Ann. Anim. Sci., Vol. 16, No. 4 (2016) 1115–1125 DOI: 10.1515/aoas-2016-0038

DE GRUYTER
OPEN

THE EFFECT OF DIETARY ESSENTIAL OIL MIXTURE SUPPLEMENTATION ON PERFORMANCE, EGG QUALITY AND BONE CHARACTERISTICS IN LAYING HENS*

Osman Olgun

Selcuk University, Faculty of Agriculture, Department of Animal Science, 42075, Konya, Turkey
Corresponding author: oolgun@selcuk.edu.tr

Abstract

The aim of this study was to investigate the effect of seven different levels (0, 25, 50, 100, 200, 400, and 600 mg/kg) of a phytogenic feed additive containing a mixture of essential oils from thyme, black cumin, fennel, anise and rosemary on performance, eggshell quality, bone biomechanical properties and bone mineralization in laying hens. This study consisted of a total of 112, 21-week-old Super Nick laying hens, which were randomly distributed into seven experimental groups. During the 12-week experimental period, each experimental group of four replicates of four birds each was fed with seven treatment diets. Egg weight and egg mass were positively linearly affected by essential oil mixture supplementation. Also, eggshell thickness was increased quadratically by essential oil mixture supplementation. The biomechanical properties and tibia mineral content were adversely affected by essential oil mixture supplementation at the level of 600 and 400 mg/kg, respectively. These results demonstrated that dietary supplementation with a low or medium concentration of essential oils improved bone parameters, while at high levels were adversely affected in laying hens.

Key words: mixture essential oils, laying hens, bone, eggshell quality, performance

The use of phytogenic feed additives such as plant extracts and essential oils in poultry nutrition has gained recent interest, through the ban of using antibiotics. Generally, the interest in essential oils as feed additive has mainly focused on the effect on performance (Çabuk et al., 2006; Bozkurt et al., 2012 a, b; Bölükbaşı et al., 2008; Kaya et al., 2013), eggshell quality traits (Bölükbaşı et al., 2008; Bozkurt et al., 2012 a, b; Kaya et al., 2013; Olgun and Yıldız, 2014), antimicrobial activity (Cross et al., 2007; Jang et al., 2007; Bölükbaşı et al., 2008; Cao et al., 2010), antioxidants (Botsoglou et al., 2005; Krause and Ternes, 1999), nutrient digestibility (Hernandez et al., 2004; Cao et al., 2010; Amad et al., 2011) or lipid metabolism (Bölükbaşı et

* This research was supported by Selcuk University BAP. Project No: 15401024.

al., 2008; Kaya et al., 2013). Bozkurt et al. (2012 a) reported the beneficial effects of supplementation with feed-grade essential oil mixture (EOM; 24 mg/kg) on the egg production and egg weight of Lohmann LSL White and Lohmann Brown layers. In addition, Kaya et al. (2013) indicated that supplementation of a mixed herbal product containing *Origanum vulgare*, *Thymus vulgaris*, thyme oil, origanum oil, garlic oil, anise oil and fennel oil (500, 750, and 1,000 mg/kg) to diets of laying hens improved eggshell stiffness and thickness.

The bone in laying hens is an important mineral source for metabolic requirements and eggshell formation, which has a major influence on the economics of the egg industry. In a very limited number of studies, the effects of phytogenic feed additives or essential oils in the diet on the bone have been studied. Świątkiewicz et al. (2014) stated that the dietary addition of 200 mg/kg of herb extract mixtures (*Taraxaci siccum*, *Urticae siccum* and *Salviae siccum*) increased the breaking strength of femur and tibia in laying hens. Similarly, Mühlbauer et al. (2003) demonstrated that essential oils (sage, rosemary and thyme) and monoterpenes are efficient inhibitors of bone resorption in rats.

