Consequences of various housing systems and dietary supplementation of thymol, carvacrol, and euganol on performance, egg quality, blood chemistry, and antioxidant parameters

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ABSTRACT The present work was carried out to investigate the influences of housing system and dietary essential oils (**EOs**) supplementation to laying hens on the productive performance, egg quality, immunity, antioxidant parameters, and hematology. A factorial arrangement (2×4) was performed, including 2 housing systems and 4 different types of EOs (without EOs, thymol, carvacrol, and euganol) during the production stages (from 28–78 wk of age). Birds were randomly divided into 2 groups with each of 2,000 birds. The first group was moved to laying cages while the second group was a floor reared. Each group was randomly divided into 4 groups (5 replicates of 100 birds each): The first were considered as a control group, and the second, third, and fourth groups were treated with thymol, carvacrol, and euganol EO, respectively. The results showed that hens reared in cage system had higher egg weight ($P < 0.05$), egg production, egg mass, and feed intake and better feed conversion ratio ($P < 0.001$) than those reared in the floor system. Blood picture values (except white blood cells), phagocytic index, phagocytic activity, and blood chemistry parameters (except calcium, phosphorus, and urea values) of laying hens were not affected ($P > 0.05$) by housing system. The groups fed EOs showed a rapid improvement ($P < 0.001$) in the egg production%, egg weight, egg mass, and egg quality. Thymol group had the highest egg production ($P < 0.001$). Thymol and eugenol groups had the highest egg weight, egg mass, and egg quality ($P < 0.001$). The groups fed diets containing thymol or eugenol consumed lower feed and had better feed conversion ratio ($P < 0.001$) than the control group. Immunity indices (phagocytic activity [$P < 0.05$], avian influenza [AIH5 and AIH9], $P < 0.001$) were improved with the presence of EOs in the laying hen diet. These results strongly suggest that dietary EO supplementation could be a successful attempt to improve the productive performance, egg quality, and immunity of laying hens.

Key words: essential oil, egg production, hen, housing system, blood

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INTRODUCTION

The general trend of poultry industry is to provide safety for birds, whether in the feed or the environmental conditions (Alagawany et al., 2019a,b; Elnesr et al., 2019; Reda et al., 2019; Ismail et al., 2020). Housing and nutrition are 2 main factors of a successful poultry farming business. Poultry products and their quality can be affected seriously by housing systems. Any housing system has advantages and disadvantages with regard to the bird performance, health, and welfare. The appropriate housing system for layer chickens should be considered to maximize egg quality traits and egg production. Laying hen's performance and production indices such as feed consumption, feed efficiency, egg weight, and egg production may be influenced by the various housing systems (Batkowska et al., 2014).

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Essential oils (**EOs**) are aromatic oily liquids extracted from the plant products such as seeds, buds, flowers, leaves, roots, fruits, and bark (Abd El-Hack et al., 2015, 2018a,b and 2019; Reda et al., 2020). These oils are secondary metabolites rich in many compounds (more than 3,000). Recently, increasing attention to EOs was paid in the poultry industry. Thymol is a main constituent of commonly used EOs, such as oregano and thyme oils (Bassolé and Juliani, 2012). Carvacrol is a constituent of several medicinal plants, such as thyme, black cumin, oregano, and savory (*Satureja hortensis*) (Alagawany et al., 2015). The eugenol is extracted from numerous plants such as cinnamon, lemon grass, cloves, and tulsi (Mak et al., 2019). These EOs (thymol, carvacrol, and eugenol) play an important role in metabolism and physiology of animal due to their role in stimulating digestion (Luna et al., 2012; Bozkurt et al., 2014).

Several studies have shown that EOs may improve animal performance and health status by many approaches such as via anti-inflammatory, anthelmintic, antimicrobial, and antioxidant properties as well as stimulation of digestive secretions and immune modulation (Saki et al., 2014; Abd El-Hack et al., 2016; Abo Ghanima et al., 2020). The EOs can be used in poultry feeding for boosting health and performance (Alagawany et al., 2015, 2019c; Changxing et al., 2019). Besides, the effects of EOs on the digestive physiology have been revealed and used in poultry nutrition (Alagawany et al., 2018; Mahgoub et al., 2019; Mohamed et al., 2019). Several studies have demonstrated the positive effects of EOs on egg production and egg quality in laying hens (Bozkurt et al., 2012; Özek, 2012). The dietary supplementation with EOs has been suggested as a strategy to augment poultry productivity in parameters such as egg laying rate, egg quality, and feed conversion ratio (**FCR**) (Abd El-Hack et al., 2016). In commercial egg-laying farming projects, the success depends on the total number and size of eggs produced. The suitable housing system and good feed additives for laying hens can increase production performance and product quality. Studies showing the effect of EOs on laying hens under different housing systems are still rare.

The present study hypothesized that housing systems (floor and cage) under different diets supplemented with EOs (thymol, carvacrol, and euganol) could affect laying hens. Therefore, the aim of the present study was to examine the effects of housing system and dietary EO supplementation to laying hens on the productive performance, egg quality, hematology, immunity, and antioxidant parameters.

MATERIALS AND METHODS

All procedures were implemented according to the Local Experimental Animal Care Committee and approved by the ethics committee of Damanhour University, Egypt, and the ethical code is DMU/VetMed-2019-/0145. All procedures used in this study were in accordance with international ethical standards. The research involved no human participants.

Birds and Experimental Design

Four thousand ISA brown laying hens (27 wk old) were obtained from Al Waha poultry industry (Damo-El Basyounia-El Fayoum–Egypt). A factorial arrangement (2×4) including 2 housing systems and 4 different types of EOs (without EOs, thymol, carvacrol, and euganol) was used during the production stages (from 28–78 wk of age). Birds were randomly housed in laying cages and floor reared with 2,000 birds each. The birds in each housing system were randomly divided into 4 groups with 5 replicates of 100 birds each: 0 mg/kg EOs, 300 mg of thymol EO/kg diet, 300 mg of carvacrol EO/kg diet, and 300 mg of euganol EO/kg diet. Each group was divided into equal. The diets were formulated to meet or exceed NRC (1994) recommendations (Table 1). The hens were fed diets in mash form during the experiment (28-76 wk). Thyme EO was added in a dose of 300 mg/kg diet in thr form of 100% pure therapeutic grade essential thymol oil obtained from Xi'an Geekee Biotech Co., Ltd., Shaanxi, China. Carvacrol EO was added in a dose of 300 mg/kg diet in the form of pure 100% oil obtained from Sigmachem crop company, Fujian, China. Euganol EO was added in a dose of 300 mg/kg diet in the form of pure 100% oil obtained from Jiangxi Senhai Natural Plant Oil Co., Ltd. Jiangxi, China (Mainland).

