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PAPER

Effects of dietary mannan oligosaccharide and herbal essential oil blend supplementation on performance and oxidative stability of eggs and liver in laying hens

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Abstract

The role of dietary supplemental mannan oligosaccharide (MOS) and an essential oil blend (EOB) on performance of laying hens, and susceptibility of egg yolk and hen liver to lipid oxidation were examined. Four hundred and thirty-two 52-week old Lohmann laying hens were divided into three groups and fed a basal diet containing no antioxidant as control (CNT), basal diet plus 1 g/kg MOS and basal diet with 24 mg/kg EOB, for a 10-week experimental period. Supplementation of diet with MOS and EOB improved egg production rate and eggshell weight, but did not influence other performance or egg quality traits. MOS and EOB provided higher antioxidant activity in egg yolk than the control regimen at all storage time periods. EOB also retained the oxidative stability of liver by measuring malondialdehyde (MDA) levels. Liver antioxidant enzymes, including superoxide dismutase (SOD) and glutathion peroxidase (GSH-Px), were higher in birds fed the additives. MOS and EOB tended to increase serum glucose concentration (6.2% and 8.8%, respectively) while they slightly decreased triglycerides (11.0% and 4.8%, respectively) without affecting cholesterol level. Relative weight of pancreas and spleen were not affected by dietary treatments whereas diet supplemented with EOB significantly increased liver weight. The findings of this study suggest that EOB and MOS could act as free radical scavengers that enhance performance and also increase eggshell weight.

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Introduction

Over the past decade, essential oils (EOs) of some herbs have received growing attention as a possible means of stimulating growth in farm animals. Research has mainly been directed to broilers and pigs, and the scientific data on the dietary effects of EOs in laying hens are still very limited (Windisch *et al.* 2008; Brenes and Roura, 2010). Promising results were reported on performance and eggshell quality of laying hens fed diets supplemented with EOs (Çabuk *et al.*, 2006; Bölükbaşi *et al.*, 2007; 2008; Bozkurt *et al.*, 2012). Furthermore, there is evidence that adding medicinal herbs and their extracts to the diet of laying hens affects the oxidative stability of egg yolks and this might be of interest to the egg processing industry (Galobart *et al.*, 2001; Botsoglou *et al.*, 2005). However, this aspect of nutrition has still not been fully investigated in laying hens.

Several comprehensive reviews on the use of commercially available MOS, yeast cell wall derivate of *saccharomyces cerevisiae*, in layer and broiler breeder diets reported that MOS can improve performance and eggshell quality as well as immunity in several ways (Berry and Lui, 2000; Stanley *et al.*, 2000; Shashidhara and Devegowda, 2003; Gürbüz *et al.*, 2011). MOS may improve digestive enzyme activity, gastrointestinal microflora and intestinal morphology, and thereby enhance digestion and absorption of nutrients (Spring *et al.*, 2000; Yang *et al.*, 2008).

The highly unsaturated nature of n-3 fatty acids predisposes the egg yolk and poultry meat to lipid oxidation (Botsoglou *et al.*, 1997, 2002, 2003). By increasing the degree of unsaturation of the yolk lipid oxidation increases, and toxic oxidation products lower the quality and nutritional and organoleptic values of the animal product (Cherian *et al.*, 1996). Extracts and EOs of medicinal herbs are also of interest to the poultry industry to improve the oxidative stability of chicken meat and eggs (Botsoglou *et al.*, 1998; Galobart *et al.*, 2001; Basmacıoğlu *et al.*, 2004).

Much research on the use of α-tocopherol, an efficient free radical scavenger, in hen feed has shown that the high efficiency of this vitamin in improving the oxidative stability of eggs (Cherian *et al.*, 1996; Bölükbaşi *et al.*, 2007) and poultry meat (Botsoglou *et al.*, 2002, 2003). However, there has not been much research on the use of aromatic plants in hen feeding. But there is some *in vivo* scientific evidence available to suggest that herbal EOs could serve as an antioxidant feed additive. Recent experimental studies have shown that extract and EOs of oregano and rosemary could increase the oxidative stability of chicken and turkey meat (Botsoglou *et al.*, 2002; Basmacıoğlu *et al.*, 2004) and also of egg yolk (Galobart *et al.*, 2001; Botsoglou *et al.*, 2005) when incorporated into the diet. Unfortunately, definition of the antioxidant action of EOs and MOS in bird liver has been problematic.