Although only few studies investigate the effect of phytogenic feed additives or essential oils on bones, more studies have been performed to determine the effect on minerals necessary for normal bone and eggshell formation in poultry (Lim and Paik, 2003; Świątkiewicz and Koreleski, 2008). Numerous reports have shown that dietary addition of phytogenic feed additives or essential oils resulted in an increase in serum phosphorus (Akbarian et al., 2015) and plasma zinc (Zn) (Torki et al., 2015), and a decrease in plasma calcium (Ca) (Capkovicova et al., 2014), or were ineffective on mineral levels in poultry (Ali et al., 2007; Cao et al., 2010; Lokaewmanee et al., 2014). In contrast, Olgun and Yıldız (2014) noted that the dietary addition of EOM (400 or 600 mg/kg) containing a mixture of essential oils from thyme, black cumin, fennel, anise and rosemary reduced the excretion of minerals (Ca, P, magnesium (Mg), manganese (Mn) and Zn) in breeder quails. The findings of Amad et al. (2011) showed that the phytogenic feed additive as thyme and star anise oils to the broiler diets caused a linear increase in the apparent ileal digestibility of Ca and P, respectively. Also, they concluded that the improvement in the digestibility of nutrients could be due to the stimulation effect of the phytogenic feed, which increased absorption surface area in the intestine and endogenous digestive enzymes.

The aim of this study was to determine the effect of different levels of dietary EOM supplementation on the performance, egg quality, tibia biomechanical properties and mineral contents of bone in laying hens.

Material and methods

A total of 112, 21-week-old, Super Nick laying hens were randomly allotted to seven equal groups according to dietary treatments. Each group included four replicates with four laying hens. The hens were fed with a basal diet based on corn-soy-

bean meal, containing 17.10% crude protein, and corresponding to a metabolisable energy of 11.52 MJ/kg (Super Nick, 2012; Table 1). All hens in the first group were fed on a basal diet and considered as control, while those in the 2nd, 3rd, 4th, 5th and 6th groups were fed the same diet but supplemented with 25, 50, 100, 200, 400 and 600 mg EOM/kg diet throughout the experimental period of 12 weeks. The EOM was composed with equal amounts of five totally different essential oils (i.e., thyme oil, black cumin oil, fennel oil, anise oil, and rosemary oil (as active ingredients; thymol, p-Cymene, α- and β-pinene, anethole, and cineole, respectively)). The birds were housed in an environmentally controlled room equipped with 28 metal battery cages (50×40×50 cm), while they were fed and watered *ad libitum* throughout the experimental period. Also, all hens were daily exposed to 16 lighting hours from 06:00 to 22:00 with light intensity of 8 lux/m² throughout the experimental period. Criteria specified by the NIH (National Institute of Health Guide for the Care and Use of Laboratory Animals) were followed during the study period.

Table 1. Composition of the basal diet (g/kg as feed)

Item	Content
Ingredients	
corn	520.00
barley	70.00
soybean meal	210.00
sunflower meal	63.00
sunflower oil	26.70
limestone	87.50
dicalcium phosphate	13.80
salt	4.00
premix[1]	2.50
DL methionine	1.50
sawdust[3]	1.00
	1000.00
Chemical composition	
metabolisable energy (MJ/kg)	11.52
crude protein	17.10
lysine	8.32
methionine	4.13
methionine + cysteine	7.38
calcium[2]	37.74
total phosphorus[2]	4.49
non-phytate phosphorus	3.81

[1]Supplied per kg diet, Manganese: 80 mg, Iron: 60 mg, Zinc: 60 mg, Copper: 5 mg, Selenium: 0.15 mg, Cobalt: 0.20 mg, Iodine: 1 mg, Trans-retinol: 3.6 mg, Cholecalciferol: 0.1 mg, Menadione: 5 mg, α-tocopherol acetate: 75 mg, Thiamine: 3 mg, Riboflavin: 6 mg, Pyridoxine: 5 mg, Cyanocobalamin: 0.03 mg, Nicotinic acid: 40 mg, Pantothenic acid: 10 mg, Folic acid: 0.75 mg, D-biotin: 0.075 mg, Choline chloride: 375 mg,

[2] Analyzed value as feed.

[3] The essential oil premixes used 975, 950, 900, 800, 600 and 400 g of zeolite, respectively, as a carrier for 25, 50, 100, 200, 400 and 600 g, respectively, of essential oil.

