



Effect of Dietary Supplementation with a Herbal Extract on Growth Performance and Meat Quality in Quails Raised under Thermal-Neutral and Heat Stress Conditions

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Abstract

The present study was conducted to evaluate the effect of dietary supplementation of the herbal extract on growth performance, carcass yield, and meat quality in quails raised under thermal-neutral (TN) and heat stress (HS) conditions. For this purpose, a total of 384 one-day-old Japanese quail chicks (*Coturnix coturnix japonica*) were randomly allocated to 4 treatments in a 2 × 2 factorial arrangement with two levels of dietary herbal extract (Digestarom® Poultry; 0 and 100 mg/kg of diet) and two thermal environments (TN and GS). A corn-soybean meal-based diet was prepared for starter and grower phases according to NRC (1994) recommendations. Growth performance along with carcass and meat quality characteristics including color, pH levels, L*, a*, and b* values, cooking loss, and water holding capacity were recorded. Heat stress negatively affected the feed intake of birds during the second phase of the experiment ($P < 0.001$). Meat pH levels were decreased at the 15th minute postmortem in quails subjected to HS ($P < 0.05$), while other meat quality parameters were not affected. The HS also had no significant effect on blood stress indicators such as heterophil/lymphocyte ratio or malondialdehyde concentration. The herbal extract could not extremely improve growth performance and meat quality in quails, but it helped to some extent to buffer the negative effects of HS. In conclusion, it seems quails have a high tolerant capacity and the beneficial effect of dietary herbal extract addition might be achieved when quails are subjected to higher thermal conditions.

Introduction

Poultry production has an important role in meeting the protein requirement of the ever-growing world population. In modern poultry farming, many factors such as nutrition, diseases, and also stress can affect the performance and health of birds via modulating their blood hormone and metabolite levels, gut microbiome, and immune system (Taşkın *et al.*, 2015). Therefore, getting under control of these factors is extremely critical for every period of poultry breeding (Pinchasov and Noy, 1993). One of the most important factors in poultry farming is the environmental temperature (Kaplan *et al.* 2006). In the Mediterranean climate zone, high temperature negatively affects the productive performance of poultry (Beyazıtöglü, 2009). Domestic birds are in the class of homeothermic animals and their acceptable thermo-neutral environmental temperature is between 14-25°C. When the environmental temperature exceeds more than high critical zone

birds' body temperature balance begins to deteriorate and this condition is called heat stress (Tonbak and Çiftçi, 2012; Şimşek *et al.*, 2013).

Although broiler chickens are more susceptible to heat stress because of high production level (Olanrewaju *et al.*, 2010), all class of poultry hardly regulate their body temperature when environmental temperature exceeding 25°C, as they lack sweat glands, 95% of their bodies are covered with feathers, and their skin contains a large layer of fat (Karlı and Dönmez, 2007). Reducing feed intake but increasing water consumption are the first physiological responses as a result of rising the environmental temperature. This decrease in feed consumption naturally reflects on the productivity of the animal. Tonbak and Çiftçi (2012) stated that feed consumption decreased by 4-5% with an increase of 1°C above 30°C in quails. In birds exposed to heat stress, the lack of thermal balance or deterioration of homeostasis accompany increased respiratory rate,

decreased physical activity, feed consumption, feed utilization, and product quality decreases (Caurez and Olo, 2013).

Following a ban on the use of antibiotics as feed additives in 2006, researchers focused to find other alternatives that are natural and safe, and also do not leave harmful residues in animal products (Bilal *et al.*, 2008). In this context, the use of various herbal extracts has become popular in recent years to increase productivity in poultry farming and to eliminate the stress-related negative impacts (Christaki *et al.*, 2012). Herbal extracts are natural compounds derived from various plants that Lange (2005) previously confirmed their effectiveness on feed characteristics and also the productive performance of farm animals. These effects are mediated by increasing the flavor and aroma of feed, stimulating feed consumption and secretion of digestive enzymes, preventing pathogenic microorganisms from settling in the digestive system, and increasing the use of nutrients in feed composition (Jamroz *et al.*, 2003; Karasu and Öztürk, 2014). In the present study, we aimed to investigate the effects of the addition of herbal extract (Digestarom® Poultry) to diets on the growth performance and meat quality in quails raised in different thermal environments.