Several scientific studies have examined the antioxidant properties of some selected EOs as natural antioxidant feed additives. However, in spite of this, there is hardly any information comparing EOs and MOS. Given this, a commercial essential oil blend (EOB) derived from six selected herbs that grow wild in Turkey was added to laying hen diet and its antioxidant activity was evaluated. Our aim was to assess the dietary effects of EOB and MOS on the performance and egg quality of laying hens, as well as on the oxidative stability of table eggs and hen liver. Serum metabolic profile and relative weight of some organs were also evaluated.

Materials and methods

Birds and housing

A total of 432 Lohmann LSL-Classic white line laying hens, aged 52 weeks, were placed in cages (6 birds per cage) in a naturally ventilated poultry house with windows and with a light

regimen of 16 h light to 8 h dark. The average daily mean temperature during the experiment in this region was 27.7°C (mean of highest temperatures 30.3°C and of the lowest 24.9°C). Three groups of 144 laying hens (6 replicates per group) were randomly assigned to one of three dietary treatments. Four adjacent cages with a total of 24 hens made up the replicate group. Hens were housed 6 per cage, corresponding to 500 cm^2 per hen, in wire-bottomed cages (60×50×56 cm) each equipped with a feeder and nipple. Birds were offered feed and water *ad libitum*. The experimental period lasted ten weeks. Diets containing no feed additive were fed to all hens before starting the trial.

Feed supplements and diets

The isocaloric-isonitrogenic basal diet based on corn, soybean meal, wheat and sunflower meal was formulated according to the recommendations of the breeder (Lohmann Tierzucht GmbH, 2011). Hens within the control group (CNT) were fed a commercial basal diet supplemented with 30 mg α-tocopheryl acetate/kg, according to standard recommendations, derived from a pre-mix. The ingredients and the nutrient composition of the basal diet are presented in Table 1. The remaining two groups were given the same basal diet supplemented with a commercial essential oil blend at 24 mg/kg diet (EOB) or mannan oligosaccharide at 1 g/kg diet (MOS). The EOB including carvacrol, thymol, 1,8-cineole, p-cymene, and limonene as major active component was composed of 6 totally different essential oils, *i.e.* oregano oil (*Origanum sp.*), laurel leaf oil (*Laurus nobilis*), sage leaf oil (*Salvia tribola*), myrtle leaf oil (*Myrus communis*), fennel seed oil (*Foeniculum vulgare*), and citrus peel oil (*Citrus sp.*). The essential oil premix used contained 976 g of zeolite as a carrier for 24 g of EOB. Hydrodistillation was used to extract the essential oils. Both these additives were added at the expense of sawdust as inert filler. The commercial preparations of MOS (Bio-Mos®; Alltech, Inc., Nicholasville, KY, USA) and EOB (Heryumix™, Herba Ltd. Co. Seferihisar, İzmir, Turkey) were mixed in a carrier which was then added at 1 kg per ton to the basal diet.

The experimental basal diet was also analyzed for dry matter, crude protein, ether extract, crude ash, crude fiber, starch, sugar, calcium, and total phosphorus content using methods outlined by the Association of German Agricultural Analysis and Research Institutes (VDLUFA) for the chemical analysis of feedstuff (Naumann and Bassler, 1993). Fatty acid composition was determined by gas chromatography (Agilent Technologies 6890N, Germany, 2004) according to IUPAC (1987) recommendations (Table 2).

