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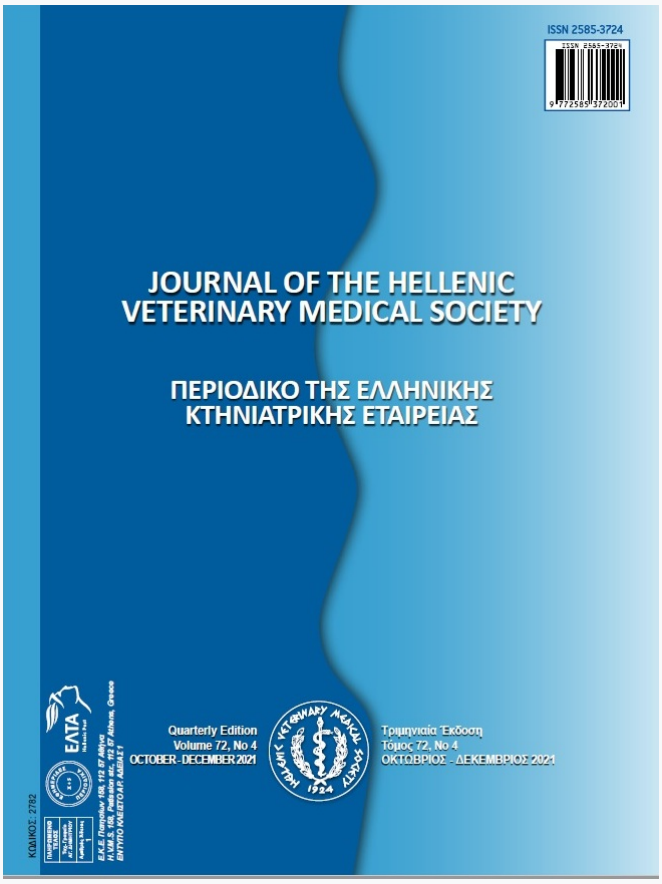

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Research article
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Effects of *Alchemilla vulgaris* on haematology and antioxidant status of heat-stressed quails during the late laying period

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ABSTRACT: The effects of *Alchemilla vulgaris* (AV) on haematology and serum, liver, and ovarian antioxidant status of heat-stressed quail in the late laying period were observed in this study. A 2×3 factorial design was used with 0, 1 and 3% AV fed in thermoneutral (TN) and heat stress (HS) conditions. A total of 150 quails were randomly assigned to six groups. The quails were located in temperature controlled rooms. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and platelet distribution width (PDW) obtained in quail fed 1% AV were higher than in 3% AV under both TN and HS conditions. Comparing 3% AV to 1% AV, the concentration of MCH obtained for 1% AV was higher in HS and lower in TN conditions. Besides, quails fed for 1% AV had a lower procalcitonin (PCT) value in HS than 3% AV but this PCT value was the same in TN. The serum malondialdehyde (MDA) was lower in 1% AV than 3% AV in both HS and TN. The ovarian MDA was lower in TN than HS. In both TN and HS conditions, the ovarian MDA value was determined higher for 1% AV than for 3% AV. The liver glutathione (GSH) value was higher in 1% AV than 3% AV in both TN and HS conditions. The Total Oxidant Capacity (TOS) value was found higher for 3% AV in TN and 1% AV in HS. The serum GSH, TOS, and oxidative stress index (OSI) values were lower for 3% AV compared to 1% AV for both TN and HS conditions, whereas for MDA value this was the opposite. The ovarium MDA and TOS values were lower for 3% AV than for 1% AV in both TN and HS. Also, the liver MDA, GSH, and Total Antioxidant Capacity (TAS) values were lower for 3% AV than for 1% AV in both TN and HS conditions. Finally, dietary AV has been shown to have a partial antioxidative effect on the defense system and also has effect on red blood cell profiles and platelet counts rather than white blood cell profiles.

Keywords: Blood parameters, *Coturnix coturnix japonica*, lady's mantle, oxidative stress

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INTRODUCTION

Eggs have an important place in human nutrition (Tunsaringkarn et al., 2013). High environmental temperature causes severe problems in poultry eggs' production (Melesse et al., 2011). Heat stress (HS) arising from high environmental temperature not only adversely affects the production of laying hens but it also inhibits their immune function (Mashaly et al., 2004) compromises their welfare, and impinges upon food quality and safety (Shane, 1988; Lara and Rostagno, 2013). Thus, understanding and controlling environmental conditions is crucial to success in the meat and egg production in the poultry breeding systems (Lara and Rostagno, 2013).

