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Effects of *Alchemilla vulgaris* on growth performance, carcass characteristics and some biochemical parameters of heat stressed broilers

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ABSTRACT. This study aimed to investigate the effects of *Alchemilla vulgaris* (*A. vulgaris*) on growth performance, carcass characteristics, and biochemical parameters in broilers exposed to high environmental temperature conditions. A total of 45 broilers of 21 days of age (Ross 308) were used and grouped as the control group (C), and the groups with the addition of 1% (G1), or 3% (G2) *A. vulgaris* to chicken diet, respectively. In this study, the body weights of heat stressed broilers were significantly different on the 35th day and onwards. Feed intake was higher in the control group. Feed conversion ratio (FCR) was better in groups G1 and G2 compared to control on the 36th and 42nd day, the FCR was better on the 21st- 42nd days. The highest hot and cold carcass performance were observed in group G2 (79.72±0.93% and 78.02±0.99, respectively), and the lowest values were observed in group C (76.26±1.13% and 75.70±1.20%, respectively). *A. vulgaris,* reduced serum malondialdehyde (MDA) levels as numerically, and suppressed lipid peroxidation in a dose-dependent manner. It had significant effects on monocytes (MON %, MON count), platelets (PLT), and plateletcrit (PCT) parameters only. In conclusion, the deleterious effects of high environmental temperature in broilers could be partially reversed by *A. vulgaris* addition to the diets between days 21 and 42.

Keywords: Alchemilla vulgaris, Broiler, Carcass characteristics, High environmental temperature, Growth performance

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INTRODUCTION

Heat stress has become a serious problem in poultry production along with global warming. High environmental temperature has harmful effects on the physiology and immunology of poultry and reduces efficiency. The harmful effects include delayed growth rate, reduced feed intake, and impaired blood biochemistry and immunity. It was reported that feed intake of poultry decreased over 25 ℃, and that heat stress-related reduced capacity reached a serious level (Bollengier-Lee, 1999; Imik *et al*., 2013).

It was recommended to increase either feed intake or the nutrient ingredients within the diet to compensate for the reduced feed intake due to high environmental temperature (Ayhan *et al*., 2000). Studies have been conducted to investigate the effects of various herbal ingredients into the diets to increase heat stress-related reduced feed intake, especially in poultry (Karslı and Dönmez, 2007) and to reduce the other negative effects of heat stress. Thus, in the study of Akhavan-Salamat and Ghasemi (2016), the effects of betaine (Bet) and dried turmeric rhizome powder (TRP) on leukocyte profile, humoral immunity and antioxidant status alone or in combination in broilers exposed to heat stress were studied, and it was observed that the negative effects of heat stress on body weight, feed intake, FCR, and death rate were partially eliminated with dietary Bet and TRP. Likewise, it was observed in the study of Hassan *et al.* (2018) that addition of rutin (is a flavonol glycoside) into the rations resulted in increased body weight, protein efficiency rate, leukocyte and lymphocyte count, but did not affect the total blood protein, albumin, globulin and alanine transaminase levels in broilers. It was also determined in the study that high rutin concentration (0.5 and 1 g/kg) added into the rations significantly reduced the serum total cholesterol (TC), concentrations of triacylglycerol, low density lipoprotein (LDL) cholesterol ($p<0.001$) and malondialdehyde (MDA) ($p=0.001$), and increased the activities of superoxide dismutase, catalase, and glutathione peroxidase.

Alchemilla vulgaris is a biologically active herb as an immunomodulatory agent, which is rich in phyto components. It is widely referred to as Lady's Mantle, Bear's Foot or Lion foot, and used as a public medicine in European countries to a significant extent (Al-osaj, 2016). It is a member of the Rosaceae family and is rich primarily in flavonoids such as quercetin and kaempferol (Al-osaj, 2016), tannins, and chemical compounds such as gallic acid and ellagic acids (Neagu *et al*., 2015; Kostadinović *et al*., 2019). Dried lion's foot leaves have rich in total polyphenolic and flavonoids content (395.65 and 183.10 mg/100g, respectively) (El-Hadidy *et al*., 2018). The antioxidant, anti-inflammatory, anti-microbial and the anti-carcinogenic effects of the *A. vulgaris* have been attributed to its flavonoid components (9.8 mg/g) (Havsteen, 2002; Pietta, 1998).