Materials and Methods

Compliance with ethical standards

This study was approved by the Animal Ethics Committee of Aydın Adnan Menderes University with decision number of 2016/73, Aydın, Turkey.

Animals and trail pattern

The study was conducted at Poultry Research Unit of Faculty of Veterinary Medicine, Adnan Menderes University, and Aydın, Turkey. The study was conducted as a completely randomized design in a 2 × 2 factorial arrangement of dietary herbal extract supplementation (0 and 100 mg/kg of diet) and different temperature levels (thermal-neutral and high). A total of 384 one-day-old Japanese quail chicks (*Coturnix coturnix Japonica*) were randomly allocated to four experimental groups, each comprising of 8 replicate pens (each of them 25 × 44 × 90 cm size with individual heaters, feeders, and drinkers).

Housing

Automatic heaters with adjustable thermostats in each compartment and air conditioners in the test rooms were used to keep the ambient temperature at the desired level. Heat stress (HS) was applied by providing 35 ± 2°C temperature and 60 ± 5% humidity throughout the whole experiment, while temperature for the other groups was decreased gradually by 2 - 3°C weekly to the final level of 23 - 24°C by the end of the trial (thermal-neutral, TN). The level of temperature values of each cage was measured and recorded 3 times a day and the determined temperature values were kept under control in the relevant groups throughout the study. The lighting was provided 24 hours a day, with daylight and bulbs day and night.

Table 1. Composition and calculated value of basal diets for starter and grower (g/kg as fed basis)

Feedstuff	Diets	
	Starter (0-14 th days)	Grower (15-42 nd days)
Corn (8% CP)	51.40	58.35
Soybean meal (48 % CP)	41.45	36.00
Vegetable oil	3.00	1.50
Limestone	1.25	1.25
Dicalcium phosphate	1.60	1.60
Salt	0.35	0.35
DL-Methionine	0.30	0.30
L-Lysine HCL	0.15	0.15
Vitamin and Mineral premix*	0.50	0.50
Calculated values**		
Metabolically energy, kcal/kg	2910	2900
Crude protein. %	24.00	22.00
Lysine, %	1.48	1.34
Methionine + Cystine, %	1.10	1.05
Calcium. %	0.98	0.96
Available phosphor. %	0.42	0.41
Sodium, %	0.17	0.17

* For vitamin and mineral premix per kg of diet: retinol acetate 1706 mg, cholecalciferol 41 mg, DL- α -tocopherol 27 mg, menadion 0.99 mg, cobalamin 0.015 mg, folic acid 0.8 mg, D-pantothenic acid 15 mg, riboflavin 5.4 mg, niacin 45 mg, thiamine 2.7 mg, D-biotin 0.07 mg, pyridoxine 5.3 mg, manganese 90 mg, zinc 83 mg, iron 121 mg, copper 12 mg, iodine 0.5 mg, selenium 0.3 mg.

** The level of herbal extract (Digestarom® Poultry; a blend of 8% peppermint, 2% eugenol or clove, 3.4% anethole or anise and thyme and sodium chloride as a carrier up to 100%; Micro-Plus Konzentrate GmbH, Germany) addition to diets was 100 mg per kg of feed.

Dietary regimes

A corn-soybean meal basal diet was prepared based on the recommendations of NRC (1994) for starter (0 to 14 d) and grower (15 to 42 d) periods (Table 1). The herbal extract Digestarom® Poultry (consisting 8% peppermint, 2% eugenol or clove, 3.4% anethole or anise, thyme, and sodium chloride as a carrier up to 100%; Micro-Plus Konzentrate GmbH, Germany) in two levels of 0 and 100 mg/kg was added to the basal diet. In the study water and feed were given *ad libitum* to birds.

Growth performance

On the days of 7th, 14th, 21st, 28th, 35th, and 42nd of the study, the birds and feeds from feeders were weighed for body weight (BW) and feed intake (FI) determination by subgroups. Body weight gain (BWG) was calculated by using the body weight difference between weighing periods. Feed conversion ratio (FCR) was calculated by dividing FI by BWG.

