Effects of dietary herbal essential oil mixture and/or mannan-oligosaccharide supplementation on laying performance, some serum biochemical markers and humoral immunity in laying hens exposed to heat

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SUMMARY

This study investigates the effects of dietary supplementation with essential oil mixture (EOM) and/or mannan-oligosaccharides (MOS) in laying hens reared under heat stress conditions on body weight gain, laying performance and egg quality as well as on some serum biochemical markers (glucose, cholesterol and triglycerides) and on humoral immunity. A total of 192 laying hens, 52 week old at the beginning of the experiment, exposed to high ambient temperatures during the summer season, were randomly allotted into 4 equal groups according to the diet regimens: 3 groups were supplemented with EOM (included essential oils from Oregano, laurel, sage, myrtle, fennel seeds and citrus peels, 36 mg/kg/day) or with MOS (1 g/kg/day) or with EOM and MOS (with the same dosages) for 16 weeks whereas the last one was not supplemented (negative control). The weight gain tended to be higher (but not significantly) in the 3 supplemented groups. The dietary supplementation with EOM alone or combined with MOS has significantly increased the egg weight and the Haugh unit and the albumen height were markedly enhanced in eggs from all supplemented hens whereas only eggs from hens treated with EOM and MOS combination exhibited significantly higher eggshell thickness. The intestinal pH was dramatically depressed in MOS and in EOM supplemented hens, whereas the intestinal viscosity was significantly reduced only in birds treated with both 2 additives and the small intestine weight was significantly decreased in MOS supplemented ones. On the other hand, no significant alterations in blood biochemistry or in titres of circulating antibodies against the Newcastle disease, infectious bronchitis and infectious bursal disease viruses were evidenced in supplemented hens compared to the controls. These results show that MOS and/or EOM supplementation in laying hens exposed to high temperatures improves some laying performances (egg weight) and egg qualities and partly promotes the digestive function.

Keywords: Laying hens, essential oils, mannan-oligosaccharide, heat exposure, laying performance, egg quality, digestive system characteristics, humoral immunity.

RÉSUMÉ

Effets d’une supplémentation de la ration par un mélange d’huiles essentielles végétales et/ou par des mannanoligosaccharides sur les performances de ponte, quelques paramètres biochimiques sanguins et l’immunité humorale chez les poules pondeuses exposées à la chaleur

Cette étude a pour objectifs d’analyser les effets d’une supplémentation de la ration par un mélange d’huiles essentielles (MHE) et/ou par des mannanoligosaccharides (MOS) chez des poules pondeuses exposées à de fortes chaleurs sur la croissance, les performances de ponte, la qualité des œufs ainsi que sur quelques paramètres biochimiques sanguins (glucose, cholestérol et triglycérides) et sur l’immunité humorale. Au total, 192 poules pondeuses, âgées de 52 semaines au début de l’expérimentation et exposées à de fortes températures ambiante durant l’été, ont été aléatoirement réparties en 4 groupes égaux en fonction du régime alimentaire : 3 groupes ont été supplémentés soit par un mélange d’huiles essentielles (issus de l’origan, du laurier, de la sauge, de la myrte, des graines de fenouil et des écorces d’agrumes, 36 mg/kg/jour), soit par des mannanoligosaccharides (1 g/kg/jour), soit par les 2 (aux mêmes dosages) pendant 16 semaines alors que le dernier n’a reçu aucune supplémentation. Le gain de poids est apparu plus élevé (mais non significativement) pour les 3 groupes supplémentés. La supplémentation de la ration par les huiles essentielles seules ou combinées aux MOS a augmenté significativement le poids des œufs, et les unités de Haugh et la hauteur de l’albumen des œufs issus de toutes les poules supplémentées a été nettement accrue, alors que seulement les œufs issus des oiseaux traités à la fois par les huiles essentielles et les MOS ont présenté une augmentation significative de l’épaisseur de la coquille. Le pH intestinal a notament baissé chez les poules supplémentées par les MOS seuls ou combinés aux huiles essentielles, tandis que la viscosité intestinale a été significativement réduite seulement chez les poules traitées par les 2 additifs et que le poids de l’intestin grêle a été significativement diminué seulement chez celles supplémentées par les MOS. Par ailleurs, aucune modification significative de la biochimie sanguine ou des titres des anticorps sériques dirigés contre les virus de la maladie de Newcastle, de la bronchite infectieuse ou de la maladie de la bourse n’a été mise en évidence chez les poules supplémentées. Ces résultats montrent qu’une supplémentation par des huiles essentielles et/ou les MOS améliore certaines performances de ponte (poids des œufs) ainsi que la qualité des œufs et favorise, au moins partiellement, la fonction digestive.