Initial and final body weight (BW) of hens were recorded by weighing the hens using a 1.0 g precision scale. Egg production rate (EPR) was recorded daily. Feed intake (FI) and egg weight (EW) were recorded bi-weekly. Egg mass (EM) was calculated from the bi-weekly EPR and EW data using the formula: *EM = (EPR× EW)/Period (days)*. The feed conversion ratio (FCR) was calculated using the formula: FCR = FI/EM. The egg quality characteristics (specific gravity, shell breaking strength, shell weight, and shell thickness) were evaluated using random samples of 10 eggs from each replicate, thereby totaling 40 eggs from each treatment. Specific gravity was determined on the day of collection using graded salt solutions ranging from 1.080 to 1.090, with gradations of 0.005 (Holder and Bradford, 1979). Eggshell breaking strength was measured using a cantilever system by applying increasing pressure to the broad pole of the shell using an Egg Force Reader (Orka Food Technology Ltd., Ramat Hasharon, Israel). The eggs were broken to determine eggshell, albumen and yolk weights. The eggshells were rinsed in running water and dried in an oven at 60°C for 12 h. Eggshells were weighed using a 0.01 g precision scale. Eggshell percentage was calculated using the formula: *eggshell weight (% EW) = [eggshell weight (g)/EW (g)]*. Eggshell thickness (including the membranes) was determined at three points on the eggs (one point on the air cell and two randomized points on the equator) using a micrometer (Mitutoyo Inc., Kawasaki, Japan).

Hens (one hen per replicate and four hens per treatment group) were killed by cervical dislocation, and then the left and right tibias with some attached flesh were collected. Bones were excised from all flesh and proximal cartilages were removed. While the left tibias were used for the determination of mineral contents, the right ones were used for measuring the bone mechanical properties. The sample tibias were placed in a plastic container and stored at −20ºC until analysis. The bone samples were thawed at room temperature for 6 h in an air-conditioned room before the measurements began. The bone mechanical properties were determined from the load-deformation curve generated from a three-point bending test (ASAE Standard S459, 2001) using an Instron Universal Testing Instrument (Model 1122; Instron, Canton, MA) and the Test Works 4 software package (version 4.02; MTS System Corporation, Eden Prairie, MN). The crosshead speed was constant at 5 mm per min. The full-scale load of the load cell was 5,000 Newton (N). Shear tests were performed on the tibia using a double-shear block apparatus. The shear force was exerted over a 6.35-mm (0.25-inch) section located at the centre of the diaphysis. These tests enabled the ultimate shear force and shear stress to be evaluated for each bone. The mean wall thickness (cortex thickness) of the tibia was measured using digital calipers (precision of 0.001 mm) at two points on the central axis of the broken tibia that was used to determine the mechanical properties. These mechanical properties of the bone are described by Wilson and Ruszler (1996) and Armstrong et al. (2002).

Tibia mineral contents were determined using MarsXpress Technology Inside and an Inductively Coupled Plasma Atomic Emission Spectrometer (Vista AX CCD Simultaneous ICP-AES, Varian, Mulgrave, Australia). Approximately 0.20 g dried sample (bone with marrow removed) was introduced into a burning cup and 5 mL nitric acid, 3 mL perchloric acid and 2 mL hydrogen peroxide were added. The sample was incinerated in a MARS 5 Microwave Oven (CEM, Corp., Mathews, NC, USA)

at 190°C and 1.207 kPa pressure, and subsequently diluted to 50 mL with distilled water. The mineral concentrations were determined using an ICP-AES (Skujins, 1998).

Data were analyzed by one-way ANOVA using the SPSS 18.0 software package (SPSS Inc., Chicago, IL, USA), using the cage mean as an experimental unit. A probability value of $P < 0.05$ was considered statistically significant. Orthogonal polynomial contrasts were used to assess the significance of linear and quadratic models to describe the response of the dependent variable to a rising EOM level.