Slaughtering Process

On the 28th and 42nd days of the experiment, all the birds were weighed individually and from each subgroup (32 in total for the 28th d; 96 in total for 42nd d) were randomly separated for the slaughtering process (the quail heads and feet were separated and the internal organs were removed). The carcasses were weighed just after and 24 h later (kept at 4°C) of the slaughtering for the determined hot and cold carcass yields.

Blood sample analysis

To determine the heterophil/lymphocyte ratio (H/L) and biochemical parameters, blood samples were taken from each subgroup (32 in total for the 28th d; 96 in total for 42nd d). At least 5 mL of blood was collected in EDTA tubes during the slaughter and then centrifuged at 704 × g for separation of plasma for 10 minutes and staining with Pappenheim panoptic staining method (May Grunwald-Giemsa). A total of 100 leukocytes were counted in each sample for the determined ratio (Gross and Siegel, 1983). To determine biochemical blood parameters, blood samples taken into tubes were centrifuged and serum was removed. The method reported by Yoshioka *et al.* (1979) was used to measure serum malondialdehyde (MDA) level. Briefly, 250 µL thiobarbituric acid TBA (0.67%), 625 µL TCA (20%), and 125 µL sample (blood serum) were added and boiled at 95°C for 30 minutes. It was kept in a container full of ice to cool. After cooling, 1 mL of a butonal was added and centrifuged for 10 minutes at 704 × g.

Meat quality analysis

At 15 min and 24 h postmortem, the final pH was

measured with a previously calibrated portable pH meter. The pH of each sample was measured in the pectoralis major muscle at about 1-cm depth. Similarly, the color values were measured from three different regions of the skinless breast meat in which was preserved at +4°C at 24, 72, and 120 h postmortem. Color intensities with the colorimeter (Minolca CR-200, Japan) based on L*, a*, and b* values (L* = 0 black, L* = 100 white; a* = + 60 red, a* = - 60 green and b* = + 60 yellow, b* = - 60 blue). The pH levels were also examined on the meat samples after 15 min and 24 h (kept at +4°C) of slaughtering. cooking loss analysis was performed according to the method reported by Honikel (1998) on the 1st and 3rd days after slaughtering (samples were kept at +4°C). Accordingly, samples (20-25 grams) taken from breast meat were weighed and placed in a nylon bag. The sample bags were tightly sealed to prevent water from entering. The meat sample (20 g) was placed in a polyethylene bag and heated in a water bath at 80°C to achieve an internal temperature of 75°C. After cooking, meat samples were cooled under running water and then cooled at 4°C. The cooled samples were removed from their bags, dried with a paper towel, and weighed again. Cooking loss was calculated as the ratio of the difference between the weight of meat samples before and after cooking divided by the initial weight. In the same manner, water holding capacity (WHC) is also determined by the pressure method specified by Hamm (1986). Approximately 5 g of meat sample was weighed in 5 pieces between two pre-determined strainer papers (10 × 10 cm). These filter papers were taken between two glass sheets (15 × 15 cm) and 2250 g weight was applied to them. After the waiting time (5 minutes) has expired, the pieces of meat were removed from the filter paper and the filter papers were weighed again. By calculating the difference between the first and the last weight, the WHC was determined as % (Barton-Gade *et al.*, 1993).

The total number of mesophilic aerobic bacteria (TMAB) was determined in meat samples from slaughtering. The meat samples were stored at +4°C and evaluated on days 0, 2, and 5 after slaughtering. 10 grams of quail carcass samples were weighed under aseptic conditions and placed in stomacher bags and 90 mL of sterile physiological peptone water was added to it. Afterward, the samples were homogenized for 2 minutes in the stomacher device. Serial dilutions were prepared from the homogenate obtained, parallel cultivation was done using the surface spreading method and TMAB numbers were determined as log cfu/g. From the decimal dilutions prepared to determine the number of TMAB, Plate Count Agar was inoculated using surface spreading technique, and Petri dishes were evaluated after 24-48 hours incubation at 37°C (Halkman, 2005).

