GSC Biological and Pharmaceutical Sciences, 2020, 13(02), 136-145

Available online at GSC Online Press Directory

GSC Biological and Pharmaceutical Sciences

e-ISSN: 2581-3250, CODEN (USA): GBPSC2

Journal homepage: https://www.gsconlinepress.com/journals/gscbps

(RESEARCH ARTICLE)

Effect of oregano (*Oreganum syriacum* L.) essential oil and cage density on performance parameters, egg quality criteria, some blood biochemical parameters, blood antioxidant capacity, and intestinal histopathology in laying hens

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Publication history**:** Received on 10 November 2020; revised on 18 November 2020; accepted on 19 November 2020

Article DOI: https://doi.org/10.30574/gscbps.2020.13.2.0370

Abstract

Although many methods have been developed in order to reduce the negative effects in laying hens reared at high cage density, researches on effects of dietary supplementation of oregano (*Oreganum syriacum L.*, OEO) essential oil as a feed additive to the diets are extremely sparse. This study investigated the effects of OEO and caging density on performance parameters, egg quality criteria, some blood biochemical parameters, blood antioxidant capacity, and intestinal histopathology of laying hens. We used 276 white Lohmann laying hens aged 38–40 weeks in the experiment. The animals were divided to positive control (600 cm^2 hen/cage, PC), negative control (360 cm^2 hen/cage, NC), negative control+ 200 mg/kg OEO (NC+O2), negative control+ 400 mg/kg OEO (NC+O4), and negative control+ 600 mg/kg OEO (NC+O6). In this study, it was determined that egg weight and egg shell weight increased at higher caging density, but they did not change with dietary supplementation of OEO. In addition, the plasma calcium and phosphorus decreased with the higher caging density and dietary supplementation of OEO. Supplemented OEO to the diets of the laying hens reduced lipid peroxidation and improved antioxidant capacity and intestinal histopathology of laying hens. As a result, it was determined that OEO decreased the negative effects of high caging density. While this positive effect was found to be at a maximum dose at 400 mg/kg of OEO, it was determined that a dose of 600 mg/kg of OEO had a toxic effect, if even numerically.

Keywords: Caging Density; Duodenum; Egg; Laying Hens; Oregano.

1. Introduction

Egg production demand is constantly rising as increasing global population. As a result, the number of hen cages is increasing to meet the demand. However, an increase in the number of poultry cages has had a negative impact on economic efficiency because they can not be used effectively during periods of lower consumption and demand. Higher caging densities are one of the important stress factors that adversely affect poultry. The ideal caging area requirements has been estimated at a minimum 450 cm^2 per adult hen [1]. Unfavorable caging conditions may adversely affect the laying hens' yield performance parameters, some blood biochemical parameters, blood antioxidation capacity,

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intestinal histopathology, and certain physiological behaviors [2-8]. Although many methods and feedstuffs are used to reduce the negative effects of high caging densities in poultry [9-12], research on the specific effects of oregano/thyme essential oil is extremely sparse [13]. Oregano essential oil (OEO) is an essential oil derived from plants. Effects of OEO on poultry are variable. Previous studies have shown that it generally improved feed conversion ratio (FCR) and increases affect feed intake (FI), egg weight (EW), and egg production (EP) and but does not the damaged egg ratio (DER) [14,15]. But, there are studies that dietary supplementation of OEO does not effect on performance parameters in poultry [16-17]. Although there are study that indicate otherwise of this study [14], it has no effect on egg quality criteria such as the egg shape index (ESI), eggshell strength (ESS), eggshell thickness (EST), the albumin index (AI), the yolk index (YI), and the Haugh unit (HU) [15]. Like other essential oils, OEO has antioxidant properties. It generally decreases malondialdehyde (MDA) in poultry and also increases the plasma superoxide dismutase (SOD) and the plasma glutathione peroxidase (GPx) activities poultry [16,18]. However, no study has examined the antioxidative enzymes that the plasma catalase (CAT) and glutathione (GSH) in laying hens. Also, OEO is known to affect some blood biochemical parameters [15]. Studies on different types of oregano have found that it generally increases the villus height (VH), but does not have change the villus width (VW) and the crypt depth (CD) [17]. This study aimed determine whether dietary supplementation of OEO in laying hens can reduce the negative effects of higher caging density on the hens' performance parameters, egg quality, some blood biochemical parameters, blood antioxidant capacity, and intestinal histopathology.

