Performance, egg quality, and immune response of laying hens fed diets supplemented with mannan-oligosaccharide or an essential oil mixture under moderate and hot environmental conditions

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ABSTRACT In total, 432 thirty-six-week-old laying hens were fed a basal diet supplemented with mannan-oligosaccharide (MOS) or an essential oil mixture (EOM) from 36 to 51 wk of age. Hens were divided into 3 equal groups replicated 6 times with 24 hens per replicate. No significant difference was observed among the dietary treatments in terms of performance indices. Different from the dietary manipulation, high environmental temperatures negatively influenced all of the laying performance traits except the feed conversion ratio in association with the diminished feed consumption. The MOS, and particularly the EOM, tended to alleviate the deleterious effect of heat stress on BW gain. Mortality was higher in MOS-fed hens than with other treatments. A supplementation diet with MOS or EOM provided increments in eggshell weight ($P < 0.01$). Relative albumen weight was significantly decreased ($P < 0.05$) in response to EOM or MOS supplementation; however, this was not the case in the yolk weight rate. The MOS decreased albumen height and Haugh unit ($P < 0.05$). High environmental temperatures hampered entire egg quality characteristics except for the eggshell breaking strength and egg yolk weight. These results indicated that heat stress adversely affected both productive performance and egg quality. As for the results of this study, neither MOS nor EOM was efficacious in improving efficiency of egg production and stimulating humoral immune response in laying hens reared under moderate and hot climatic conditions. However, the ameliorative effect exerted by MOS and EOM on eggshell characteristics is conclusive.

Key words: essential oil, mannan-oligosaccharide, layer performance, egg quality, immune response

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INTRODUCTION

Temperatures fluctuating in a very wide range in commercial laying hen houses that are located in hot climatic regions pose a serious threat to health, productive performance, and egg quality of modern layer hybrids (Al-Saffar and Rose, 2002; Balnave and Brake, 2005; Lin et al., 2006). High environmental temperatures have been reported to lower egg production and decrease egg weight and eggshell thickness while deteriorating efficiency of egg production (de Andrade et al., 1976, 1977; Emery et al., 1984; Mashaly et al., 2004). Several dietary strategies such as increasing nutrient density and supplemental fat level and vitamins A, C, E, and mineral additions to the diet have been applied for many decades to alleviate the harmful effects of heat stress on bird health and productivity (Leeson, 1986; Lin et al., 2006).

The general health status and performance of layer hens exposed to heat-stressed environmental conditions positively responded to supplementation diets with antibiotic growth promoters (Miles et al., 1985; Männer and Wang, 1991; Çabuk et al., 2006). Extracts and essential oils of some herbs and the yeast cell wall derivate of saccharomyces cerevisea, that is, mannan-oligosaccharide (**MOS**), received considerable interest in poultry feeding as a novel feed additive alternative to AGP. In addition to substantial antimicrobial properties proven in vitro (Cowan, 1999; Dorman and Deans, 2000; Rolfe, 2000; Fernandez et al., 2002; Baurhoo et al., 2009), various modes of action of those additives have attracted more interest recently (Spring, 1999; Hertrampf, 2001; Hooge, 2004; Brenes and Roura, 2010).

Improved egg production performance was observed in laying hens (Berry and Lui, 2000; Çabuk et al., 2006; Gürbüz et al., 2011) and broiler breeders (Shashidhara and Devegowda, 2003). Berry and Lui (2000)

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and Shashidhara and Devegowda (2003) reported the beneficial effects on eggshell quality traits when older breeder females were fed MOS. Several comprehensive reviews on the use of commercially available MOS on layer and broiler breeder diets reported that MOS can assist immunity in several ways (Cotter et al., 2000; Shashidhara and Devegowda, 2003).

Over the past decade, essential oils (**EO**) of some herbs also received increased attention as possible growth enhancers for farm animals, especially broilers (Brenes and Roura, 2010; Wallace et al., 2010). Unfortunately, the scientific information related to the dietary effects of EO in terms of layer hens is still very limited. Some hopeful signs were reported in performance, immune response, and eggshell quality of laying hens fed diets supplemented with EO of thyme, sage, and rosemary (Bölükbaşı et al., 2008, 2009) and an essential oil mixture (**EOM**; Çabuk et al., 2006). A commercial EOM of 6 herbs promoted productive and reproductive performance in broiler breeders (Bozkurt et al., 2009).

