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The impact of dietary tarragon (*Artemisia dracunculus*) on serum apelin, brain-derived neurotrophic factor, cardiac troponin concentrations and histopathology of liver tissue in laying hens housed at different stocking densities

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Abstract: Due to its association with several other stress factors (poultry house gases, inadequate ventilation, heat, cold and poor hygiene), the high stocking density is a major stress factor that adversely affects the health and performance of poultry and the quality of the poultry products. Therefore, this experimental study was aimed at analysing the impact of different doses of dietary tarragon (*Artemisia dracunculus*) on the serum apelin, plasma brain-derived neurotrophic factor (p-BDNF), and cardiac troponin I (cTnI) concentrations, and the correlation between these indicators in laying hens housed at different stocking densities. The aim of this study is to investigate the effects of adding tarragon in different ratios to laying hen rations in the 2nd ovulation period on the cTnI, apelin, and BDNF hormone concentrations and the liver histopathology. The experiment was carried out over a period of eight weeks, with 192 Lohman Brown commercial hybrids at 50 weeks of age. Eight groups (four replicates each), composed of laying hens of equal body weight, which were housed at stocking densities of 580 cm^2/hen and 810 cm^2/hen and received 0, 1, 5 and 10 mmol/kg of tarragon (*Artemisia dracunculus*) in the feed, were established. At the end of the trial, 96 of the housed egg-laying hens (3 birds in each subgroup, a total of 12 birds in each group) were randomly selected and blood samples were taken from the *vena subcutanea ulnaris*. The samples collected were analysed for the apelin, p-BDNF, and cTnI contents. The analysis results demonstrated that tarragon supplementation had no effect on the serum apelin, p-BDNF and cTnI concentrations ($P > 0.05$). The Sub-Groups ST1, ST1.2, and ST6 presented with severe hyperaemia of the sinusoidal, portal and acinar blood vessels, whilst the hyperaemia of these blood vessels was moderate in Sub-Group ST12. Apelin, BDNF, and cTnI can act as protective factors against negative consequences of stress (e.g., stocking density or heat stress).

Keywords: diet; diet supplementation; apelin; BDNF; cardiac troponin I (cTnI)

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Poultry farming has a strategic importance in the livestock sector because of its role in meeting the demand for animal protein (meat, eggs) brought about by the continuous increase of the world's human population (Bayraktar and Tekce 2019). Commercial egg production is a major livestock sector, which faces multiple risks throughout the production and management processes, starting from the establishment of the farm, and extending to aspects such as disease prevention, animal nutrition and animal husbandry. Laying hen farms aim to maximise the profit per animal unit. A higher number of hens per square meter reduces the production costs, yet an excessive stocking density has a negative impact on the performance. The welfare of laying hens kept in conventional battery cages has been well scrutinised. Although conventional battery cages have been perceived as the most profitable housing system for layers, they are not considered to be welfare-friendly (Appleby 2004; Appleby et al. 1993; Craig and Swanson 1994). A high stocking density, resulting from the housing of a greater number of hens per square meter of usable area, is a major stress factor which causes overcrowding, behavioural disorders, reduced access to feed and water, increased rates of wounding and diseases, and eventually, poor welfare (Appleby 2004; Kang et al. 2016). A high stocking density has also been reported to cause dermatitis (Sorensen et al. 2000; Matkovic et al. 2019), abdominal wounds, skin lesions (Weimer et al. 2019) and thoracic oedema (Meseret 2016). The cage stocking density is classified under three categories as follows: high (384 cm^2/hen), medium (464 cm^2/hen), and low (580 cm^2/hen) (McGlone 2010; Nicol et al. 2017). A high stocking density is reported to cause sudden behavioural changes and sudden death syndrome as well as several direct and indirect effects on the physiology and anatomy of the animals (Bessei 2006). The hypothalamic-pituitary-adrenal (HPA) axis, comprised of the hypothalamus (H), pituitary gland (P) and adrenal glands (A), regulates several physiological processes in the body, including digestion, immunity and energy storage among others. In response to a stressful stimulus, the hypothalamus releases vasopressin and a corticotropin-releasing hormone (CRH), which initiate the adrenocorticotropic hormone (ACTH) synthesis in the pituitary gland. Furthermore, the release of stress hormones, referred to as corticotropins, as well as cortisol, and glucocorticoids, such as corticosterone (CORT), used as stress indicators in animals, activate the HPA axis. Plasma cortisol or corticosterone is the primary glucocorticoid, the level of which is used as an indicator of stress and the endocrine response of the HPA axis to the stress (Charmandari et al. 2005).