2. Material and methods

Approval for this study was obtained from the Local Ethics Committee of Ataturk University (No:36643897-000-E.1800064346).

2.1. Essential oil, zeolite, and preparation

The oregano herbs (*Origanum syriacum L.*) were collected in the season (autumn and winter) and dried in the shade for 3–4 days. The dried plant materials were chopped and placed in a beaker to obtain the essential oil using steam distillation. The components of the essential oil were identified using ISQ Single Quadrupole model gas (Thermo Fisher Scientific Inc., USA) chromatography (Table 1).

Table 1 Chemical Composition of Oregano (Origanum syriacum L.) Essential Oil

Components*	**Rate (%)**	**Components***	**Rate (%)**
Alpha-Pinene	1.30	3-Cyclohexen-1-ol	1.27
Alpha-Thujene	1.62	Thymol	18.38
Myrcene	2.95	Carvacrol	18.23
Terpineol	4.78		
Caryophyllene	2.21		
Gamma-Terpinene	21.89		
P-Cymene	17.90		
1-Octan-3-ol	2.83		
Trans-Sabinene hydrate	2.04		
Total			97.49

* ≥ 0.5

The structure of each compound was identified using mass spectrophotometry with Xcalibur software [19]. The zeolite (clinoptilolite) used in the hens' diets was purchased from a commercial company (Rota Mining Corp., Turkey). The zeolite particle sizes ranged from 0.3 to 0.7 mm. The lowest absorption potential of the zeolite was determined to be 40mL/100 kg [20]. Later, OEO was absorbed in zeolite (0.15%) in 200, 400, and 600 mg/kg doses in beaker. Before being added to the hen' diets, oregano essential oil absorbed in zeolite was stored 24 hours at +4°C in the fridge to minimize the volatility of its.

2.2. Animals, diets, and management

For the purpose of this study, 276 white Lohmann laying hens aged 38–40 weeks were used, kept in cages sized (Width: 59 × Length: 61 × Height: 60 cm). The animals were divided into randomly 5 groups according to body weight (average,

1501 ± 58.4 g) including positive control (600 cm^2 hen/cage, PC), negative control (360 cm^2 hen/cage, NC), negative control+ 200 mg/kg OEO (NC+O2), negative control+ 400 mg/kg OEO (NC+O4), negative control+ 600 mg/kg OEO (NC+O6). Standard feeder and drinker spaces were identical in each pen, and the animals were kept in 12-h light/dark cycles at a temperature of 24°C± 3°C [22]. Feed and water were supplied ad libitum and hens were fed on a soybean/maize-based diet. The animal experiment lasted 8 weeks and was preceded by a 7-day adaption period. AOAC [23] and Van Soest and Robertson [24] methods were used to determine the nutrient contents of the diets (Table 2).

2.3. Performance parameters and egg quality criteria

At the end of each week, the remaining feeds in front of animals were weighed to determine the FI. Egg samples were daily collected to calculate the EP and DER and also to measure the EW on a weekly basis. The FCR was calculated as the amount of feed consumed (in kg) per

Table 2 Nutrient Content and Ingredients of All Diets (g/kg)

Ingredients[6]				**Analyzed nutrient levels[6]**		
	Control Group	Trial Groups[3]			Control Group	Trial Groups[3]
Maize	567.00	566.74		DM %	88.55	88.70
SBM[4]	172	171.8		CP %	17.50	17.47
Maize Gluten	92.2	92.2		ME[2]	12.17	12.15
Wheat Bran	22.1	21.1		EE%	5.48	5.47
Vegetable Oil	36.4	36.5		Ash %	19.48	19.51
Marble Powder	88.85	88.82		CF %	2.81	2.81
DL-Mehtionine	0.71	0.72		Ca %	4.40	4.40
L-Lysine	0.92	0.93		Av. P %	0.37	0.37
DCP[5]	14.4	14.3				
Salt	2.51	2.51				
Premix[1]	2.91	2.91				
Zeolite	0.00	1.50				