Mots clés : Poules pondeuses, huiles essentielles, mannanoligosaccharides, exposition à la chaleur, performances de ponte, qualité des œufs, caractéristiques du système digestif, immunité humorale.
Introduction

Heat stress has deleterious effects on productive performance of different poultry species [32]. For example, in laying hens, heat stress reduces egg production [17, 32, 33], egg weight [4, 18, 38, 43], eggshell thickness and strength [15, 20, 31, 32]. Furthermore, birds reduce food intake in high temperatures because of reducing metabolic heat production [47]. On the other hand, EMERY et al. [15] reported that high temperature did not affect egg production. In addition, MuIRUR and HARRISON [33] observed that heat stress did not affect egg weight and food conversion. Nutritional manipulations are preferred to alleviate the negative effects of heat stress on performance of laying hens. Therefore, antioxidants, lactic acid, antibiotic, probiotic, prebiotic, vitamins and minerals as a nutritional manipulation tools are commonly added to the diets of birds reared under heat stress [29, 42, 48].

Essential oils extracted from herb and spices are a complex mixture of various compounds, which consist of aromatic and volatile substances [1, 5, 27]. Essential oils have some antimicrobial [14], antioxidant [6], enzymatic [22], digestion stimulating [11] biological properties. They also have anti-heat stress effects and they activate immune system [12, 39]. Prebiotics are non digestible carbohydrates. Two of the most commonly studied prebiotics are fructo-oligosaccharides and mannan-oligosaccharides (MOS) which are obtained from the cell wall of yeast (Saccharomyces cerevisiae) [19]. The yeast cell wall has powerful antigenic stimulating properties [44] but MOS do not enrich for beneficial bacterial populations [9, 19, 37]. Instead, they are thought to act by binding and removing pathogens from the intestinal tract and stimulation of the immune system [8, 37]. Moreover, a study carried out recently [12] showed that MOS have beneficial effects on reducing the negative effects of high temperatures. Recently, the effects of essential oils in broiler diets have been largely demonstrated [1, 2, 8, 11, 24, 25, 28]. However, the effects of essential oils and MOS in laying hen diets have not been extensively studied, especially in summer season.

The aim of this experiment was to assess the effects of dietary supplements such as an essential oil mixture (EOM) and/or MOS in laying hen diets in hot summer season on laying traits, digestive system characteristics, some serum biochemical markers, and humoral immunity of laying hens.

Material and Methods

BIRDS AND EXPERIMENTAL DESIGN

In this trial, one hundred ninety two 52 week-old ATAK-S laying hens were used. The trial was conducted in a layer pen designed for commercial during the summer season, between June and September 2010, in Ankara, located in central Turkey. The experimental diets and drinking water were offered ad libitum. A photoperiod of 17 hours/day was maintained. The hens were housed in individual layer cages. The layer cages were three floored and 25 x 47 cm in size. Hen house temperature was recorded all hours of the day during the experiment. Mean house temperature for each month was calculated from these records. Mean hen house temperatures (°C) for June, July, August and September were 26, 29, 30 and 28, respectively.

