxidation products in atherosclerosis. In: Scanlan, R., Taylor, S.L. (Eds.), Food Toxicology. Marcel Dekker, New York, NY, pp: 297-330.

AOAC, 1990. Official Methods of Analysis of the Association of Official Analytical Chemists, Herlich K. (Ed.), A.O.A.C. International, Arlington, USA.

Aymond, W.M. and M.E.Van Elswyk, 1995. Thiobarbituric acid reactive substances and n-3 fatty acids in response to whole and ground flaxseed. Poult. Sci., 74: 1388-1394.

Botsoglou, N.A., E. Christaki, D.J. Fletouris, P. Florou-Paneri and A.B. Spais, 2002b. The effect of dietary oregano essential oil on lipid oxidation in raw and cooked chicken during refrigerated storage. Meat Sci., 62: 259-265.

Botsoglou, N.A., D.J. Fletouris, P. Florou-Paneri, E. Christaki and A.B. Spais, 2003a. Inhibition of lipid oxidation in long-term frozen stored chicken meat by dietary oregano essential oil and α-tocopheryl acetate supplementation. Food Res. Int., 36: 207-213.

Botsoglou, N.A., P. Florou-Paneri, E. Christaki, D.J. Fletouris and A.B. Spais, 2002a. Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. Br. Poult. Sci., 43: 223-230.

Botsoglou, N.A., D.J. Fletouris, G.E. Papageorgiou, V.N. Vassilopoulos, A.J. Mantis and A.G. Trakatellis, 1994. A rapid, sensitive, and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissues, food, and feedstuff samples. J. Agri. Food Chem., 42: 1931-1937.

Botsoglou, N.A., P. Florou-Paneri, E. Christaki, I. Giannenas and A.B. Spais, 2004. Performance of rabbits and oxidative stability of muscle tissues as affected by dietary supplementation with oregano essential oil. Arch. Anim. Nutr., 58: 209-218.

Botsoglou, N.A., A. Govaris, E.N. Botsoglou, S.H. Grigoropoulou and G. Papageorgiou, G. 2003b. Antioxidant activity of dietary oregano essential oil and α-tocopheryl acetate supplementation in long-term frozen stored turkey meat. J. Agri. Food Chem., 51: 2930-2936.

Botsoglou, N.A., S.H. Grigoropoulou, E.N. Botsoglou, A. Govaris and G. Papageorgiou, 2003c. The effects of dietary oregano essential oil and α-tocopheryl acetate on lipid oxidation in raw and cooked turkey during refrigerated storage. Meat Sci., 65: 1193-1200.

Botsoglou, N.A., A.L. Yannakopoulos, D.J. Fletouris, A.C. Tserveni-Goussi and P. Fortomaris, 1997. Effect of dietary thyme on the oxidative stability of egg yolk. J. Agri. Food Chem., 45: 3711-3716.

Cherian, G., F.W. Wolfe and J.S. Sim, 1996. Feeding dietary oils with tocopherols: effects on internal qualities of eggs during storage. J. Food Sci., 61: 15-18.

Economou, K.D., V. Oreopoulou and C. Thomopoulos, 1991. Antioxidant properties of some plant extracts of the Labiatae family. J. Am. Oil Chem. Soc., 68: 109-113.

Galobart, J., A.C. Barroeta, M.D. Baucells, R. Codony and W. Ternes, 2001. Effect of dietary supplementation with rosemary extract and α-tocopheryl acetate on lipid oxidation in eggs enriched with ω3-fatty acids. Poult. Sci., 80: 460-467.

Jiang, Y.H., R.B. McGeachin and C.A. Bailey, 1994. α-Tocopherol, β-carotene, and retinal enrichment of chicken eggs. Poult. Sci., 73: 1137-1143.

Krause, E. and W. Ternes, 2000. Bioavailability of the antioxidative Rosmarinus officinal is compound carnosic acid in eggs. Eur. Food Res. Tec., 3: 161-164.

Lopez-Bote, C.J., J.I. Gray, E.A. Gomaa and C.J. Flegal, 1998. Effect of dietary administration of oil extracts from rosemary and sage on lipid oxidation in broiler meat. Br. Poult. Sci., 39: 235-240.

Marshall, A.C., A.R. Sams and M.E. Van Elswyk, 1994. Oxidative stability and sensory quality of stored eggs from hens fed 1.5% menhaden oil. J. Food Sci., 59: 561-563.

Milos, M., J. Mastelic and I. Jerkovic, 2000. Chemical composition and antioxidant effect of glycosidically bound volatile compounds from oregano (*Origanum vulgare* L. ssp. hirtum). Food Contr., 71: 79-83.

National Research Council, 1994. Nutrient requirements of poultry, 9th Ed. National Academy Press, Washington, D.C.

Offord, E., F. Guillot, R. Aeschbach, J. Loliger and A. Pfeifer, 1997. Antioxidant and biological properties of rosemary components: Implications for food and health. In F. Shahidi (Ed.), Natural Antioxidants. Chemistry, Health Effects and Applications, pp: 88-96, Washington: AOCS, Press.

Qi, G.H. and J.S. Sim, 1998. Natural tocopherol enrichment and its effect in n-3 fatty acid modified chicken eggs. J. Agri. Food Chem., 46: 920-1926.

Raharjo, S., J.N. Sofos and G.R. Schmidt, 1993. Solid-phase acid extraction improves thiobarbituric acid methods to determine lipid oxidation. J. Food Sci., 58: 921-932.

Simopoulos, A.P. and N. Salem, 1992. Egg yolk as a source of long-chain polyunsaturated fatty acids in infant feeding. Am. J. Clin. Nutr., 55: 411-414.

Sivropoulou, A., E. Papanikolaou, C. Nikolaou, S. Kokkini, T. Lanaras and M. Arsenakis, 1996. Antimicrobial and cytotoxic activities of *Origanum* Essential oils. J. Agri. Food Chem., 44: 1202-1205.

Tarladgis, B.G., A.M. Pearson and L.R. Dugan, 1964. Chemistry of the 2-thiobarbituric acid test for determination of oxidative rancidity in Foods. II. Formation of the TBA-malonaldehyde complex without acid-heat treatment. J. Sci. Food Agri., 15: 602-607.

Tsimidou, M., E. Papavergou and D. Boskou, 1995. Evaluation of oregano antioxidant activity in mackerel oil. Food Res. Int., 28: 431-433.

Vekiari, S.A, V. Oreopoulou, C. Tzia and C. Thomopoulos, 1993. Oregano flavonoids as lipid antioxidants. J. Am. Oil Chem. Soc., 70: 483-487.

Yanishlieva, V., N. Marinova, H. Gordon and G. Raneva, 1999. Antioxidant activity and mechanism of action of thymol and carvacrol in two food lipid systems. Food Chem., 64: 59-66.