Results

The changes in BW, EPR, EW, EM, FI, and FCR results are presented in Table 2. However, EW linearly increased with increasing EOM supplementation, especially at 200 or 400 mg/kg ($P \leq 0.001$). Also, the egg mass linearly increased ($P \leq 0.05$) in laying hens supplemented with 50 mg/kg EOM compared to those supplemented with 0, 25, and 100 mg/kg EOM. Addition of EOM had no effects on the body-weight change, EPR, FI, FCR and mortality (data not shown). The eggshell quality traits, such as specific gravity, egg breaking strength, eggshell weight and eggshell thickness are presented in Table 3. There was a quadratic effect of EOM on eggshell thickness ($P \leq 0.001$); therefore, the eggshell thickness was positively influenced by the additional dietary EOM.

Table 2. Effect of dietary supplementation of essential oil mixture on the performance in laying hens

	Essential oils mixture (mg/kg)							SEM[1]	P-value of contrast	
	0	25	50	100	200	400	600		linear	quadratic
Body weight change (g)	73.8	73.1	10.2	65.1	77.9	10.4	56.1	38.7	0.638	0.759
Egg production (eggs/100 birds/d)	84.33	87.79	91.25	84.38	87.25	87.08	88.47	1.76	0.519	0.630
Egg weight (g)	56.40	56.06	58.51	57.16	58.70	59.95	58.05	0.49	0.001	0.109
Egg mass (g/bird/d)	47.58	49.21	53.39	48.22	51.21	52.18	51.37	1.04	0.028	0.295
Feed intake (g/bird/d)	99.04	94.88	93.85	99.11	95.24	99.41	95.68	4.19	0.988	0.817
Feed conversion ratio (g feed/g egg)	2.08	1.93	1.76	2.06	1.86	1.90	1.86	0.07	0.208	0.433

[1]Each value represents the mean of four replicates.

The biomechanical properties of the bones in the laying hens supplemented with EOM are shown in Table 4. The shear force and shear stress increased quadratically ($P \leq 0.01$ and $P \leq 0.05$, respectively) with the addition of EOM, but they were minimized by the addition of 600 mg/kg of EOM. The Ca concentration in the tibia increased quadratically ($P \leq 0.01$) by dietary EOM supplementation, while the highest level was recorded for hens supplemented with 100 mg EOM/kg diet (Table 5). The Zn content of the bones was influenced quadratically ($P \leq 0.01$) by dietary EOM

supplementation and a minimum value was observed at a supplementation level of 400 mg/kg EOM. The cortex thickness, cortex cross-section area, and P, Mg or Mn contents of tibia were not affected by EOM supplementation at different levels.

Table 3. Effect of dietary supplementation of essential oil mixture on the eggshell quality in laying hens

	Essential oils mixture (mg/kg)								P-value of contrast	
	0	25	50	100	200	400	600	SEM[1]	linear	quadratic
Specific gravity (g/cm^3)	1.097	1.096	1.096	1.097	1.099	1.094	1.098	0.001	0.516	0.778
Egg breaking strength (kg)	4.82	4.71	4.55	4.73	4.70	4.65	4.64	0.09	0.386	0.472
Eggshell weight % (g/100 g egg)	10.67	10.33	10.14	10.62	10.80	10.17	10.52	0.13	0.891	0.637
Eggshell thickness (μm)	307.9	328.3	324.2	331.1	338.0	330.0	327.6	3.1	0.001	0.001

[1]Each value represents the mean of four replicates.

Table 4. Effect of dietary supplementation of essential oil mixture on tibia biomechanical properties

	Essential oils mixture (mg/kg)							SEM[1]	P-value of contrast	
	0	25	50	100	200	400	600		linear	quadratic
Cortex thickness (mm)	0.660	0.643	0.688	0.668	0.723	0.655	0.688	0.035	0.498	0.655
Cortex cross section area (mm^2)	11.54	10.73	11.71	10.87	12.06	11.10	11.26	0.54	0.937	0.886
Shear force (N)	744	813	852	707	755	799	534	39	0.006	0.008
Shear stress (N/mm^2)	64.67	76.72	73.6	65.38	62.57	72.31	47.64	4.33	0.011	0.022

[1]Each value represents the mean of four replicates.