In poultry species, egg production is an energy-intensive process. Excessive energy consumption reduces the laying capacity of the hens. Similar to the case in mammals, alterations in the lipid synthesis and metabolism cause granulosa cell apoptosis, altered immune functions and hormonal synthesis in chickens, and, thereby, place ovarian functions under risk (Walzem and Chen 2014). Apelin, which was first isolated from bovine stomach extracts and is known to originate from a 77 amino acid-precursor (preproapelin), is an adipokine involved in energy regulation (Tatemoto et al. 2001). Apelin, which is the endogenous ligand of the apelin (APJ) receptor, occurs in various isoforms, such as apelin-12, -13, -17, and -36. In terms of biological activity, isoform apelin-13 is 8 times stronger than apelin-17 and 60 times stronger than apelin-36 (Tatemoto et al. 1998). Therefore, the majority of recent research has focused on apelin-13, which has a higher biological activity than the other apelin isoforms and contains N-terminal pyroglutamate residues (Beltowski 2006). Cardiac troponin I (cTnI), which is an indicator of stress-related cardiac arrhythmia and myocardial damage, has found common use in the past decade as a highly specific cardiac marker (Adams et al. 1994; Wu et al. 1996; Brown and Bertolet 1997). Cardiac troponins are proteins which control the calcium-mediated interaction between actin and myosin and allow contraction at the sarcomere level. Troponins are major structural proteins, which, together with tropomyosin, have an important role in regulating the contraction of the skeletal and cardiac muscles (Hi et al. 2019). The troponin complex, which consists of three protein subunits, namely, troponin I, troponin T (cTnT), and troponin C (cTnC), and is located on the thin filament of the contractile apparatus, plays a significant role in the transmission of the intracellular calcium signal into the actin-myosin interaction. The amount of cardiac troponin, which passes into the blood circulation, depends on the type, duration and severity of the myocyte damage. The monitoring of the blood cTnI and cTnT concentrations is a biochemical method used to determine acute coronary syndromes

https://doi.org/10.17221/9/2020-VETMED

(Alpert et al. 2000). In the event of myocardial damage, troponins pass from the myocytes into the blood circulation. Depending on the severity of the myocardial damage, blood troponin levels elevate within 1–4 h on average, and are detectable for a period of 7–14 days, which makes it possible to assess and quantify the damage to the myocardium (Apple 1999).

As a growth mediator responsible for neurogenesis, the brain-derived neurotrophic factor (BDNF) regulates the growth, survival, and differentiation of the neurons and prevents ischaemia-induced cell death, and thereby, maintains the continuity of the cell activity and repair (Kertes et al. 2017). BDNF induces and controls the generation of neurons from stem cells (neurogenesis) (Zigova et al. 1998; Benrasis et al. 2001). Not only does it show a neuroprotective effect in the presence of stress (Spina et al. 1992), BDNF also has a fundamental role in energy homeostasis (Bothwell 1995). Stress is a neuronal damage factor, which reduces the BDNF levels (Lee et al. 2008; Fuchikami et al. 2009) and triggers degenerative cellular processes in the limbic system (Adlard and Cotman 2004; McEwen 2006). Brain lateralisation is reported to be of relevance to the assessment of animal welfare (Rogers et al. 2010).

