



Tarragon (*Artemisia Dracunculus L.*) Could Alleviate Negative Effects of Stocking Density in Laying Hens

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ABSTRACT

The study was conducted to determine the effects of stocking density (SD, 5 or 7 layers/cage) and tarragon (*Artemisia dracunculus L.*) diet supplementation at four levels (0, 1.2, 6 and 12 g/kg feed) on performance, certain egg characteristics, serum, liver, egg yolk and small intestine bacteria parameters in laying hens. The experiment was carried out over a period of 8 weeks, with 192 Lohman Brown commercial hybrids at 50-w-age. The results showed that an increased SD reduced feed intake (FI; $p < 0.01$) and egg production ($p < 0.05$), but had no effect on the weight gain, feed conversion ratio, damaged egg ratio, egg weight and egg quality ($p > 0.05$) parameters. The supplementation of tarragon to the diet reduced the FI and damaged egg ratio ($p < 0.01$), and improved egg production and FCR ($p < 0.01$). While an increased SD reduced serum total antioxidants ($p < 0.05$), it elevated corticosterone (CORT) and total oxidant serum (TOS) ($p < 0.05$). Tarragon was found to enhance total immunoglobulin ($p < 0.05$), but to decrease the CORT and TOS of the serum ($p < 0.05$). An increased SD raised the malondialdehyde (MDA) in the serum ($p < 0.001$), liver ($p < 0.05$) and yolk ($p < 0.001$). Tarragon supplementation reduced MDA of the serum ($p < 0.05$), liver ($p < 0.001$) and yolk ($p < 0.001$). *E. coli* and total Mesophilic Aerobic Bacteria counts in the small intestine were raised ($p < 0.001$) with increased SD. Tarragon decreased ($p < 0.05$) mesophilic aerobic bacteria. It was thus found that, tarragon supplementation can be considered generally effective in improving performance parameters, alleviating stress-induced negativities, reducing lipid peroxidation, regulating the immune system and controlling some intestinal microorganisms.

INTRODUCTION

A high (H) stocking density (SD) is one approach to increasing profitability in conventional poultry production, although HSD decreases animal welfare and increases stress. In the event of stress, the adrenocorticotrophic hormone secreted from the anterior pituitary affects the adrenal cortex for the synthesis of glucocorticoids (corticosterone [CORT] and cortisol). The primary glucocorticoid released from the hypothalamic-hypophysis-adrenal (HPA) axis with the effects of stress factors is corticosterone in poultry (Ralph & Tilbrook, 2016). An increase in the CORT hormone in poultry has been found to decrease performance (Kutlu & Forbes, 1993; Mirfendereski & Jahanian, 2015; Sahin *et al.*, 2002; and immunity parameters (Mirfendereski & Jahanian, 2015; von Eugen *et al.*, 2019). Stress factors effect total serum antioxidant (TAS) and oxidant (TOS) concentrations (Sohail *et al.*, 2011). While light and moderate stressors can be easily handled, severe stressors can cause pathological conditions (Moberg & Mench, 2000). That said, contradictory data has been presented with regards to the reaction of the HPA axis in connection with the period and intensity of the stressors in chickens (Ericsson, 2016).



Basic free radicals deriving from oxygen in living systems play a role in oxidative stress and oxidative rancidity (Conforti *et al.*, 2006). Oxidative stress damages important biological molecules, and when the body's ability to rid itself of radicals is reduced, at least one of the pathological findings in animals (Fellenberg & Speisky, 2006) lipid peroxydation in animal products (Galobart *et al.*, 2001) occur. Today, medicinal aromatic plants with high phenolic and flavonoid content, as well as their extracts, are used to alleviate the negative effects of stress factors and synthetic antioxidants. It has been stated that the dietary supplementation of antioxidant substances regulates corticosteroid synthesis in the adrenal glands (Mirfendereski & Jahanian, 2015; Von Eugen *et al.*, 2019) enhances immunity (Mirfendereski & Jahanian, 2015) and decreases the lipid peroxidation of egg yolk (Botsoglou *et al.*, 2005; Karaalp *et al.*, 2018). In addition, dietary supplementations of anti-microbial substances suppress pathogenic microflora in the digestive system (Wenk, 2000).

Artemisia dracunculus L., which is one of the 22 types of the *Artemisia* species grown in Turkey, has both antioxidant and antibacterial features. The dominant ingredients in the oil of *Artemisia dracunculus* L. plant, which is a multi-year woody plant, are (Z)-anethole (81%), (Z)- β -ocimene (6.5%), (E)- β -ocimene (3.1%), limonene (3.1%) and methyleugenol (1.8%) (Kordali *et al.* 2005). In another study conducted in the same period and in the same region, the β -ocimene (1.237.21 arbitrary units, $AU \times 10^{-6}$), α -pinene (114.4 $AU \times 10^{-6}$), β -thujene (166.92 $AU \times 10^{-6}$), D-limonene (366.2 $AU \times 10^{-6}$), γ -terpinene (187.27 $AU \times 10^{-6}$), Terpinolene (129.92 $AU \times 10^{-6}$) and Estragole (11 242 $AU \times 10^{-6}$) levels were stated (Yilmaz *et al.* 2019). There have to date been several studies examining the effects of *Artemisia annua* (Brisibe *et al.*, 2008) *Artemisia sieberi* (Khalaji *et al.*, 2011; Kheirabadi *et al.*, 2014) and *Artemisia dracunculus* (Gharetappe *et al.*, 2015; Hosseinzadeh & Moghaddam, 2014), all of which are *Artemisia spp.*, in broiler chickens; and while there have been a few studies examining effects of *Artemisia annua* in laying hens (Brisibe *et al.*, 2008; Li *et al.*, 2016), no study has been found with specific focus on *Artemisia dracunculus*.