Extremely high environmental temperatures occur during 4 mo (from June to September) in the subtropics zone of the world, and these temperatures induce devastating effects on performance, egg quality, general health status, and nutrient utilization in laying hens. However, few comparative studies attempted to examine the influence of MOS and herbal EO as novel feed additives on layer performance under those harsh climatic conditions. From this perspective, the objective of our study was to investigate the potential of dietary MOS or EOM supplementation as a means of ameliorative additive strategies against the adverse effects of seasonal heat stress on overall egg production performance, egg quality, and specific immunity of laying hens throughout the post-peak production period.

MATERIALS AND METHODS

Birds and Housing

In total, 432 commercial white layer hens (Lohmann LSL-classic), 36 wk old and with uniform BW, were assigned to 3 equal groups replicated 6 times with 24 hens per replicate. The experimental house was a 3-tier cage facility and hens were housed 6 per cage (60 × 50 × 56 cm). The hens in 4 adjacent cages were considered an experimental replicate. Hens were exposed to natural environmental conditions in an open-sided hen house situated between 36° and 38° northern latitude and 26° and 28° eastern longitude, in Aydın, in western Turkey. The average daily mean temperature during the experiment in this region was 24°C (mean of highest temperatures 34°C and of the minimum 16°C). A tunnel ventilation fan with a flow rate of 2.0 m/s was run between 0800 h to 2000 h to prevent the mass mortalities due to the high ambient temperatures experienced before in this region.

The chicks were vaccinated against infectious bursal disease virus (**IBD**), Newcastle disease virus (**NDV**), and infectious bronchitis virus (**IBV**) via drinking water or intramuscular injection at different stages of the growing period, as suggested by the local commercial hatchery. The Ministry of Agriculture, General Directorate of Research Institutional Animal Care and Use Committee approved the techniques and procedures involved in the animal care and handling.

The experiment lasted between April 6 and July 27, 2010, for a 16-wk period throughout the post-peak egg production period. The whole experimental period consisted of 2 time periods that each lasted 8 wk. The flock reached peak egg production (97.53%) at 29 wk of age. The first phase lasted between 36 and 43 wk of age (period I), which represented moderate climatic conditions in April and May. The second period (period II) ran through June and July while hens aged between 44 and 51 wk, which represented high environmental temperatures. The ambient temperature and humidity values were recorded using an electronic data recorder. In period I, the average daily mean temperature was 24.6°C (mean of highest temperatures 26.9°C and of the minimum 22.1°C). In period II, the average daily mean temperature was 29.2°C (mean of highest temperatures 33.2°C and of the minimum 25.1°C). The mean humidity values were 53 and 55% in periods I and II, respectively. The lighting regimen was 16 h of continuous light per day from 0600 h to 2200 h.

Experimental Diets

Hens in the control group (**CNT**) were given a corn-soybean-based basal diet supplemented with no performance enhancer additive. Table 1 shows the ingredients and the nutrient composition of the basal diet. The remaining 2 groups were given the same basal diet supplemented with an additional 1 g of mannan-oligosaccharide/kg (MOS) or 24 mg of essential oil mixture/kg (EOM). Both additives were added at the expense of saw dust as inert filler. The commercial preparations of MOS and EOM were mixed in a carrier, which was then added to the basal diet at 1 kg per ton. An EOM, including carvacrol, thymol, 1:8-cineole, *p*-cymene, and limonene as active components, was composed of 6 different essential oils: oregano oil (*Origanum* sp.), laurel leaf oil (*Laurus nobilis*), sage leaf oil (*Salvia triloba*), myrtle leaf oil (*Myrtus communis*), fennel seed oil (*Foeniculum vulgare*), and citrus peel oil (*Citrus* sp.). Hydro distillation was used to extract the essential oils. The essential oil premix (i.e., EOM) used 976 g of zeolite as a carrier of 24 g of essential oil.