When the environmental temperature (ET) exceeds the limits of the thermoneutral zone (TN) (16-25 °C), the body temperature of poultry increases (Melesse et al., 2011). High environmental temperature causes oxidative stress (OS) in the body, causing the depletion of antioxidant substances. Moreover, imbalance in the body due to excessive production of free radicals and excessive lack of antioxidants in the event of OS leads to damaged proteins, lipids, and DNA, as well as numerous diseases (Sakac & Sakac, 2000; Dalle-Donne et al., 2006). As a result, under the HS conditions yield losses, high mortality rates, and nominal profitability can occur (Sahin & Kucuk, 2003; Akdemir et al., 2015).

Antioxidant substances or natural herbs can be added to poultry diets for protecting the cells from the damaging effects of oxidative stress (Shane, 1988). Indeed, it has been demonstrated by many previous studies (Oktyabrskay et al., 2009; Akdemir et al., 2015). The degree of oxidative stress is indicated by some specific parameters such as malondialdehyde (MDA), total antioxidant capacity (TAS), total oxidant capacity (TOS), and oxidative stress index (OSI). Also, MDA is the specific biomarker of lipid peroxidation (Lykkesfeldt, 2007; Singh et al., 2014). Therefore, antioxidant parameters can be used as a meaningful indicator in poultry exposed to HS.

Alchemilla vulgaris (AV) is a biologically active herb important for humans as an immunomodulatory agent, which is rich in phyto components. It is widely referred to as Lady's mantle, Bear's foot, or Lion's foot, and used as a public medicine in European countries to a significant extent (Al-Osaj, 2016). The bioactive composition of AV is given in Table 1.

Table 1. The amounts of selected phenols in *Alchemilla vulgaris* (Vlaisavljevic et al., 2019).

Compounds	Amounts (μg/g dry weight)
p-Hydroxybenzoic acid	135.45
Protocatechuic acid	255.94
2,5-Dihydroxybenzoic acid	45.74
p-Coumaric acid	470.21
Gallic acid	2465.79
Esculetin	353.03
Caffeic acid	1138.54
Ferulic acid	346.87
Genistein	94.01
Apigenin	501.97
Naringenin	15.40
Luteolin	638.09
Kaempferol	364.00
Catechin	8144.98
Chrysoeriol	222.63
Quercetin	4541.70
Chlorogenic acid	23.13
Apigenin-7-O-b-glucoside	141.6
Luteolin-7-O-b-glucoside	329.71
Quercitrin	70.37
Kaempherol-3-O-glucoside	1038.52
Quercitrin-hexosides	2274.85

*Phenolic compounds were quantified in ethyl-acetate.

AV is a member of plants with antioxidant properties, has important biological efficacy such as antimicrobial, anticancer, antidiarrheal, antiarthritis, diuretic, depurative, intestinal antiseptic, and improving menopausal irregularities (Spiridonov et al., 2005; Neagu et al., 2015). AV is widely used by the public as a phytotherapeutic agent for the treatment of various disorders (Havsteen, 2002; Spiridonov et al., 2005; Neagu et al., 2015). Moreover, Havsteen (2002) reported that the biological effect of AV is largely due to its content of flavonoids. They are the most forceful and abundant ingredients of AV, which activate the antioxidant enzymes in OS conditions (D'agostino et al., 1998; Oktyabrskay et al., 2009).

AV has antioxidant, antibacterial, antifungal, and anti-inflammatory activities, also the remarkable biological activity of its extracts, as well as their full biocompatibility with fibroblasts and keratinocytes (Vlaisavljevic et al., 2019). The high amount of phenolic compounds in methanolic and ethyl-acetate extracts of above ground parts and roots of AV especially they were rich in condensed tannins (Boroja et al., 2018; Vlaisavljevic et al., 2019). AV is recommended to protect against hepatotoxicity in rats, and this effect is dependent on the antioxidant content of AV and its free radical scavenging effect (El-Hadidy et al., 2018). It is reported that the Rutin (is a member of flavonoids) contained in AV, increases the lymphocyte levels of broiler under normal environmental conditions (Hassan et al., 2018). Besides, there are some studies on the effects of flavonoids or flavonoid-containing plants on poultry defense systems and antioxidant parameters (Surai 2013; Ma et al., 2014).

Oxidative stress accelerates the aging process by causing changes in body functions, and aging-related diseases and complications are prevented by changes in antioxidant support or antioxidant enzyme systems (Kregel et al., 2007). The serum total antioxidant capacity of elderly individuals is lower than that of adult subjects. Serum oxidant status and oxidative stress index of elderly subjects were found to be significantly higher in adults (Yalçın, 2018).