Artemisia dracunculus L., known as estragon or tarragon, is a small, shrubby, perennial plant, which belongs to the family Asteraceae and is native to Anatolia (Ceylan 1996; Kordali et al. 2005). Tarragon has a wide range of pharmacological activity, including antioxidant, antimicrobial, carminative, digestive, anti-inflammatory, antipyretic, antiseptic, antispasmodic, antiparasitic, anthelmintic, and fungicidal effects (Volak and Stodola 1998; Duke 2002; Hassanzadeh et al. 2016). Furthermore, this plant has also been reported to show effects on cerebral and gastrointestinal functions (Aglarova et al. 2008). There is no legal restriction on the use of tarragon as its commercially available form is classified as a nontoxic essential oil (Voitkevich 1999). Dried tarragon contains 24% protein, 45% carbohydrates, 7% fat and 7% fibre (Attokaran 2011). Tarragon also contains an essential oil (0.4–0.8%), bitter substance and tannin. Tarragon leaves are reported to contain 4% of an aromatic essential oil, the composition of which includes various substances such as camphor, artemisia ketone, and cineole (Mansuroglu and Gurel 2001).

Our hypothesis was that the addition of tarragon to the diet of laying hens would elicit a response in the form of serum and plasma adipokines (Apelin, BDNF, and cTnI).

The study was aimed at the investigation of the impact of different doses of dietary tarragon (*Artemisia dracunculus*) on the serum apelin, plasma brain-derived neurotrophic factor (p-BDNF), cardiac troponin I (cTnI) concentrations and the histopathology of the liver tissue and the correlation between these variables in the egg-laying hens housed at different stocking densities.

MATERIAL AND METHODS

Animals, experimental design and feed

The study was conducted at the Kelkit Organic Agriculture Research and Application Centre of Gümüşhane University, and the animal material comprised 50-week-old, 192 hybrid commercial layers of the Lohmann Brown strain. After a 2 week-acclimatisation period, the study was carried out for a period of 8 weeks. Replicate groups composed of five and seven hens were established for the normal and high stocking densities, respectively. The size of the cages, in which the replicate groups were housed, was 90 × 45 × 35 cm, and the floor area per bird was 810 cm^2 at the normal stocking density and 580 cm^2 at the high stocking density. The feed ration provided to the laying hens was supplemented with ground homogenous tarragon at doses of 0, 1.2, 6 and 12 g/kg in the Groups T_0, $T_{1.2}$, T_6 and T_{12}, respectively. The study was conducted in compliance with the animal welfare rules and principles, pursuant to the approval, dated May 10, 2017 and numbered 2017/1-07, of the Local Ethics Board for Animal Experiments of Gümüşhane University.

Temperature, humidity and lighting of the poultry house

The laying hens were maintained on a 16:8 light-dark cycle and were provided with water and feed *ad libitum*. The temperature and humidity inside the poultry house were adjusted according to the needs of the animals. The composition of the commercial feed provided to the birds is presented in Table 1. The feed was analysed in accordance

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Table 1. Basal diet ration nutrient content and analysis (g/kg)

Ingredients	%
Corn	35.00
Triticale	17.50
Wheat	7.50
Soybean meal (34%)	11.46
Sunflower meal (33%)	10.36
Hazelnut (42%)	3.50
Corn gluten meal (60%)	2.50
Vegetable oil	0.83
Limestone	9.55
Dicalcium phosphate (18%)	0.72
NaCl	0.32
Premix[1]	0.25
L-lysine HCl	0.15
Naturabind-S	0.12
Chemical analysis of feed (%)	
Crude matter	89.22
Crude protein	16.75
Ether extract	4.66
Crude fibre	5.29
Crude ash	13.17
Starch	33.99
Calculated contents of feed	
Metabolizable energy (kcal/kg)	2 735
Methionine (%)	0.37
Methionine + cystine (%)	0.67
Lysine (%)	0.75
Linoleic acid (%)	2.21
Ca (%)	3.90
Available P (%)	0.35
Na (%)	0.15