Performance parameters

All hens were weighed individually at 52 and 61 weeks of age. Hen/day egg production (%) and shell-less egg rate were recorded daily from 52 to 61 weeks of age. The shell-less egg rate (%) was calculated by dividing the total number of eggs without shells (an egg without a shell but with an intact membrane) by the total number of eggs in each treatment. During this period, a random sample of 36 eggs per treatment per day was collected on two consecutive days every week (6 eggs per replicate per day). The feed consumption and feed conversion ratio were determined at 7-day intervals. The feed conversion ratio was expressed as kilograms of feed consumed per kilogram of egg produced (kg feed/kg egg). Egg mass was calculated by multiplying egg weight by egg production rate. All production variables were determined per replicate. The magnitude of production variables such as feed intake and egg production was adjusted for hen mortalities. Deaths were recorded daily as they occurred. An additional sample of 24 eggs was randomly collected from each experimental group (4 eggs per replicate) every 14 days to assess eggshell quality parameters such as eggshell weight, strength and thickness. Eggshell thickness (without inner and outer shell membranes) is defined as an average of three different thickness measurements (top, middle and bottom) of the egg. This was measured using an ultrasonic micrometer (SANOVO; SANOVO Technology A/S, Odense NV, Denmark) without cracking the eggshell. Eggshell strength was measured using electronic eggshell tester equipment (Egg Force Reader, SANOVO) and expressed as unit of compression force exposed to unit eggshell surface area (kg/cm^2). Eggs were then cracked, carefully separating the eggshell, and albumen height was obtained using a micrometer with

Table 1. Ingredients and nutrient composition of the experimental basal diet (as fed).

Ingredients, g/kg		Nutrient composition	
Corn	475.00	Dry matter, %	88.98
Wheat	100.00	Crude protein N x 6.25, %	17.87
Soybean meal, 48 % CP	257.00	Ether extract, %	5.39
Sunflower meal	26.00	Crude ash, %	12.56
Soybean oil	30.50	Crude fiber, %	3.03
Di-calcium phosphate	14.50	Starch, %	36.55
Ground limestone	87.00	Sucrose, %	2.67
NaCl	2.50	Calcium, %	3.84
DL-Methionine 99%	1.00	Total phosphorus, %	0.67
Vitamin pre-mix°	2.50	Phosphorus, nonphytate,^ %	0.38
Mineral pre-mix#	1.00	Methionine+cysteine,^ %	0.68
$NaHCO_3$	0.50	Lysine,^ %	0.80
Choline chloride	0.50	Threonine,^ %	0.66
Saw dust§	1.00	Metabolizable energy,^ kcal/kg	2826

°Provides per kg of diet: vitamin A (retinyl acetate), 12.000 U; vitamin D3 (cholecalciferol), 2400 U; vitamin E (DL-α-tocopheryl acetate), 30 mg; vitamin K_3, 3 mg; vitamin B_1, 3 mg; vitamin B_2, 7 mg; vitamin B_6, 4 mg; vitamin B_{12}, 0.02 mg; nicotine amid, 40 mg; Ca-D-pantothenate, 8 mg; folic acid, 1 mg; biotin, 0.045 mg; vitamin C, 50 mg; choline chloride, 125 mg. #Provides per kg of diet: Mn, 80 mg; Fe, 40 mg; Zn 60 mg; Cu, 5 mg; I, 0.4 mg; Co, 0.1 mg; Se 0.15 mg. §Sawdust was substituted by MOS or EOB preparations. ^Calculated composition.

Table 2. Fatty acids composition of basal diet.

Fatty acids, %	
Caprilic	0.34
Myristic	0.36
Palmitoleic	10.88
Margaric	0.26
Stearic	0.25
Oleic	24.08
Linoleic	0.42
Linolenic	49.97
Eicosenoic	5.02
Arachidic	0.28
Eicosapentaenoic	2.59
Behenic	0.43
Erucic	1.10
SFA	2.00
MUFA	38.01
PUFA	59.99

SFA, Saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

ultrasonic wave system (SANOVO). Eggshell weight is defined as a percentage of the eggshell weight. Albumen height, percentage weight of albumen and yolk were described as internal egg quality parameters. The weight of albumen and yolk were divided into whole egg weight and then multiplied by 100 to determine percentage weight.