In a study on the potential for mitigating effects of heat stress through dietary AV supplementation during the late laying period of Japanese quail (Coturnix coturnix japonica); in HS quail supplemented with 1% AV, egg production was reduced and FCR was increased compared with the other treatments. Dietary AV was found to reduce egg production in TN conditions, but 3% AV supplementation in the HS group prevented decreased egg production and improved FCR. Various indicators of egg quality were significantly affected by supplementation with AV at certain times during the experiment. Most effects of HS on egg quality were manifest in the first 15 days of ET regimes. Although HS significantly decreased eggshell weight until 31-45 days, AV supplementation improved it on the 45th day and then maintained it through the end of the experiment. Thus, AV may mitigate some effects of HS by partially preventing decreased egg production and increased FCR during the late laying period of Japanese quail (Akdemir et al., 2019).

Although there have been many studies on the use of AV in the field of biological and metabolic disturbances, no studies have been found on the effects on the haematological parameters and serum, liver, and ovarian antioxidant parameters in poultry. Therefore, considering that antioxidant capacity decreases more with age, the present study aimed to determine the effects of dietary AV supplementation on the haematological parameters and serum, liver, and ovarian antioxidant status in the late laying period of heat stressed quails.

MATERIALS AND METHODS

One hundred and fifty 20-wk old Japanese quails (purchased from Insanay Kanatli Hayvan Uretim Paz. Tic. Inc., Elazig, Turkey) were used in this 75 days experiment. After an adaptation period (10 days), birds with an average body weight of 197.8 ± 2.3 g were randomly assigned to 6 groups of 25 birds, and each group was subdivided into 5 replicates with 5 birds per cage. The quails in TN group were housed at 22 ± 2 °C/24 hours/day, and the quails in HS group were housed at 22 ± 2 °C /8 hours/day between 09:00-17:00 hours, at 34 ± 2 °C /16 hours/day for the rest of the day, in temperature-controlled rooms throughout the experiment. The experiment was approved (approval document no 2018/A-22) by the Committee on Animal Research at Inonu University, Malatya, Tyrkey, and conducted to Akcadag Vocational School Division of Inonu University.

In this study, 2 (ET; TN and HS) x 3 (basal diet supplemented with AV at 0, 1, and 3 %) factorial design was used. These doses have been determined considering the use of herbal additives used in similar studies in close or similar doses. Also, it was deemed appropriate to handle low doses since the effect of AV was not fully known. Quails were fed one of three

diets (Table 2), namely a basal diet or the basal diet supplemented with 1% or 3% of *Alchemilla vulgaris* in powder form (Altinterim Co., Elazig, Turkey). They were housed in cages providing 100-120 cm^2 floor area per bird. The birds were exposed to a 16 L:8 D illumination cycle for 75 days. Diets and freshwater were offered for *ad libitum* consumption throughout the experiment.

Table 2. Ingredients and nutrient composition of the basal diet (%)[1] fed to Japanese quail during the late laying period

Ingredient	Amount (%)
Corn	54.34
Soybean meal	28.91
Soy oil	4.96
Salt	0.31
DL-methionine	0.19
Limestone	9.26
Dicalcium phosphate	1.68
Vitamin and mineral premix[2]	0.35
Nutrient composition (%, dry matter basis)	
Crude protein	18.09
Calcium	3.73
Phosphorus	0.63
Methionine[3]	0.42
Lysine[3]	1.04
Calculated metabolizable energy kcal/kg[3]	2912

[1]1% or 3% of *Alchemilla vulgaris* was added to basal diet at the expense of corn for the supplemented diets

[2] Per kilogram, retinyl acetate: 1.8 mg, cholecalciferol: 0.025 md, dl-tocopheryl acetate: 1.25 mg, menadione sodium bisulfite: 2,5 mg, thiamine-hydrochloride: 1.5 mg, riboflavin: 3 mg, niacin: 12.5 mg, d-pantothenic acid: 5 mg, pyridoxine hydrochloride: 2.5 mg, vitamin B12: 0,0075 mg, folic acid: 0.25 mg, choline chloride: 125 mg, manganese ($MnSO_4$-H_2O): 50 mg, iron ($FeSO_4$-$7H_2O$): 30 mg, zinc (ZnO): 30 mg, copper ($CuSO_4$-$5H_2O$): 5 mg, cobalt ($CoCl_2$- $6H_2O$): 0.1 mg, iodine as KI: 0.4 mg, selenium (Na_2SeO_3): 0.15 mg

[3]Calculated value according to tabular values listed for the feed ingredients (Jurgens, 1996).