In research, of all studied extracts, ethyl acetate extract from Lady's Mantle roots characterized by the highest content of catechins in comparison with other samples demonstrated the highest activity *in vitro* towards the studied viruses (neutralization index for vaccinia and ectromelia viruses were 4.0 and 3.5 lg, respectively) (Filippova, 2017). *A. vulgaris* (leaves and flowers) have the highest effect on lipase activity and α –amylase activity comparative with the extracts of Sophora Japonica and Crataegus Azarolus (leaves and fruits) which suggest that the chemical content of polar extracts of these plants might be of therapeutic interest concerning the treatment of obesity (Samah *et al*., 2018).

And it is determined that 3% *A. vulgaris* supplementation in the heat-stressed quail prevented decreased egg production and improved FCR (Akdemir *et al*., 2019). *A. vulgaris* L. can be proposed to protect the toxicity induced by Carbon tetrachloride (CCl_4) in rats, also to help inhibit the improvement of cardiovascular diseases and cystic fibrosis (El-Hadidy *et al*., 2019). Another research results demonstrate that the wound-healing properties of *A. vulgaris* associated with promitotic activity in epithelial cells and myofibroblasts (Shrivastava *et al*., 2007).

Efforts to eliminate stress-related productivity in broiler chickens are promising for the future of poultry production. Therefore, adding antioxidant plants to the diets of broilers is considered a simple, effective, and profitable application in the field due to its oxidative stress reduction effects. However, in the literature, there is no study on the effect of A. vulgaris use on the growth, feed intake, carcass characteristic parameters, and blood and serum stress parameters in the chickens raised under heat stress conditions. As reported in the above studies, A. vulgaris has the potential to be used as a natural remedy due to its antioxidant and anti-inflammatory properties. The present study aimed to determine the effects of dietary A. vulgaris supplementation on growth performance, carcass characteristics, and blood and serum stress parameters in 21-42 day heat stressed broilers.

MATERIALS AND METHODS

This study was conducted in Akçadağ Vocational School Division of Malatya Turgut Ozal University. The experiment was approved (approval document no 2018/A-34) by the Committee on Animal Research at Inonu University, Malatya, Turkey. Broilers (total of forty-five 21-days old, Ross 308), purchased from a commercial company (Seher Tavukçuluk, Malatya, Turkey), with an initial average body weight of 956.4 ± 23.4 g were used in this experiment. Furthermore, the birds were randomly assigned to 3 groups (15 birds in each group) defined as the control group (C) fed with basal diet (Table 1), and a basal diet supplemented with 1% (G1) (1) or 3% (G2) (2) of *A. vulgaris* (purchased from Altinterim Co., Elazig, Turkey as a powdered form). The chicks were selected from all groups in the study in terms of male and female gender-balanced. Broilers were housed 10-12 birds per $1m^2$ floor area/10-12 bird. The birds were also exposed to a 23L:1D illumination cycle. Diets and freshwater were offered for *ad libitum* consumption throughout the experiment. The 21-day-old broilers classified were kept at 34±2 °C until 42 days of age. The birds were fed with a finishing diet (Table 1) were obtained from a commercial feed factory (Seher Tavukçuluk, Malatya, Turkey).

Table 1. Ingredient and nutrient composition of the basal diet[a]

Ingredients, %	Finisher (21-42 d)
Corn	61.13
Soybean meal (48 %)	30.6
Soy oil	4.2
Limestone	1.51
Dicalcium phosphate	1.53
Sodium chloride	0.41
DL-Methionine	0.12
Vitamin-mineral premix[b]	0.5
Chemical analyses, dry matter basis, %	
Crude protein	20.50
Crude fat	6.13
Crude fiber	3.89
Calcium	0.97
Phosphorus	0.42
Calculated compositions[c]	
Metabolizable energy, Kcal/kg	3130
Lysine, %	1.12
Methionine + cysteine, %	0.85