Estimation of Laying Performance Parameters and Egg Quality

Hen-day egg production (**HDEP**), feed consumption, and egg weight were recorded daily on a replicate basis. FCR was calculated as grams of feed intake per gram of egg mass produced. Average egg mass (per hen per day in grams) = per cent HDEP $\times$ average egg weight in grams. The parameters related to egg quality were evaluated at 72 wk of age. Fifteen eggs were randomly collected per treatment to determine these parameters. The collected eggs were weighed, and each egg was then exposed to a pressing force by using an eggshell strength meter. On breaking, the egg contents were poured. Eggshell thickness (without the shell membrane) was measured by using a micrometer at the middle part of the egg. The Haugh unit (**HU**) value was calculated using the egg weight and albumen height. The higher value indicates the better egg quality. Shell, albumin, and yolk percentage were calculated as a percentage of egg weight. Yolk index% = (yolk height/yolk diameter) $\times$ 100. Egg shape index% = (egg width/egg length) $\times$ 100. Yolk diameter, egg width, and egg length measured using an electronic digital caliper. The tri-legged micrometer was used for measuring the height of yolk and albumen.

Blood Biochemical Parameters

At 76th week of age, 5 birds from each replicate were randomly selected, and blood samples were collected

Table 1. Ingredients and calculated analysis of layer basal diet.

Item	%
Ingredients	
Yellow corn	61.23
Soybean meal (44% protein)	19.02
Corn gluten meal (60% protein)	7.02
Vitamins and minerals premix[1]	0.30
Wheat bran	0.46
Calcium carbonate	1.36
Di-calcium phosphate	8.96
DL-methionine	0.05
NaCl	0.40
Lysine	1.20
Chemical analysis (%)[2]	
Crude protein	18.01
Metabolic energy (Kcal/kg)	2,800
Crude fiber	2.85
Calcium	3.81
Phosphorus	0.63

[1]Each diet was supplied with 3 kg/ton vitamin & minerals mix (commercial source B. p. Max). Each 3 kg contains, vitamin A 10,000,000 MIU, vitamin D 2,000,000 MIU, vitamin E 10,000 mg, vitamin K3 1,000 mg, vitamin B1 1,000 mg, vitamin B2 5,000 mg, vitamin B6 1,500 mg, biotin 50 mg, butylated hydroxytoluene 10,000 mg, pantothenic 10,000 mg, folic acid 1000 mg, nicotinic acid 30,000 mg, Mn 60 g, zinc 50 g, Fe 30 g, Cu 4 g, I 3 g, selenium 0.1 g, and Co 0.1 g.

[2]The diets were formulated to meet or exceed NRC (1994) recommendations.

from the wing vein. Then, blood sample tubes were left in slope position till serum samples were separated through centrifugation at 3,000 rpm for 15 min. The serum was collected and preserved in a deep freezer at (−20°C) until the time of analysis. The serum constituents (cholesterol, total protein, calcium, phosphorus, urea, creatinine, aspartate aminotransferase [**AST**], and alanine aminotransferase [**ALT**]) were determined using commercial kits purchased from Biodiagnostic Company.

Blood Picture

Heamatological parameters (red blood cells [**RBCs**], hemoglobin [**Hb**], packed cell volume [**PCV**], and white blood cells [**WBCs**]) were determined in the whole blood that contained anticoagulants. The blood film was prepared according to the method described by Lucky (1977) to determine the differential leukocytes count. Ten drops from May-Grunwald stain stock solution on a dry, unfixed smear were added to an equal amount of distilled water, then mixed and left for 1 min for staining. The dye was decanted without rinsing. Diluted Giemsa's solution (10 drops of the dye were added to 10 mL of distilled water) was poured over the film as counter stain and left for 20 min then rinsed in water current and examined by the oil immersion lens. The percentage and absolute value for each type of cells were calculated according to the study by Schalm et al. (1986).

Antioxidant Parameters

Determination of malondialdehyde (**MDA**) concentration was measured by the scheme of Jo and Ahn (1998). Estimation of glutathione peroxidase (**GPx**) activity was measured using the Paglia and Valentine (1967) spectrophotometry method based on the Northwest Life Science Specialties GPx assay kits protocol NWK-GPX01. Determination of superoxide dismutase (**SOD**) activity was performed using the Northwest Life Science Specialties SOD activity assay, which provided a simple rate method for determining SOD activity. This method is based on monitoring the auto-oxidation rate of hematoxylin as originally described by Martin et al. (1987).

Estimation of Phagocytic Index and Phagocytic Activity and Cellular Immunity

Blood and serum samples were collected at 76th day of age (5 samples per replicate and total 25 samples per each group) and used for determination of phagocytic activity (**PA**) and phagocytic index (**PI**) according to Kawahara et al. (1991). Fifty micrograms of *Candida albicans* culture was added to 1 mL of citrated blood from each sample and incubated in a water bath at 25°C for 5 h, and then blood smears from each tube were stained with Giemsa stain. Phagocytosis was estimated by determining the proportion of macrophages, which contained intracellular yeast cells in a random count of 300 macrophages and expressed as percentage of PA. The number of phagocytized organisms was counted in the phagocytic cells and called PI.

PA = percentage of phagocytic cells containing yeast cells.

$$\text{Phagocytic index (PI)} = \frac{\text{Number of yeast cells phagocytized}}{\text{Number of phagocytic cells}}$$

Serology for Newcastle Disease Virus and Avian Influenza Virus (AIH5 and AIH9)

Serum samples were also used for hemagglutination inhibition (**HI**) test against ND virus and avian influenza (AI) virus (AIH5 and AIH9). These tests were performed by using a standard protocol described for HI titers (Beard, 1989).

Statistical Analysis

Data were analyzed by statistical analysis system (SAS, 2002). A 2 × 4 factorial design was used to analyze data of performance as a response to 2 housing systems and 4 different types of EOs. Differences among means were detected using 2-way analysis of variance. The differences among means were determined using Duncan test.

RESULTS

Egg Production

Egg production of hens in cage system was higher ($P < 0.001$) than that in the floor system (Table 2). The

Table 2. Egg production of laying hens as affected by different housing systems, essential oils, and their interaction during the experiment.