Chemical analyses of the basal diet for crude protein (988.05), ether extract (932.06), crude fiber (962.09), crude ash (936.07), Ca (968.08), and P (965.17) were done in triplicate using the methods described by the AOAC International (1990). Energy and amino acid (methionine and lysine) ingredients were computed from tabular values for the feedstuffs (Jurgens, 1996). Feed consumption was measured on the 25th, 50th, and 75th days of the experiment.

At the end of the study, a total of 36 birds were killed by cervical dislocation, including 6 birds per group. One part of the blood samples was put into additive-free vacutainers. They were centrifuged (Remi, R-8C BL R-8M) at 3,000 g for 10 min at 4 °C and aliquots were transferred to microfuge tubes. Serum, liver, and ovary samples were kept on ice and protected from light to avoid oxidation during sampling and were then stored at −80 °C until analyses. The other part of the blood samples was taken into anticoagulant tubes. Haematological parameters; white blood cell (WBC), lymphocytes (LYM), monocytes (MON), granulocytes (GRAN), red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT) and Platelet larger cell ratio (P-LCR) was immediately measured with haematological analysis device (PROCAN pe-6800 VET).

MDA and GSH were determined according to Mihara & Uchiyama (1978) and Ellman (1979). The serum, liver, and ovarium TAS and TOS were determined according to Erel (2005) with a spectrophotometer (Konika Minolta CM-5). The OSI was defined as the ratio of the TOS level to the TAS level. Specifically, OSI (arbitrary unit) = TOS (μmol H2O2 Eq/L)/TAS (μmol Trolox Eq/L).

Data were analyzed by two-way ANOVA using the GLM procedure (SPSS, 2015). The following model was applied: $y_{ijk} = \mu + ET_i + AV_j + (\text{ET*AV})_{ij} + e_{ijk}$, where y = response variable, μ = population mean, ET = effect of environmental temperature (1: 22 ± 2

°C for 24 h/day (TN), 2: 34 ± 2 °C for 8 h/day (HS; between 09:00-17:00 hours) followed by 22 ± 2 °C for 16 h/day throughout the experiment), AV = effect of AV supplementation (0: 0% AV, 1: 1% AV *and 3:* 3% AV*),* (ET*AV) = effect of interactions between E and AV*,* and e = residual error [*N* (σ, μ; 0, 1)]. The differences among treatments were evaluated by Duncan Multiple Range Test. Also, statistical significance was considered at P < 0.05 (SPSS, 2015).

RESULTS AND DISCUSSION

The study aims to determine the effects of dietary AV supplementation on the haematological parameters and serum, liver, and ovarian antioxidant status in the late laying period of heat stressed quails. High environmental temperature primarily causes oxidative stress in animals. It has been largely known that increased environmental temperature causes significant yield losses, increased mortality rates, and low profitability in poultry (Lu et al., 2007). It is emphasized that the supplementation of natural herbal additives to animal feed can effectively reduce these negative effects of heat stress.

In the present study, white and red blood cell profile and platelets were analyzed to determine the effect of AV in quail under different ET conditions (Table 3). The effects of ET on PDW and PCT were significant (P <0.05). In the HS, PDW level was lower but PCT level was higher than TN. In terms of the 0% AV, GRAN was the lowest (0.53, 10^3/μL) in TN and the highest (1.20, 10^3/μL) in HS. MCH and PCT values were the highest (32.82, Pg and 0.06, %, respectively) in TN, MCHC was the lowest (43.62, g/dl) in HS. MCV, and PDW were the highest (74.00, fL and 18.83, %, respectively) in HS. In comparisons between 1% AV and 3% AV the GRAN for 1% AV was higher (1.27, 10^3/μL) in the TN than the 3% AV (0.70, 10^3/μL), but was similar (0.80, 10^3/μL and 0.80, 10^3/μL, respectively) in the HS (Table 3).