The initial humoral and cellular immune response takes the form of cytokines released from activated T cells. These cytokines are transformed into plasma cells that are capable of producing antibodies (IgG, IgA and IgM) through the stimulation of B lymphocytes (Taheri *et al.*, 2005). There are three main antibody

classes containing IgM, IgA and IgG (IgY) that have been identified in chickens (Ayaz *et al.*, 2008). IgG in chickens accounts for approximately 75% of the total serum antibodies (Carlander *et al.*, 2000).

One of the more common bacteria found in animal intestines is *Escherichia coli* (*E. coli*) (Omidpanah *et al.*, 2016). Some serotypes of *E. coli* are pathogens for poultry (Dho-Moulin & Fairbrother, 1999; and some serotypes are pathogens for humans (Kassaify and Mine 2004). Aerobic mesophilic bacteria and their metabolites are the main pathogenic microorganisms causing environmental pollution (Witkowska & Sowińska, 2013).

The present study evaluates the effects of the dietary supplementation of *Artemisia dracunculus* L. in commercial laying hens housed in different SD on performance, certain egg quality characteristics, peroxidation, immune response and some intestinal bacteria.

MATERIALS AND METHODS

Animals, Experimental Desing and Feed

All procedures were approved by the Animal Experiments Local Ethics Committee of Gumushane University (Approval date: 10.05.2017; Decision No: 01-07). A total of 192 Lohman Brown layer hens (at 50-w old) were used in a 2x4 factorial (with 4 replications) arrangement of treatments that included two cage densities [5, normal (N) SD and 7 (HSD) hens per cage; 90x45x35 cm], and four ratios of ground tarragon (0, 1.2, 6 and 12 g/kg diet, T0, T1.2, T6 and T12, respectively). The SDs were 810 and 580 cm² area per hen in the NSD and HSD groups, respectively. The ingredients and chemical compositions of the commercial feed are presented in Table 1. The tarragon was obtained from the village of Yedigözeler in the Bayburt Province, and was kept stored in a cool and dry environment. The total antioxidant amount of tarragon was measured as 0.833 mmol/g according to Erel (2004). The dietary supplementation of 0, 1.2, 6 and 12 g/kg of ground tarragon corresponded to 0, 1, 5 and 10 mmol of additional antioxidant, respectively. The first 2-w (48 to 50-w old) was the adaptation period. The main trial lasted for a total of 8-w, from 50 to 58-w old. The hens were exposed to 16 h light and 8 h darkness, feed and water were provided *ad libitum*, and the accommodation temperature was maintained at 21 to 24 °C throughout the trial period. The diet was analyzed according to standard AOAC (2007), procedures for chemical composition.



Table 1 – Composition of experimental diet.

Ingredients	g/kg
Corn	352.40
Triticale	175.00
Wheat	75.00
Soybean meal, 340 g CP/kg	114.60
Sunflower meal, 330 g CP/kg	103.60
Hazelnut, 420 g CP/kg	35.00
Corn gluten meal, 600 g CP/kg	25.00
Vegetable oil	8.30
Limestone	95.50
Dicalcium phosphate	7.20
NaCl	3.20
Premix	2.50
L-Lysine HCl	1.50
Naturabind-S	1.20
Chemical analysis of feed	
Crude matter	892.20
Crude protein	167.50
Ether extract	46.60
Crude fiber	52.90
Crude ash	131.70
Starch	339.90
Calculated contents of feed (.../kg)	
Metabolizable energy, MJ ²	11.45
Methionine, g	3.70
Methionine + Cystine, g	6.70
Lysine, g	7.50
Linoleic acid, g	22.10
Ca, g	39.00
Available P, g	3.50
Na, g	1.50

¹Premix provided per kilogram of diet: vitamin A (retinyl acetate), 6.7 mg; vitamin D₃ (cholecalciferol), 1.6 mg; vitamin E (α -tocopherol), 30 mg; vitamin K₃ (menadione), 2.5 mg; vitamin B₁ (thiamine), 3 mg; vitamin B₂ (riboflavin), 7 mg; vitamin B₃ (niacin), 40 mg; vitamin B₅ (Ca-D-pantothenate), 8 mg; vitamin B₆ (pyridoxine), 4mg; vitamin B₈ (D-biotin), 0.1 mg; vitamin B₉ (folic acid), 1 mg; vitamin B₁₂ (cyanocobalamin), 0.02 mg; vitamin C (ascorbic acid), 50 mg; choline chloride, 125 mg; Mn, 80 mg; Fe, 60 mg; Zn, 60 mg; Cu, 5 mg; Co, 0.10 mg; Se, 0.15 mg.

²Metabolizable energy (ME) is calculated according to the method provided by TSE (1991).

Performance and Egg Quality Parameters

Weight gain (WG), feed intake (FI) and feed conversion ratio, FCR were determined every two weeks; the damaged egg ratio (DER), average egg weight (AEW) and egg weight (EW) were determined daily. In order to determine such egg quality parameters such as yolk height (YH), yolk color index (YCI), Haugh unit (HU), shell strength (SS), shell thickness (ST), shape index (SI) and shell weight (SW), three random egg samples were taken from each replication (12 from each group; total 96) at 4 and 8 w. After the samples were kept at room temperature for 24 hours, they were subjected to analysis with an advanced laser light method branded NABEL DET6000.