	Egg production % during					
Items	28–36 wk	36–44 wk	44–52 wk	52–60 wk	60–68 wk	68–76 wk
Housing system						
Cage	88.52	89.40	85.47	80.45	72.97	63.52
Floor	84.40	85.42	80.50	74.72	68.27	57.70
Essential oils (EOs)						
0	84.20^{b}	84.80^{c}	79.95^{c}	73.05^{c}	65.75^{b}	56.50^{b}
Thymol	87.85^{a}	89.70^{a}	86.65^{a}	81.45^{a}	72.92^{a}	62.65^{a}
Carvacrol	86.55^{a}	87.30^{b}	82.35^{b,c}	77.95^{b}	71.95^{a}	61.85^{a}
Eugenol	87.25^{a}	87.85^{b}	83.01^{b}	78.30^{b}	71.90^{a}	61.45^{a}
Housing × EOs						
Cage						
0	85.90	86.60	82.20	76.01^{b}	67.30^{d}	59.00^{b}
Thymol	89.60	92.30	90.01	84.70^{a}	75.50^{a}	64.90^{a}
Carvacrol	88.40	88.60	83.70	78.90^{b}	73.01^{b}	63.70^{a}
Eugenol	90.20	90.10	86.01	82.20^{a}	76.10^{a}	66.50^{a}
Floor						
0	82.50	83.01	77.70	70.01^{d}	64.20^{e}	54.01^{c}
Thymol	86.10	87.10	83.30	78.20^{b}	70.30^{c}	60.40^{b}
Carvacrol	84.70	86.01	81.01	77.01^{b}	70.90^{c}	60.01^{b}
Eugenol	84.30	85.60	80.01	74.40^{c}	67.70^{d}	56.40^{c}
SEM	0.67	0.65	0.90	0.98	0.94	0.88
Portability						
Housing system	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
EOs	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Housing × EOs	0.224	0.237	0.148	0.032	0.011	0.004

[a-d]Means in the same column within each classification bearing different superscript lowercase letters are significantly different ($P < 0.05$ or 0.01).

groups fed EOs showed a rapid increase ($P < 0.001$) in the egg production during all experimental periods except the period from 44 to 52 wk of age compared with the control group. During this period, the groups fed thymol or eugenol had higher ($P < 0.001$) egg production than the control and carvacrol groups. Thymol group had the highest egg production at all experimental periods. There were significant differences in egg production during some periods (52–60, 60–68, and 68–76 wk) among the groups due to the interaction effect.

Table 3. Egg weight of laying hens as affected by different housing systems, essential oils, and their interaction during the experiment.

	Egg weight (g) during					
Items	28–36 wk	36–44 wk	44–52 wk	52–60 wk	60–68 wk	68–76 wk
Housing system						
Cage	44.65	52.15	56.67	58.20	59.62	60.77
Floor	43.57	50.92	55.45	57.05	58.65	59.77
Essential oils (EOs)						
0	41.65^{c}	50.10^{b}	54.70^{b}	55.95^{c}	57.90^{c}	59.15^{b}
Thymol	44.20^{b}	51.85^{a,b}	55.65^{b}	57.40^{b}	59.20^{b}	60.05^{b}
Carvacrol	43.60^{b}	51.40^{a,b}	55.50^{b}	57.15^{b,c}	58.55^{b,c}	59.55^{b}
Eugenol	47.00^{a}	52.80^{a}	58.40^{a}	60.00^{a}	60.90^{a}	61.90^{a}
Housing × EOs						
Cage						
0	42.20	50.80	54.90	56.20	58.00	59.20
Thymol	45.10	52.10	56.50	58.40	60.30	61.10
Carvacrol	44.10	52.10	55.50	57.50	58.80	59.70
Eugenol	47.20	53.60	59.80	60.70	61.40	62.20
Floor						
0	41.10	49.40	54.50	55.70	57.80	59.10
Thymol	43.30	51.60	54.80	56.40	58.10	59.00
Carvacrol	43.10	50.70	55.50	56.80	58.30	59.40
Eugenol	46.80	52.00	57.00	59.30	60.40	61.60
SEM	0.570	0.839	0.732	0.506	0.476	0.560
Portability						
Housing system	0.012	0.047	0.024	0.003	0.007	0.59
EOs	<0.001	0.024	<0.001	<0.001	<0.001	<0.001
Housing × EOs	0.861	0.914	0.228	0.443	0.185	0.288

[a-c]Means in the same column within each classification bearing different superscript lowercase letters are significantly different ($P < 0.05$ or 0.01).

Table 4. Egg mass of laying hens as affected by different housing systems, essential oils, and their interaction during the experiment.

	Egg mass (g) during					
Items	28–36 wk	36–44 wk	44–52 wk	52–60 wk	60–68 wk	68–76 wk
Housing system						
Cage	43.40	48.11	47.83	45.56	40.82	18.77
Floor	40.38	44.93	43.53	41.71	37.39	16.55
Essential oils (EOs)						
0	38.63^{c}	44.23^{b}	42.03^{c}	39.63^{c}	35.94^{b}	16.04^{b}
Thymol	42.89^{a,b}	48.02^{a}	47.81^{a}	45.38^{a,b}	39.99^{a}	18.43^{a}
Carvacrol	41.29^{b}	45.97^{a,b}	45.14^{b}	43.72^{b}	39.53^{a}	17.82^{a}
Eugenol	44.74^{a}	47.85^{a}	47.75^{a}	45.83^{a}	40.95^{a}	18.34^{a}
Housing × EOs						
Cage						
0	39.94	45.37	44.09^{b}	40.99^{b,c}	36.84^{b,c}	16.98
Thymol	44.61	50.21	50.45^{a}	47.96^{a}	42.17^{a}	19.41
Carvacrol	42.65	46.79	45.97^{b}	44.58^{a,b}	40.23^{a,b}	18.66
Eugenol	46.38	50.08	50.82^{a}	48.72^{a}	44.04^{a}	20.03
Floor						
0	37.32	43.10	39.96^{c}	38.27^{c}	35.05^{c}	15.10
Thymol	41.17	45.82	45.17^{b}	42.81^{b}	37.81^{b}	17.46
Carvacrol	39.94	45.15	44.30^{b}	42.85^{b}	38.83^{b}	16.98
Eugenol	43.10	45.63	44.67^{b}	42.95^{b}	37.86^{b}	16.65
SEM	0.713	0.810	0.682	0.660	0.682	0.350
Portability						
Housing system	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
EOs	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Housing × EOs	0.918	0.210	0.015	0.012	0.004	0.080

[a-c]Means in the same column within each classification bearing different superscript lowercase letters are significantly different ($P < 0.05$ or 0.01).