[a] *A. vulgaris* was added into the basal diet at a dosage of 0, 1 and 3 %.
[b] Vitamin premix provides the following per kg: all-trans- retinyl acetate, 1.8 mg; cholecalciferol, 0.025 mg; all-a-tocopherol acetate, 1.25 mg; menadione, 1.1 mg; riboflavin, 4.4 mg; thiamine, 1.1 mg; pyridoxine, 2.2 mg; niacin, 35 mg; Ca-pantothenate, 10 mg; vitamin B_{12}, 0.02 mg; folic acid, 0.55 mg; d-biotin, 0.1 mg. Mineral premix provides the following per kg: Mn (from MnO), 40 mg; Fe (from FeSO4), 12.5 mg; Zn (from ZnO), 25 mg; Cu (from $CuSO_4$), 3.5 mg; I (from KI), 0.3 mg; Se (from NaSe), 0.15 mg; choline chloride, 175 mg.
[c]Calculated value according to tabular values listed for the feed ingredients (20).

The broilers in each group were weighed following 8-12 hours of fasting time on the 28th, 35th and 42nd days, and the bodyweight gains were calculated. Additionally, the feed intake per week was measured, and FCR was determined (consumed feed, g/gained weight, g).

The blood samples (5 ml) were collected from *vena cutanea ulnaris* of 6 chickens by random sampling from each group into both EDTA (anticoagulant tubes) and biochemistry tubes (additive-free vacutainer) on the 42nd day. Blood was rapidly transferred to the laboratory for serum separation and other analyses.

The blood samples were taken into the anticoagulant tubes and thereafter hematological parameters (WBC; white blood cell, LYM; lymphocytes, MON; monocytes, GRAN; granulocytes, RBC; red blood cell, HGB; hemoglobin, HCT; hematocrit, MCV;

mean corpuscular volume, MCH; mean corpuscular hemoglobin, MCHC; mean corpuscular hemoglobin concentration, PLT; platelets, MPV; mean platelet volume, PDW; platelet distribution width, PCT; plateletcrit and P-LCR; Platelet larger cell ratio) were measured with hematological analysis device. Also, the blood samples collected into the additive-free vacutainers were centrifuged at 5000 rpm/min for 5 minutes and serum separation was performed for measurement of some stress parameters. Reduced glutathione (GSH) and malondialdehyde (MDA) were determined according to Uchiyama and Mihara (1978). The serum TAS (Total Antioxidant Status) and TOS (Total Oxidant Status) levels were determined by previously reported methods (Erel, 2004; Erel, 2005) with a spectrophotometer. The OSI (Oxidative Stress Index) was defined as the ratio of the TOS level to the TAS level. Specifically, OSI (arbitrary unit) = TOS (μmol H_2O_2 Eq/L)/TAS (μmol Trolox Eq/L).

In the groups, the slaughter weight was detected for all chickens for the detection of carcass characteristics and then the chickens were sent for slaughter.

Before slaughter, chickens were subjected to a total feed withdrawal of 8 hours. The previously weighed chickens were cut using the cervical dislocation method and then were plucked and eviscerated. Post-slaughter hot and cold carcass weights were measured and the carcass characteristics were determined.

For these aims, the organ weights (breast, leg, wing, neck, and back) were recorded. The carcass weight was obtained by removing the head, neck, shanks, and abdominal fat from bled, plucked, and eviscerated chickens. Then, the hot carcass, breast, leg (thigh+drumstick), and abdominal fat weights were recorded. Carcass yields were determined according to the main commercial parts of the breast meat (including pectoralis major and pectoralis minor muscles) and the leg (including thigh and drumstick meat) (Cömert, et al. 2016). The cold carcass weights were determined by waiting at +4 °C for 24 hours. The weights of the pieces were determined using a 1 g scale. Heart, liver, spleen, and abdominal fat weight was determined proportionally with a precision scale of 0.01 g.