Table 3. Effects of dietary *Alchemilla vulgaris* supplementation (%) on haematological parameters in late laying period of heat-stressed quails (Mean ± SE)

Variables	n	WBC (10^3/μL)	LYM (10^3/μL)	MON (10^3/μL)	GRAN (10^3/μL)	RBC (10^6/μL)	HG (g/dl)	HCT (%)	MCV (fL)
					ET[a]				
TN[b]	18	36.91±0.95	34.64±0.35	1.50±0.12	0.83±0.09	3.78±0.10	12.00±0.28	27.59±0.72	71.48±1.21
HS[c]	18	37.38±0.50	34.18±0.40	1.46±0.08	0.93±0.09	3.98±0.09	12.54±0.32	27.35±0.61	70.51±1.43
					AV[d]				
	0	6	34.88±1.44	35.17±0.56	1.28±0.10	0.53±0.02^{A}	3.63±0.22	11.88±0.58	28.50±1.71	72.75±2.03^{BC}
TN	1	6	39.07±1.45	34.30±0.64	1.83±0.13	1.27±0.19^{C}	3.82±0.14	12.07±0.36	27.98±0.73	73.72±1.53^{C}
	3	6	36.77±1.81	34.47±0.69	1.38±0.28	0.70±0.6^{A}	3.88±0.15	12.05±0.55	26.28±0.12	67.97±2.19^{AB}
	0	6	38.97±0.58	33.82±0.49	1.53±0.13	1.20±0.22^{BC}	4.09±0.12	13.20±0.38	28.93±0.52	74.00±0.62^{C}
HS	1	6	36.88±0.77	33.67±0.99	1.41±0.17	0.80±0.13^{AB}	3.95±0.19	12.78±0.47	27.77±0.91	73.65±1.66^{C}
	3	6	36.31±0.94	35.07±0.44	1.43±0.13	0.80±0.09^{AB}	3.89±0.15	11.65±0.66	25.35±0.20	63.87±2.09^{A}
ET			-	-	-	-	-	-	-	-
AV			-	-	-	*	-	-	-	*

Variables	n	MCH (Pg)	MCHC (g/dl)	PLT (10^3/μL)	MPV (fL)	PDW (%)	PCT (%)	P-LCR (%)
					ET			
TN	18	31.83±0.46	44.71±0.46	43.50±1.93	11.99±0.19	17.64±0.50	0.05±0.00	28.58±0.71
HS	18	31.51±0.44	44.89±0.49	43.61±1.53	12.17±0.14	16.23±0.37	0.06±0.00	29.85±0.49
					AV			
	0	6	32.82±0.59^{B}	45.30±1.00^{BC}	43.17±2.52	12.00±0.15	17.35±0.66^{BC}	0.06±0.01	28.25±1.09
TN	1	6	31.65±0.61^{AB}	43.05±0.31^{A}	40.33±1.76	12.35±0.19	17.75±0.33^{BC}	0.04±0.01	30.23±0.32
	3	6	31.03±1.03^{AB}	45.77±0.45^{C}	41.83±2.34	11.62±0.52	16.35±1.14^{AB}	0.04±0.01	27.25±1.71
	0	6	32.25±0.39^{B}	43.62±0.52^{AB}	48.33±4.70	12.52±0.10	18.83±0.68^{C}	0.06±0.01	31.37±0.67
HS	1	6	32.45±0.60^{B}	44.20±0.69^{BC}	39.33±2.84	12.20±0.11	16.45±0.55^{AB}	0.05±0.01	28.67±0.99
	3	6	29.82±0.76^{A}	43.85±0.75^{AB}	48.33±1.05	11.78±0.34	14.88±0.23^{A}	0.06±0.01	29.52±0.50
ET			-	-	-	-	*	*	-
AV			*	*	-	-	*	-	-

-:P >0.05, *: P <0.05, ^{A,B,C}: Means with a common superscript do not differ at P<0.05).

ET: Environmental temperature; TN:: Thermoneutral; HS: Heat stress; AV: *Alchemilla vulgaris*.

MCV, MCH and PDW values for 1% AV were higher in both TN (73.72, fL, 31.65, Pg and 17.75, %, respectively) and HS (73.65, fL, 32.45, Pg and 16.45, %, respectively). The MCHC value for 1% AV was higher (44.20, g/dl) in the HS and lower (43.05, g/dl) in the TN (Table 3).

PCT value for 1% AV was lower (0.05, %) in the HS and same (0.04, %) with %3 AV in the TN. Also, GRAN, MCV, PDW in terms of 1 and 3% AV and MCH in terms of 3% AV was calculated lower under HS conditions (P <0.05; Table 3).

No statistically significant difference was observed in WBC, LYM, MON, RBC, HG, HCT, PLT, MPV, and P-LCR levels of the groups 1 and 3% AV supplementation under TN and HS conditions. Also, the effects of interaction between ET x AV levels were not significant on the blood haematological profiles in quails exposed to heat stress in the study (P>0.05) (Table 3).