Feed and water were provided ad libitum. The hens were given 2 wk to acclimate to the experimental diets. All the diets were isonitrogenous and isocaloric and were fed in mash form. The experimental diets were formulated to meet the nutrient requirements for layer hens according to the recommendations of the breeder

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

Item	Amount
Ingredient (g/kg)	
Corn	475.00
Wheat	100.00
Soybean meal (48% CP)	257.00
Sunflower meal	26.00
Soybean oil	30.50
Dicalcium phosphate	14.50
Ground limestone	87.00
NaCl	2.50
DL-Methionine (99%)	1.00
Vitamin premix[1]	2.50
Mineral premix[2]	1.00
$NaHCO_3$	0.50
Choline chloride	0.50
Sawdust[3]	1.00
Nutrient composition (%)	
Analyzed	
DM	88.98
CP (N × 6.25)	17.87
Ether extract	5.39
Crude ash	12.56
Crude fiber	3.03
Starch	36.55
Sucrose	2.67
Calcium	3.84
Phosphorus, total	0.67
Calculated	
Phosphorus, nonphytate	0.38
Lysine	0.80
Methionine + cysteine	0.68
Threonine	0.66
Tryptophan	0.19
ME, kcal/kg	2,826

[1]Provides per kilogram of diet: vitamin A (retinyl acetate), 12,000 IU; vitamin D_3 (cholecalciferol), 60 μg; vitamin E (DL-α-tocopheryl acetate), 32.96 IU; 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; nicotinic acid, 40 mg; Ca-D-pantothenate, 8 mg; folic acid, 1 mg; biotin, 0.045 mg; vitamin C, 50 mg; choline chloride, 125 mg.

[2]Provides per kilogram 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.

[3]Sawdust was substituted by mannan-oligosaccharide or essential oil mixture preparations.

(Lohmann LSL, Commercial Management Guide, 2007, Lohmann Tierzucht, Cuxhaven, Germany).

Collection and Analyses

All hens were weighed individually at 36, 43, and 51 wk of age. Hen/day egg production (%) and shell-less egg ratio were recorded daily from 36 to 51 wk of age. The shell-less egg ratio (%) 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/treatment per day was collected on 2 consecutive days every week (6 eggs per replicate per day). Therefore, a total of 1,152 eggs was weighed in each treatment to determine the average egg weight throughout the trial. The feed consumption and feed conversion ratio were determined at 7-d intervals. The feed conversion ratio was expressed as kilograms of feed consumed per kilogram of egg produced. Egg mass was calculated by multiplying egg weight by egg production rate. All production variables were determined on replicate basis. The magnitude of production variables such as feed intake and egg production were adjusted for hen mortalities. The deaths of hens were recorded daily as they occurred.

An additional sample of 18 eggs was randomly collected from each experimental group (3 eggs per replicate) every 14 d to assess eggshell quality parameters. Therefore, 432 eggs in total were analyzed for egg quality. Eggshell quality characteristics were eggshell weight, strength, and thickness. Eggshell thickness (without inner and outer shell membranes) was measured at 3 different points (top, middle, and bottom) using an ultrasonic micrometre (Sanovo Technology A/S, Odense NV, Denmark) without cracking the eggshell. Eggshell thickness is defined as an average of 3 different thickness measurements of an egg. Eggshell strength was measured by 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). Then, eggs were cracked, carefully separating the eggshell, and albumen height was obtained using a micrometer with ultrasonic wave system (Sanovo). Eggshell weight is defined as a percentage of the eggshell weight. Albumen height, Haugh unit, 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. The Haugh unit was calculated using Rousch's formula (Rousch, 1981).

Blood samples were taken by puncturing the wing vein from one bird per cage (a total of 24 birds per group) before feeding regimens were initiated (36 wk of age) and at the end of the experiment (51 wk of age). The serum was isolated and stored at −20°C. Individual serum samples were analyzed for antibody responses against IBD, NDV, and IBV by the ELISA technique using commercial kits (Kirkegaard and Perry Laboratories, Gaithersburg, MD). The plates were read at 405 nm on an ELISA reader (Labsystems Multiscan MS, Labsystems, Helsinki, Finland).

The nutrient content of the diets was determined by proximate analysis (Naumann and Bassler, 1993). The experimental basal diets were analyzed for DM, CP, ether extract, crude ash, crude fiber, starch, sugar, total Ca and P 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). The metabolizable energy content of the diets was calculated based on chemical composition (TSE, 1991).