Statistical Analysis

Descriptive statistics were calculated for each parameter according to the data obtained. The Kruskall Wallis variance analysis was used for the intergroup comparisons to determine the effect of *A. vulgaris* addition into rations of the broilers on the investigated parameters. The Duncan Multiple Range Test (DMRT) as post hoc test was used for parameters that demonstrated significance (Akgül, 2005). Analyses were performed using the SPSS 22.0 version program package (SPSS, 2015).

RESULTS

The effects of *A. vulgaris* added at different amounts into the diets of broilers exposed to heat stress on body weights and body weight gains have been presented in Table 2 and that on feed intake and FCR have been presented in Table 3. Effects of *A. vulgaris* on the carcass weights (g) and ratios, and various carcass part weights of broilers exposed to heat stress are presented in Table 4.

Table 2. Effects of ***A. vulgaris*** added at different amounts into the rations of broilers exposed to high environmental temperature conditions on body weight and body weight gain

		Bodyweight, g			
Groups	**n**	**day 21** $\bar{X} \pm S_{\bar{X}}$	**day 28** $\bar{X} \pm S_{\bar{X}}$	**day 35** $\bar{X} \pm S_{\bar{X}}$	**day 42** $\bar{X} \pm S_{\bar{X}}$
Control	15	953.20±24.97	1486.67±37.58	2100.00±68.87^{B}	2688.00±97.45
G1	15	960.40±18.46	1434.67±24.18	2001.33±35.91^{AB}	2569.33±63.39
G2	15	955.20±26.58	1382.67±34.02	1892.00±56.33^{A}	2430.67±67.72
p		-	-	*	-
		Bodyweight gain, g			
Groups	**n**	**day 21-28**	**day 29-35**	**day 36-42**	**day 21-42** $\bar{X} \pm S_{\bar{X}}$
Control	15	76.20	87.60	73.50	79.10±4.32
G1	15	67.80	81.00	71.00	73.27±3.98
G2	15	61.10	72.80	67.30	67.07±3.38
P		-	-	-	-

-:p>0.05, *:p<0.05, $^{A, B}$: Differences between the values of the different letters in the same column are important (p <0.05).

Table 3. Effects of *A. vulgaris* added at different amounts into the rations of broilers exposed to high environmental temperature conditions on feed conversion ratio (FCR) and feed intake

Groups	n	Feed intake, g			
		day 21-28	day 29-35	day 36-42	day 21-42 $\bar{X} \pm S_{\bar{X}}$
Control	15	120.20	147.60	154.00	140.60±10.37
G1	15	114.10	137.30	145.80	132.40±9.47
G2	15	107.00	128.60	139.30	124.97±9.50
p		-	-	-	-
Groups	**n**	**FCR**			
		day 21-28	day 29-35	day 36-42	day 21-42 $\bar{X} \pm S_{\bar{X}}$
Control	15	1.58	1.68	2.10	1.78±0.16
G1	15	1.68	1.70	2.05	1.81±0.12
G2	15	1.75	1.77	2.07	1.86±0.10
p		-	-	-	-

-:p>0.05

Table 4. Effects of *A. vulgaris* on the carcass weights and yields, and various carcass part weights of broilers exposed to high environmental temperature conditions

Groups	n	Hot carcass weight, g $\bar{X} \pm S_{\bar{X}}$	Cold carcass weight, g $\bar{X} \pm S_{\bar{X}}$	Hot carcass yield, % $\bar{X} \pm S_{\bar{X}}$	Cold carcass yield, % $\bar{X} \pm S_{\bar{X}}$
Control	15	2076.73±96.56	2034.80±96.61	76.26±1.13	75.70±1.20
G1	15	1983.80±59.95	1945.27±59.75	77.21±0.69	75.71±0.70
G2	15	1937.80±67.14	1896.47±67.58	79.72±0.93	78.02±0.99
p		-	-	-	-
Groups	**n**	**Breast, g** $\bar{X} \pm S_{\bar{X}}$	**Leg, g** $\bar{X} \pm S_{\bar{X}}$	**Wing, g** $\bar{X} \pm S_{\bar{X}}$	**Neck+Back, g** $\bar{X} \pm S_{\bar{X}}$
Control	15	786.67 ± 39.66^{B}	817.78 ± 44.63^{B}	218.89 ± 9.72^{B}	260.44 ± 13.64^{B}
G1	15	771.14 ± 40.56^{B}	749.14 ± 30.21^{AB}	199.43 ± 5.99^{AB}	230.00 ± 11.55^{B}
G2	15	652.00 ± 24.23^{A}	664.44 ± 25.66^{A}	180.00 ± 5.93^{A}	195.11 ± 8.86^{A}
p		*	*	**	**