[1] Per kg of feed: trans-retinol, 6 000 000 IU; cholecalciferol, 1 200 000 IU; dl-α-tocopheryl acetate, 15 000 mg; menadione, 2 000 mg; thiamine, 1 500 mg; riboflavin, 3 500 mg; niacin; 12 500 mg; d-pantothenate, 5 000 mg; pyridoxine, 2 500 mg; cobalamin, 7.5 mg; d-biotin, 22.5 mg; folic acid, 500 mg; choline chloride, 62 500 mg; ascorbic acid, 25 000 mg; Mn, 40 000 mg; Fe, 30 000 mg; Zn, 30 000 mg; Cu, 2500 mg; Co, 100 mg; I, 500 mg; Se, 75 mg. [2] The value was calculated and converted, MJ/kg (**21**). [3] Experimental groups include all diets containing 0.15% zeolite.[4] Soybean Meal. [5]Dicalcium phosphate.[6] g/kg

quantity of eggs (in kg). On days 7, 14, 28, and 56 of the treatment, one egg from each subgroup was randomly collected to determine the egg quality criteria. The ESS, ESI, EST, eggshell weight (ESW), yolk color (YC), AI, YI, and HU were determined as per the formulas used by Ergün et al. [25].

2.4. Sampling and measurements

At the end of the experiment, 5 hens that the average of the group in terms of body weight randomly selected from each treatment group (total 40 hens) and were slaughtered. Blood samples were collected into vacuum tubes anticoagulant to determine the lipid peroxidation, blood antioxidant capacity and some blood biochemical parameters. In order to obtain the plasma, the blood samples were centrifuged at 3000 rpm at 4°C for 10 min and the plasma samples were stored at -20°C until analysis. Plasma levels of malondialdehyde (MDA); activities of superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPx), and catalase (CAT) were measured using Biotek μQuant MQX200 Elisa Reader (Biotek Corp., USA) [26]. Blood plasma parameters were analyzed with a Beckman AU500 autoanalyzer (Beckman Coulter Inc., USA), and the hormones were analyzed with a Beckman Access 2 Immunoassay System autoanalyzer (Beckman Coulter Inc., USA). Intestinal samples obtained from them were fixed, waxed, and stained according to the method of Li et al. [27]. The VH, VW, CD, tunica mucosa width (TMW), and lamina propria (LP) were

measured from samples selected from five different regions of the duodenum by the oculometer of a microscope equipped with a micrometer at 10x magnification.

2.5. Statistical analysis

Data were subjected to two-way ANOVA analysis procedure of SPSS 25.0 statistical program (SPSS Inc, Chicago, IL, USA) and the data are presented as mean ± standard error. Significant differences were determined using Duncan's multiple range test. P-values less than 0.05 were considered as statistically significant.

3. Results and discussion

3.1. Performance parameters

In the study, it was observed that the EW increased in the groups with higher caging density. In addition, although it was observed that a numerical increase/improvement the FI, EP, FCR, and DER, there was no statistically significant difference among the groups for these parameters (Table 3). In studies carried out by Geng et al. [8] in laying hens, they were reported that although FI increased higher caging density, the EP, EW, and FCR were not affected by the difference caging densities. In the studies investigating the effects of higher caging density in poultry reported that although FI, EW, and EP decrease [6,7,28] and also DER increase [9], there are studies reporting that it has no effect [4,5,12]. The higher caging density in poultry causes to increasing of their activities (access to feed and water and also individual defense), and consequently, poultry spend more energy Al-Rawi et al. [2]. Cost of this energy is difficult to quantify because the types of activity is affected by many factors and it varies between 4-35% [29]. The elevate in energy needs of poultry causes to increase of feed intake. In a study conducted by Leeson and Summers [30] in laying hens, EW was reported to be increased by elevated of cage density. The main reason for the enhanced in EW may be related the OEO intake of laying hens exposed to higher caging density. Although OEO has variable effects on performance in laying hens, it is know that usually does not affect performance parameters. In this study, it was observed that dietary supplementation of OEO had no effect on performance parameters except for EW in laying hens reared higher caging density. Similarly, Florou-Paneri et al. [14] found that 50 and 100 mg/kg of OEO had no effect on laying hens' FI, EP, or DER. Contrary to these study, was reported that increased FI [16,18] and EP [15] and also improved FCR with supplementation of oregano essential oil/oregano herb to the layer's chicken diets [18]. The cause of this difference may be associated with the extraction method and the amounts added to the diets as well as the procedure and duration of feed storage. Therefore, OEO may not be more effective in poultry exposed to stress.

Table 3 Performance parameters of all groups

Groups	FI (g/day)	EW (g)	EP (%)	FCR (kg,feed/egg)	DER (%)
PC	106	58.6[b]	89.6	2.04	0.84
NC	110	59.9[a]	89.7	2.05	0.77
NC+O2	108	60.2[a]	87.7	2.07	1.29
NC+O4	107	60.3[a]	92.1	1.94	0.98
NC+O6	111	59.7[a]	86.3	2.21	1.32
SEM	1.64	0.32	2.69	0.06	0.35
p	0.232	0.047	0.617	0.111	0.722

a,b: Statistical differences between different letters in the same column averages shown that it is significant (P<0.05). FI: Feed intake; EW: Egg weight; EP: Egg production; FCR: Feed conversion rate; DER: Damaged egg rate; SEM: Standard error means.