Serum CORT, IgG, TAS and TOS

At the end of the experiment, a total of 80 samples, 10 from each group, were taken for blood, yolk and tissue analysis. CORT, TAS, TOS and IgG in the blood; malondialdehyde (MDA) in the blood, yolk and liver; and *E. coli* and total mesophilic aerobic bacteria in the small intestine were determined. At the end of the experimental period, serum was separated by centrifuging approximately 7 ml blood samples taken from the vena jugularis after 12-hours of fasting, and kept at -82°C until the analysis. CORT concentrations were determined using commercially available radio immune assay sets (Byk-Sangtec Diagnostica, Dietzenbach-Germany; Immulite 2000, DPC, LA) (Sahin *et al.*, 2003). Serum IgG concentrations were determined using the ELISA method, as mentioned by Li *et al.* (2000). linearity of the standard IgG was calculated using the Excel program. Serum TOS was measured spectrophotometrically and calibrated with hydrogen peroxide (Erel, 2005). Serum TAS level was measured using the colorimetric method developed by Erel (2004). The eggs that were selected for yolk MDA analysis were stored for 21 days at +6 °C, after which, 10 samples were taken from the liver and yolk, and stored until the time of analysis at -82 °C after being homogenized. The MDA analyses were carried out based on the method put forward by .

Small Intestine *E. coli* and Total Mesophilic Aerobic Bacteria

A total of 10 small intestine content samples were collected from each experiment group for total mesophilic aerobic bacteria and *E.coli* counts. The total *E.coli* and mesophilic aerobic bacteria counts reproduced at the end of the incubation period were determined in line with.

Statistical analysis

The data was subjected to an analysis of variance (General Linear Model procedure) with a completely randomized design using SPSS software (2002). Data on the egg quality parameters was analyzed according to the 2x2x4 factorial design. A Duncan multiple range test was applied to compare the effects of the different doses of tarragon.

RESULTS

Performance and Egg Quality Criteria

Effects on performance of SD and of different doses of tarragon in the diet are given in Table 2. SD and



Table 2 – The effects of tarragon supplementation to diet on performance parameters.

S	T, g/kg feed	BWBE, g	BWEE, g	WG, g	FI, g/hen/d	FCR, kg /kg	EP, %	AEW, g	DE, %
N	0	1617	2123	506	127.0 ^a	2.59 ^b	73.5 ^{bc}	66.8	4.42 ^{bc}
	1.2	1659	2088	429	123.5 ^b	2.38 ^{ab}	78.5 ^{ab}	66.4	3.77 ^{ab}
	6	1621	2050	429	124.5 ^b	2.26 ^a	84.0 ^a	65.9	3.07 ^a
	12	1662	2107	445	127.5 ^a	2.55 ^b	76.3 ^{bc}	65.8	4.17 ^{bc}
H	0	1659	2133	473	115.8 ^{cd}	2.57 ^b	71.3 ^c	63.4	4.85 ^c
	1.2	1661	2058	398	113.8 ^d	2.19 ^a	79.0 ^{ab}	65.9	4.02 ^{abc}
	6	1629	2004	374	114.3 ^{cd}	2.39 ^{ab}	75.0 ^{bc}	63.9	3.66 ^{ab}
	12	1646	2000	354	116.5 ^c	2.43 ^{ab}	73.3 ^{bc}	65.6	4.51 ^{bc}
	SEM	9	15	18	1.0	0.04	0.04	0.4	0.13
S	N	1640	2092	453	125.6 ^a	2.44	78.1 ^a	66.2	3.86
	H	1649	2049	400	115.1 ^b	2.40	74.6 ^b	64.7	4.26
T, g/kg feed	0	1638	2128	490	121.4 ^a	2.58 ^c	72.4 ^b	65.1	4.64 ^c
	1.2	1660	2073	413	118.6 ^b	2.28 ^a	78.8 ^a	66.2	3.90 ^{ab}
	6	1625	2027	402	119.4 ^b	2.33 ^{ab}	79.5 ^a	64.9	3.36 ^a
	12	1654	2053	400	122.0 ^a	2.49 ^{bc}	74.8 ^{ab}	65.7	4.34 ^{bc}
<i>p</i> -value									
S		NS	NS	NS	0.01	NS	0.05	NS	NS
T		NS	NS	NS	0.01	0.01	0.01	NS	0.01
S×T		NS	NS	NS	NS	NS	NS	NS	NS

S, stocking; N, normal; H, high, T, tarragon; BWBE, body weight at the beginning of the experiment; BWEE, Body weight at the end of the experiment; WG, weight gain; FI, feed intake; EP, egg production; AEW, average egg weight; FCR, feed conversion ratio; DE, damaged egg; NS, not significant.

^{a-d} Values within a column with different superscripts differ significantly (Duncan's test).

tarragon doses had no effect on body weight between the beginning and end of the test, WG and AEW ($p>0.05$). An increase in SD had no effect on FCR and DER ($p>0.05$), but decreased the daily FI ($p<0.01$) and EP ($p<0.05$). T1.2 and T6 diets decreased FI and DER ($p<0.01$) and improved FCR ($p<0.01$).

The effects on egg quality characteristics of SD and different tarragon doses in the diet are given in Table 3. It was determined that age, SD and tarragon dietary supplementation had no significant effect on the YH, HU, SS, SI or SW egg quality parameters. Age and SD had no effect on YCI ($p>0.05$), while YCI was decreased by tarragon supplementation ($p<0.01$). The interactions of D×S and D×S×T on YCI, on the other hand, were significant ($p<0.01$), and SD and changes in the tarragon doses in the diet had no effect on ST ($p>0.05$), but decreased as the age increased ($p<0.05$).