Table 5. Effect of dietary supplementation of essential oil mixture on the mineral contents of bone in laying hens

	Essential oils mixture (mg/kg)							SEM[1]	P-value of contrast	
	0	25	50	100	200	400	600		linear	quadratic
Calcium (g/kg)	272.0	284.3	277.3	286.4	282.3	273.7	277.6	2.7	0.968	0.010
Phosphorus (g/kg)	116.5	117.6	114.5	116.9	115.7	113.5	113.9	1.2	0.390	0.593
Magnesium (g/kg)	4.44	4.39	4.39	4.31	4.35	4.36	4.40	0.11	0.699	0.505
Zinc (mg/kg)	159.5	164.9	163.1	159.7	162.2	144.1	150.2	2.4	0.001	0.010
Manganese (mg/kg)	2.77	2.84	3.29	2.95	2.87	2.99	2.51	0.28	0.604	0.215

[1]Each value represents the mean of four replicates.

Discussion

In this study, dietary supplementation with 400 mg/kg EOM significantly improved EW, while the supplementation with 50 mg/kg of EOM significantly improved EM as compared with the control group. Similar to the results of this study, the addition of EOM (24 mg/kg) to the diet had a positive effect on EW in laying hens in the study of Bozkurt et al. (2012 a). Also, Bölükbaşı et al. (2008) found that EW was improved by supplementation of the diet by thyme, sage, and rosemary oil in laying hens. However, previous studies showed that essential oil supplementation, either individually or as a blend, did not affect performance in laying hens (Ali et al., 2007; Bozkurt et al., 2012 b; Kaya et al., 2013), broiler breeders (Bozkurt et al., 2009) or quails (Çabuk et al., 2014; Olgun and Yıldız, 2014). The essential oils might improve the functions of the ovary and/or the digestibility of nutrients in the intestine and consequently increase EW and EM in laying hens.

The present study distinctly demonstrated that the eggshell thickness was quadratically increased when laying hens were fed to the diets supplemented with EOM. The specific gravity, egg breaking strength and eggshell weight were not affected by adding EOM. These results are partially supported by the findings of Kaya et al. (2013), who indicated that supplementation of feed mixtures with plant extracts of *Origanum vulgare* and *Thymus vulgaris* and oils of thyme, origanum, garlic, anise and fennel (500, 750, and 1,000 mg/kg) improved eggshell thickness, but did not affect the eggshell weight of laying hens. However, Bozkurt et al. (2012 a) reported that supplementation with 24 mg/kg EOM had no effect on the specific gravity or eggshell thickness, and increased the eggshell weight of laying hens. In addition, Botsoglou et al. (2005) demonstrated that the dietary supplementation of laying hens by rosemary (5 g/kg), oregano (5 g/kg), and saffron (20 mg/kg) had no effect on eggshell thickness. Another study reported that supplementation with 0.25% thyme and 0.25% anise did not affect eggshell weight or thickness in native laying hens (Ali et al., 2007). On the other hand, Olgun and Yıldız (2014) noted that eggshell weight was not affected by supplementation with EOM, but that eggshell thickness was significantly reduced by supplementation with 400 or 600 mg/kg EOM in quail breeders.

Essential oils, which possess lipophilic properties, can easily cross cell membranes and affect bone cell functions by stimulating or inhibiting specific molecular pathways, and can increase osteoblast proliferation by mitogen-activated protein kinase, which is critical for cell survival, thereby increasing bone mineral density and bone strength (Sabbieti et al., 2011). The present study clearly demonstrated that the shear force and shear stress of the tibia significantly decreased for hens that were fed with the highest level (600 mg/kg) of EOM; nevertheless, the highest shear force and shear stress were obtained in hens fed with 50 and 25 mg/kg EOM, respectively. Few studies have been performed to determine the effect of phytogenic feed additives on the biomechanical properties of bone, and the dosage, species and combinations of herbs used in these studies also differed greatly. Świątkiewicz et al. (2014) stated that the dietary addition of 200 mg/kg of a blend of herbal extracts (*Taraxaci siccum*, *Urticae siccum* and *Salviae siccum*) with a high dried