Statistical Analyses

The experiment used a completely randomized design, and each experimental unit was a replicate consisting of 6 groups of adjacently caged layer hens fed as one group. Data were analyzed on a 2-factorial ANOVA

using the SAS Institute's GLM procedure (SAS Institute, 2001). The main effects of diet, period, and the diet-by-period interaction were tested. Arcsin transformation was applied to the percentage values before testing for differences. Duncan's multiple-range test was carried out to detect differences among treatments. All differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Laying Hen Performance

Table 2 presents the BW at 36, 43, and 51 wk of age and overall mortality of hens. The final BW did not differ among treatments, but corresponding values pertaining to hens fed MOS or EOM tended to increase near to statistical significance ($P = 0.06$). These results imply that hens fed on MOS and EOM strive to cope with the detrimental effects of heat stress without sacrificing performance while keeping BW over the untreated hens. Confirming our findings, the final BW of layer hens fed EOM or MOS diets was significantly higher than those receiving an unsupplemented diet reared under severe hot climatic conditions (Çabuk et al., 2006).

Indeed, the overall BW gain in the CNT group (8 g) was markedly lower than the recommended value (70 g) for hens reared under optimal conditions (Lohmann, 2007) during the age period of 36 to 51 wk. This clearly indicates the negative effect of high environmental temperatures on BW gain of hens particularly in the later phase of the trial in association with marked decline in feed consumption.

During the course of the study, hens given CNT diet or treated with EOM maintained better survival, whereas birds receiving the MOS-added diet failed to do so (Table 2). Mortality was 1.38, 3.47, and 0.69% for CNT, MOS, and EOM treatments, respectively ($P < 0.05$). The majority of the total hen mortalities (6 of the 8) occurred in July, the hottest period of the experiment (number of hen mortalities are 1, 1, and 4 in CNT, EOM, and MOS treatments, respectively).

Overall performance characteristics including egg weight, egg mass, feed consumption, feed conversion ratio (g of feed/g of egg), and shell-less egg ratio did not differ significantly among treatment groups ($P > 0.05$) (Table 3). Attempts to improve egg production performance and egg quality through supplementation diet with common performance enhancers, particularly prebiotics and phytobiotics, have remained restricted compared with broilers (Steiner, 2009). Several positive responses have been observed for laying hens and broiler breeders when fed on diets added with MOS (Chukwu and Stanley, 1997; Berry and Lui, 2000; Stanley et al., 2000; Shashidhara and Devegowda, 2003). Still others have reported benefits in performance with respect to EOM supplementation in layer hen diet (Çabuk et al. 2006; Bölükbaşı et al. 2008; Özek et al. 2011; Bozkurt et al. 2012) or observed no effect (Florou-Paneri et al., 2005; Bölükbaşı et al., 2009, 2010).

Today, there are remarkable in vitro and in vivo proofs that show that either EO (Cowan, 1999; Dorman and Deans, 2000; Mitsch et al., 2004) or MOS (Rolfe, 2000; Fernandez et al., 2002; Baurhoo et al., 2009) could limit growth of the common intestinal pathogens. It appears that those specific mechanisms of in-feed MOS and EOM were not sufficient enough to prevent drastic decreases in overall performance when layer hens underwent seasonal heat stress. In general, neither MOS nor EOM compensated for the sharp reductions in egg weight and the egg production rate during period II. A contradictory observation (Çabuk et al., 2006) indicating that the beneficial responses to those MOS or EOM in aged brown layer hens (54 to 74 wk) under similar environmental conditions implies that strain or age of the hen may affect the effectiveness of those additives.

Nevertheless, all of the performance traits, with the exception of feed conversion efficiency, were affected from the experimental period with a very high statistical significance ($P < 0.01$). The increase in mean ambient temperature by 4.6°C impaired all performance indices except the feed conversion ratio. High environmental temperatures reaching up to 33°C at midday throughout the summer season (period II) induced remarkable reductions in egg production rate, egg weight, and egg mass compared with values measured in moderate conditions. The drastic reduction in feed intake over 10% during period II and associated marked decreases in

Table 2. Body weight and mortality of layer hens fed on diets added with mannan-oligosaccharide (MOS) and essential oil mixture (EOM)

	BW (g)			Mortality (%)
Item	36 wk	43 wk	51 wk	(36 to 51 wk)
Diet[1]				
CNT	1,638	1,661	1,630	1.38[b]
MOS	1,654	1,682	1,661	3.47[a]
EOM	1,660	1,691	1,686	0.69[b]
Pooled SEM[2]	11.02	15.27	13.84	0.64
P-value	0.2945	0.3244	0.0659	0.0092

[a,b]Means within columns with different superscripts are different at $P < 0.05$.