Serum CORT, IgG, TAS and TOS

The effect of SD and tarragon doses on serum CORT, IgG, TAS and TOS quantities are presented in Table 4. Serum CORT, which was high ($p<0.001$) in HSD, decreased ($p<0.001$) in a parallel with tarragon dietary supplementation. Furthermore the S×T interaction on serum CORT was significant ($p<0.001$). SD had no effect on the serum IgG ($p>0.05$). T6 and T12 increased the serum IgG more than in the control, and the T12 effect was higher ($p<0.001$). HSD decreased serum TAS ($p<0.01$), and tarragon supplementation

increased the TAS amount only numerically ($p>0.05$). While SD increased serum TOS ($p<0.001$), the tarragon doses decreased this parameter ($p<0.001$). T12 was more effective ($p<0.05$) in decreasing serum TOS when compared to T1.2 and T6.

Serum, Liver and Egg MDA

The effects of SD and tarragon doses on serum, liver and egg MDA quantities are presented in Table 5. HSD raised the MDA in the serum ($p<0.001$), liver ($p<0.05$) and egg yolk ($p<0.001$). T6 and T12 diets decreased serum MDA. All doses of tarragon decreased the liver MDA ($p<0.001$), and T6 and T12 were more effective than T1.2 on this parameter. Moreover S×T interaction was significant in the quantity of liver MDA ($p<0.05$). The T6 and T12 diets decreased the yolk MDA ($p<0.001$), and T12 was more effective on the yolk MDA when compared to T6 ($p<0.05$).

Small Intestine *E. coli* and Total Mesophilic Aerobic Bacteria

The effects of SD and tarragon doses on *E. coli* and total mesophilic aerobic bacteria counts in the small intestine are presented in Table 5. HSD raised the counts of *E. coli* and the total Mesophilic Aerobic Bacteria in the small intestine ($p<0.001$). Tarragon doses decreased *E. coli* only numerically ($p>0.05$); and T6 and T12 decreased significantly ($p<0.05$) the total mesophilic aerobic bacteria count when compared to the control.



Table 3 – The effect of tarragon supplementation to diet on the egg shell quality parameters.

D	S	T, g/kg feed	EW, g	YH, mm	YCI	HB	SS, Kg	ST, mm	SI	SW, g
28	N	0	65.8 ^{ab}	6.3	9.7 ^a	75.7	2.19 ^{ab}	0.37 ^{ab}	73.6	8.0 ^{ab}
		1.2	64.4 ^{ab}	6.1	6.8 ^{bc}	74.1	2.43 ^a	0.38 ^{ab}	73.4	8.0 ^{ab}
		6	65.3 ^{ab}	6.1	6.6 ^{bc}	74.3	2.46 ^a	0.38 ^{ab}	74.8	8.1 ^a
		12	64.5 ^{ab}	5.9	6.6 ^{bc}	73.3	2.34 ^{ab}	0.34 ^{ab}	73.9	7.8 ^{ab}
	H	0	61.9 ^{ab}	6.2	7.5 ^{bc}	76.9	2.38 ^a	0.36 ^{ab}	74.4	7.7 ^{ab}
		1.2	65.4 ^{ab}	6.6	7.1 ^{bc}	77.5	2.42 ^a	0.39 ^a	73.5	7.9 ^{ab}
		6	61.3 ^b	5.7	6.6 ^{bc}	71.5	2.35 ^{ab}	0.38 ^{ab}	74.1	7.7 ^{ab}
		12	63.3 ^{ab}	6.0	7.6 ^{bc}	74.9	2.39 ^a	0.35 ^{ab}	74.3	7.7 ^{ab}
56	N	0	68.2 ^a	5.6	7.5 ^{bc}	71.7	2.49 ^a	0.33 ^b	73.9	7.6 ^{ab}
		1.2	65.3 ^{ab}	6.4	6.8 ^{bc}	76.9	2.40 ^a	0.34 ^{ab}	73.3	7.7 ^{ab}
		6	61.8 ^{ab}	6.1	5.8 ^c	73.8	2.33 ^{ab}	0.35 ^{ab}	73.7	7.6 ^{ab}
		12	65.2 ^{ab}	5.7	6.7 ^{bc}	71.7	2.37 ^a	0.36 ^{ab}	73.8	7.2 ^b
	H	0	64.8 ^{ab}	5.8	10.4 ^a	73.4	1.97 ^b	0.34 ^{ab}	74.0	7.4 ^{ab}
		1.2	65.4 ^{ab}	6.4	6.4 ^{bc}	77.1	2.38 ^a	0.36 ^{ab}	73.1	8.0 ^{ab}
		6	62.1 ^{ab}	5.6	5.8 ^{bc}	73.1	2.43 ^a	0.36 ^{ab}	74.1	8.0 ^{ab}
		12	65.8 ^{ab}	5.6	6.6 ^{bc}	70.4	2.04 ^a	0.35 ^{ab}	72.9	7.7 ^{ab}
SEM			0.5	0.1	0.2	0.8	0.03	0.01	0.2	0.1
D	28		64.0	6.1	7.3	74.8	2.37	0.37 ^a	74.0	7.9
	56		64.8	5.9	7.1	73.5	2.35	0.35 ^b	73.6	7.6
S	N		65.0	6.0	7.0	73.9	2.38	0.36	73.8	7.7
	H		63.7	6.0	7.4	74.3	2.34	0.36	73.8	7.7
T, g/kg feed	0		65.1	6.0	8.8 ^a	74.4	2.26	0.35	74.0	7.7
	1.2		65.1	6.3	6.8 ^b	76.4	2.41	0.37	73.3	7.9
	6		62.6	5.8	6.4 ^b	73.2	2.39	0.37	73.2	7.8
	12		64.7	5.8	6.9 ^b	72.6	2.37	0.35	73.7	7.6
<i>p</i> -value										
D			NS	NS	NS	NS	NS	0.05	NS	NS
S			NS	NS	NS	NS	NS	NS	NS	NS
T			NS	NS	0.01	NS	NS	NS	NS	NS
DxS			NS	NS	NS	NS	NS	NS	NS	NS
DxT			NS	NS	NS	NS	NS	NS	NS	NS
SxT			NS	NS	NS	NS	NS	NS	NS	NS
DxSxT			NS	NS	0.01	NS	NS	NS	NS	NS