Birds were randomly divided into four treatment groups of 48 birds each. The dietary treatments used in this study were as follow: 1. basal diet (negative control), 2. basal diet supplemented with 36 mg/kg EOM (Heryumix™, Herba Ltd. Co., İzmir, Turkey), 3. basal diet supplemented with 1 g/kg MOS (Bio-Mos, Altech Inc) and 4. basal diet supplemented with 36 mg/kg EOM and 1 g/kg MOS. The commercial product Heryumix™ was a mixture of 6 different essential oils derived from selected herbs [Oregano oil (Origanum sp.), laurel leaf oil (Laurus nobilis L.), sage leaf oil (Salvia triloba L.), myrtle leaf oil (Myrtus communis), fennel seeds oil (Foeniculum vulgare), and citrus peel oil (Citrus sp.)] and included carvacrol, thymol, 1,8-cineole, p-cymne, and limonene as active components. The commercial product Bio-Mos contains mannan-oligosaccharide (MOS). Essential oil premix used 964 g of zeolite as a carrier for 36 g of essential oil mixture. Essential oil premix and MOS were added as supplements to the basal diet (i.e., 1 kg of supplement/1,000 kg of feed was added in place of the sawdust conventionally found in the feed mix). The ingredients and chemical composition of the basal diets are presented in Table I.

Before the experiment, the test animals were fed with starter (0-4 weeks), grower (5-10 weeks), developer (11-16 weeks), egg starter (17-20 weeks), layer stage 1 (21-40 weeks) and layer stage 2 (41-52 weeks) diets and during the experiment, the basal layer stage 2 (52-68 weeks) was used. The diets were formulated to meet or exceed NRC [35] specifications. The basal diet was analyzed for dry matter, crude ash, crude protein, crude oil, crude fibre, sugar, starch, according to chemical analytical methods recommended by the Association of German Agricultural Analysis and Research Institutes (VDLUFA) [34]. The metabolisable energy content of the diet was calculated based on chemical composition [3] and the ingredients and chemical composition of the basal diets are presented in Table I. The experimental diets were isonitrogenic and isocaloric. Any substance was not added to the diet for improving egg yolk colour in order to determine the treatment effects on egg yolk colour.

The experimental protocol was approved by the Ethic Committee from Ankara Poultry Research Institute.

ANALYSIS OF GROWTH PERFORMANCE AND LAYING TRAITS

Individual body weights of all hens were measured at the beginning and at the end of the experiment, when hens were 52 week old and 68 week old respectively. Diets were in mash form. Egg production performance was expressed as a percentage of hen-day egg production. Egg production, broken-cracked eggs and mortality were recorded daily. During the entire experimental period, 30 randomly sampled eggs from each treatment group were collected on two consecutive days biweekly. Hence, 960 eggs were weighed to determine average egg weight. Food intake was recorded on a weekly basis. Egg mass and food conversion ratios were calculated as following:

Daily egg mass (g/100) = Hen-day egg production (%) x egg weight (g)/100.

Food conversion ratio (g food/g egg) = Daily food intake (g/100) / Daily egg mass (g/100).
The pH value of the intestinal (duodenum and jejunum) content was determined with a pH meter (Hanna Instruments-8413). Several aliquots were filled with each sample, labelled, and centrifuged (4000 g for 10 minutes at 4°C). The supernatant was withdrawn and the viscosity of a 0.5 mL aliquot was measured using a Brookfield Digital Viscometer (Model DV-II+PRO, Brookfield Engineering Laboratories, Stoughton, MA, USA) maintained at 40°C and expressed in cp.

**BIOCHEMICAL AND SEROLOGICAL ANALYSES**

At the end of the study, blood samples (10 mL) were drawn from the jugular vein of 6 birds from each treatment group in sterile microtubes without anticoagulant. Food was not withdrawn from front of the hens before blood was drawn. After clotting for 2 hours at room temperature, serum was separated by centrifugation at 3000 g for 10 minutes at room temperature, and stored at -20°C until biochemical analyses (determination of the serum concentrations of glucose by the glucose oxidase method [49], of triglycerides by the glycerol-3-phosphate-oxidase para-aminophenazone GPO-PAP colorimetric enzymatic method [49] and of total cholesterol by the cholesterol-oxidase para-aminophenazone CHOD-PAP colorimetric enzymatic method [41]).

As a conventional vaccine program was used for the experimental animals, serum samples were collected from 6 hens from each treatment group at the beginning and at the end of the experiment in order to investigate the antibody titres against the Newcastle disease (ND), Infectious bronchitis (IB) and Infectious bursal disease (IBD) viruses by ELISA technique using commercial kits (IDEXX Flockcheck). Plates were read at 650nm on an ELISA reader (Labsystems Multiscan MS, Labsystems, Helsinki, Finland).