A study on broilers showed that; AV has been shown to significantly affect certain blood parameters (MON (%), MON (10^3/μL), PLT, and PCT) (p <0.05, p <0.01). It was noteworthy that monocyte levels increased significantly because monocytes are phagocytic cells of the blood and support the immune system of the body by killing pathological microorganisms. This suggests that the addition of AV to the diets may contribute to the immune system and the resistance to diseases (Köseman et al., 2020).

Acute HS caused changes in the proportions of circulating leucocyte components but it was determined that acute HS did not affect the hematocrit levels or eosinophil proportion (Altan et al., 2000). In another study, it was found that hematocrit levels decreased in birds exposed to heat stress (Altan et al., 2003). The hematocrit level obtained in our study was consistent with Altan et al., (2000), but did not similar to Altan et al., (2003). During the acclimation period, some blood parameters such as basophil, heterophil, and H/L ratio increased in the high temperature group. Exposure to an acute heat temperature of chickens at 42 d resulted in a significant increase in basophil, heterophil, and H/L ratio in both groups. High temperatures caused a decrease in monocyte and lymphocyte proportions, whereas the proportion of eosinophil was not affected (Erköse and Akşit, 2009).

In the present study, suggested that GRAN (eosinophils, basophils, neutrophils) were lower in HS than the control group in supplemented with 1 and 3% AV because of neutropenia is the low occurrence. Neutropenia occurs in congenital and immune/nonimmune reasons and it is less than 1500/mm^3 neutrophil count in humans (Kaya, 2013). The level of neutropenia in quails is unknown. Therefore, it is considered that the decrease in GRAN may be a positive feature of AV since WBC, LYM, and MON levels determined in this study do not show a statistically significant change.

The lower blood MCV, MCH, and PDW levels in stressed quails supplemented with AV suggest that AV supplementation may affect red blood cell profiles. MCV is a measure of the average size of RBC and is also biochemically associated with MCH and MCHC. Pathologically low MCV level is occurred parallel with microcytic erythrocyte, while low MCH and MCHC levels have occurred parallel with hypochromic erythrocyte cases. Low levels are caused by severe blood loss, hemoglobinopathy, microcytic anemia, and iron deficiency (Kaya, 2013). However, there was no decrease in RBC, HG, and HCT levels or statistical significance related to this study. Therefore, low MCV and MCH levels are thought to be caused by a factor other than AV supplemented.

The PDW test is used to measure the difference between the sizes of the platelets and is interpreted taking into account the other levels obtained from the test. An unstable distribution may indicate serious deficiencies in the body or various diseases. Only low platelet count; platelet agglutination (EDTA-dependent), platelet satellitism (EDTA-dependent and polymorph or around the other cell), platelet neutrophil agglutination (EDTA-dependent), giant platelet, clotted sample, over-filled tube results from the study (Kaya, 2013). In this study, the PDW levels for 1% AV were higher in both TN and HS from 3% AV.

Besides in the present study, the effect of AV on ET conditions was determined by analyzing MDA, GSH, TAS, TOS, and OSI levels in blood serum of quail fed ration supplemented with 1 and 3% AV (Table 4). The effect of AV on serum MDA levels was determined significant (P <0.05; Table 4). The differences were not found significantly for GSH, TAS, TOS, and OSI levels in the blood serum of the quails fed with 1 and 3% AV supplementation under TN and HS conditions (P >0.05; Table 4). However, in terms of AV; MDA in the blood serum was significant (P <0.05; Table 4). The effects of interaction between ET x AV factors were not significant on the serum antioxidant parameters in quails exposed to heat stress (P>0.05) (Table 4).

Table 4. Effects of dietary *Alchemilla vulgaris* supplementation (%) on serum antioxidant status in late laying period of heat-stressed quails (Mean ± SE)