[1]Premix provided per kilogram of diet: vitamin A, 10 000 IU; vitamin D3, 2 400 IU; vitamin E, 30 mg; vitamin K3, 2.5 mg; vitamin B1, 3 mg; vitamin B2, 7 mg; vitamin B6, 4 mg; vitamin B12, 0.02 mg; niacin, 40 mg; Ca-D-pantothenate, 8 mg; folic acid, 1 mg; D-biotin, 0.1 mg; vitamin C, 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

with the methods adopted by the Association of Official Analytical Chemists (AOAC). During the acclimatisation period, a 16:8 light-dark cycle (60 W) was maintained and the temperature was adjusted to 22 °C.

Content of the tarragon (*Artemisia dracunculus*) leaves

The tarragon leaves added to the feed of the animals were obtained from farmers, who grew tarragon in the Yedigözeler village of the Bayburt province. After washing and removing the soil, plants and also non-usable parts of the herb, it was placed on a clean floor, and dried under the appropriate room temperature. The dried tarragon samples were powdered in a mill, and were added to the experimental diets (Hosseinzadeh and Moghaddam 2014). *Artemisia dracunculus* also has important compounds attributed to it, such as: methyl chavicol, ocimene, myrcene, camphor, camphene, *p*-anisic acid, limonene, linalool, *p*-methoxy cinnamic aldehyde, flavonoid, coumarin and minerals (Gulpinar 2012). The total antioxidant level of the tarragon plant used in this study was determined by the colorimetric method described by Erel (2004), using dianisidine dihydrochloride as a substrate, at the laboratory of the Biochemistry Department of Atatürk University, Faculty of Veterinary Medicine. The total antioxidant amount of the tarragon plant was determined as 0.833 mmol/g. In another study conducted in the same period and in the same region, the β-ocimene (1 237.21 arbitrary units, AU × 10^{-6}), α-pinene (114.4 AU × 10^{-6}), β-thujene (166.92 AU × 10^{-6}), D-limonene (366.2 AU × 10^{-6}), γ-terpinene (187.27 AU × 10^{-6}), Terpinolene (129.92 AU × 10^{-6}) and Estragole (11 242 AU × 10^{-6}) levels were stated (Yilmaz et al. 2019).

Collection of serum samples

At the end of the trial, 96 laying hens (three birds in each subgroup, a total of twelve birds in each group) were randomly selected for the blood sampling. The samples were collected from the *vena subcutanea ulnaris* to 2 ml tubes during the cervical dislocation. A refrigerated centrifuge (NF 1200, CORE, Ankara, Turkey) at +4 °C for 12 min was used to obtain the serum samples (Tekce and Gul 2015).

Measurement of serum apelin-13 and plasma BDNF concentrations

The serum apelin, and plasma brain-derived neurotrophic factor (BDNF) were measured us-

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ing an Enzyme-Linked Immuno Sorbent Assay (ELISA) (R&D Systems, Minneapolis, MN, USA) and the values were read using an ELISA reader (Mindray MR-96 A, P.R. China).

The minimum detectable concentration used to measure the apelin concentration in the blood serum obtained from the research was < 18.75 pg/ml. An ELISA kit type-specific for chicken apelin (FineTest, Product code: ECH0078, P.R. China) from 31.25–2 000.00 pg/ml, an intra-assay coefficient of 8.0%, and an inter-assay coefficient of 10.0% was utilised in accordance with the manufacturer's protocol.

The results were evaluated by reading the absorbance values at 450 nm in accordance with the procedure reported in the kit (Dai et al. 2018). The minimum detectable concentration to measure serum cTnI concentrations in the blood serum obtained from the study was < 9.4 pg/ml. An ELISA kit type-specific for chicken cTnI (FineTest, Product code: ECH0069, P.R. China) from 15.6–1 000.00 pg/ml, an intra-assay coefficient of 8.0%, and an inter-assay coefficient of 10.0% was utilised in accordance with the manufacturer's protocol. The results were evaluated by reading absorbances at 450 nm in accordance with the procedure reported in the kit (Bayraktar and Tekce 2019). A commercially available chicken BDNF (Product code: 201-16-1172; Sunred, P.R. China) ELISA kit was utilised by reading the 450 nm absorbance values in accordance with the procedure reported in the kit (Dai et al. 2018).