-:p>0.05, *:p<0.05, **:p<0.01. ^{A,B}: Differences between the values of the different letters in the same column are important (p <0.05).

Effects of *A. vulgaris* on serum antioxidant levels in the blood used as stress parameters (MDA, GSH, TAS, TOS and OSI) of broilers exposed to heat stress have been presented in Table 5. Although no significant difference was observed in the serum levels of MDA, GSH, TAS, TOS and OSI, the MDA and GSH levels showed a tendency to decrease following dietary *A. vulgaris* supplementation, and TOS and OSI levels showed a tendency to increase (Table 5).

Table 5. Effects of *A. vulgaris* on serum stress parameters of broilers exposed to high environmental temperature conditions

Groups	n	MDA[a] $\bar{X} \pm S_{\bar{X}}$	GSH[b] $\bar{X} \pm S_{\bar{X}}$	TAS[c] $\bar{X} \pm S_{\bar{X}}$	TOS[d] $\bar{X} \pm S_{\bar{X}}$	OSI[e] $\bar{X} \pm S_{\bar{X}}$
Control	6	4.34±0.52	39.87±6.19	1.59±0.14	27.71±7.12	18.25±4.38
G1	6	3.73±0.08	32.32±0.99	1.34±0.12	20.67±1.79	16.38±2.34
G2	6	3.63±0.15	36.25±2.04	1.60±0.12	31.95±5.13	20.03±2.56
p		-	-	-	-	-

-: p>0.05. [a]Malondialdehyde, µmol/L; [b]Reduced Glutathione, µmol/L; [c]Total Antioxidant Status, mmol Trolox Equiv./L; [d]Total Oxidant Status, µmol H_2O_2 Equiv/L; [e]Oxidative Stress Index, mmol Trolox/µmol H_2O_2.

Also, effects of *A. vulgaris* on hematological parameters (WBC, LYM (%), MON (%), GRAN (%), LYM (10^3/µL), MON (10^3/µL), GRAN (10^3/µL), RBC, HGB, HTC, MCV, MCH, MCHC, RDV-SD, RDV-CV, PLT, MPV, PDW, PCT, and P-LCR) have been presented in Table 6. As demonstrated in Table 6, the effect of *A. vulgaris* on some blood parameters was observed to be significant only for MON (%), MON (10^3/µL), PLT, and PCT ($p<0.05$, $p<0.05$, $p<0.01$, $p<0.05$, respectively). MON (%) and MON (10^3/µL) were highest in group G2 (5.55±0.24 % and 1.42±0.08 10^3/µL, respectively), and PLT and PCT were highest in group C (53.00±2.96 10^3/µL and 0.06±0.01%, respectively) (Table 6).

Table 6. Effects of *A. vulgaris* on various blood parameters of broilers exposed to high environmental temperature conditions