Egg Weight

Weight of eggs produced by hens in the cage system was higher ($P < 0.05$) than that of the hens in the floor system, during all experimental periods except the late period (68–76 wk; Table 3). During the first period (28–36 wk), the group supplemented with EOs had significantly higher egg weight ($P < 0.001$) than the control group. Supplementation of eugenol caused significantly higher egg weight than other treatments and control during the periods from 36 to 44 ($P < 0.05$), 44 to 52, and 68 to 76 wk ($P < 0.001$). Weight of eggs produced from the groups fed eugenol or thymol was significantly higher ($P < 0.001$) than that in the control and carvacrol groups during 52–60 and 60–68 wk of age. Egg weight was not affected by the interaction between housing system and EOs.

Egg Mass

Egg mass of hens in the cage system was higher ($P < 0.001$) than that of hens in the floor system (Table 4). Hens supplemented with EOs had significantly higher ($P < 0.001$) egg mass than those recorded in the control group during entire periods, except during the period from 36–44 wk. The addition of carvacrol did not affect egg mass ($P > 0.05$). No significant interaction effect ($P > 0.05$) on egg mass was detected between housing system and EOs during some periods (28–36, 36–44, and 68–76 wk). However, during the other periods (44–52, 52–60, and 60–68 wk), egg mass significantly ($P < 0.05$) increased in response to the interaction between EOs and housing system.

Feed Intake

As shown in Table 5, hens that were reared in floor system consumed higher feed ($P < 0.001$) than those reared in cage system. Feed intake of hens fed diet containing thymol was significantly lower ($P < 0.05$) than that of the control group during the early period (28–36 wk). During other periods (36–44, 44–52, 52–60, 60–68, and 68–76 wk), the groups fed diets containing thymol or eugenol consumed lower feed ($P < 0.001$) than the control group. No significant interaction influence ($P > 0.05$) from housing system and EOs was detected on feed intake during the early periods (28–36 and 36–44 wk). However, during the other periods (44–52, 52–60, 60–68, and 68–76 wk), feed intake was significantly ($P < 0.05$) decreased in response to the interaction between EOs and cage system.

Feed Conversion Ratio

As shown in Table 6, hens reared in the cage system had better FCR ($P < 0.001$) than those housed in the floor system. The groups fed diets supplemented with EOs had significantly ($P < 0.001$) better FCR than the control group at all experimental periods. The results at all experimental periods showed that FCR of hens was not affected ($P > 0.05$) by the interaction between EOs and housing system.

Egg Quality

The best values of egg quality were obtained from hens reared in cage system compared with those reared in the

Table 5. Feed intake (g) of laying hens as affected by different housing systems, essential oils, and their interaction during the experiment.

	Feed intake (g) during					
Items	28–36 wk	36–44 wk	44–52 wk	52–60 wk	60–68 wk	68–76 wk
Housing system						
Cage	89.15	109.20	119.30	126.35	121.55	115.40
Floor	95.77	113.12	125.57	134.30	129.25	117.20
Essential oils (EOs)						
0	93.80^{a}	112.95^{a}	123.80^{a}	132.45^{a}	127.25^{a}	117.60^{a}
Thymol	91.25^{b}	109.95^{b}	121.35^{b}	128.20^{c}	124.50^{b,c}	115.75^{c}
Carvacrol	92.40^{a,b}	111.65^{a,b}	123.00^{a,b}	131.35^{a,b}	126.00^{a,b}	116.85^{a,b}
Eugenol	92.40^{a,b}	110.10^{b}	121.60^{b}	129.30^{b,c}	123.85^{c}	115.00^{b,c}
Housing × EOs						
Cage						
0	89.60	110.70	119.50^{d}	127.10^{e}	122.20^{e}	116.10^{b}
Thymol	88.70	107.70	119.10^{d}	125.60^{f}	120.90^{f}	114.70^{c}
Carvacrol	89.10	110.10	119.40^{d}	127.20^{e}	121.90^{e}	115.30^{b}
Eugenol	89.20	108.30	119.20^{d}	125.50^{f}	121.20^{e}	115.50^{b}
Floor						
0	98.00	115.20	128.10^{a}	137.80^{a}	132.30^{a}	119.10^{a}
Thymol	93.80	112.20	123.60^{c}	130.80^{d}	128.10^{c}	116.80^{b}
Carvacrol	95.70	113.20	126.60^{b}	135.50^{b}	130.10^{b}	118.40^{a}
Eugenol	95.60	111.90	124.00^{c}	133.10^{c}	126.50^{d}	114.50^{c}
SEM	0.639	0.730	0.663	0.781	0.785	0.545
Portability						
Housing system	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
EOs	0.004	0.001	0.002	<0.001	0.001	<0.001
Housing × EOs	0.101	0.717	0.011	0.013	0.035	0.002

[a-f]Means in the same column within each classification bearing different superscript lowercase letters are significantly different ($P < 0.05$ or 0.01).

floor system ($P < 0.05$) (Table 7). Data show that shell thickness, egg index, and HU were influenced ($P < 0.001$) by the addition of EOs in the diet, where layers fed EOs had the highest values of these parameters ($P < 0.001$) compared with the control. The higher values of shell and yolk ($P < 0.001$) were obtained for hens fed diets containing thymol or eugenal than for those in the control and carvacrol groups. However, the lower values of albumin% and yolk index ($P < 0.001$) were obtained for hens fed diets containing

Table 6. Feed conversion of laying hens as affected by different housing systems, essential oils, and their interaction during the experiment.

	Feed conversion ratio (g feed/g egg) during					
Items	28–36 wk	36–44 wk	44–52 wk	52–60 wk	60–68 wk	68–76 wk
Housing system						
Cage	2.26	2.34	2.47	2.71	2.80	3.01
Floor	2.61	2.60	2.82	3.15	3.24	3.42
Essential oils (EOs)						
0	2.68^{a}	2.66^{a}	2.84^{a}	3.25^{a}	3.35^{a}	3.53^{a}
Thymol	2.35^{b,c}	2.37^{b}	2.52^{c}	2.75^{c}	2.90^{b,c}	3.09^{b}
Carvacrol	2.45^{b}	2.49^{b}	2.69^{b}	2.95^{b}	2.99^{b}	3.18^{b}
Eugenol	2.25^{c}	2.38^{b}	2.52^{c}	2.77^{c}	2.84^{c}	3.05^{b}
Housing × EOs						
Cage						
0	2.47	2.51	2.65	2.98	3.13	3.33
Thymol	2.19	2.24	2.34	2.54	2.65	2.89
Carvacrol	2.28	2.39	2.57	2.80	2.84	3.03
Eugenol	2.09	2.24	2.32	2.51	2.59	2.79
Floor						
0	2.88	2.81	3.03	3.53	3.57	3.74
Thymol	2.51	2.50	2.71	2.97	3.14	3.28
Carvacrol	2.62	2.60	2.81	3.10	3.15	3.33
Eugenol	2.42	2.51	2.73	3.02	3.09	3.30
SEM	0.045	0.053	0.048	0.050	0.052	0.068
Portability						
Housing system	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
EOs	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Housing × EOs	0.699	0.860	0,339	0.073	0.245	0.474

[a-c]Means in the same column within each classification bearing different superscript lowercase letters are significantly different ($P < 0.05$ or 0.01).