Histopathologic assessment

At the end of the trial, cervical dislocation was performed in 80 birds in total, randomly selected as ten out of each group, for the histopathological assessment and necropsy. The liver tissue samples taken for the histopathological assessment were then fixed for 48 hours in a 10% formalin solution. They were embedded in paraffin blocks according to the routine histological follow-up procedures. Cross-sections were taken from each block at a 4 µm thickness (Bancroftet al. 2012). The microscopic findings of the hepatic tissue (parenchyma, serosa) samples were assessed for each study group (Leica DM 1000, Germany). In the sub-groups, which were housed at the normal stocking density and received different doses of the dietary tarragon (Groups NT_1, $NT_{1.2}$, NT_6, NT_{12}), the microscopic examination of the hepatic tissue showed that the histological structure of the parenchyma and serosa was normal. In the sub-groups, housed at the high stocking density, and receiving different doses of the dietary tarragon (Groups ST_1, $ST_{1.2}$, ST_6, ST_{12}), the histopathological examination of the hepatic tissue samples revealed the hydropic degeneration of the hepatocytes, ranging from very severe, severe, and moderate to mild, and steatosis of the hepatocytes, ranging from very severe and moderate to mild. The sub-groups ST_1, $ST_{1.2}$, and ST_6 presented with severe hyperaemia of the sinusoidal, portal and acinar blood vessels, whilst in sub-group $ST_{12,}$ the hyperaemia of these blood vessels was moderate (Figure 1).

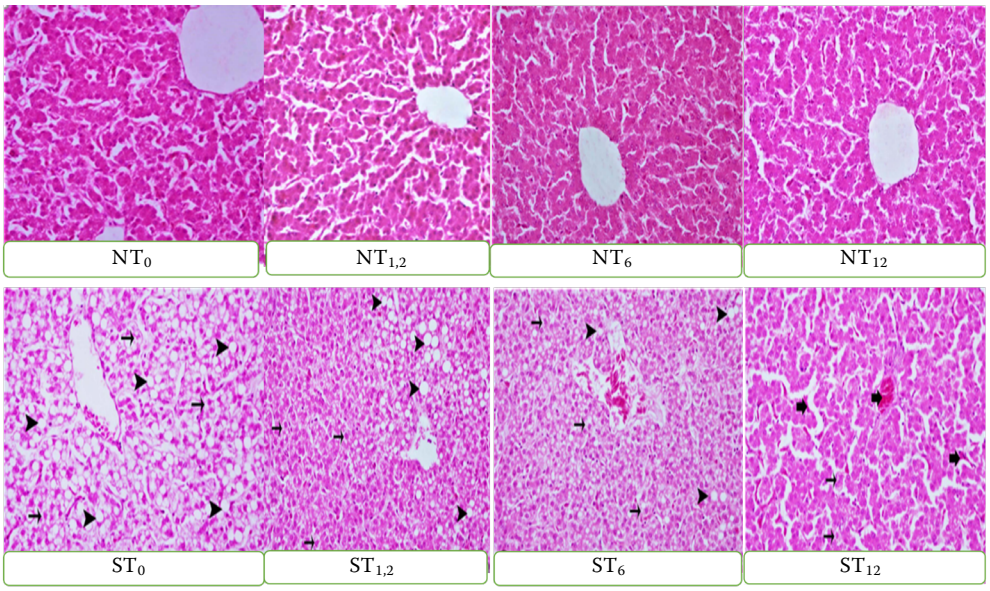

Figure 1. Histopathological view of the liver tissue (× 40, H&E)