[1]Diets: CNT = control (no additive); MOS = mannan-oligosaccharide at 1 g/kg of diet; EOM = an essential oil mixture at 24 mg/kg of diet.

[2]Data are means of 6 replicates of 4 adjacent cages with 24 hens each per treatment.

Table 3. Performance of hens fed diets administrated with mannan-oligosaccharide (MOS) and essential oil mixture (EOM)

Period	Diet[1]	Egg production rate (%)	Egg weight (g)	Egg mass (g/d)	Feed consumption (g/hen/d)	Feed conversion ratio (kg of feed/kg of egg)	Shell-less egg rate (%)
I, April–May	CNT	96.41	64.26	61.92	114.99	1.857	0.41
	MOS	97.55	64.44	62.85	115.52	1.837	0.21
	EOM	96.72	64.29	62.19	114.56	1.842	0.38
II, June–July	CNT	91.05	62.18	56.65	103.49	1.834	0.35
	MOS	90.41	61.95	56.06	103.00	1.849	0.27
	EOM	90.71	62.20	56.46	102.36	1.820	0.36
Pooled SEM[2]		0.35	0.18	0.46	0.77	0.014	0.07
Main effect[3]							
Diet							
CNT		93.73	63.22	59.28	109.24	1.845	0.38
MOS		93.98	63.19	59.45	109.26	1.843	0.24
EOM		93.71	63.24	59.32	108.46	1.831	0.37
Period							
I		96.89[a]	64.33[a]	62.32[a]	115.02[a]	1.845	0.33
II		90.72[b]	62.11[b]	56.39[b]	102.95[b]	1.834	0.33
P-value							
Diet		0.5738	0.9028	0.7924	0.1257	0.2561	0.2769
Period		0.0001	0.0001	0.0001	0.0001	0.4390	0.8515
Diet × period		0.0762	0.7991	0.3479	0.6368	0.7200	0.5415

[a,b]Means within columns, within main effects, with different superscripts are different at $P < 0.05$.

[1]Diets: CNT = control (no additive); MOS = mannan-oligosaccharide at 1 g/kg of diet; EOM = an essential oil mixture at 24 mg/kg of diet.

[2]Data are means of 6 replicates of 4 adjacent cages with 24 hens each per treatment.

[3]Data were analyzed as a 3 × 2 arrangement.

performance traits measured ($P < 0.01$) signifies the detrimental effect of heat stress on hen performance rather than the age-related effect.

In this study, feed consumption was adversely affected by high ambient temperature with more than 12 g per hen per day ($P < 0.01$), whereas dietary treatments showed no influence ($P > 0.05$). The deterioration in productive performance under the hot climatic conditions compared with moderate conditions was most likely due to the decline in feed consumption that restricted intake of available nutrients for egg formation. The sharp decline in feed consumption underlines the considerable susceptibility of modern layer hybrids to moderate increases of 4.6°C over the thermo neutral zone.

The assumption that extract and EO of some herbs might improve the palatability of feed due to their aromatic characteristics could promote feed consumption when added to diets of poultry (Williams and Losa, 2001; Windisch et al., 2008). Nevertheless, such an assumption has not yet been confirmed with consistent and repeatable evidences in laying hens. A significant stimulation of feed intake was not obtained in any of the studies when laying hens administered with EO of oregano (Florou-Paneri et al., 2005), thyme, sage, and rosemary (Bölükbaşı et al., 2008) and a blend of EO (Özek et al., 2011; Bozkurt et al., 2012). Available limited results do not also support the promotion in feed intake in layer hens with respect to dietary treatment with MOS (Berry and Lui, 2000; Stanley et al., 2000; Zaghini et al., 2005; Çabuk et al., 2006).