Variables		n	MDA (µmol/L)	GSH (µmol/L)	TAS (mmol Trolox Equiv/L)	TOS (µmol H_2O_2 Equiv/L)	OSI (TOS/TAS)
					ET[a]		
TN[b]		18	7.70±0.57	18.97±1.11	1.11±0.10	13.95±1.22	13.64±1.07
HS[c]		18	8.61±0.70	18.57±0.55	1.00±0.07	12.44±0.71	13.72±1.77
					AV[d]		
TN	0	6	8.09±1.20^{A}	17.18±0.97	1.06±0.18	11.25±1.02	12.83±1.60
	1	6	7.33±0.66^{A}	20.93±2.70	1.06±0.18	15.52±2.36	15.45±2.01
	3	6	7.69±1.16^{A}	19.14±2.01	1.20±0.21	14.07±2.75	12.65±2.03
HS	0	6	11.27±0.92^{B}	16.60±1.12	0.89±0.11	12.75±0.92	17.35±4.88
	1	6	6.89±1.26^{A}	19.07±10.6	1.15±0.11	13.27±0.83	12.12±1.44
	3	6	7.69±0.65^{A}	19.03±0.68	0.97±0.14	11.30±1.79	11.70±1.47
ET			-	-	-	-	-
AV			*	-	-	-	-

-:P >0.05, *: P <0.05, ^{A,B}: Means with different superscripts are significantly different (P <0.05).
[a]: Environmental temperature; [b]: Thermoneutral; [c]: Heat stress; [d]: *Alchemilla vulgaris*.

A study on broilers showed that; AV known to have anti-inflammatory, antioxidant, and anti-microbial effects did not affect serum MDA, GSH, TAS, TOS, and OSI values (p> 0.05). Although not statistically significant, MDA levels and suppressed lipid peroxidation in a dose-dependent manner decreased in the serum (Köseman et al., 2020).

In terms of the 0% AV group, the MDA value in the blood serum was the highest in both TN (8.09, µmol/L) and HS (11.27, µmol/L) (Table 4). In the study, when comparing MDA levels in the blood serum in 1% AV and 3% AV, MDA levels were found lower in both HS and TN in 1% AV groups (Table 4). The GSH, TOS and OSI values in blood serum for 3% AV were lower in both TN (19.14, µmol/L, 14.07, µmol H_2O_2 Equiv/L and 12.65, TOS/TAS, respectively) and HS (19.03, µmol/L, 11.30, µmol H_2O_2 Equiv/L and 11.70, TOS/TAS, respectively) whereas MDA was higher (7.69, µmol/L for TN, 7.69, µmol/L for HS) (Table 4).

The effects of AV on ET conditions were presented for MDA, GSH, TAS, TOS, and OSI levels in the liver of the quails in Table 5. The effect of ET on TOS in liver was significant (P <0.05; Table 5). The effect of AV on the liver GSH, and TOS levels were found significant (P <0.05; Table 5). In terms of AV; GSH and TOS levels in the liver, and also in terms of ET; TOS levels in the liver were significant (P <0.05; Table 5).

Table 5. Effects of dietary *Alchemilla vulgaris* supplementation (%) on liver antioxidant status in late laying period of heat-stressed quails (Mean ± SE)

Variables		n	MDA (nmol/g wet tissue)	GSH (nmol/g wet tissue)	TAS (mmol Trolox Equiv/L)	TOS (µmol H_2O_2 Equiv/L)	OSI (TOS/TAS)
					ET[a]		
TN[b]		18	87.61±5.02	894.22±43.12	1.34±0.04	24.94±1.51	23.27±1.43
HS[c]		18	90.89±3.68	835.50±28.73	1.30±0.05	31.30±2.17	19.56±1.27
					AV[d]		
TN	0	6	90.00±12.01	901.67±36.27^{AB}	1.45±0.09	28.57±2.30^{AB}	24.32±2.46
	1	6	88.00±9.41	830.33±61.05^{A}	1.41±0.03	20.83±1.60^{A}	25.25±2.65
	3	6	84.83±4.62	774.50±41.83^{A}	1.35±0.07	25.42±3.06^{A}	20.27±2.23
HS	0	6	100.17±6.27	814.67±35.60^{A}	1.26±0.08	30.61±3.31^{AB}	20.05±2.01
	1	6	87.50±7.06	1042.50±101.54^{B}	1.30±0.10	35.72±3.88^{B}	16.40±1.48
	3	6	85.00±4.75	825.50±27.94^{A}	1.15±0.06	27.57±3.89^{AB}	22.23±2.61
ET			-	-	-	*	-
AV			-	*	-	*	-

-:P >0.05, *: P <0.05, ^{A,B}: Means with different superscripts are significantly different (P <0.05).
[a]: Environmental temperature; [b]: Thermoneutral; [c]: Heat stress; [d]: *Alchemilla vulgaris*.