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Statistical analysis

The apelin, p-BDNF and cTnI concentrations were controlled for normal distribution and all of them were distributed normally. The statistical analyses of the diet and stocking densities effects on the apelin, p-BDNF and cTnI were performed using the General Linear Model (GLM) that is given below:

$$Y_{ijk} = \mu + D_i + T_j + (D \times T)_{ij} + e_{ijk} \quad (1)$$

where:

Y_{ijk} – an observation;
μ – the overall mean;
D_i – the diet effect;
T_i – the stocking densities effect;
$(D \times T)_{ij}$ – the interaction effect;
e_{ijk} – the experimental error.

A one-way analysis of variance (ANOVA) was used for a 2 (two stocking densities levels) × 3 (three dose levels) factorial design. The Duncan multiple comparison test was used for comparing the group means. The statistical analyses were performed by using IBM SPSS statistics v22.0.

RESULTS

In this study, the impact of ground tarragon (*Artemisia dracunculus*), added at different doses (0, 1.2, 6 and 12 g/kg in Groups T_0, $T_{1.2}$, T_6 and T_{12}) in a homogenous form to the feed of egg-laying hens housed at different stocking densities, was investigated on the apelin, BNDF, and cTnI concentrations. The data pertaining to the study groups are presented in Table 2.

In the group, which was housed at the normal stocking density (810 cm^2/hen) and was not exposed to stress, the apelin concentration was the highest in sub-group $NT_{1.2}$ (1.397 ng/ml) and the lowest in sub-group NT_{12} (0.641 ng/ml). Furthermore, while the p-BDNF concentration was the highest in subgroup NT_6 (2.086 ng/ml) and the lowest in subgroup NT_0 (1.965 ng/ml), the cTnI concentration was the

Table 2. Hormone levels of apelin, BDNF, cTnI due to the tarragon administration in the laying hens found in the stocking density (ng/ml)

	Apelin (ng/ml)		BDNF (ng/ml)		cTnI (ng/ml)	
	non stress	stress	non stress	stress	non stress	stress
T_0	0.878 ± 0.315	0.874 ± 0.315	1.965 ± 0.031	2.004 ± 0.031	2.110 ± 0.052	1.981 ± 0.052
$T_{1,2}$	1.397 ± 0.315	1.082 ± 0.315	2.029 ± 0.031	2.025 ± 0.031	2.092 ± 0.052	2.057 ± 0.052
T_6	1.038 ± 0.315	0.679 ± 0.315	2.086 ± 0.031	2.083 ± 0.031	2.082 ± 0.052	2.084 ± 0.052
T_{12}	0.641 ± 0.315	1.447 ± 0.315	2.046 ± 0.031	2.009 ± 0.031	1.930 ± 0.052	2.064 ± 0.052
Source of variation (*P*-values)						
Diet	0.602		0.030		0.363	
Stress	0.883		0.953		0.851	
Stress × diet	0.249		0.363		0.116	
Main effect means diet						
T_0	0.878 ± 0.223		1.984 ± 0.022^{b}		2.045 ± 0.037	
$T_{1,2}$	1.239 ± 0.223		2.027 ± 0.022^{ab}		2.074 ± 0.037	
T_6	0.858 ± 0.223		2.085 ± 0.022^{a}		2.083 ± 0.037	
T_{12}	1.044 ± 0.223		2.028 ± 0.022^{ab}		1.997 ± 0.037	
Stress						
Non stress	0.988		2.032		2.053	
Stress	1.022		2.030		2.046	
SEM	0.158		0.015		0.026	

$^{a–c}$The means with the same letters in a column are statistically different ($P > 0.05$)

https://doi.org/10.17221/9/2020-VETMED

highest in sub-group NT_0 (2.110 ng/ml) and the lowest in sub-group NT_{12} (1.930 ng/ml).