Malondialdehyde measurement in egg yolk

Chemicals and calibration

We dissolved 5 g of trichloroacetic acid (TCA) (Merck, Darmstadt, Germany) in 100 mL of H_2O (Stock TCA Solution). We then dissolved 0.8 g of butylated hydroxytoluen (BHT) (Sigma Chemical, St. Louis, MO, USA) in 100 mL of hexan (Stock BHT Solution) and 0.8 g of thiobarbituric acid (TBA) (Sigma Chemical) was dissolved in 100 mL of H_2O (Stok TBA solution). We used 1,1,3,3-tetraethoxypropane (Sigma Chemical) for the calibration curve.

Egg collection and analysis

To investigate the effects of dietary treatments on lipid oxidation of table egg yolks during refrigerated storage (4±1°C), 36 eggs were collected from each group during the last week of the trial (6 eggs from each replicate). Eggs were placed in a refrigerated cabinet to detect yolk malondialdehyde (MDA) content. Egg yolks were analyzed for oxidative stability alterations at 0, 3, 6, 9, 12 and 15 days of storage (n=3).

Spectrophotometry

MDA was determined in egg yolks according to our modified method (Tokuşoğlu, 2009) based on Botsoglou *et al.* (1994) and Mateos *et al.* (2005). The absorbance measurements were made at the wavelength of the spectrophotometer (Shimadzu, Model UV-2550 UV-VIS) by scanning at 400-650 nm. MDA concentration of samples was measured by calibration solutions based on 1,1,3,3-tetraethoxypropane (Sigma Chemical) at 521.5 nm via tertiary numerical differentiation including slope and intercept data.

HPLC method and HPLC conditions

An aliquot of 250 μL of egg yolk homogenate was placed in a 1.5 mL Eppendorf vial and 50 μL of 6M NaOH was added to this mixture. Alkaline hydrolysis of protein-bound, was provided by incubating this mixture in a 60°C water bath (Nüve, BM 402) for 30 min. The protein part of the mixture was precipitated with 125 μL of 35% (v/v) per chloric acid, then this mixture was centrifuged (Nüve, NF 800R) at 5000 × g for 5 min. We transferred 250 μL of supernatant (upper phase) to another Eppendorf vial and mixed this with 25 μL of 2,4-dinitrophenylhydrazine (DNPH) prepared as a 5 mM solution in 2 M hydrochloric acid (HCl) (Merck). The reaction mixture was incubated in darkness for 30 min at 25±1°C. We injected 50 μL of reaction mixture onto the HPLC. A Nucleosil 100 RP-18 column (4.0×125 mm, 5 μm particle size) and a Lichrospher guard column (4.0×4.0 mm) were used. All samples were eluted as isocratic using a mobile phase mixture of 0.2% (v/v) acetic acid in bidistilled water and acetonitrile (ACN) (62:38, v/v). The flow rate was 0.6 mL/min at 25±1°C of column temperature and HPLC chromatograms of MDA in egg yolk samples were obtained as 310 nm of detection.

Analytical validation

Confirmation analysis was carried out to validate the HPLC method (n=3). Chromatographic parameters for linearity, detection limits, quantification, reproducibility and recovery were obtained. The calibration curves were acquired by using the standard MDA solution that was prepared by hydrolysis of tetraethoxypropane in 1% sulphuric acid (Merck) [y= 2.33 x + 1.05 (R^2=0.9998)], [y= 2.82 x + 3.46 (R^2=0.9998)]. Concentrations were expressed as ng MDA/100 mg lipid in egg yolk sample.