Statistical Analysis

The data were analyzed using a statistical software package SPSS (version 22.0 Armonk, NY) to assess the effect of dietary herbal extract supplementation on performance, carcass yield, and meat quality in quails subjected to TN or HS. A two-way analysis of variance was applied in the GLM procedures of SPSS. Confidence interval of 95% (P -value < 0.05) was considered as significant for interactions and

main effects. The interaction means were differentiated using Tukey's HSD as a post hoc test in case of significant interactions.

Results

There was no significant interaction between dietary herbal extract supplementation and heat stress for growth parameters of quails except FI at 6th, and FCR at the 1st week (P < 0.05; Table 2 to 4).

Table 2. Body weight of quails subjected to normal or high ambient temperature fed a basal diet supplemented with the herbal extract

Applications		Body weight (g)						
Heat Stress	Herbal Extract	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
-	-	8.13	30.43	72.30	112.06	156.06	182.63	202.41
-	+	8.15	29.21	72.21	114.03	154.28	182.88	203.76
+	-	8.15	28.96	69.81	110.40	153.28	180.83	199.78
+	+	8.13	29.31	71.44	115.34	157.04	182.63	202.79
<i>SEM</i>		0.19	0.56	1.16	1.50	1.63	2.56	3.17
Heat Stress								
-		8.14	29.82	72.26	113.04	155.17	182.75	203.09
+		8.14	29.14	70.63	112.87	155.16	181.73	201.28
Herbal Extract								
-		8.14	29.69	71.06	111.23 ^b	154.67	181.73	201.09
+		8.14	29.26	71.83	114.68 ^a	155.66	182.75	203.28
ANOVA		P						
Heat Stress		0.99	0.23	0.17	0.91	0.99	0.69	0.57
Herbal Extract		0.66	0.45	0.51	0.03	0.55	0.69	0.50
Heat Stress × Herbal Extract		0.31	0.17	0.47	0.33	0.10	0.76	0.80

Heat stress condition, $35 \pm 2^\circ\text{C}$ temperature, and $60 \pm 5\%$ humidity level.

Herbal extract supplemented (Digestarom[®] Poultry) at the level of 0 and 100 mg/kg of diet

^{a,b}: Means bearing different superscripts within the same column are statistically significant (P < 0.05).

Table 3. Body weight gain of quails subjected to normal or high ambient temperature fed a basal diet supplemented with the herbal extract

Applications		Body Weight Gain (g)							2-6 th weeks	0-6 th weeks
Heat Stress	Herbal Extract	1 st week	2 nd week	3 rd week	4 th week	5 th week	6 th week	0-2 nd weeks		
-	-	22.30	41.89	39.78	43.99	26.54	19.83	64.19	130.14	194.30
-	+	21.06	42.99	41.81	40.26	28.63	20.86	64.05	131.59	195.61
+	-	20.83	40.85	40.58	42.89	27.56	18.93	61.66	129.96	191.61
+	+	21.18	42.13	43.89	41.70	25.58	20.15	63.31	131.35	194.65
<i>SEM</i>		0.56	0.80	1.34	1.44	1.77	1.54	1.16	3.09	3.17
Heat Stress										
-		21.68	42.44	40.79	42.13	27.58	20.34	64.12	130.86	194.98
+		21.00	41.49	42.23	42.29	26.57	19.54	62.49	130.66	193.15
Herbal Extract										
-		21.56	41.37	40.18	43.44	27.05	19.38	62.93	130.05	192.96
+		21.12	42.56	42.85	40.98	27.10	20.51	63.68	131.47	195.13
ANOVA		P								
Heat Stress		0.23	0.25	0.29	0.91	0.57	0.60	0.17	0.95	0.21
Herbal Extract		0.43	0.15	0.056	0.10	0.98	0.47	0.52	0.65	0.55
Heat Stress × Herbal Extract		0.16	0.91	0.64	0.39	0.26	0.95	0.45	0.99	0.92

Heat Stress has been applied, $35 \pm 2^\circ\text{C}$ temperature and $60 \pm 5\%$ humidity level constantly.