**STATISTICAL ANALYSIS**

All data were subjected to analysis of variance in the general linear model using the SPSS 15.0 [46] statistical package. The experimental unit was a cage containing 1 hen. Post-hoc analyses were carried out to separate the significant differences between treatment means using the Duncan’s test. Differences were considered as significant when $P$ value was less than 0.05.

**Results**

The productivity and egg quality data of laying hens are summarized in Table II. There were no significant differences in hen-day egg production, egg mass, cracked-broken egg ratio, food intake, food conversion ratio, liveability and live weight gain (LWG) between the treatment groups, although the body weight gains, egg masses and food intakes tended to

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**ANALYSIS OF THE DIGESTIVE FUNCTION**

At the end of the experimental period, 6 hens were selected from each treatment group, weighed and slaughtered severing the bronchial vein to determine some digestive system characteristics. After evisceration, pancreas, liver and small intestine without digestive content were weighed individually and the whole small intestine length was measured. The weights of these internal organs were expressed as percentages of live body weight.

The productivity and egg quality data of laying hens are summarized in Table II. There were no significant differences in hen-day egg production, egg mass, cracked-broken egg ratio, food intake, food conversion ratio, liveability and live weight gain (LWG) between the treatment groups, although the body weight gains, egg masses and food intakes tended to
ÖZEK (K.)

be higher in the supplemented groups than in the controls and the best food conversion ratio was obtained in the EOM group. Furthermore, the egg weights were significantly higher in the EOM and (MOS + EOM) groups than in the control and MOS groups ($P = 0.026$). Essential oil mixture and MOS supplementations did not significantly affect the outer egg quality parameters (eggshell weight, eggshell weight ratio and eggshell strength) except the eggshell thickness which was markedly increased in hens simultaneously supplemented with EOM and MOS ($P = 0.002$). However, dietary EOM and/or MOS supplementations have significantly modified some inner egg quality parameters, such as the albumen height and Haugh units which were dramatically elevated compared to the hen controls ($P = 0.013$ and $P = 0.022$, respectively). By contrast, the yolk colour intensity was not affected by the dietary treatments.

The effects of the dietary additives on the relative weight of some internal organs (liver and pancreas), small intestine characteristics and on some biochemical and serological markers are reported in Table III. The intestinal pH was significantly decreased when MOS were added to the diet alone or coupled to the EOM ($P < 0.001$) compared to the 2 other groups. The intestinal viscosity and the small intestine weight tended also to be affected by the MOS supplementation alone or combined with EOM but these 2 parameters were significantly lowered only in the MOS + EOM group ($P = 0.006$) and in the MOS group ($P = 0.005$), respectively. As shown in Table III, biochemical and immune parameters were not affected by the various supplementations but total cholesterol concentrations tended to increase in treated birds (from 8% in MOS supplemented hens to 23% in EOM supplemented ones) compared to the controls.

**Table II:** Effects of essential oil mixture (EOM, 36 mg/kg), mannan-oligosaccharide (MOS, 1 g/kg) and essential oil mixture (EOM, 36 mg/kg) plus mannan-oligosaccharide (MOS, 1 g/kg) included in the basal diet (layer stage 2) of laying hens for 16 weeks on productivity and egg quality parameters. Results are expressed as mean ± pooled standard error of the mean (SEM).

**Discussion**

Essential oil mixture, MOS and EOM + MOS supplementation in layer diets resulted in no detrimental effects on general health status. Essential oil mixture, MOS and EOM + MOS supplementation in laying hen diets did not affect any productivity parameters except the egg weight which was significantly increased in hens supplemented with EOM alone or combined to MOS. Moreover, the body weight gains tended to be higher in supplemented birds than in the controls. Recent studies indicated that supplementation with plant extracts in broiler diets result in growth promotion, nutrient digestibility enhancement, and improvement of food efficacy in broilers [1, 2, 8, 11]. But some author reported that essential oil supplementation to broiler diets did not improve growth [24, 28]. SIM et al. [45] showed that food efficiency was significantly improved in turkeys fed with diets including 0.2% MOS for the first 6 weeks of life and then 0.05% for the remainder of the trial compared to the not supplemented controls.