Malondialdehyde, superoxide dismutase and glutathion peroxidase measurements in liver

Briefly, the tissues were washed in 1.15% KCl (+4°C) (w/v) and were then stored at -80°C until further analysis. The liver tissues were homogenized in ice-cold 1.15% KCl at 1300 rpm for 3 min with homogenizator (Yellow Line Ost Basic, Germany). Lipid peroxidation was determined using the procedure described by Yoshoiko *et al.* (1979) in which MDA, an end product of fatty acid peroxidation, reacts with TBA to form a colored complex with a maximum absorbance at 532 nm. The homogenized substance was centrifuged at 5000 g for 60 min.

The activities of total superoxide dismutase (SOD) (Biovision, K335-100, USA) and glutathion peroxidase (GSH-Px) (OxisResearch, Bioxytech, Gpx-340, 21017, USA) were determined in liver tissue supernatant. Homogenizate and supernatant protein concentrations were determined using the Lowry *et al.* (1951) protein assay method; the results are given for MDA (μmol/g protein), SOD (% inhibition/g protein), GSH-px (U/g protein).

Serum metabolic profile

Blood samples were allowed to stand for 2 h at room temperature to allow for appropriate clotting. The samples were then centrifuged at 1700 g for 10 min. Serum glucose concentration (mg/dL) was determined (A2191, Archem, Istanbul, Turkey) by spectrophotometry (UV-1601, Shimadzu, Rydalmere, Australia) and the serum samples were stored at -20°C until analysis. Serum total cholesterol (Archem, A2091) and triglyceride (A2311, Archem) levels (mg/dL) were analyzed by spectrophotometry.

Sampling of birds and measuring organ weight

At the end of the experiment (at 61 weeks of age), 18 hens whose body weight was similar to the group average (±10%) were selected from each feed group (3 hens per replicate) and wing banded. Sampled birds were then electrically stunned, slaughtered and eviscerated to weigh the liver, pancreas and spleen. The individual weight of these organs was divided by their own slaughter weights to obtain the respective proportions. Liver samples were vacuum-packed and stored at -70°C until required. Blood samples were collected by cardiac puncture and placed into non-additive blood collection tubes in order to separate the serum.

Statistical analysis

Statistical analyses were performed using JMP (version 5.1.2, SAS Institute Inc., Cary, NC, USA). Data were analyzed as a one-way ANOVA using the General Linear Models procedure. All data were checked for normality before analysis. Analysis of variance was used in a completely randomized design. Significant differences between treatment means were separated using Duncan's multiple range test with a 5% probability. $P<0.05$ was considered statistically significant.

Results and discussion

MOS and EOB supplementation did not affect ($P>0.05$) the body weight and mortality rate of hens at the end of the 10-week experimental period (Table 3). Previous studies had also reported no beneficial effects on the body weight of hens when they were fed diets supplemented with MOS (Stanley *et al.*, 2000; Gürbüz *et al.*, 2011) and oregano EO (Florou-Paneri *et al.*, 2005).

Table 3 shows the dietary effects of MOS and EOB on the performance parameters of laying

hens. The general health status of laying hens was good during the experimentation period (52-61 weeks of age) while the productive performance exceeded the performance goals recommended by the breeder (Lohmann Tierzucht GmbH, 2011). Under these circumstances, dietary supplementation with MOS improved the laying rate with a high statistical significance while EOB resulted in numerical increases. MOS increased the egg production rate by 1.94% (P<0.01) compared to the untreated CNT group. Feed additives had no impact on egg weight, egg mass, feed consumption, feed conversion rate, and shell-less egg rate (P>0.05).

These results were in agreement with studies that supplemented the diet of laying hens with MOS (Berry and Lui, 2000; Stanley *et al.*, 2000; Gürbüz *et al.*, 2011) and EOs from several medicinal herbs (Çabuk *et al.*, 2006; Bölükbaşı *et al.*, 2007; Bozkurt *et al.*, 2012). The available scientific evidence demonstrates that MOS and EOB can improve the digestive enzyme activity, the gastrointestinal microflora and the intestinal morphology, thereby enhancing the digestion and absorption of nutrients (Jang *et al.*, 2007; Gürbüz *et al.*, 2011); this further supports the increased laying rate.