Table 6. Effects of dietary *Alchemilla vulgaris* supplementation (%) on ovarium antioxidant status in late laying period of heat-stressed quails (Mean ± SE)

Variables		n	MDA (nmol/g wet tissue)	GSH (nmol/g wet tissue)	TAS (mmol Trolox Equiv/L)	TOS (μmol H_2O_2 Equiv/L)	OSI (TOS/TAS)
				ET[a]			
TN[b]		18	63.78±2.56	837.78±41.97	0.97±0.08	17.16±1.29	27.51±8.25
HS[c]		18	64.94±4.35	797.94±37.31	1.04±0.09	17.75±1.29	22.86±4.95
				AV[d]			
TN	0	6	61.50±4.19^{A}	888.67±85.06	1.01±0.12	16.91±2.76	28.28±2.92
	1	6	69.17±5.05^{AB}	903.67±57.28	1.02±0.08	20.29±1.52	20.50±2.46
	3	6	60.67±3.86^{A}	721.00±57.72	0.91±0.15	14.30±1.84	23.75±1.22
HS	0	6	79.50±9.23^{B}	880.50±55.71	0.96±0.18	18.16±2.35	18.88±2.88
	1	6	62.83±5.44^{A}	730.50±47.41	0.99±0.13	18.12±2.63	20.70±4.72
	3	6	52.50±1.93^{A}	782.83±80.82	1.11±0.23	16.97±2.07	28.98±1.41
ET			-	-	-	-	-
AV			*	-	-	-	-

-:P >0.05, *: P <0.05, ^{A,B}: Means with different superscripts are significantly different (P <0.05).

[a]: Environmental temperature; [b]: Thermoneutral; [c]: Heat stress; [d]: *Alchemilla vulgaris*.

The GSH value in the liver was highest (901.67, nmol/g wet tissue) in TN, and the lowest (814.67, nmol/g wet tissue) in HS (Table 5). In the study, while TOS levels in the liver in 1% AV in TN were found to be lower but it was found to be higher in 1% AV in HS (P <0.05) (Table 5). MDA, GSH, and TAS values for 3% AV in the liver were lower in both TN and HS (Table 5). The effects of interaction between ET x AV factors were not significant on the liver antioxidant parameters in quails exposed to heat stress (P>0.05) (Table 5).

In this study, the effects of AV on ET conditions for MDA, GSH, TAS, TOS, and OSI levels in ovarium of quail fed ration supplemented with 1 and 3% AV were determined (Table 6). The effect of AV on ovarian MDA levels was significant (P <0.05; Table 6). However, the differences were not found significant for GSH, TAS, TOS, and OSI levels in the ovaries of the quails fed with 1 and 3% AV supplementation under TN and HS conditions (P >0.05; Table 6). The differences between 1% AV and 3% AV for MDA levels in the ovarium were significant in both HS and TN (P <0.05; Table 6).

In the study, TOS was the highest (28.57, μmol H_2O_2 Equiv/L) in TN. MDA value in the ovary was found to be the highest value (79.50, nmol/g wet tissue) in HS. MDA levels were found higher in 1% AV groups in both HS and TN (Table 6). The effects of interaction between ET x AV factors were not found significant on the ovarium antioxidant parameters in quails exposed to heat stress (P>0.05) (Table 6).

The decrease in MDA levels in plasma or tissues indicates decreased lipid peroxidation. In the case of OS, GSH activity decreases due to excessive consumption of GSH like other antioxidant enzymes (Singh *et al*., 2014). In light of these findings, it can be said that the different ratio of AV has a different effect on stress parameters. Different levels of the same stress parameters in the blood serum, liver, and ovarium are evaluated as the effect of AV on different organs is different. Also, the lack of statistical significance in most parameters is thought to be the destruction of polyphenols and flavonoids in AV content.

If all of the data are evaluated together, data express that the HS caused general OS in the quails and dietary AV supplementation reduced the OS by exhibiting antioxidant effect. Besides, there is no record coping with dietary AV supplementation on antioxidant status in late laying periods of heat stressed quails for comparing our data. But flavonoids were determined that antioxidant activity. Besides, flavonoids significantly increased some antioxidant enzymes such as GSH, while lowering MDA levels (Mahmoud *et al*., 2012). The outcomes of the present study are in agreement with our study.

CONCLUSIONS

In conclusion, some hematological profiles and serum, liver and ovarian antioxidant parameters are impressed in a heat stress exposured in late laying period of quails. Supplementation 1% and 3% AV is partially improved the antioxidant defense system with its antioxidant effect and also, incompletely positively affected these parameters. Besides, 3% AV supplementation is more effective than 1% AV on some haematological and antioxidant parameters. However, more research including different doses and durations is needed to better define the effects of AV on quails exposed to heat stress.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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