3.2. Egg quality criteria

Egg quality criteria are crucial for both egg producers and consumers. Poor quality causes major economic losses in the egg industry [25]. The effect of caging density and dietary supplementation of essential oils on egg quality criteria varies [4, 5, 8, 9, 14]. The effect of caging density in laying hens on egg quality criteria was indicated in Table 4. In this study, it was observed that higher caging density had effect ESW. The results of this study, except for ESW are similar to Hayırlı et al. [9], Kang et al. [5], and Geng et al. [8]. The formation of the egg shell is a long process that lasting 20 hours on average and occured more during the night times (especially during the last 14-16 hours). It was reported that hens receiving their daily supply of Ca in the afternoon, rather than morning, utilized significantly more of that day's dietary Ca for shell formation, excreted significantly less Ca via feces, and had significantly improved shell quality [31]. There is

a negative relationship between Ca absorption from the digestive tract and Ca mobilization from bone during egg shell formation [32]. Due to the increase in the activity of NF-kB cells during stress, it was reported that more Ca mobilization from bone was achieved [33]. It was observed that supplementation of OEO in laying hen's diets did not affect the egg quality criteria (Table 4).

Table 4 Egg quality criteria of all groups

Groups	ESI (%)	ESS (kg/cm²)	EST (mm)	ESW (g)	YC	AI (%)	YI (%)	HU
PC	72.2	4.79	0.370	6.56^{b}	10.4	8.01	42.7	77.3
NC	73.9	4.57	0.361	7.03^{a}	10.5	8.38	42.5	78.2
NC+O2	73.1	4.51	0.350	7.07^{a}	10.7	8.29	42.0	79.4
NC+O4	73.6	4.87	0.359	7.23^{a}	10.4	7.81	41.3	78.0
NC+O6	73.2	4.40	0.365	7.19^{a}	10.3	7.83	42.0	75.4
SEM	0.53	0.17	0.01	0.11	0.20	0.44	0.63	1.94
p	0.250	0.333	0.357	0.001	0.799	0.838	0.637	0.687

a,b: Statistical differences between different letters in the same column averages shown that it is significant (P<0.05).

ESI: Egg shape index; ESS: Egg shell strength; EST: Egg shell thickness; ESW: Egg shell weight; YC: Yolk color; AI: Albumin index; YI: Yolk index; HU: Haugh unit; SEM: Standard error means.

3.3. Biochemical analysis

Some blood biochemical parameters of laying hens exposed to the different caging densities are given in Table 5. Higher caging density negatively affected the plasma Ca and P While plasma Ca increased with the dietary supplementation (400 and 600 mg/kg), plasma P increased with the dietary supplementation (200 and 600 mg/kg). In this study, Chol, Trgl, AST, ALT, HDL, LDL, Ins, Glu, TP, Alb, and Crea was not affect by dietary supplementation of OEO. Although Asghar Saki et al. [3] reported that the blood calcium levels decreased in laying hens exposed to the higher caging density, there are also the study indicating that Ca and P increase [9].

Table 5 Plasma biochemical analysis results of all groups

Groups		PC	NC	NC+O2	NC+O4	NC+O6	SEM	*p*
Chol	(mg/dL)	86.8	83.8	103	98.3	87.8	8.08	0.406
Trgl	(mg/dL)	1182	1244	1485	1205	1285	177.7	0.761
AST	(U/L)	181	199	200	211	167	11.68	0.116
ALT	(U/L)	2.75	3.25	7.25	2.5	4.5	1.89	0.413
HDL	(mg/dL)	24.3	27.5	32.8	36.8	34.8	3.36	0.098
LDL	(mg/dL)	42.8	42.5	41.6	52	42.8	5.48	0.659
Ins	(uIU/mL)	0.35	0.51	0.53	0.44	0.59	0.07	0.189
Glu	(mg/dL)	159	158	159	192	175	14.79	0.442
TP	(g/dL)	4.43	4.9	5.76	5.78	5.55	0.39	0.249
Alb	(g/dL)	1.53	1.6	1.68	1.59	1.53	0.06	0.563
Ca	(mg/dL)	27.8^{b}	25.5^{c}	25.2^{c}	29.5^{ab}	32.9^{a}	1.13	0.05
P	(mg/dL)	6.25^{ab}	5.30^{c}	6.70^{ab}	5.86^{bc}	6.93^{a}	0.38	0.043
Crea	(mg/dL)	0.1	0.151	0.134	0.181	0.18	0.02	0.152