Groups	n	WBC, 10^3/µL	LYM, %	MON, %	GRAN, %	LYM, 10^3/µL
		$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$
Control	6	25.25±0.65	93.68±0.84	3.95±0.35^{A}	2.37±0.54	23.65±0.44
G1	6	23.62±0.69	93.67±0.91	3.97±0.42^{A}	2.37±0.50	21.38±1.05
G2	6	25.87±0.91	89.07±1.20	5.55±0.24^{B}	4.90±1.04	23.82±1.35
p		-	-	*	-	-
Groups	**n**	**MON, 10^3/µL**	**GRAN,10^3/µL**	**RBC,10^6/µL**	**HGB, g/dl**	**HCT, %**
		$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$
Control	6	1.02±0.12^{A}	0.58±0.15	3.40±0.09	9.72±0.24	19.08±0.36
G1	6	0.92±0.07^{A}	0.48±0.11	3.13±0.13	8.83±0.32	17.77±0.61
G2	6	1.42±0.08^{B}	0.62±0.05	3.42±0.14	9.57±0.47	18.92±0.78
p		*	-	-	-	-
Groups	**n**	**MCV, fL**	**MCH, Pg**	**MCHC,g/dl**	**RDW-SD, fL**	**RDW-CV, %**
		$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$
Control	6	56.42±1.23	28.53±0.33	50.87±0.61	27.57±0.57	9.62±0.27
G1	6	57.02±0.52	28.22±0.22	49.68±0.25	28.20±0.30	9.77±0.10
G2	6	55.40±0.49	27.85±0.30	50.43±0.45	26.95±0.42	9.52±0.18
p		-		-	-	-
Groups	**n**	**PLT, 10^3/µL**	**MPV, fL**	**PDW, %**	**PCT, %**	**P-LCR, %**
		$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$	$\bar{X} \pm S_{\bar{X}}$
Control	6	53.00±2.96^{B}	11.07±0.21	11.87±0.58	0.06±0.01^{B}	21.43±0.66
G1	6	36.00±2.33^{A}	11.40±0.22	12.53±0.81	0.04±0.01^{A}	22.22±1.70
G2	6	46.50±3.85^{B}	11.57±0.23	12.12±0.40	0.05±0.01^{AB}	23.20±1.73
p		**	-	-	*	-

-:$p>0.05$, *: $p<0.05$, **:$p<0.01$. ^{A,B}: Differences between the values of the different letters in the same column are important ($p<0.05$).

DISCUSSION

Effect of *A. vulgaris* on the growth performance of broilers

It was observed in this study that the body weights of broilers exposed to heat stress were significantly different on the 35th day and onwards ($p<0.05$), and body weights in the control group were higher compared to the remaining groups (G1 and G2) (Table 2). No chickens died during the study.. Increases in feed intake were observed to be higher in the control group as well (Table 3). Although it was observed that FCR was better in groups G1 and G2 compared to control on the 36th and 42nd day of the study, the FCR was better on the 21st – 42nd days (Table 3). No comparison could be performed with similar studies since no study could be detected on the effects of *A. vulgaris* on the growth performance of the broilers exposed to heat stress in the literature. However, in a study comparing body weights of chickens not exposed to heat stress and fed with a mixture containing 4 different herbs including *A. vulgaris* as well, the body weights were found to be lower compared to the control group; and it was emphasized that it regulated activities of digestive enzymes in rats, and increased the metabolic rate and fat burning (Said *et al*., 2011). *A. vulgaris* has been reported to be rich in flavonoids and shown action via this biomolecule (Havsteen, 2002). Similar to the outcomes of our study, Karslı and Dönmez (2007) reported that the flavonoid-rich herb mixtures added into the broiler diets to reduce heat stress has negative effects on feed intake. Likewise, no improvement was reported in feed intake, carcass

weight, and FCR parameters of broilers exposed to heat stress and fed with propolis, which is known to be rich in flavonoids, compared to the control group (Chegini *et al*., 2018). On the other hand, flavonoids added to the rations such as quercetin, genistein, hesperidin and rutin have been reported to have no effect on feed intake, carcass weight and FCR of broilers exposed to heat stress, whereas high dose flavonoids have been reported to have a partial effect on CW on 22nd - 42th days (Kamboh *et al*., 2013).

Effect of *A. vulgaris* on the carcass characteristics of the broilers

No significant difference was observed between the groups concerning hot and cold carcass weight or both carcass yield (p>0.05). However, although not statistically significant, the highest hot and cold carcass performance values were observed in group G2 (79.572±0.93% and 78.02±0.99, respectively), and the lowest values were observed in group C (76.26±1.13% and 75.70±1.20%, respectively) (Table 4). Similar to our study, Karslı and Dönmez (2007) reported no negative effect of flavonoid-rich herb mixture added into the diets of broilers exposed to heat stress on carcass weight and reported the highest carcass weight in the control group and the lowest in the group fed with herbal extract mixture (P<0.05). This indicates that *A. vulgaris* added into the diets had no effect on the carcass weight, but showed a tendency to improve the carcass performance.