The egg quality characteristics of hens fed a diet supplemented with MOS or EOB are shown in Table 3. None of the egg quality properties were affected by either MOS or EOB, except for eggshell weight (%). Dietary EOB significantly (P<0.05) increased the eggshell weight proportion compared to the control group while the MOS treatment gave intermediate results. This increase in eggshell weight agreed with the numerical increase in the eggshell thickness and eggshell breaking strength. Several studies have already documented the positive effect of EOs (Çabuk *et al.*, 2006; Bölükbaşı *et al.*, 2008) and MOS (Berry and Lui, 2000) on eggshell quality, but the underlying mechanism whereby EOs and MOS affect the eggshell structure are still not well understood. However, it could be that EOs may lead to higher nutrient retention and nutrient availability through the intestines during shell formation.

The effects of dietary treatments on the oxidative stability of eggshells measured at different time periods are shown in Table 4. The extent of lipid oxidation, as measured by MDA formation, differed (P<0.01) among dietary treatments during refrigerated storage on Days 0, 3, 6, 9, 12 and 15, respectively. Both the MOS and EOB dietary treatments significantly lowered the yolk MDA concentration throughout the course of storage (P<0.01). The mean

Table 3. Productive performance, egg quality, body weight and mortality of layer hens fed on experimental diets.

Parameters	Experimental diets				
	CNT	MOS	EOB	SEM	P
Productive performance					
Egg production rate, %	88.77^{b}	90.71^{a}	89.45^{ab}	0.32	0.009
Egg weight, g	62.72	62.63	62.74	0.16	0.902
Egg mass, g/d	55.21	56.44	55.82	0.42	0.392
Feed consumption, g/hen/d	101.65	103.50	101.22	0.71	0.102
Feed conversion rate, kg feed/kg egg	1.84	1.83	1.81	0.01	0.438
Shell-less egg rate, %	0.81	0.91	0.82	0.05	0.676
Egg quality					
Shell thickness, μ	397	396	404	2.64	0.090
Shell breaking strength, kg/cm^2	4.20	4.37	4.39	0.13	0.112
Shell weight, %	9.80^{b}	9.99^{ab}	10.17^{a}	0.06	0.000
Yolk weight, %	26.91	27.08	26.95	0.20	0.178
Albumen weight, %	63.27	62.91	62.87	0.22	0.073
Albumen height, mm	6.65	6.63	6.60	0.08	0.601
Body weight, g					
Initial weight, 52 weeks	1641	1618	1639	15.16	0.699
Final weight, 61 weeks	1693	1698	1715	16.27	0.315
Mortality, % 52-61 weeks	0.34	0.17	0.00	0.12	0.463

CNT, untreated basal diet; MOS, mannan oligosaccharide at 1 g/kg diet; EOB, an essential oil blend at 24 mg/kg diet; ^{a,b}means within rows with different superscripts are different at P<0.05.

Table 4. Effects of mannan oligosaccharide and essential oil blend on lipid oxidation of eggs and hepatic antioxidant enzyme activities, serum metabolic profile and relative organ weight of laying hens.

Parameters	Experimental diets				
	CNT	MOS	EOB	SEM	P
Lipid oxidation of eggs (TBARS ng MDA/100 mg lipid) at different storage time					
0 d	0.88^{a}	0.62^{b}	0.54^{c}	0.003	0.0001
3 d	1.26^{a}	0.73^{b}	0.75^{b}	0.006	0.0001
6 d	1.44^{a}	0.84^{c}	0.90^{b}	0.007	0.0001
9 d	1.64^{a}	1.03^{c}	1.11^{b}	0.011	0.0001
12 d	1.90^{a}	1.29^{b}	1.27^{b}	0.010	0.0001
15 d	2.81^{a}	1.50^{b}	1.40^{c}	0.018	0.0001
0-15 d, mean	1.66^{a}	1.00^{b}	0.99^{b}	0.004	0.0001
Lipid oxidation (MDA) and antioxidant enzyme activities of liver					
MDA, μmol/g protein	9.25^{a}	7.61^{a}	5.24^{b}	0.592	0.0001
SOD, % inhibition/g protein	0.126^{c}	0.303^{b}	0.427^{a}	0.004	0.0001
GSH-Px, U/g protein	36.34^{b}	36.23^{b}	46.62^{a}	1.661	0.0001
Serum metabolic profile					
Glucose, mg/dL	306	325	333	8.572	0.0820
Triglycerides, mg/dL	1658	1475	1577	58.771	0.0992
Cholesterol, mg/dL	187	188	173	11.037	0.5701
Relative organ weight					
Liver, %	1.98^{b}	1.98^{b}	2.21^{a}	0.064	0.0171
Pancreas, %	0.18	0.17	0.18	0.005	0.9368
Spleen, %	0.07	0.07	0.08	0.002	0.1494