Table 7. Egg quality of laying hens as affected by different housing systems, essential oils, and their interaction during the experiment.

Items	Shell thickness (μm)	Shell %	Yolk %	Albumin %	Egg shape index%	Yolk index%	Haugh unit
Housing system							
Cage	0.376	8.92	29.02	62.04	77.74	22.47	83.67
Floor	0.360	8.65	28.85	62.49	76.83	22.49	81.30
Essential oils (EOs)							
0	0.359^{c}	8.52^{b}	28.53^{b}	62.94^{a}	75.59^{c}	23.54^{a}	79.72^{d}
Thymol	0.371^{a,b}	8.92^{a}	29.10^{a}	61.96^{c}	77.98^{a}	22.29^{b}	83.38^{b}
Carvacrol	0.368^{b}	8.69^{b}	28.77^{b}	62.53^{b}	77.20^{b}	22.43^{b}	81.53^{c}
Eugenol	0.374^{a}	9.01^{a}	29.33^{a}	61.64^{c}	78.37^{a}	21.66^{c}	85.32^{a}
Housing × EOs							
Cage							
0	0.363^{c}	8.71	28.60	62.67	76.01	23.28^{a}	80.80
Thymol	0.381^{a}	9.07	29.05	61.87	78.59	22.09^{b}	84.21
Carvacrol	0.377^{b}	8.85	28.82	62.30	77.53	22.77^{b}	82.73
Eugenol	0.382^{a}	9.05	29.60	61.33	78.84	21.75^{c}	86.95
Floor							
0	0.356^{d}	8.32	28.45	63.21	75.19	23.81^{a}	78.64
Thymol	0.362^{c}	8.78	29.15	62.05	77.38	22.08^{b}	82.56
Carvacrol	0.359^{d}	8.52	28.72	62.75	76.86	22.08^{b}	80.34
Eugenol	0.366^{c}	8.98	29.06	61.95	77.89	21.57^{c}	83.68
SEM	0.01	0.07	0.117	0.13	0.16	0.11	0.44
Portability							
Housing system	<0.001	<0.001	0.039	<0.001	<0.001	0.853	<0.001
EOs	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Housing × EOs	0.001	0.222	0.07	0.420	0.413	<0.001	0.339

[a-c]Means in the same column within each classification bearing different superscript lowercase letters are significantly different ($P < 0.05$ or 0.01).

EOs than for those in the control group. Shell, yolk, albumin%, egg index, and HU were not affected ($P > 0.05$) by the interaction between EOs and housing system. There were significant differences in shell thickness ($P = 0.001$) and yolk index ($P < 0.001$) among the groups due to the interaction effect.

Blood Profiles

Blood profiles were not affected by housing system ($P > 0.05$), except that WBC level was significantly ($P < 0.001$) increased in hens reared in cage system compared with those in the floor system (Table 8). RBCs, PCV, and Hb were not influenced by the inclusion of EOs in the diets. Dietary inclusion of thymol oil increased WBCs ($P < 0.001$) compared with other oils and control groups. The interaction between EOs and housing system was significant with respect to WBCs ($P < 0.001$) and RBCs ($P < 0.05$) after dietary inclusion of EOs in each of the housing system.

Eosinophils, lymphocytes, basophils, and monocytes% were significantly ($P < 0.05$) increased with EOs supplementation compared with those in the control group. However, heterophils% was declined with carvacrol and thymol supplements ($P < 0.001$) compared with the eugenol and control groups. No significant interaction ($P > 0.05$) was observed in WBC differential between housing system and EOs.

Immunity and Antioxidant Parameters

PI was not affected ($P > 0.05$) by EOs and housing system or their interaction. PA was not affected by housing system, but it was affected only by EOs; the group fed thymol recorded the highest value ($P < 0.05$). Obtained results exhibited a significant ($P < 0.05$) increase in values of ND and AIH9 for hens reared in the cage compared with those reared in the floor system. Values of AIH5 and AIH9 were improved ($P < 0.001$) by inclusion of EOs in the laying hen diet. Values of ND were increased ($P < 0.001$) with thymol and eugenol supplements compared with the control group. No significant interaction between EOs and housing system was observed in immunity indices. As shown in Table 9, the MDA level and SOD activity were significantly ($P < 0.001$) decreased in hens reared in the cage compared with the floor system. GPx activity was not affected ($P > 0.05$) by the housing system. Antioxidant indices (MDA, GPx, and SOD) were decreased ($P < 0.001$) with thymol and eugenol compared with the control group. These indices were significantly ($P < 0.05$) decreased by the interaction between EOs and housing system compared with that of the control group.

Blood Chemistry

As indicated in Table 10, no change was noticed in blood chemistry parameters ($P > 0.05$) between the hens reared in the cage and floor systems, except that calcium, phosphorus, and urea values were higher ($P < 0.05$) in hens of floor system. Supplementation of EOs in laying hen's diet significantly ($P < 0.001$) decreased levels of cholesterol, urea, creatinine, ALT, and AST. However, the groups fed diets containing EOs had higher calcium and phosphorus levels ($P < 0.001$) than those of the control group. Blood

Table 8. Blood picture of laying hens as affected by different housing systems, essential oils, and their interaction during the experiment.