Herbal extract supplemented (Digestarom[®] Poultry) at the level of 0 and 100 mg/kg of diet

Table 4. Feed intake of quails subjected to normal or high ambient temperature fed a basal diet supplemented with the herbal extract

Applications		Feed Intake (g)								
Heat Stress	Herbal Extract	1 st week	2 nd week	3 rd week	4 th week	5 th week	6 th week	0-2 nd weeks	2-6 th weeks	0-6 th weeks
-	-	31.11	79.01	112.11	138.95 ^b	184.08	163.88 ^a	110.18	599.02	682.36
-	+	31.10	77.33	109.05	145.98 ^a	177.40	152.93 ^b	108.43	585.36	695.51
+	-	33.46	79.04	106.67	138.67 ^b	165.99	143.59 ^b	112.50	554.91	682.49
+	+	30.68	76.31	105.88	137.86 ^b	165.04	146.63 ^b	107.00	555.40	672.38
<i>SEM</i>		0.77	1.61	1.62	1.99	5.04	3.13	2.10	7.51	10.07
Heat Stress										
-		31.10	78.17	110.58 ^a	142.46 ^a	180.74 ^a	158.41 ^a	109.27	592.19 ^a	688.94
+		32.07	77.68	106.27 ^b	138.27 ^b	165.51 ^b	145.11 ^b	109.75	555.16 ^b	677.44
Herbal Extract										
-		32.28	79.03	109.39	138.81	175.03	153.73	111.31	576.96	682.43
+		30.90	76.82	107.46	141.92	171.22	149.78	107.72	570.38	683.95
ANOVA		P								
Heat Stress		0.22	0.76	0.013	0.04	0.005	<0.001	0.82	<0.001	0.26
Herbal Extract		0.08	0.18	0.24	0.13	0.46	0.27	0.10	0.39	0.91
Heat Stress × Herbal Extract		0.08	0.75	0.49	0.059	0.57	0.03*	0.37	0.35	0.42

Heat Stress has been applied, 35 ± 2°C temperature and 60 ± 5% humidity level constantly.

Herbal extract supplemented (Digestaron[®] Poultry) at the level of 0 and 100 mg/kg of diet

^{a,b}: Means bearing different superscripts within the same column are statistically significant ($P < 0.05$).

Heat stress had no significant effect on BW, BWG, and FCR. However, heat stress reduced feed intake in quails between 15 to 42 days and also the 6th week of the study compared to those subjected to thermo-neutral conditions ($P < 0.05$; $P < 0.01$, and $P < 0.001$, respectively). Dietary herbal extracts supplementation had an increasing effect on BW (P

< 0.05) and BWG ($P < 0.056$) on the 21st day and of the study. However, herbal extract added to the diet had no significant effect on feed intake of quails except the 4th week ($P < 0.059$). On the other hand, dietary herbal extract supplementation worsened FCR at the 4th week ($P < 0.05$; Table 5).

Table 5. The feed conversion ratio of quails subjected to normal or high ambient temperature fed a basal diet supplemented with the herbal extract

Applications		Feed Conversion Ratio								
Heat Stress	Herbal Extract	1 st week	2 nd week	3 rd week	4 th week	5 th week	6 th week	0-2 nd weeks	2-6 th weeks	0-6 th weeks
-	-	1.44 ^b	1.89	2.77	3.22	6.71	7.88	1.73	4.40	3.51
-	+	1.49 ^{ab}	1.81	2.59	3.50	6.09	7.63	1.70	4.37	3.56
+	-	1.54 ^a	1.89	2.65	3.25	6.30	7.65	1.73	4.35	3.56
+	+	1.47 ^{ab}	1.86	2.54	3.42	7.21	8.29	1.77	4.47	3.45
<i>SEM</i>		0.03	0.03	0.11	0.10	0.41	0.50	0.03	0.08	0.17
Heat Stress										
-		1.46	1.85	2.68	3.36	6.40	7.75	1.72	4.39	3.54
+		1.51	1.87	2.59	3.34	6.75	7.97	1.75	4.41	3.51
Herbal Extract										
-		1.49	1.89	2.71	3.24 ^b	6.50	8.08	1.75	4.37	3.54
+		1.48	1.83	2.57	3.46 ^a	6.65	7.64	1.71	4.42	3.51
ANOVA		P								
Heat Stress		0.11	0.50	0.44	0.80	0.39	0.67	0.23	0.76	0.57
Herbal Extract		0.66	0.10	0.21	0.03	0.72	0.39	0.15	0.55	0.61
Heat Stress × Herbal Extract		0.02	0.43	0.74	0.57	0.07	0.70	0.77	0.35	0.76

Heat Stress has been applied, 35 ± 2°C temperature and 60 ± 5% humidity level constantly.