Effect of *A. vulgaris* on some blood and serum stress parameters in broilers

Different outcomes have been observed in numerous studies investigating the effects of stress on serum antioxidant levels, enzyme activities, and blood parameters of broilers raised at high temperature conditions. Erköse and Akşit (2009) reported significantly reduced serum cholesterol, alkaline phosphatase, and uric acid and hematocrit levels in broilers exposed to acute heat stress. Indeed, acute heat stress has been related to many problems in broilers, such as myopathy, blood acid/base imbalance, and increase in cholesterol, uric acid, alkaline phosphatase, and plasma creatine kinase (CK) levels (Sandercock *et al*., 2001). Overproduction of reactive oxygen species (ROS) related to heat stress has been known to cause lipid peroxidation and increase the level of MDA (Altan *et al*., 2003). Furthermore, in a study conducted on 44-day-old broilers, exposure to 39±1 °C heat stress for 2 hours was shown to result in an increase in heterophile and basophil ratios, reduction in monocyte and lymphocyte ratios, and no effect in the eosinophil count and hematocrit values (Altan *et al*., 2000). In the study of Dönmez and Atalay (2007), an increase was reported in the heterophile and basophil counts of broilers, and a decrease in eosinophil and lymphocyte counts. On the other hand, some studies have reported that antioxidants added to the diets have partially reduced or not affected the negative outcomes of heat stress. It was observed in our study that, *A. vulgaris,* which is known to have anti-inflammatory, antioxidant and anti-microbial effects, did not affect serum MDA, GSH, TAS, TOS and OSI values (p>0.05) (Table 5), but reduced serum MDA levels and suppressed lipid peroxidation in a dose-dependent manner, although not statistically significant. We believe that its lipid suppressing effect would be stronger if the rate added to the rations could be increased and if the duration of the study could be delayed. *A. vulgaris* was demonstrated to significantly affect certain blood parameters (MON (%), MON (10^3/µL), PLT, and PCT) (p<0.05, p<0.01) (Table 6). Among those, it was notable that the monocyte levels were significantly increased with the change in the dose of *A. vulgaris* added into the diets, 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 *A. vulgaris* to the diets may contribute to the immune system and the resistance of the chickens to diseases. It was observed in the study of Akhavan-Salamat and Ghasemi (2016) investigating the effect of dried turmeric powder addition into diets of broilers exposed to heat stress alone or in combination, which included betaine and flavonoids, that the serum SOD and GPx activities were higher in the study groups compared to the control group (P <0.05).

No effect of rutin addition of *A. vulgaris* into the diets of broilers was reported on total protein, albumin, globulin, and alanine transaminase levels in the study of Hassan et al. (2018), whereas increased leukocyte and lymphocyte counts were observed. Furthermore, as an ingredient, the routine was demonstrated to significantly reduce the levels of total serum cholesterol, triglyceride, LDL cholesterol, and malondialdehyde concentrations, and to increase the CAT, SOD, and GPx activity. Hasheimi et al. (2013) investigated the effect of Zingiber addition on broilers exposed to heat stress and observed increased plasma corticosterone concentration and heterophil/lymphocyte ratio independent from the diet on the 42nd day.

CONCLUSION

Especially 3% A. vulgaris supplement to heat stress broiler diets, although not statistically significant, decreases feed intake while increasing body weight and hot and cold carcass performance. This is important for broiler-producing farms to provide more profit. A. vulgaris also has significant effects on MON (%), MON (count), PLT, and PCT parameters. Also, addition of A. vulgaris to ration reduced MDA levels as numerically and suppressed lipid peroxidation partially, too. This result is important for farms growing broilers in hot climates. Besides, there may be a solution that will contribute to an effective broiler breeding today, where there is a global warming problem. However, to better define the effects of A. vulgaris on poultry exposed to heat stress, studies with different doses and durations should be conducted.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

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