D, day; S, stocking; N, normal; H, high; T, tarragon; EW, egg weight; YH, yellow height; YCI; yellow color index NS, not significant; HB, haugh birimi; SS, shell strength; ST, shell thickness; SI, shape index; SW, shell weight.^{a-c}

Values within a column with different superscripts differ significantly (Duncan's test).

DISCUSSION

Performance

In the present study, SD and tarragon doses had no effect on body weight between the beginning and end of the test, WG and AEW ($p>0.05$). There are also studies indicating that the HSD has a negative effect on the WG (Onbaşilar & Aksoy, 2005; Nahashon *et al.*, 2006; Sahin *et al.*, 2007; Sarica *et al.*, 2008) and has no effect as in the present study (Jalal *et al.*, 2006; Onbaşilar *et al.*, 2009; Fidan, 2010). The differences between the results of the present study and those in literature could be attributed to the lack of standardization of area allocated per hen. In the present study, the addition of 40% more space per animal to the HSD group than in the NSD group was not sufficient to affect weight parameters ($p>0.05$). It is stated that

the dietary supplementation of 1% *Artemisia sieberi* (Khalaji *et al.*, 2011), 2 and 4% *Artemisia annua* (Cherian *et al.*, 2013) and 0.4% *Artemisia dracunculus* to broilers (Gharetappe *et al.*, 2015) had no effect on WG. (Hosseinzadeh & Moghaddam, 2014) reported that the dietary supplementation of 0.125 and 0.25% of *Artemisia dracunculus* did not affect WG, while a 0.5% dose decreased it. Kheirabadi *et al.* (2014) reported that the incorporation of 5 mg/kg of *Artemisia sieberi* granulated extract (GEAS) into the diet reduced live weight at 42 days when compared to control group. It was reported that the incorporation of 10 and 20% of *Artemisia annua* (Brisibe *et al.*, 2008); 0.5, 1 and 1.5% of *Artemisia annua* leaf powder with 2000 and 4000 ppm methanolic extract (Gholamrezaie *et al.*, 2013); 1.5% of *Artemisia annua* (Drăgan *et al.*, 2014); and 100, 150 and 200 g/kg of *Artemisia*



Table 4 – The effect of the tarragon supplementation on the blood serum CORT, IgG, TAS and TOS.

S	T, g/kg feed	CORT nmol/L	IgG mg/dL	TAS μmol/L	TOS μmol/L
N	0	43 ^e	1.09 ^{bc}	761 ^{ab}	21.9 ^d
	1.2	39 ^e	1.13 ^{bc}	776 ^{ab}	19.1 ^e
	6	39 ^e	1.20 ^{ab}	787 ^{ab}	19.8 ^{de}
	12	37 ^e	1.30 ^a	798 ^a	18.4 ^e
H	0	102 ^a	1.08 ^c	710 ^b	30.8 ^a
	1.2	93 ^b	1.13 ^{bc}	728 ^{ab}	28.9 ^{ab}
	6	71 ^c	1.16 ^{bc}	735 ^{ab}	27.7 ^{bc}
	12	61 ^d	1.20 ^{ab}	749 ^{ab}	26.1 ^c
SEM	SEM	3	0.01	9	0.6
S	N	40 ^b	1.18	781 ^a	19.8 ^b
	H	82 ^a	1.14	730 ^b	28.4 ^a
T	0	73 ^a	1.08 ^c	735	26.4 ^a
	1.2	66 ^b	1.13 ^{bc}	752	24.0 ^b
	6	55 ^c	1.18 ^b	761	23.7 ^{bc}
	12	49 ^d	1.25 ^a	773	22.3 ^c
<i>p</i> -values					
S		0.001	0.109	0.004	0.001
T		0.001	0.001	0.467	0.001
S×T		0.001	0.541	1.000	0.550

S, stocking; N, normal; H, high; T, tarragon; CORT, corticosterone; TlgG, total immunoglobulin G; TAS, total antioksidant of serum; TOS, total oxidant of serum; SEM, standart error of the mean.

^{a-e} Values within a column with different superscripts differ significantly (Duncan's test).

absinthium (Kostadinović *et al.*, 2015) into broiler diets increased WG. In the present study, the incorporation of different levels of tarragon into diets of laying hens had no significant effect on live weight or live weight gain ($p>0.05$).

In addition to the findings that SD had no effect on the FI of laying hens (Anderson *et al.*, 2004; Simsek &

Kilic, 2006), similar findings to those of our study that it decreased FI have been reported ($p<0.01$) (Sohail *et al.*, 2004; Jalal *et al.*, 2006; Sahin *et al.*, 2007; Onbaşlılar *et al.*, 2009; Fidan, 2010; Mirfendereski & Jahanian, 2015). It is considered that the reduction in feeder space per bird in HSD is the main factor reducing FI. T1.2 and T6 diets reduced FI when compared to the control

Table 5 – The effect of the tarragon supplementation on the MDA and intestine microorganisms.