The feed conversion ratio was not influenced by feed additives or high environmental temperatures. A surprising observation in the present investigation was the lack of negative effect of heat stress on feed conversion ratio. Indeed, the effect of high ambient temperature on feed conversion ratio appears rather controversial. Whereas several reports indicated either detrimental (Peguri and Coon, 1991) or useless (Balnave and Muheereza, 1997; Mashaly et al., 2004) effects, some others showed beneficial effects (Emery et al., 1984, Tanor et al., 1984).

In the present study, neither MOS nor EOM were effective in promoting feed conversion efficiency of modern layer breeds under hot and moderate climatic conditions. An important claim often made of phytogenic feed additives is improving efficiency of feed conversion thereby enhancing the intestinal availability of essential nutrients for absorption (Hertrampf, 2001; Williams and Losa, 2001); however, the specific experimental verification in laying hens is rather limited. Although the considerable potential to support gut health (Rolfe, 2000; Sims et al., 2004; Baurhoo et al., 2009), relatively little information is available as to application and potential benefits of MOS in laying hen nutrition. However, the superior productivity of the hen strain used and lack of room for improvement in performance, even under harsh climatic conditions, is conclusive.

Egg Quality

All of the egg quality parameters, excluding eggshell strength, were significantly affected either by feed additives or environmental temperature or both (Table 4). Eggshell quality was the property most adversely affected by heat stress (Tanor et al., 1984; Balnave and Muheereza, 1997; Mashaly et al., 2004). In the present study, exposure of laying hens to high envi-

Table 4. Egg quality as influenced by dietary supplementation with mannan-oligosaccharide (MOS) and essential oil mixture (EOM)

Period	Diet[1]	Shell thickness (μm)	Shell breaking strength (kg/cm^2)	Shell weight (%)	Yolk weight (%)	Albumen weight (%)	Albumen height (mm)	Haugh unit (score)
I, April–May	CNT	405	4.180	10.06	26.33	63.60	7.24	83.34
	MOS	410	4.199	10.24	26.45	63.30	7.03	81.83
	EOM	412	4.392	10.23	26.87	62.88	7.21	83.40
II, June–July	CNT	396	4.168	9.92	26.84	63.22	6.96	82.10
	MOS	399	4.298	10.20	27.49	62.30	6.61	79.92
	EOM	398	4.464	10.11	26.87	63.00	6.75	80.66
Pooled SEM[2]		0.35	2.84	0.136	0.07	0.20	0.21	0.09
Main effect[3]								
Diet								
CNT		400	4.174	9.99^b	26.58	63.41^a	7.10^a	82.72^a
MOS		404	4.249	10.22^a	26.97	62.80^b	6.82^b	80.87^b
EOM		405	4.428	10.17^a	26.87	62.94^b	6.98^{ab}	82.03^a
Period								
I		409^a	4.257	10.18^a	26.55^b	63.26^a	7.16^a	82.86^a
II		398^b	4.310	10.08^b	27.07^a	62.84^b	6.77^b	80.89^b
P-value								
Diet		0.1058	0.0911	0.0002	0.1789	0.0073	0.0406	0.0414
Period		0.0001	0.8205	0.0122	0.0051	0.0463	0.0001	0.0001
Diet × period		0.5177	0.9590	0.4869	0.1529	0.1451	0.4677	0.4423

[a,b]Means within columns, within main effects, with different superscripts are different at $P < 0.05$.

[1]Diets: CNT = control (no additive); MOS = mannan-oligosaccharide at 1 g/kg of diet; EOM = an essential oil mixture at 24 mg/kg of diet.

[2]Data are means of 6 replicates of 4 adjacent cages with 24 hens each per treatment.

[3]Data were analyzed as a 3 × 2 arrangement.

ronmental temperatures decreased eggshell thickness ($P < 0.01$) and eggshell weight ($P < 0.05$). Notably, eggshell strength was not influenced by the heat stress despite the deteriorations in eggshell thickness and eggshell weight. Confirming our observations, Roberts and Brackpool (1994) reported that in high environmental temperatures, eggshell structure is modified in favor of shell strength, even when shell thickness is reduced.