Items	WBCs (10^3/μL)	RBCs (10^6/μL)	PCV%	Hb%	Eosinophils %	Lymphocytes %	Heterophiles %	Basophiles %	Monocytes %
Housing system									
Cage	24.28	3.21	29.16	14.10	8.43	36.34	48.68	1.09	5.45
Floor	23.80	3.21	29.16	14.13	8.39	35.82	49.30	1.07	5.41
Essential oils (EOs)									
0	23.82^{b}	3.20	29.16	14.12	8.29^{b}	35.10^{b}	50.30^{a}	1.05^{b}	5.24^{c}
Thymol	24.56^{a}	3.20	29.23	14.10	8.46^{a,b}	36.84^{a}	47.96^{b}	1.08^{a,b}	5.65^{a}
Carvacrol	23.80^{b}	3.22	29.16	14.02	8.54^{a}	36.40^{a}	48.58^{b}	1.10^{a}	5.37^{b,c}
Eugenol	23.99^{b}	3.21	29.10	14.22	8.35^{a,b}	35.98^{a,b}	49.10^{a,b}	1.09^{a}	5.47^{a,b}
Housing × EOs									
Cage									
0	23.82^{b,c}	3.23^{a}	29.12	14.12	8.38	35.76	49.44	1.07	5.34
Thymol	25.40^{a}	3.16^{b}	29.28	14.03	8.40	37.26	47.58	1.08	5.68
Carvacrol	23.90^{b}	3.23^{a}	29.16	14.06	8.58	36.48	48.45	1.11	5.38
Eugenol	24.01^{b}	3.21^{a}	29.08	14.18	8.36	35.86	49.25	1.10	5.42
Floor									
0	23.82^{b}	3.16^{b}	29.20	14.12	6.20	34.44	51.16	1.03	5.15
Thymol	23.73^{b,c}	3.24^{a}	29.19	14.18	8.52	36.42	48.34	1.09	5.62
Carvacrol	23.70^{c}	3.22^{a}	29.16	13.98	8.50	36.32	48.72	1.09	6.36
Eugenol	23.97^{b}	3.21^{a}	29.12	14.26	8.34	36.10	48.96	1.07	5.52
SEM	0.10	0.02	0.14	0.07	0.09	0.36	0.45	0.01	0.07
Portability									
Housing system	<0.001	0.975	0.932	0.499	0.536	0.052	0.064	0.126	0.440
EOs	<0.001	0.651	0.832	0.090	0.043	<0.001	<0.001	0.022	<0.001
Housing × EOs	<0.001	0.022	0.949	0.481	0.422	0.163	0.176	0.355	0.304

[a-c]Means in the same column within each classification bearing different superscript lowercase letters are significantly different ($P < 0.05$ or 0.01).
Abbreviations: Hb, hemoglobin; PCV, packed cell volume; RBCs, red blood cells; WBCs, white blood cells.

chemistry parameters, except calcium and creatinine levels, were not significantly affected ($P > 0.05$) by the interaction between EOs and housing system.

DISCUSSION

The production rates of poultry have improved significantly over recent decades as the result of improved nutrition and housing system (Saeed et al., 2019). The housing system is an external factor that influences the egg production of hens (Englmaierová et al., 2014). In the present study, better results (egg production, egg weight, and egg mass) were achieved in the cage system. These results are in agreement with studies by Yakubu et al. (2007) who clarified the superiority of laying performance in birds kept in cages compared with the birds reared on the litter. Voslářová et al. (2006) obtained a higher number of eggs and a higher egg mass in hens housed in the cage system than in any system. HDEP was significantly higher for the cage system than for floor system (Anderson and Adams, 1994; Stanley et al., 2014). The results herein could be supportive for deciding which rearing system is more appropriate and brings less adverse consequence to the laying performance.

Bozkurt et al. (2012) revealed that EO mixture (including thymol and carvacrol) supplementation to the laying hen diet significantly augmented the egg weight and egg production rate in comparison with the control diet. Egg weight and egg mass were positively linearly affected by EOs supplementation (Bölükbasi et al., 2008; Olgun, 2016). The addition of an EO mixture (36 mg/kg) boosted egg weight in the experiment of Özek et al. (2011). Supplementation of thymol (250 mg/kg) resulted in improved productive performance of laying hens (Abdel-Wareth, 2016). Improvements in egg production may be attributed to increased dietary nutrients digestibility and the digestive capacity that induce the intestinal availability of these nutrients for the benefit of the body (Windisch et al., 2008). Olgun (2016) reported that EOs might improve the ovary functions and the nutrients digestibility in the intestine and consequently increase egg weight and egg mass in laying hens.

Current findings about feed intake comply with the results of previous studies, indicating higher feed consumption for the floor system than for the cage system. Layers kept in litter system consumed more feed than the layers housed in cage systems (Adam, 2017). Feed consumption was higher by 10% per day for the floor system than for the cage system (Tauson et al., 1999). This was in the line with the findings of Preisinger (2000) who reported that birds in floor system tended to eat more feed than those in cage systems. The lowest daily feed consumption and the best FCR were observed in cages compared to the litter system (Englmaierová et al., 2014). The FCR in layers kept in the cage system was better than that in floor housing systems (Gerzilov et al., 2012).

In the present study, hens fed diets containing EOs consumed lower feed than the control group, in agreement with Bölükbasi et al. (2010) who stated that feed intake was reduced by dietary supplementation with EOs including thyme oil. In a study using 200 mg/kg of EOs (Bölükbasi et al., 2008), they found that all the treatments lowered feed intake for hens when compared

Table 9. Immunity and antioxidant parameters of laying hens as affected by different housing systems, essential oils, and their interaction during the experiment.

Items	Phagocytic index	Phagocytic activity	ND 60 W	AI H5 60 W	AI H9 60 W	MDA (nmoles/mL)	GPx (U/gHb)	SOD (U/gHb)
Housing system								
Cage	1.62	16.62	2.90	2.74	2.66	1.95	21.01	67.55
Floor	1.63	16.32	2.77	2.67	2.48	2.27	21.75	76.25
Essential oils (EOs)								
0	1.55	15.90^{b}	2.63^{c}	2.40^{c}	2.22^{b}	2.50^{a}	24.30^{a}	81.01^{a}
Thymol	1.67	17.25^{a}	2.91^{a,b}	2.82^{a,b}	2.63^{a}	1.93^{c}	19.40^{c}	63.70^{b}
Carvacrol	1.70	16.45^{a,b}	2.73^{b,c}	2.68^{b}	2.60^{a}	2.16^{b}	22.10^{a,b}	75.70^{a}
Eugenol	1.59	16.10^{b}	3.06^{a}	2.91^{a}	2.82^{a}	1.87^{c}	19.70^{b,c}	67.20^{b}
Housing × EOs								
Cage								
0	1.60	16.50	2.75	2.52	2.39	2.48^{a}	22.80^{b}	76.40^{b}
Thymol	1.62	17.30	2.96	2.86	2.71	1.74^{c}	18.20^{d}	51.40^{e}
Carvacrol	1.70	16.40	2.79	2.68	2.64	1.96^{b}	22.20^{b}	72.60^{c}
Eugenol	1.58	15.90	3.09	2.89	2.89	1.64^{c}	20.80^{c}	69.80^{c}
Floor								
0	1.50	15.30	2.52	2.27	2.04	2.52^{a}	25.80^{a}	85.60^{a}
Thymol	1.72	17.20	2.86	2.79	2.56	2.12^{b}	20.60^{c}	76.00^{b}
Carvacrol	1.70	16.50	2.68	2.68	2.56	2.36^{a}	22.01^{b}	78.80^{b}
Eugenol	1.60	16.30	3.03	2.93	2.75	2.11^{b}	18.60^{d}	64.60^{d}
SEM	0.06	0.33	0.07	0.07	0.09	0.078	0.078	2.47
Portability								
Housing system	0.913	0.400	0.026	0.205	0.009	<0.001	0.249	<0.001
EOs	0.093	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Housing × EOs	0.495	0.106	0.734	0.278	0.472	0.045	0.025	<0.001

[a-e]Means in the same column within each classification bearing different superscript lowercase letters are significantly different ($P < 0.05$ or 0.01).