In the group, which was housed at the high stocking density (580 cm^2/hen) and exposed to stress, the apelin concentration was the highest in sub-group ST_{12} (1.447 ng/ml) and the lowest in sub-group ST_6 (0.679 ng/ml). While the p-BDNF concentration was the highest in sub-group ST_6 (2.083 ng/ml) and the lowest in sub-group ST_0 (2.004 ng/ml), the cTnI concentration was the highest in sub-group ST_6 (2.084 ng/ml) and the lowest in sub-group ST_0 (1.981 ng/ml). The serum apelin concentrations in the groups housed at the normal and high stocking density were determined to fall within the normal reference range, and did not show any statistically significant differences ($P > 0.05$). The analyses demonstrated that the dietary tarragon supplementation had no effect on the apelin, BDNF and cTnI concentrations under stressful or stress-free conditions ($P > 0.05$).

The histopathological findings detected in the study groups are shown in Figure 1. The examinations demonstrated that the groups that received the dietary tarragon under the stress-free conditions did not differ from the control group. On the other hand, when compared to the control group, the study groups that were exposed to the stress presented with very severe hepatocytic steatosis, and the presence of vacuolised lipid degenerations in the cytoplasm of the hepatocytes, which resulted in the peripheral positioning of the nucleus in these cells. The hepatocytes showed very severe hydropic degeneration, their cytoplasm was swollen, and the cells were stained a pale colour. The sinusoidal, portal and acinar blood vessels were severely hyperaemic. When compared to the control group, it was determined that, in the sub-groups, which were exposed to stress and received dietary tarragon, depending on the dose of tarragon, the hepatocyte degeneration, sinusoidal dilatation and hyperaemia of the sinusoidal, portal and acinar blood vessels decreased. Thus, the tarragon was determined to show a regulatory effect.

DISCUSSION

As is the case in several other animal species, in poultry, the body responds to stress with endocrine and biochemical alterations. Increasing the stocking density of birds in cages elevates the plasma corticosterone concentrations (Eugen et al. 2019), inhibits the locomotor development (Puron et al. 1995; Feddes et al. 2002; Dawkins et al. 2004), and due to the inefficiency of the body heat loss mechanism, results in heat stress, which, in return, reduces the yields and deteriorates the health status of the animals (Onbasilar and Aksoy 2005). While cholesterol (CHOL), glucose (GLU), high-density lipoprotein (HDL), and triglyceride (TRI) concentrations are accepted as stress markers for all poultry species, plasma corticosterone concentrations are not always considered to be an indicator of stress for egg-laying hens (Mumma et al. 2006). In response to the increased metabolic demand, the glucocorticoids mobilise energy reserves, thus, while increased plasma CORT concentrations have positive outcomes in the short-term (Turner et al. 2012), they may cause adverse effects in the long-term (Turner et al. 2010). The data obtained for the plasma CORT concentrations in the present study agree with some literature reports (Fitko et al. 1993; Eugen et al. 2019), but disagree with some other studies (Downing and Bryden 1999; Davis et al. 2000).

Different from mammals, in avian species, lipogenesis mainly occurs in the hepatic tissue and is observed at a very limited level in the adipose tissue (Hermier 1997). Glucocorticoids, released in response to stress, firstly mobilise the adipose tissue. The adipose tissue is the main body source of reactive oxygen species (ROS), which cause oxidative stress when their plasma concentrations are elevated (Furukawa et al. 2004). The hormone apelin, released from the adipose tissue, owing to its interaction with the apelin receptor (APJ), supresses the production of the reactive oxygen species in the adipocytes. Furthermore, apelin is reported to enhance the expression of the antioxidant enzymes through the activation of the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated protein kinase (ERK) and AMP-activated protein kinase (AMPK) pathways, to inhibit the expression of the pro-oxidant enzyme via the AMPK pathway, and to reduce the oxidative stress-induced disorders of the pro- and oxidant enzymes, mitochondrial biogenesis and functional expression, and the release of proactive and anti-inflammatory adipocytokines (Than et al. 2014). It is accepted that, through the release of various adipocytokines and metabolic factors, the adipose tissue regulates the metabolic homeostasis (Trayhurn