S	T, g/kg feed	SMDA mmol/L	LMDA mmol/L	EMDA mmol/L	<i>E. coli</i> Log, TBX	TMAB Log, PCA
N	0	3.60 ^c	95 ^{bc}	221 ^{cd}	3.65 ^{bc}	6.2 ^{bcd}
	1.2	3.49 ^c	94 ^{bc}	218 ^{cd}	3.61 ^c	6.0 ^{cd}
	6	3.50 ^c	91 ^{bcd}	216 ^d	3.52 ^c	5.9 ^d
	12	3.46 ^c	85 ^d	211 ^d	3.42 ^c	5.8 ^d
H	0	4.25 ^a	107 ^a	254 ^a	4.05 ^a	6.8 ^a
	1.2	3.99 ^{ab}	96 ^b	248 ^a	4.03 ^a	6.7 ^{ab}
	6	3.73 ^{bc}	90 ^{bcd}	237 ^b	3.94 ^{ab}	6.4 ^{abc}
	12	3.79 ^{bc}	88 ^{cd}	227 ^{bc}	3.92 ^{ab}	6.4 ^{abc}
	SEM	0.05	1	2	0.04	0.1
S	N	3.51 ^b	91 ^b	217 ^b	3.55 ^b	6.0 ^b
	H	3.94 ^a	95 ^a	242 ^a	3.98 ^a	6.6 ^a
T	0	3.92 ^a	101 ^a	237 ^a	3.85	6.5 ^a
	1.2	3.74 ^{ab}	95 ^b	233 ^{ab}	3.82	6.3 ^{ab}
	6	3.61 ^b	90 ^c	227 ^b	3.73	6.1 ^b
	12	3.62 ^b	87 ^c	219 ^c	3.67	6.1 ^b
<i>p</i> -values						
S		0.001	0.011	0.001	0.001	0.001
T		0.019	0.001	0.001	0.280	0.040
S×T		0.234	0.022	0.056	0.972	0.976

S, stocking; N, normal; H, high; T, tarragon; STP, serum total protein; LTP, liver total protein; SMDA, serum malondialdehyde; LMDA, liver malondialdehyde; EMDA, egg malondialdehyde; TMAB, total mesophilic aerobic bacteria; SEM, standart error of the mean.

^{a-d} Values within a column with different superscripts differ significantly (Duncan's test).



group ($p < 0.01$), while the T12 diet had no effect on FI ($p > 0.05$) in the present study, which concurs with the findings of Gholamrezaie *et al.* (2013), who reported that the incorporation of *Artemisia annua* extract and leaf powder mixture into broiler diets reduced FI. The incorporation of *Artemisia sieberi* (Khalaji *et al.*, 2011), *Artemisia dracunculus* (Gharetappe *et al.*, 2015; Hosseinzadeh & Moghaddam, 2014), *Artemisia annua* (Cherian *et al.*, 2013) and GEAS (Kheirabadi *et al.*, 2014) into broiler diets did not affect FI, although Brisibe *et al.* (2008) reported that the incorporation of *Artemisia annua* leaves increased FI.

There have been several studies indicating that SD affected (Onbaşilar *et al.*, 2009), had no effect (Anderson *et al.*, 2004; Simsek & Kilic, 2006; Fidan, 2010; Mirfendereski & Jahanian, 2015) or improved (Sahin *et al.*, 2007) FCR in laying hens. In the present study, HSD had no effect on FCR ($p > 0.05$), while T1.2 and T6 diets improved FCR when compared to control diet ($p < 0.05$). There have been studies that the incorporation of different *Artemisia* species into the diets of broilers improved FCR (Gholamrezaie *et al.*, 2013; Drăgan *et al.*, 2014; Kheirabadi *et al.*, 2014; Kostadinović *et al.*, 2015) or not affected (Khalaji *et al.*, 2011; Cherian *et al.*, 2013; Gharetappe *et al.*, 2015). Hosseinzadeh & Moghaddam (2014) reported that the incorporation of 0.5% tarragon into the diet affected FCR negatively, while lower levels (0.125% and 0.25%) had no effect.

In our study, it was found that HSD reduced EP as in previous studies (Onbaşilar & Aksoy, 2005; Jalal *et al.* 2006; Sarica *et al.*, 2008; Onbaşilar *et al.*, 2009; Fidan, 2010; Mirfendereski & Jahanian, 2015) ($p < 0.05$), and reduced feeder space per bird (Onbaşilar & Aksoy, 2005; Jalal *et al.*, 2006) and stress conditions caused by high stocking densities (Sarica *et al.*, 2008; Mirfendereski & Jahanian, 2015;) were found to be responsible for this result. Mirfendereski & Jahanian (2015) found a reduction of EP in HSD to be associated with blood CORT levels. In some studies, it was reported that SD had no effect on EP (Simsek & Kilic, 2006; Sahin *et al.*, 2007). Brisibe *et al.* (2008) reported that the incorporation of 20% *Artemisia annua* leaves into laying hen diets increased EP. It has further been reported that the incorporation of an extract composed of 70% pine needle and 30% *Artemisia annua* into laying hen diets (Li *et al.*, 2016) improved EP. In the present study, the incorporation of tarragon into the diet increased EP in the T1.2 and T6 diets when compared to the control diet ($p < 0.01$). This improvement can be explained by the fact that tarragon, which has antioxidant properties, reduces

serum CORT and TOS levels, thus reducing stress. It has further been reported that the incorporation of *salvia officinalis*, *thymbra spicata*, *menthae piperitae* extracts, vitamin E (Kaya & Turgut, 2012) and *Coriander Oil* extract (Çiftci & Macit, 2018) into laying hen diets increased EP, while *Rosmarinus officinalis* L. (Çimrin & Demirel, 2016) incorporation did not affect EP.