CNT, untreated basal diet; MOS, mannan oligosaccharide at 1 g/kg diet; EOB, an essential oil blend at 24 mg/kg diet; MDA, malondialdehyde; SOD, superoxide dismutase; GSH-Px, glutathion peroxidase; ^{a,b}means within rows with different superscripts are different at P<0.05.

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antioxidant activity of the MOS group was equivalent to that of the EOB group. The MDA values on Days 3, 6, 9, 12 and 15 were progressively higher (P<0.01) with refrigerated storage compared with their initial status on Day 0, which indicated a temporal response increase in the oxidative deterioration in eggs. MDA formation was actually initiated after zero days of storage and it continued during the latter stages of storage (Botsoglou *et al.*, 1997). The fundamental antioxidant activity and mechanism of action of many spices, plant extracts and herbal essential oils have been analyzed in numerous in vitro studies (Baratta *et al.*, 1998; Lee and Shibamoto, 2002; Pizzale *et al.*, 2002). However, reports are limited on the dietary use of aromatic plants to increase the oxidative stability of shell eggs. Dietary supplementation of oregano EO (Florou-Paneri *et al.*, 2005) and ground herbs, such as rosemary, oregano, and saffron (Botsoglou *et al.*, 2005), were found to increase the oxidative stability of eggshells refrigerated for up to 60 days.

Phenolic compounds found in oregano EO, *i.e.* carvacrol and thymol, are responsible for the antimicrobial and antioxidant properties of different oregano species, where they make up over 78% of total volatile oils (Economou *et al.*, 1991; Pizzale *et al.*, 2002; Botsoglou *et al.*, 2003). The effectiveness of the main antioxidant constituent of sage oil, carnosic acid, was markedly higher than of traditional chemical antioxidants (Lopez–Bote *et al.*, 1998). We, therefore, suggest that the major antioxidant constituents of oregano and sage EOs, i.e. carvacrol, thymol and 1,8-cineol, might be exploited for their additive or synergistic antioxidant effects. The current study appeared to confirm a suggestion by Lopez-Bote *et al.* (1998) that vitamin E combined with rosemary EO as antioxidant additives had a synergistic effect compared with their individual application.

Some information is available concerning the antioxidant activity of extracts and EOs from some herbs in the diets of laying hens, but there is little scientific evidence on the use of MOS as a dietary supplement. It has been widely reported that histidine, an antioxidant amino acid that forms a major constituent of the yeast cell wall, may play a role in the overall antioxidant activity (Wade and Tucker, 1998). Another suggestion is that different mechanisms may be involved in their protective effect, *i.e.* an antioxidant action in the case of ofloxacin-induced DNA damage and a direct adsorption of acridine orange to mannan conjugates as possible mechanisms of protection, based on spectrophotometric measurements (Križková *et al.*, 2006).