However, there is a lack of scientific information pertaining to the supplemental effects of performance-enhancer feed additives such as MOS or EOM on egg quality under heat stress. Noticeable improvements in shell quality of broilers breeders (Berry and Lui, 2000) and decreased eggshell deformation in layer hens (Çabuk et al., 2006) were reported when hens were fed diets supplemented with MOS. Consistent with those reports, in the present study, both MOS and EOM increased eggshell weight ($P < 0.01$), eggshell thickness ($P = 0.10$), and shell breaking strength ($P = 0.09$) compared with no added procedure (Table 4). The better results obtained for the eggshell quality parameters in hens fed MOS could be due to the prebiotic influence on the metabolic activity of the beneficial bacteria colony within the layers' intestine, which positively influences mineral absorption rate, especially those of Ca^{2+} and Mg^{2+} (Roberfroid et al., 2000). However, the mode of action of in-feed EO to achieve better eggshell quality is not completely clear.

In contrast to those beneficial effects on eggshell quality indices, neither MOS nor EOM enhanced the internal egg quality traits examined, while they decreased relative albumen weight and albumen height (Table 4). The MOS adversely affected Haugh unit. This implies that hens could not be able to keep up with the pace of the eggshell quality and internal egg quality when promoted by EOM and MOS. Egg yolk weight did not differ among treatments. The suggestion that carvacrol mediated protection mechanism in the liver may improve the production or transportation of egg yolk precursors from the liver and therefore increase egg yolk percentage over those birds that were not treated with EO such as thyme or sage (Botsoglou et al., 1997, 2002). However, this was not the case in our study; even the EOM used in this study consisted mainly of thyme and sage which provide carvacrol as a bioactive component. Similar failures in terms of improving albumen and egg yolk were reported by other researchers when hens were fed on diets with EO of thyme and rosemary (Bölükbaşı et al., 2008).

The albumen height and Haugh unit, 2 major indicators of egg quality, were adversely affected by elevated environmental temperatures. The significant reduction in albumen weight was in accordance with the decrease in albumen height (Table 4). The yolk weight was the only egg quality criterion that was not negatively influenced by heat stress. This implies that the increase in yolk weight may be relevant to decreased albumen weight through a natural physiological pathway rather than heat stress effect.

Immune Response

Antibody titers against IBD, ND, and IBV expressing the immune response of hens to viral infections were measured in this study. No significant changes in antibody responses were observed between 3 groups at the end of the 16-wk experimental period. Indeed, antibody responses have been used as measures of humoral

immune status of birds (Davis and Sell, 1989; Sklan et al., 1994). From this perspective, none of the additives used in this study, whether yeast-based or botanical originated, could support the immune system and boost antibody titers measured. Without special attention on heat stress, the mode of action of MOS on the immune response is relatively well documented to EO. The results showed that MOS served as an immune modulator in laying hens (Cotter et al., 2000) and in broiler breeders (Shashidhara and Devegowda, 2003). Our findings agree with Shafey et al. (2001), who reported no improvement in the antibody titers against IBDV and NDV in broilers fed MOS.

A very recent report by Özek et al. (2011) demonstrated that an EO blend was not effective in improving the humoral immune response of layer hens as measured serum IBDV, NDV, and IBV titers. In fact, the anti-oxidation properties of some plant bioactives (e.g., extracts and essential oils of some medicinal herbs) have been thought to have a role in the development of immune response in birds via protecting cells from oxidative damage and enhancing the function and proliferation of these cells (Sun et al., 1983; Franchini et al., 1991; Ma et al., 2005). However, it appears that the EOM used in this study, which consisted mainly of carvacrol and 1,8-cineole, could not promote systemic antibody response although they were regarded as remarkable antioxidant constituents (Botsoglou et al., 1997, 2005). Present findings showed that the antibody responses of laying hens is influenced by age-related decline rather than feed additive-based interference. Data relevant to antibody titers suggested that none of these additives has a function in stimulating humoral immune response in laying hens even though they were reared under seasonal heat stress for a 2-mo period.

In conclusion, differences in the nature of laying hen performance parameters could be attributed to the severity of the seasonal heat stress rather than dietary additive regimens applied. Neither MOS nor EOM, novel additives with a natural origin, showed any performance enhancer potential as a means of ameliorating the adverse effect of high environmental temperatures in layer hens throughout the post-peak production period, but they increased eggshell weight.

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