distillers' grains and solubles increased the breaking strength of the femur and the breaking strength and yielding load of the tibia in laying hens. Similarly, Zhou et al. (2009) found that supplementation with 1 g/kg of a mixture of four Chinese herbs (*Herbal Epimedii*, *Rhizoma Drynariae*, *Rhizoma Atractylodis*, and *Radix Astragali*) increased the tibia breaking strength in older laying hens. Mühlbauer et al. (2003) demonstrated that various monoterpene components present in sage oil strongly inhibited bone resorption *in vivo* and *in vitro*. These results indicate that the addition of phytogenic feed additives or essential oils to the diet have a positive influence on bone in laying hens. In the present study, dietary addition by the two lowest concentrations of EOM non-significantly increased the shear force and shear stress of the tibia by 14.5% and 17.7%, respectively, compared to those in hens fed with the control diet. However, the highest level (600 mg/kg) of EOM supplementation to the diet had a negative effect on the biomechanical properties of the tibia.

In the present study, the Ca and Zn contents of tibia were quadratically influenced by dietary EOM supplementation. The Ca concentration of the tibia was increased by the addition of EOM to the diet and reached the highest value following supplementation by 100 mg/kg EOM ($P \leq 0.01$). The Zn content in the tibia non-significantly increased with the addition of EOM, but was significantly decreased by the addition of the 400 mg/kg EOM, in comparison with that in the control group (0 mg/kg). To our knowledge, no data concerning the effect of essential oils or herbs on the contents of Ca, Zn or other minerals in bone are available in animals; nevertheless, Olgun and Yıldız (2014) reported that supplements of 400 or 600 mg/kg EOM decreased the excretion of minerals, including Ca, and Zn in breeder quails. Similarly, Amad et al. (2011) and Mountzouris et al. (2011) indicated that supplementation of essential oils to the diet increased ileal Ca bioavailability in broilers. However, some studies reported that the dietary addition of essential oils or herbs did not affect plasma (Ali et al., 2007) or ileal (Cao et al., 2010; Maenner et al., 2011) Ca concentrations. Capkovicova et al. (2014) also reported that the addition of sage extract (0.05 and 0.01%) decreased the plasma Ca content in layer chickens. Vali et al. (2013) found that supplementation of thyme and cinnamon to the diet increased the egg mineral content (iron, Zn, and copper) compared to that in control quails. Similarly, Taranu et al. (2012) noted that the contents of plasma P and Mg, spleen micro-minerals, and liver Mn and Zn were not affected in piglets fed diet containing 0.04% EOM.

The increase in Ca and Zn concentrations in the bone could be attributed to the stimulation of endogenous digestive enzymes or may be due to an increased surface area in the intestine (Amad et al., 2011), which can decrease intestinal pH (Denli et al., 2004; Cao et al., 2010), inhibit the population of harmful microorganisms in the intestine (Bölükbaşı et al., 2008; Mountzouris et al., 2011; Jiang et al., 2015), and positively influence bone-cell function (Sabbieti et al., 2011).

From these results it could be concluded that the dietary supplement with EOM significantly affected EW and EM. The best eggshell thickness was obtained from eggs produced from hens fed with 200 mg/kg EOM. Low or medium doses of EOM added to the diet improved the biomechanical properties and mineral contents of the

tibia, but the addition of high doses (600 and 400 mg/kg, respectively) was detrimental to these parameters in laying hens.

References

Akbarian A., Golian A., Kermanshahi H., De Smet S., Michiels J. (2015). Antioxidant enzyme activities, plasma hormone levels and serum metabolites of finishing broiler chickens reared under high ambient temperature and fed lemon and orange peel extracts and *Curcuma xanthorrhiza* essential oil. J. Anim. Physiol. Anim. Nutr., 99: 150–162.

Ali M.N., Hassan M.S., Abd El-Ghany F.A. (2007). Effect of strain, type of natural antioxidant and sulphate ion on productive, physiological and hatching performance of native laying hens. Int. J. Poult. Sci., 6: 539–554.

Amad A.A., Manner K., Wendler K.R., Neumann K., Zentek J. (2011). Effects of a phytogenic feed additive on growth performance and ileal nutrient digestibility in broiler chickens. Poultry Sci., 90: 2811–2816.

Armstrong T.A., Flowers W.L., Spears J.W., Nielsen F.H. (2002). Long-term effects of boron supplementation on reproductive characteristics and bone mechanical properties in gilts. J. Anim. Sci., 80: 154–161.