In the present study, the egg production was not significantly improved in EOM or MOS supplemented hens during...
the summer season. By contrast, SHASHIDHARA et al. [44] observed that MOS supplementation to broiler breeder diets significantly increased egg production between 60-67 weeks of age and in a recent study carried out in the summer season [12], the hen-day egg production of brown layers, 54 to 74 weeks old, receiving diets supplemented with an EOM and MOS was significantly higher compared to the control (P < 0.01). Additionally, the beneficial effects of the herbal additives on the egg production rate of laying hens during heat stress were observed in another recent study [30]. On the other hand, BOZKURT et al. [10] showed that dietary EOM supplementation did not affect egg production and egg weight from broiler breeders. In another study [7] investigating the effects of dietary aromatic plant extracts on the laying performance of 32 to 40 weeks old hens, no significant differences in egg production and egg weight were found among the treatment groups. Previous studies reported that dietary MOS and plant extract supplementation exhibited different effects on egg production and egg quality of laying hens. These discrepancies would be related to the amounts of MOS and plant extracts added to the diets and to the nature and source of such additives.

The results of the present study showed no significant differences in eggshell weight, eggweight weight ratio, eggshell strength, and egg yolk colour among treatments. However, the eggshell thickness was markedly enhanced in EOM and MOS supplemented hens and the albumen height and Haugh unit were significantly increased in all supplemented birds compared to the controls. As heat stress reduces egg quality (eggshell thickness and strength) in laying hens [15, 20, 31, 32], the heat exposure may result to an increase in cracked-broken egg number leading to large economic losses in the laying hen sector. In this context, it is very important that combined supplementation of EOM with MOS in diet increased eggshell thickness in current study. In addition, both dietary EOM and MOS supplementations increased albumen height and Haugh unit. The Haugh unit is known as an indicator of egg freshness and is related to shelf life. The improvement in these parameters may indicate that essential oil and MOS supplementation can improve egg quality by increasing its shelf life. However, these results were in disagreement with those of BOTSOGLOU et al. [7] and FLOROU-PANERI et al. [16] who reported that Haugh unit, eggshell thickness and albumen height were not affected by oregano essential oil supplementation to diet. Significant differences were observed in intestinal pH and viscosity, and small intestine weight among treatments. The MOS supplementation to diet alone or combined with EOM significantly decreased intestinal pH. Low pH in stomach and intestine suppress growth of acid-intolerant bacteria. Low gastric pH accelerates the conversion of pepsinogen to pepsin, which enhances the absorption rate of proteins and minerals [36].