a,b,c: Statistical differences between different letters in the same column averages shown that it is significant (P<0.05). Chol: Cholesterol; Trgl: Triglyceride; AST: Aspartate transaminase; ALT: Alanine transaminase; HDL: High density lipoprotein; LDL: Low density lipoprotein; Ins: Insulin; Glu: Glucose; TP: Total protein; Alb: Albumin; Ca: Calcium; P: Phosphorus; Crea: Creatinine; SEM: Standard error means.

In poultry, the absorption mechanism of phosphorus and calcium are interrelated and the absorption decreases if any of them disrupt balance [34]. It is thought that stress conditions may negatively affect the absorption of calcium and

phosphorus by negatively affecting the intestinal villi properties [27]. There is no consensus yet about the effect of oregano essential oil on some blood biochemical parameters in poultry because it is quite variable. The results demonstrated that OEO improved bio-availability Ca and P in laying hens (Table 5). In a study of broiler chickens, it was observed that the increase in blood Ca and P was not statistically significant with the dietary supplementation of OEO [35]. In similar the study, Levkut et al. [36] stated that the blood Ca level increased with the dietary supplementation of OEO. However, it is reported that the ground thyme diets has an effect on blood Ca and P in broilers [37]. Thyme/oregano essential oil can positively effect on plasma Ca and P probably by improving intestinal morphology and oxidative stress or by increasing intestinal pH [16, 38].

3.4. Antioxidant enzymes and MDA level in the plasma

Antioxidant enzymes plays an important role in protecting cells from damage caused by reactive oxygen species, but this process requires dietary supply of the appropriate nutrients [16]. Although higher caging density increased lipid peroxidation and deteriorate antioxidant capacity, OEO reduced lipid peroxidation and improved antioxidant capacity and intestinal histopathology in laying hens (Table 6). It was observed that the dietary supplementation (200 and 400 mg/kg) of OEO to the diets decreased plasma MDA. Moreover, plasma GSH levels were increased 400 mg/kg of OEO while it was decreased with 600 mg/kg of OEO. In laying hens consuming OEO, plasma SOD tended to increase in NC + O2 and NC + O4 groups and to decrease in NC + O6 group. Although the plasma CAT increased with the dietary supplementation of OEO to the laying hens exposed to the higher caging density, it was found that the increase of GPx value only in the NC + O4 group was statistically significant. Similar to the study, in poultry, the higher caging density increased the plasma MDA [10, 13], but the dietary supplementation of thyme reduced the plasma MDA [13, 39]. However, there are studies reporting that plasma MDA levels do not change with Oregano/thyme essential oil to diets [40]. In a study by Çetin and Güçlü [11] in laying hens, it is reported that the plasma SOD, CAT, and GPx enzymes decreased at higher density. The phytogenic product may improve the antioxidative status of poultry due to the antioxidant property of thymol and carvacrol by elevating the activity of antioxidant enzymes. It is reported that the addition of oregano and thyme essential oil to the feeds increased the activity of antioxidant enzymes included CAT, SOD, and GPx. It was suggested that the high antioxidant activity of thymol (a bioactive component presents in essential oil of oregano), is due to the presence of phenolic OH groups which act as hydrogen donors to the peroxy radicals produced during the first step in lipid oxidation, thus, retarding the hydroxy peroxide formation [16,38].

Table 6 Antioxidant enzymes activity and MDA level in the plasma of all groups

Groups	MDA (nmol/l)	GSH (nmol/l)	SOD (U/l)	CAT (KU/l)	GPx (U/l)
PC	7.53^{c}	2.51^{a}	61.8^{a}	85.7^{a}	1.63^{a}
NC	14.0^{a}	1.82^{c}	56.8^{b}	67.6^{d}	1.46^{cd}
NC+O2	10.1^{b}	1.92^{bc}	60.8^{a}	81.0^{b}	1.52^{bc}
NC+O4	9.99^{b}	2.10^{b}	61.0^{a}	84.6^{a}	1.55^{ab}
NC+O6	13.2^{a}	1.22^{d}	48.8^{c}	73.1^{c}	1.41^{d}
SEM	0.48	0.08	0.86	1.05	0.03
p	<0.001	<0.001	<0.001	<0.001	<0.001

a,b,c,d: Statistical differences between different letters in the same column averages shown that it is significant (P<0.05). MDA: Malondialdehyde; GSH: Glutathione-S-transferase; SOD: Superoxide dismutase; CAT: Catalase; GPx: glutathione peroxidase; SEM: Standard error means.