ASAE (2001). ASAE Standards S459: Shear and Three Point Bending Test of Animal Bone. American Society of Agricultural Engineers, St. Joseph, USA.

Botsoglou N.A., Florou-Paneri P., Botsoglou E., Dotas V., Giannenas I., Koidis A., Mitrakos P. (2005). The effect of feeding rosemary, oregano, saffron and α-tocopheryl acetate on performance and oxidative stability of eggs. S. African J. Anim. Sci., 35: 143–151.

Bozkurt M., Alçiçek A., Çabuk M., Küçükyilmaz K., Çatli A.U. (2009). Effect of an herbal essential oil mixture on growth, laying traits, and egg hatching characteristics of broiler breeders. Poultry Sci., 88: 2368–2374.

Bozkurt M., Tokuşoğlu O., Küçükyilmaz K., Akşit H., Çabuk M., Çatli A.U., Seyrek K., Çinar M. (2012 a). Effects of dietary mannan oligosaccharide and herbal essential oil blend supplementation on performance and oxidative stability of eggs and liver in laying hens. Ital. J. Anim. Sci., 11: 223–229.

Bozkurt M., Küçükyilmaz K., Pamukcu M., Çabuk M., Alçiçek A., Çatli A.U. (2012 b). Long-term effects of dietary supplementation with an essential oil mixture on the growth and laying performance of two layer strains. Ital. J. Anim. Sci., 11: 23–28.

Bölükbaşi S.C., Erhan M.K., Kaynar O. (2008). The effect of feeding thyme, sage and rosemary on laying hen performance, cholesterol and some proteins ratio of egg yolk and *Escherichia coli* count in feces. Arch. Geflügel., 72: 231–237.

Cao P.H., Li F.D., Li F.Y., Ru Y.J., Peron A., Schulze H., Bento H. (2010). Effect of essential oils and feed enzymes on performance and nutrient utilization in broilers fed a corn/soy-based diet. Int. J. Poult. Sci., 9: 749–755.

Capkovicova A., Makova Z., Piesova E., Alves A., Faix S., Faixova Z. (2014). Evaluation of the effects of *Salvia officinalis* essential oil on plasma biochemistry, gut mucus and quantity of acidic and neutral mucins in the chicken gut. Acta Vet., 64: 138–148.

Cross D.E., McDevitt R., Hillman K., Acamovic T. (2007). The effects of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age. Brit. Poultry Sci., 48: 496–506.

Çabuk M., Bozkurt M., Alçiçek A., Çatli A.U., Başer K.H.C. (2006). Effect of a dietary essential oil mixture on performance of laying hens in the summer season. S. African J. Anim. Sci., 36: 215–221.

Çabuk M., Eratak S., Alçiçek A., Bozkurt M. (2014). Effects of herbal essential oil mixture as a dietary supplement on egg production in quail. Sci. World J., 2014: 1–4.

Denli M., Okan F., Uluocak A.N. (2004). Effect of dietary supplementation of herb essential oils on the growth performance, carcass and intestinal characteristics of quail (*Coturnix coturnix japonica*). S. African J. Anim. Sci., 34: 174–179.

Hernandez F., Madrid J., Garcia V., Orengo J., Megias M.D. (2004). Influence of two plant extracts on broilers performance, digestibility, and digestive organ size. Poultry Sci., 83: 169–174.

Holder D.P., Bradford M.V. (1979). Relationship of specific gravity of chicken eggs to number of cracked eggs and percent shell. Poultry Sci., 58: 250–251.

Jang I.S., Ko Y.H., Kang S.Y., Lee C.Y. (2007). Effect of commercial essential oils on growth performance, digestive enzyme activity and intestinal microflora population in broiler chickens. Anim. Feed Sci. Technol., 134: 304–315.

Jiang X.R., Awati A., Agazzi A., Vitari F., Ferrari A., Bento H., Bontempo V. (2015). Effects of a blend of essential oils and an enzyme combination on nutrient digestibility, ileum histology and expression of inflammatory mediators in weaned piglets. Animal, 9: 417–426.