Abbreviations: AI H5, avian influenza H5; AI H9, avian influenza H9; GPx, glutathione peroxidase; MDA, malondialdehyde; ND, Newcastle disease; SOD, superoxide dismutase.

with the untreated control group. The assumption that EOs with their aromatic constituents may promote feed intake does not seem to be justified in general in laying hens. Where, Özek et al. (2011) reported that no difference in feed intake of hens was observed when the EOs blend was supplemented in the basal diet.

In the present study, EOs can improve FCR, in agreement with the study of Çabuk et al. (2014) who concluded that EOs have beneficial effects on FCR in laying hens. Several studies have stated improvement in the egg production and FCR when diets have been supplemented with EOs (Basmacioglu-Malayoğlu et al., 2010). Micciche et al. (2019) stated that EOs can improve the absorption of nutrients in the intestine. In addition, thymol improved FCR of laying hens from 24 to 36 wk of age (Abdel-Wareth, 2016). Thymol safeguards the intestinal microvilli responsible for nutrients absorption, influencing clearly the endogenous digestible enzymes secretion (Hashemipour et al., 2013). EOs may improve the nutrient digestion and absorption through the enzymatic stimulation, and they may have positive effects on FCR when used in laying hens.

The housing system can affect egg quality in commercial laying flocks. Galic et al. (2019) decided that the housing system of laying hens has a significant effect on egg quality. Accordingly, eggs produced from hens kept in cage systems had higher yolk indices, albumen indices, and HU values than those from the floor system (Anderson and Adams, 1994). Many investigations focused on egg shell quality showed a higher quality of eggs from the cage system than from the floor system (Tůmová et al. 2009). Đukić-Stojčić et al. (2009) indicated that heavier eggs with a higher shape index and thicker shell were laid by hens housed in cage system. Caged birds produced the lowest shape index and highest percentage of yolk and albumen in the egg (Lewko and Gornowicz, 2011). In contrast to these results, Pištěková et al. (2006) exhibited that heaviest eggs with the highest yolk and albumen weight were laid by hens kept in the litter system. Also, Tůmová et al. (2011) stated that egg shape index and yolk index were higher in cage system eggs than in the litter system eggs. The different housing systems of laying hens still cause controversy among producers, researchers, consumers, and environmentalists.

In the present study, shell thickness, egg index, and HU were improved by the addition of EOs in the diet. It is known that EOs possess beneficial effects on physiology, metabolism of egg production, egg quality, and general health status of birds (Reiner et al., 2009). The dietary supplementation with EOs improved egg quality (Abd El-Hack et al., 2016). Better results obtained for eggshell quality indices could be partly due to the fact that EOs had an impact on the metabolic activity of the beneficial bacteria colonies within the intestine of laying hens, leading to positive effects on mineral absorption rate (especially Mg^{2+} and Ca^{2+}) (Ding et al., 2017). This result agrees with that of Olgun (2016) who confirmed that eggshell thickness was increased quadratically by EO supplementation. On the contrary, some studies reported that supplementation of EOs in laying hen diet had no effect on the egg quality

Table 10. Blood chemistry of laying hens as affected by different housing systems, essential oils, and their interaction during the experiment.

Items	Cholesterol (mg/dL)	Protein (g/dL)	Calcium (mmol/L)	Phosphorus (mmol/L)	Urea (mmol/L)	Creatinine (mmol/L)	ALT (U/L)	AST (U/L)
Housing system								
Cage	188	3.44	4.14	2.29	5.25	0.44	20.05	87.05
Floor	189	3.40	4.06	2.22	5.50	0.42	20.80	88.35
Essential oils (EOs)								
0	206a	3.50	3.76^{d}	2.08^{c}	5.77^{a}	0.51^{a}	22.80^{a}	100.01^{a}
Thymol	186c	3.28	4.16^{b}	2.34^{a}	5.30^{b}	0.43^{b}	20.40^{b}	86.50^{b}
Carvacrol	192b	3.45	4.02^{c}	2.26^{b}	5.45^{b}	0.45^{b}	20.40^{b}	86.10^{b}
Eugenol	171d	3.46	4.46^{a}	2.35^{a}	4.98^{c}	0.33^{c}	18.10^{c}	78.20^{c}
Housing × EOs								
Cage								
0	205	3.55	3.85^{e}	2.11	5.68	0.51^{a}	22.40	97.40
Thymol	185	3.34	4.17^{c}	2.35	5.16	0.42^{b}	20.20	86.20
Carvacrol	195	3.45	3.99^{d}	2.31	5.32	0.44^{b}	20.01	87.60
Eugenol	169	3.44	4.56^{a}	2.42	4.84	0.36^{c}	17.60	77.01
Floor								
0	208	3.45	3.67^{e}	2.05	5.86	0.50^{a}	23.20	102.60
Thymol	187	3.23	4.16^{c}	2.33	5.44	0.43^{b}	20.60	86.80
Carvacrol	190	3.46	4.05^{d}	2.21	5.58	0.45^{b}	20.80	84.60
Eugenol	173	3.48	4.35^{b}	2.29	5.12	0.31^{d}	18.60	79.40
SEM	1.76	0.10	0.045	0.02	0.05	0.01	0.60	1.43
Portability								
Housing system	0.286	0.572	0.014	<0.001	<0.001	0.123	0.091	0.208
EOs	<0.001	0.166	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Housing × EOs	0.053	0.837	0.012	0.239	0.790	0.025	0.967	0.051

[a-c]Means in the same column within each classification bearing different superscript lowercase letters are significantly different ($P < 0.05$ or 0.01).

parameters (Luna et al., 2012; Olgun and Yıldız, 2014). However, the improvements in HU in our study are important for the egg-food industry because the HU score is known as an indicator of egg freshness and is related to shelf life.

In the present study, the hematological indices were not affected by different systems in laying hens, and the values were in harmony with the normal range for healthy hens. This result was in agreement with that of Oke et al. (2017) who showed that rearing systems did not have significant effects on the hematological parameters of birds. Alabi et al. (2015) confirmed that the hematological values (PCV, Hb, and RBCs) of the hens were not significantly affected by the housing system. These results indicate that the health status of the hens was not negatively affected by the different housing systems.