It has also been reported that HSD reduced EP and increased AEW (Onbaşilar *et al.*, 2009), while HSD reduced feeder space per bird, and thus AEW (Sohail *et al.*, 2004; Sahin *et al.*, 2007; Fidan, 2010). In addition, there have been studies reporting that increasing SD has no effect on AEW (Guesdon *et al.*, 2006; Sarica *et al.*, 2008; Mirfendereski & Jahanian, 2015), as in the present study, or affected AEW (Sahin *et al.*, 2007; Onbaşilar *et al.*, 2009; Fidan, 2010). In the present study, the tarragon levels incorporated into the diet had no effect on AEW ($p > 0.05$). It has been reported that the incorporation of *Artemisia annua* leaves into laying hen diets does not affect egg weight (Brisibe *et al.*, 2008; Li *et al.*, 2016), and that the incorporation of different plant extracts (Kaya & Turgut, 2012; Çimrin & Demirel, 2016) into laying hen diets did not affect or reduced (Çiftci & Macit, 2018) egg weights. It has been stated that effects of phytochemical products on poultry performance data may differ depending on the origin, content and processing of the plant, as well as the species and age of the animal and environmental hygiene (Windisch *et al.*, 2008).

In the present study, laying hens at 50 weeks of age were kept for 8 weeks. It is stated that it can be advantageous to provide antioxidant sources to old hens (Karaalp *et al.*, 2018) whose egg shell quality may be more problematic due to many factors (Światkiewicz *et al.*, 2010). Contrary to the finding that HSD causes a significant increases in DER (Guesdon *et al.*, 2006), there have also been reports of no such effects on DER ($p > 0.05$) (Sarica *et al.*, 2008; Onbaşilar *et al.*, 2009), similar to the present study. It has been found that the incorporation of an extract composed of pine needle and *Artemisia annua* into laying hen diets reduced DER, similar to the present study (Li *et al.*, 2016). It has been further reported that the incorporation of different plant extracts into laying hen diets reduced (Kaya & Turgut, 2012) or had no affect (Çimrin & Demirel, 2016; Çiftçi & Macit, 2018) on DER. In the present study, T1.2 and T6 reduced DER when compared to the control diet ($p < 0.01$).

Egg Quality Criteria

The advancement of the trial period (50–58 weeks of age) had no effect on any egg quality criteria other



than ST ($p>0.05$). The increase in age caused a decrease in ST in the present study, concurring with the findings of and Onbaşlar *et al.* (2018). In the present study, the fact that SD did not affect egg quality parameters was consistent with earlier studies (Sahin *et al.*, 2007; Sarica *et al.* 2008; Fidan, 2010). In poultry, it was reported that SD did not affect egg YH (Karabayir *et al.*, 2010), SS (Sahin *et al.*, 2007; Sarica *et al.*, 2008; Karabayir *et al.*, 2010), ST (Onbaşlar & Aksoy, 2005; Nahashon *et al.*, 2006; Sahin *et al.*, 2007; Sarica *et al.*, 2008; Fidan, 2010), SI (Sarica *et al.*, 2008; Fidan, 2010) or SW (Sahin *et al.*, 2007; Karabayir *et al.*, 2010). That said, it has been reported that SD has different effects on parameters such as YCI (Sarica *et al.*, 2008; Fidan, 2010) and HU (Nahashon *et al.*, 2006; Sahin *et al.*, 2007; Sarica *et al.*, 2008; Fidan, 2010). It has been described that some antioxidant components of plants can improve calcium accumulation in the uterus, and thus increases shell weight and thickness (Ramírez-Tortosa *et al.*, 1999). Our study has shown that the incorporation of tarragon into the diet has no effect on egg quality parameters, aside from reducing YCI ($p<0.01$). Li *et al.* (2016) reported that the incorporation of an extract composed of pine needle and *Artemisia annua* into laying hen diets did not affect EW, ST, HU, SS or SI. The increase in tarragon levels darkened the color of the diet, which had an unexpected effect on the egg yolk, but the cause of this could not be understood.

Serum CORT, IgG, TAS and TOS

Results of the present study showed that HSD, which is a social stressor affected some stress parameters (CORT, TAS, TOS) negatively ($p<0.05$). In laying hens, it was detected that HSD lead to an increase in the blood corticosteroid level (Mirfendereski & Jahanian, 2015). The glucocorticoids (cortisol and corticosterone) released by HPA with the effect of stressors when the animal is under stress are known to be immunosuppressive, and inhibit the production and effect of antibodies, deteriorate lymphocyte function and cause leukocyte proliferation (MUnck *et al.*, 1984). Sohail *et al.* (2011) described that stress increases total oxidants and antioxidants in broiler serum. However, increases in serum antioxidants depending on the severity and duration of stressors is a controversial issue. Accordingly, in the present study, HSD increased serum CORT and TOS ($p<0.05$), but reduced TAS ($p<0.05$). The reduction in serum TAS concentration is considered to be related to the severity and duration of stressors (24 hours/day in this study) as well as the use of serum TAS in sweeping away the free radicals that are formed due to the oxidative stress that occurs.

The increases in the levels of tarragon, which contains antioxidant substances (Kordali *et al.*, 2005), as well as the continuous overall reduction of serum CORT and TOS (first reduction in the T1.2 diet) ($p<0.05$), the increase in serum IgG (first increase in T6 diet) ($p<0.05$) and the fact that it does not affect serum TAS levels ($p>0.05$) confirm our finding.