Kaya A., Kaya H., Macit M., Çelebi S., Esenbuğa N., Yörük M.A., Karaoğlu M. (2013). Effects of dietary inclusion of plant extract mixture and copper into layer diets on egg yield and quality, yolk cholesterol and fatty acid composition. J. Fac. Vet. Med., Kafkas Uni., 19: 673–679.

Krause E.L., Ternes W. (1999). Bioavailability of the antioxidative thyme compounds thymol and D-cymene-2,3-diol in eggs. Eur. Food Res. Technol., 209: 140–144.

Lim H.S., Paik I.K. (2003). Effect of supplementary mineral methionine chelates (Zn, Ca and Mn) on performance and eggshell quality of laying hens. Asia. Aust. J. Anim. Sci., 16: 1804–1805.

Lokaewmanee K., Yamauchi K.E., Komori T., Saito K. (2014). Eggshell quality, eggshell structure and small intestinal histology in laying hens fed dietary Pantoea-6® and plant extracts. Ital. J. Anim. Sci., 13: 332–339.

Maenner K., Vahjen W., Simon O. (2011). Studies on the effects of essential-oil-based feed additives on performance, ileal nutrient digestibility, and selected bacterial groups in the gastrointestinal tract of piglets. J. Anim. Sci., 89: 2106–2112.

Mountzouris K.C., Paraskevas V., Tsirtsikos P., Palamidi I., Steiner T., Schatzmayr G., Fegeros K. (2011). Assessment of a phytogenic feed additive effect on broiler growth performance, nutrient digestibility and caecal microflora composition. Anim. Feed Sci. Technol., 168: 223–231.

Mühlbauer R.C., Lozano A., Palacio S., Reinli A., Felix R. (2003). Common herbs, essential oils, and monoterpenes potently modulate bone metabolism. Bone, 32: 372–380.

Olgun O., Yildiz A.Ö. (2014). Effect of dietary supplementation of essential oils mixture on performance, eggshell quality, hatchability, and mineral excretion in quail breeders. Environ. Sci. Poll. Res., 21: 13434–13439.

Sabbieti M.G., Agas D., Maggi F., Vittori S., Marchetti L. (2011). Molecular mediators involved in *Ferulago campestris* essential oil effects on osteoblast metabolism. J. Cell. Biochem., 112: 3742–3754.

Skujins S. (1998). Handbook for ICP-AES (Varian-Vista). A short guide to Vista Series ICP-AES Operation, version 1.0. Varian Int. AG, Zug, Switzerland.

Super Nick (2012). H&N International. Super Nick white egg layer management guide. Cuxhaven, Germany.

Świątkiewicz S., Koreleski J. (2008). The effect of zinc and manganese source in the diet for laying hens on eggshell and bones quality. Vet. Med., 53: 555–563.

Świątkiewicz S., Arczewska-Włosek A., Józefiak D. (2014). Bones quality indices in laying hens fed diets with a high level of DDGS and supplemented with selected feed additives. Czech J. Anim. Sci., 59: 61–68.

Taranu I., Marin D.E., Untea A., Janczyk P., Motiu M., Criste R.D. (2012). Effect of dietary natural supplements on immune response and mineral bioavailability in piglets after weaning. Czech J. Anim. Sci., 57: 332–343.

Torki M., Akbari M., Kaviani K. (2015). Single and combined effects of zinc and cinnamon essential oil in diet on productive performance, egg quality traits, and blood parameters of laying hens reared under cold stress condition. Inter. J. Biomet., 59: 1169–1177.

Vali N., Shahin H., Vatankhah M. (2013). Determination of the effects of *Cinnamomum zeylanicum* Blume and *Thymus vulgaris* on performance and egg quality of Japanese quail (*Coturnix japonica*). Res. Op. Anim. Vet. Sci., 3: 280–284.

Wilson J.H., Ruszler P.L. (1996). Effects of dietary boron supplementation on laying hens. Brit. Poultry Sci., 37: 723–729.

Zhou Z.L., Deng Y.F., Tao Q.S., Hu Y.F., Hou J.F. (2009). Effects of Gushukang, a Chinese herbal medicine, on bone characteristics and osteoporosis in laying hens. Poultry Sci., 88: 2342–2345.

Received: 21 XII 2015
Accepted: 30 V 2016