In the present study, hematology parameters were not influenced by the inclusion of EOs in the diets. The hematological parameters tested in the study of Toghyani et al. (2010) including RBC, PCV, and Hb did not differ significantly with the addition of thyme. Unlike our result, Al-Kassie (2009) elucidated that feeding diets supplemented with oil extracted from cinnamon and thyme to birds significantly augmented Hb, PCV, and RBCs values compared with those in the control group. The addition of RepaXo (mixture of volatile oils) in poultry diet significantly improved leukocytes in terms of heterophils, lymphocytes, and eosinophil compared with the control group (Tollba et al., 2010).

The immune system of an organism could be affected by the raising system. The results of the present study exhibited significant increase in values of ND and AIH9 for hens reared in the cage than those for hens of the floor system. Kamil et al. (2012) reported similar results that the raising system significantly affected the serum Newcastle disease (**ND**) vaccine titer. That might be as the cage-housed hens could enjoy the highly regulated, protected, and controlled social and physical environment, and the environmental stressors were lower than those in floor hens. Further work is needed to study the effects of housing system on immune mechanism.

Some studies measured antibody titres against viruses of infectious bursal disease and infectious ND virus as a response to feeding diets containing medicinal plants or their EOs. In the present study, immunological indices of hens were improved by inclusion of EOs in the diet. In regard to the immunological status, HI titter of ND virus was significantly higher with addition of RepaXo (mixture of volatile oils) (Tollba et al., 2010). Laying hens showed high antibody titer levels to ND when their diets were supplemented with EOs (Özek et al., 2011). The inclusion of EOs or bioactive components in drinking water of the broilers augmented the antibody titres against infectious bursal disease, IBV, and ND vaccines (Farag and Alagawany, 2019; Hesabi Nameghi et al., 2019). The bioactive compounds of EOs might have been responsible for the raised antibody titres against the experimental antigens (Recoqillay, 2006). As described previously, improved antibody titre might be due to their effects on enhancing the proportions of systemic lymphocyte as an antibody producer and the antioxidant properties of herbal extracts (Najafi and Torki, 2010). Furthermore, herbs that are rich in such flavonoids as carvacrol and thymol extend the activity of vitamin C, act as antioxidants, and may boost the

immune function (Cook and Samman, 1996; Waheed Janabi et al., 2020). Basmacioglu-Malayoğlu et al. (2010) detected that birds fed EOs increased IgG and IgM concentrations. Hashemipour et al. (2013) clarified an improved immune response in birds fed a diet containing carvacrol or thymol, characterized by enhanced touchiness reaction and a rise of total IgG and IgG anti–sheep RBCs with reducing heterophils-to-lymphocyte ratio. The EO compounds can stimulate the synthesis of proteins and the immune system, protecting the cells against the oxidation process (Moomivand et al., 2015). The enhancements observed in the performance of laying hens fed EOs could have potentially been associated with improved immune response (Mousavi et al., 2018).

In the present results, no changes were noticed in blood chemistry parameters (cholesterol, protein, AST, ALT, creatinine) between the hens reared in the cage or floor system. This is in agreement with the study of Pavlík et al. (2007) who stated that the effects of housing systems on biochemical indicators of plasma in laying hens were not significant. Yang et al. (2014) showed that the raising system did not affect the concentrations of total protein, cholesterol, and liver enzymes.

The dietary addition of EOs resulted in an increase in serum calcium and phosphorus. The increase in concentrations of Ca and P in the blood 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). The findings of Amad et al. (2011) exhibited that the phytogenic additive thyme oil added to the broiler diets caused a linear increase in the apparent ileal Ca and P digestibility. Mountzouris et al. (2011) pointed out that EO supplementation to the chicken diet increased ileal Ca bioavailability.

The findings of this study established the important role of EOs in controlling the liver function, which is consistent with the results of Tekce and Gül (2017) who stated that the addition of a natural plant such as *Origanum syriacum* that contains carvacrol and thymol in the chicken diet reduced liver enzyme (ALT and AST) levels compared with the control group. Sharma et al. (2007) stated a significant role of plant extracts to suppress liver enzyme activity. The present study shows that EOs can significantly decrease the AST and ALT level, in agreement with the study of Zhu et al. (2014). Therefore, the addition of EOs will not damage liver cells, maybe because these oils contain antioxidants that can protect cells from DNA damage and thus are useful to animals.

EOs have beneficial influence on lipid metabolism (Acamovic and Brooker, 2005). In the present study, serum cholesterol was declined by the addition of EOs when compared with the control group. Polat et al. (2011) attributed the reduction in serum cholesterol levels to carvacrol and thymol compounds. Basmacioglu-Malayoğlu et al. (2010) demonstrated that carvacrol and thymol may exhibit hypocholesterolemic effects by 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibition. Thymol action on cholesterol synthesis is related to the inhibition on the creation of 3-hydroxy-3-methylglutaryl coenzyme A reductase, a regulatory enzyme required in the cholesterol synthesis (Bampidis et al., 2005). The decrease in serum cholesterol by EOs may explain the reduction in the MDA of the birds fed those diets.

The main field of application of natural products is in the prevention of oxidation of animals and their products (Elwan et al., 2019). The antioxidant property of EOs is assumed to protect lipids from oxidation, thereby retarding the process of lipid peroxidation (Botsoglou et al., 2002). Florou-Paneri et al. (2005) indicated that MDA was significantly decreased with the addition of oregano EO (50 or 100 mg/kg) in the diet. The EOs contribute in antioxidant activity because it decreased levels of MDA (the most important indicator of lipid peroxidation). Also, Gumus et al. (2017) stated that EOs significantly decreased MDA levels. Dietary EOs such as carvacrol and thymol could remove the excessive free radicals because of their phenolic OH groups as hydrogen donors for the proxy radicals produced during the starting lipid oxidation, thereby decreasing the hydroxyl peroxide formation (Yanishlieva et al., 1999). Based on these results, we show that EOs (thymol, carvacrol, and euganol) might play a main role as an exogenous antioxidant and could also be applied as a protective agent against the tissue damage.

CONCLUSIONS

The current results indicate that hens reared in the cage system had productive performance than hens reared in the floor system. Dietary supplementation of EOs (thymol, carvacrol, and eugenol) improved the productive performance, immunity indices, serum calcium, and phosphorus of laying hens. Also, these EOs decreased serum cholesterol, urea, creatinine, and liver enzymes. Finally, EOs represent promising feed additives for the nutrition of laying hens housed in the cage system.

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