3.5. Intestinal histopathology

VH, VW, CD, LP and TMW are related to the migration, proliferation, and death of cells that are important for intestinal health [17, 41]. The duodenum is the region where the absorption of nutrients occurs most in the intestine sections [42]. It is known that stress has an effect on intestinal morphology in hens [27]. In poultry, there are studies showing that the VH increased [10, 28, 43] and CD decreased [43]. There are also studies reporting that it obtained results supporting this study on, VH [10], VW [44], and CD [12, 45]. Findings show that VH, CD, LP, and TM were not affected by increasing the caging density, but higher caging density decreased VW (Table 7). To the best of our knowledge, previous studies that investigated the effect of caging density on laying hens' intestinal morphology has scarce. Li et al. [27] determined that different caging densities (12 and 16 hens/m^2) only affected VH in the ileum in 28^{th} day. It has been reported that in laying hens exposed to higher caging density, VH decreased in the ileum, while CD was not affected [27].

Other stress conditions effect also intestinal histopathology. In laying hens, VH increased and CD decreased with dietary supplementation of OEO. Also, TM value was found to be the highest in NC + O4 group. In a study on broilers, Fonseca-Garcia et al. [14] found that 100, 200, and 400 mg/kg doses of OEO increased VH in laying hens. Mohiti-Asli and Ghanaatparast-Rashti [41] reported that 200 mg/kg doses of OEO in broiler' diets increased VH and decreased TMW.

Table 7 Intestinal histopathology of all groups

Groups	**VH (mμ)**	**VW (mμ)**	**CD (mμ)**	**LP (mμ)**	**TM (mμ)**
PC	1591[b]	396.[a]	139[a]	269	240.[b]
NC	1512[b]	238[b]	139[a]	228	233[b]
NC+O2	2004[a]	298[b]	142[a]	204	252[b]
NC+O4	2033[a]	259[b]	132[a]	257	334[a]
NC+O6	2100[a]	223[b]	113[b]	192	252[b]
SEM	91.71	26.15	5.93	22.33	15.59
p	<0.001	0.001	0.016	0.108	0.001

a,b: Statistical differences between different letters in the same column averages shown that it is significant (P<0.05).VH: Villus height; VW: Villus width; CD: Crypt depth; LP: Lamina propria; TM: Tunica muscularis; SEM: Standard error means

In stressful conditions, intestinal microbial ecology is disturbed, leading to dysbiosis Thymol and carvacrol maintain the intestinal mucosal integrity by reducing the total number of harmful bacteria, preventing adhesion to the epithelium, and decreasing the production of toxic compounds, thus improving absorption [27]. According to the findings, it was determined that the LP was not affected to increasing both caging density and OEO doses.

4. Conclusion

Consequently, the study showed that performance parameters, egg quality criteria, some blood biochemical parameters, blood antioxidant capacity, and intestinal histopathology in laying hens negatively affected by higher caging density. Although various feed additives are supplemented in order to eliminate the adverse effects of higher caging density, the use of oregano essential oil could be used more since they are both a natural resource and abundant in nature. With the supplementation of oregano essential oil to the diets of laying hens exposed to the higher caging density, while the performance parameters and egg quality criteria were not affected, plasma Ca and P increased. In addition, lipid peroxidation decreases and antioxidant enzymes increased and intestinal histopathology showed positively affected. Although this study is similar to other studies, it has different aspects. Although performance parameters, egg quality criteria, some blood biochemical parameters, blood antioxidant capacity, and intestinal histopathology has been observed to generally improve with supplementation of 400 mg/kg OEO to laying hens' diets, supplementation of 600 mg/kg OEO to laying hens' diets generally has a toxic effect, even if numerically. Thus, more studies are required to determine the molecular mechanisms behind its effects and to better understand its effectiveness.

Compliance with ethical standards

Acknowledgments

This work was supported by the Scientific Research Projects Fund of Ataturk University under Grant number TSA-2018-6624.

Disclosure of conflict of interest

The authors declare no conflict of interest.

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