MDA concentrations in the liver of hens fed on diets supplemented with EOB and MOS are shown in Table 4. The results showed that using EOB as a dietary supplement in the diet of laying hens significantly improved the oxidative stability of the liver. The low MDA found in the liver of EOB-treated hens indicated a lower *in vivo* production and the deposition of MDA in these tissues. The possible transfer of antioxidant constituents of EOs into laying hens via feeding might inhibit the chain reaction involved in lipid oxidation, thereby reducing MDA in liver tissue (Cherian *et al.*, 1996). Unfortunately, no evidence is available on the potential antioxidant properties of aromatic herb EOs in the liver when added to poultry diets. Several recent reports have demonstrated the antioxidant properties of herbal remedies including oregano and rosemary EOs (Botsoglou *et al.*, 2002, 2003; Basmacıoğlu *et al.*, 2004), extracts of rosemary and sage (Lopez-Bote *et al.*, 1998), and a blend of several EOs (Botsoglou *et al.*, 2005), which all had the potential to increase the antioxidant capacity of poultry meat. Available studies indicate the presence of a similar protective mechanism in the liver when feed is fortified with these plant bioactives.

Table 4 shows that the liver SOD activity of birds fed EOB and MOS supplemented diets was significantly higher (P<0.01) than the control treatment. However, the liver SOD activity with the MOS treatment was lower than for EOB (P<0.01). The liver GSH-Px activity of birds fed EOB showed a similar pattern to SOD, whereas dietary MOS supplementation did not increase the liver GSH-Px concentration (P<0.01).

The antioxidant enzymes SOD and GSH-Px are considered to be the main elements of the first level of antioxidant defense in the cell because they form a major protective system against oxidative damage (Surai, 1999). The scavenger roles of these hepatic antioxidative enzymes in preventing oxidative damage to the liver, by prompting the release of SOD and GSH-Px in response to EOB supplementation, suggests that they might help reduce MDA in the liver. Nonetheless, MOS failed to have this effect. Unfortunately, little or no attention has been paid to the fact that free radical formation is reduced in body tissues when animals are fed MOS. The report of Dvorska and Surai (2001) demonstrated that dietary provision of MOS reduced the MDA level in quail liver. This only served to highlight the lack of knowledge in this area.

Birds that received a diet supplemented with EOB and MOS showed a tendency (P=0.08) towards an increased serum glucose level (6.2% and 8.8%, respectively) and a slightly decreased (P=0.09) serum triglyceride concentration (11.0% and 4.8%, respectively). No significant change was observed in serum cholesterol content in terms of these additives (Table 4). Few studies have observed the effects of MOS and EOs on serum concentrations of glucose, triglycerides and cholesterol. EOs from thyme, sage, rosemary (Bölükbaşi *et al.*, 2008) and bergamot (Bölükbaşi *et al.*, 2010) have been shown to improve the metabolic profile of laying hens throughout their peak production period.

The effects of the experimental diets on the relative weight of liver, pancreas and spleen are shown in Table 4. The percentage weight of the liver of hens fed a diet with added EOB was significantly higher than the CNT and MOS treatments (P<0.05). However, pancreas and spleen weights were not affected by the dietary treatments. Considering the higher antioxidant enzyme activities in the liver of hens fed EOB, it is feasible to suggest that the synthesis and release of these enzymes could enlarge the liver. In addition, it is also possible to postulate that the liver of EOB-fed hens might have been enlarged due to the metabolic turnover of phytochemicals in liver. Similarly, significant increases in the liver weight of broiler chicks without compromising their performance and viability was reported when birds were administered a diet with the same EOB preparation (Bozkurt *et al*., 2009). Furthermore, it is well known that toxic oxygen radicals induce the apoptosis of cells. Therefore, it is possible that the heavier livers of EOB-fed birds could be due to the decreased rate of apoptosis in liver tissues as a result of the specific antioxidant effects of EOs.

Conclusions

Supplementation of MOS and EOB was beneficial in terms of an increased egg production rate and eggshell weight. MOS and EOB reduced lipid oxidation in the shell egg at all time points during refrigerated storage. MOS had an equivalent antioxidant activity to EOB.

However, MOS was much less effective in preventing hepatic lipid oxidation than EOB. More experimental feeding trials are needed to evaluate the mechanisms of these nutritive antioxidants and to provide a better understanding of the metabolic processes in which they are involved.

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