It is being suggested that antioxidant substances regulate corticosteroid synthesis in the adrenal glands and improve certain immune responses significantly (Gholamrezaie *et al.*, 2013; Pardue & Thaxton 1984). High egg production (physiological stress) often causes a degeneration of lymphoid organs (Gray *et al.*, 1989) and the suppression of humoral and cell-mediated immune response (Murray *et al.*, 1987). Furthermore, it has been noted that cell-mediated immunity drops after 45 weeks of age (Fahey & Cheng, 2008). In the present study, the fact that SD did not affect serum IgG ($p>0.05$) suggests that reducing the space per bird is not sufficient to reduce this parameter. In addition, the T6 diet increased serum IgG ($p<0.05$). The overall increase in serum IgG with the increase of tarragon levels in the diet ($p<0.001$) can be associated with the immune-enhancing effect of tarragon. It is suggested that *Artemisia annua* leaf powder and methanolic extract affect performance by increasing the cellular and humoral immunity of broilers (Gholamrezaie *et al.*, 2013). Furthermore, it has been reported that a 1% incorporation of *Artemisia sieberi* into broiler diets has no effect on plasma lymphocytes, eosinophils, basophils, primary or secondary antibody response against sheep red blood cells, but increases monocyte percentage (Khalaji *et al.*, 2011). Gholamrezaie *et al.* (2013) reported that the effects of different natural products on the immune system were complicated, and that the stimulation of the lymphatic tissue of the digestive system was a direct effect, while the alteration of microbial population of the gastrointestinal system lumen was an indirect effect.

Serum, Liver and Egg MDA

In the present study, HSD, which is a social stressor, affected MDA, which is a lipid peroxidation indicator (in serum, liver, egg yolk), and some bacteria in the small intestine (*E. coli* and total mesophilic aerobic bacteria counts) desirably ($p<0.05$). Furthermore, HSD in the present study caused an increase in serum, liver and egg MDA ($p<0.05$) by increasing serum CORT and reducing serum TAS, resulting from the increased stress in hens. There are studies reporting that the incorporation of plants and plant products containing antioxidant substances into laying hen diets reduced



(Botsoglou *et al.*, 1997; Botsoglou *et al.*, 2005; Karaalp *et al.*, 2018; Krause & Ternes, 2000; Radwan *et al.*, 2008) or did not affect (Galobart *et al.*, 2001) egg yolk lipid peroxidation. Kostadinović *et al.* (2015) found that the incorporation of 20% *Artemisia absinthium* into the diet reduced blood MDA in broilers, while Cherian *et al.* (2013) reported that the incorporation of 2% and 4% *Artemisia annua* into broiler diets reduced MDA in the thigh and breast muscles of broilers. In the present study, serum, liver and egg MDA levels reduced ($p<0.05$) in parallel to the reduction in serum CORT and TOS under the effect of the antioxidant substances found in the tarragon incorporated into the diets (Kordali *et al.*, 2005). Serum and egg MDA started to reduce with the T6 diet, while liver MDA started to reduce with the T1.2 diet, when compared to the control diet ($p<0.05$).

Small Intestine *E. coli* and Total Mesophilic Aerobic Bacteria

The findings of the present study suggest that the increase in *E. coli* and total mesophilic aerobic bacteria counts in the small intestine due to HSD are related to contamination ($p<0.001$). In literature, the antimicrobial effects of artemisia species were mostly researched on coccidiosis, and these species were reported to be used for coccidiostatic purposes (Brisibe *et al.*, 2008; De Almeida *et al.*, 2012; Kostadinovic *et al.*, 2012 Drăgan *et al.*, 2014; Kheirabadi *et al.*, 2014). Lopes-Lutz *et al.* (2008) reported that the oils of different *Artemisia* species are effective at different levels against microorganisms such as *Escherichia coli*, *Staphylococcus epidermidis* and *Staphylococcus aureus*. The fact that the incorporation of 1% *Artemisia sieberi* into broiler diets reduces cecal coliform and *Escherichia coli* populations (Khalaji *et al.*, 2011), in addition to the findings in our study that the incorporation of different levels of tarragon into the diet has no effect on *E. coli* levels in the gut ($p>0.05$), can be associated with the difference in artemisia species used in the studies. Furthermore, the presence of 6 g or more tarragon in the diet was found to affect the reduction of total mesophilic aerobic bacteria counts when compared to the control diet ($p<0.05$).

CONCLUSIONS

With the incorporation of different levels of *Artemisia dracunculus L.* into the diets of laying hens in the second production cycle and housed under different SDs, it was found that: HSD reduced FI and EP, the T1.2 and T6 kg diets reduced FI and DER, increased EP and improved

FCR. All levels of tarragon were found to reduce YCI levels. HSD increased serum CORT and TOS, as well as MDA levels in serum, liver and egg yolk, and reduced serum TAS, which was associated with the oxidative stress occurring resulting from the increasing social stress. The reduction of serum CORT and TOS (with all diets), serum and egg yolk MDA (T6 and T12 diets) and liver MDA (T1.2 diet) with the incorporation of tarragon into the diet was associated with the antioxidant substances found in tarragon. Serum IgG (with T6 and T12 g diets) increased with the incorporation of tarragon into the diet, indicating that tarragon has immune-enhancing substances. Total mesophilic aerobic bacteria counts reduced with the T6 and T12 diets, when compared to control diets, suggesting that tarragon contains a certain level of antibacterial substances.

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