https://doi.org/10.17221/9/2020-VETMED

et al. 2006). Research on tarragon, known to have a major impact on adipogenesis, has shown that this plant enhances adipocyte development and positively affects adipocyte-related diseases by increasing the level of apelin and adiponectin released from the adipose tissue, and strengthening the effect of the insulin hormone (Richard et al. 2014). Moreover, it has been reported that tarragon shows an effect on the membrane of the skeletal cells, increases the insulin sensitivity and adipocyte differentiation in cultures (Anaya-Eugenio et al. 2014; Obanda et al. 2014; Richard et al. 2014). In a similar study conducted in rats, it was determined that the extract of *Artemisia dracunculus* leaves could potentially decrease the prevalence of coronary diseases in humans (Yazdanparast and Saei 1999; Duric et al. 2015). Stress causes damage to the hepatic parenchyma and sinusoidal structures. Perfusion and the generation of free radicals cause neutrophil leukocyte infiltration in the liver and hyperaemia in tissues. In the present study, it was ascertained that a stocking density not causing any stress to animals had no adverse effect on the liver, and produced results similar to those of the control group, as has also been suggested in previous research (Shen et al. 2007). On the other hand, when compared to the control group, in the sub-groups exposed to stress, very severe steatosis, the presence of vacuolised lipid degeneration, cytoplasmic lipid vacuoles and peripheral nuclear dislocation were observed in the hepatocytes, and the severity of these findings was observed to decrease with the increased doses of the dietary tarragon. This was attributed to the strong antioxidant property of the tarragon plant (*Artemisia dracunculus*).

When chicken flocks are housed at a high stocking density, the dominant animals take hold of first access to the feed, water and other valuable resources (Estevez 2002). Stress is known to cause arrhythmia in the ventricular system of the heart (Scorza et al. 2010). In recent years, cardiac troponin I (cTnI) has found common use as a highly specific cardiac marker for stress-induced cardiac arrhythmia, myocardial damage and myocardial disease (Adams et al. 1994; Wu et al. 1996; Brown and Bertolet 1997). Apelin-13 has a role in regulating the stress response with the BDNF by improving the HPA Axis and Hippocampal Glucocorticoid Receptor Dysfunctions (Dai et al. 2018). Apelin has a protective effect against oxidative stress in heart myocardial cells as in many tissues (Chung et al. 2016). Besides, the hormones apelin and BDNF also have regulatory roles in cardiac contraction (Szokodi et al. 2002; Fulgenzi et al. 2015). On the other hand, BDNF, one of the key adipokines involved in neurodegenerative processes, shows a neuroprotective effect (Dai et al. 2018) and acts as the direct modulator of myocardial mechanic function in the BDNF/TrkB signalling (Feng et al. 2015). To the authors' knowledge, the impact of dietary tarragon on the serum apelin levels in egg-laying hens housed at different stocking densities has not been investigated before. The results obtained in the present study for the apelin, p-BDNF, and cTnI levels are similar to those indicated in some literature reports (Feng et al. 2015; Dai et al. 2018; Shen et al. 2019). As the apelin, p-BDNF, and cTnI levels have not been investigated in poultry before, the data of the present study was compared to the results of previous research conducted in different species.

In conclusion, dietary tarragon supplementation had no effect on the apelin, BDNF and cTnI concentrations under stressful or stress-free conditions ($P > 0.05$). However, based on the results of this study, which is the first investigation on the apelin, p-BDNF, and cTnI concentrations in laying hens raised at different stocking densities, it is considered that these indicators can be used as hormonal markers of the response to stress, and thus, can aid in the prediction of the stress, in the assessment of the animal's welfare and in the development of innovative livestock management strategies. Furthermore, more detailed research is needed in this area to confirm the results of this study.

Conflict of interest

The authors declare no conflict of interest.

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