<table>
<thead>
<tr>
<th>Digestive system parameters</th>
<th>Control</th>
<th>EOM</th>
<th>MOS</th>
<th>MOS + EOM</th>
<th>SEM</th>
<th>P</th>
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<tr>
<td>Intestinal viscosity (cP)</td>
<td>1.89a</td>
<td>1.89a</td>
<td>1.72a</td>
<td>1.42b</td>
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<td>Intestinal pH</td>
<td>6.22a</td>
<td>6.30a</td>
<td>5.86b</td>
<td>5.60b</td>
<td>0.080</td>
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<td>Small intestine length (cm)</td>
<td>128.4</td>
<td>128.2</td>
<td>132.6</td>
<td>122.6</td>
<td>2.310</td>
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<td>Small intestine weight (%)</td>
<td>2.39a</td>
<td>2.46a</td>
<td>1.79b</td>
<td>2.27a</td>
<td>0.080</td>
<td>&lt; 0.01</td>
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<td>Liver weight (%)</td>
<td>1.99</td>
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<td>1.91</td>
<td>2.02</td>
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<td>Pancreas weight (%)</td>
<td>0.21</td>
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<td>0.19</td>
<td>0.18</td>
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<th>Biochemical and immune parameters</th>
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<tr>
<td>Glucose (mmol/L)</td>
<td>12.67</td>
<td>12.93</td>
<td>13.01</td>
<td>12.61</td>
<td>0.192</td>
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<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.06</td>
<td>4.99</td>
<td>4.39</td>
<td>4.54</td>
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<td>Triglycerides (g/L)</td>
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<td>2.72</td>
<td>2.35</td>
<td>2.07</td>
<td>0.194</td>
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<td></td>
</tr>
<tr>
<td>At 52 week old</td>
<td>13 756</td>
<td>12 131</td>
<td>11 820</td>
<td>13 691</td>
<td>867</td>
<td>NS</td>
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<tr>
<td>At 68 week old</td>
<td>4 291</td>
<td>6 518</td>
<td>7 116</td>
<td>3 560</td>
<td>745</td>
<td>NS</td>
</tr>
<tr>
<td>IBV antibody titres</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 52 week old</td>
<td>7 715</td>
<td>10 904</td>
<td>8 498</td>
<td>9 758</td>
<td>711</td>
<td>NS</td>
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<tr>
<td>At 68 week old</td>
<td>11 610</td>
<td>9 373</td>
<td>9 621</td>
<td>5 673</td>
<td>1 139</td>
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<td>IBDV antibody titres</td>
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<td></td>
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<tr>
<td>At 52 week old</td>
<td>9 213</td>
<td>9 245</td>
<td>8 908</td>
<td>8 379</td>
<td>239</td>
<td>NS</td>
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<td>At 68 week old</td>
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<td>8 684</td>
<td>8 623</td>
<td>7 906</td>
<td>493</td>
<td>NS</td>
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EOM: Essential oil mixture (Heryumix™, mixture of Oregano oil (Origanum sp.), laurel leaf oil (Laurus nobilis L.), sage leaf oil (Salvia triloba L.), myrtle leaf oil (Myrtus communis), fennel seeds oil (Foeniculum vulgare), and citrus peel oil (Citrus sp.)); MOS: Mannan-oligosaccharide; NDV: Newcastle Disease virus; IBV: Infectious bronchitis virus; IBDV: Infectious bursal disease virus; NS: not significant.

Different superscripts a,b in the same row indicate significant differences between groups (P < 0.05 or more).

Table III: Effects of essential oil mixture (EOM, 36 mg/kg), mannan-oligosaccharide (MOS, 1 g/kg) and essential oil mixture (EOM, 36 mg/kg) plus mannan-oligosaccharide (MOS, 1 g/kg) included in the basal diet (layer stage 2) of laying hens for 16 weeks on the digestive system traits and some blood biochemical and serological markers. Results are expressed as mean ± pooled standard error of the mean (SEM).
Combined EOM and MOS supplementation synergistically depressed the intestinal viscosity but no other study studying relationship between MOS or essential oil mixture with intestinal viscosity was available to our knowledge. Feeding with MOS supplemented diet has significantly decreased the relative small intestine weights compared to the other groups. Previous studies reported controversial effects of such supplementations on relative interne organ weights. Indeed, ALÇIÇEK et al. [2] reported that the dietary EOM supplementation significantly reduced the small intestine weight and BOZKURT et al. [8] found that dietary MOS inclusion did not affect it.

Neither dietary EOM nor MOS supplementation has significantly modified the serum glucose, triglyceride and total cholesterol concentrations. Supplementing diets with EOM, MOS and EOM + MOS had no significant advantageous effect on serum antibody titres against ND, IB and IBD viruses in the summer season. The results of the current study show that changes of antibody titres of NDV between the 52nd and the 66th weeks of age had similar trends whatever the dietary treatment. On the contrary, HU [23] and KONG et al. [26] reported that appropriate doses of Chinese herbal ingredients had positive effects on immune enhancement for chickens. Likewise, it was reported that MOS supplementation resulted to a significant improvement in antibody responses in broilers and layers [13, 40]. In addition, SHASHIDHARA and DEVEGOWDA [44] reported that MOS supplementation improved antibody responses against infectious bursal disease in broiler breeders.

In conclusion, single or combined supplementation EOM and MOS to layer diet as an anti heat stress tool showed positive effects on egg weight, eggshell thickness, albumen height and Haugh unit in summer season. Combined supplementation EOM with MOS decreased intestinal viscosity. Additionally, MOS supplementation alone or combined with EOM decreased intestinal pH, but no metabolic or immune effects were evidenced in the supplemented layer hens in the present study. More detailed studies are still needed to determine the function of EOM and MOS supplementation to laying hens in hot summer season.

References