Herbal extract supplemented (Digestaron[®] Poultry) at the level of 0 and 100 mg/kg of diet.

^{a,b}: Means bearing different superscripts within the same column are statistically significant ($P < 0.05$).

There was no interaction between dietary herbal extract supplementation and heat stress for hot and cold carcass yield, and WHC at day 42 in either group subjected to different temperature or fed

dietary herbal extract supplementation (0 or 100 mg/kg). However, there was an interaction between subjects for cooking loss percentage on the 2nd day of postmortem ($P < 0.051$). Moreover, carcass pH

decreased at the 15th minute after the slaughtering process in groups subjected to HS compared to TN ($P < 0.001$). The effect of heat stress on meat pH

level did not observe by the end of the 24th hour after slaughtering (Table 6 and 7).

Table 6. Carcass yield, meat pH values of quails subjected to normal or high ambient temperature fed a basal diet supplemented with the herbal extract

Applications		Carcass Yield (% live body weight)		pH values	
Heat Stress	Herbal Extract	Hot Carcass Yield	Cold Carcass Yield	15 th min.	24 th hour
-	-	56.53	55.93	6.36	5.67
-	+	56.14	55.65	6.36	5.70
+	-	56.60	55.85	6.17	5.69
+	+	55.71	55.64	6.23	5.71
<i>SEM</i>		0.90	0.88	0.03	0.02
Heat Stress					
-		56.34	55.79	6.36 ^a	5.67
+		56.16	55.49	6.20 ^b	5.70
Herbal Extract					
-		56.57	55.89	6.26	5.68
+		55.92	55.39	6.29	5.70
ANOVA					
		----- P -----			
Heat Stress		0.84	0.74	<0.001	0.44
Herbal Extract		0.47	0.58	0.42	0.25
Heat Stress × Herbal Extract		0.78	0.81	0.36	0.74

Heat Stress has been applied, $35 \pm 2^\circ\text{C}$ temperature and $60 \pm 5\%$ humidity level constantly.

Herbal extract supplemented (Digestarom[®] Poultry) at the level of 0 and 100 mg/kg of diet

^{a,b}: Means bearing different superscripts within the same column are statistically significant ($P < 0.05$).

Table 7. Total mesophilic aerobic bacteria (TMAB) numbers, meat water holding capacity (WHC), and cooking lost (CL) percentage of quails subjected to normal or high ambient temperature fed a basal diet supplemented with the herbal extract

Applications		Log10 (cfu/g)			%			
Heat Stress	Herbal Extract	TMAB Day 0	TMAB Day 2	TMAB Day 5	WHC Day 0	WHC Day 2	CL Day 0	CL Day 2
-	-	3.99	4.83	6.28	3.47	6.28	25.83	25.59 ^a
-	+	3.89	4.92	6.10	2.65	4.49	26.00	24.02 ^b
+	-	4.08	5.12	6.45	2.82	4.84	26.29	24.15 ^b
+	+	3.82	5.06	6.08	2.91	4.87	24.62	25.41 ^a
<i>SEM</i>		0.13	0.15	0.12	0.28	0.54	1.13	0.69
Heat Stress								
-		3.94	4.88	6.19	3.06	5.38	25.91	24.81
+		3.95	5.09	6.25	2.86	4.85	25.47	24.78
Herbal Extract								
-		4.04	4.98	6.34 ^a	3.14	5.56	26.06	24.87
+		3.86	4.99	6.09 ^b	2.78	4.68	25.31	24.71
ANOVA								
		----- P -----						
Heat Stress		0.94	0.16	0.60	0.48	0.34	0.69	0.97
Herbal Extract		0.17	0.90	0.04	0.20	0.37	0.51	0.82
Heat Stress × Herbal Extract		0.57	0.62	0.48	0.11	0.11	0.42	0.051

Heat Stress has been applied, $35 \pm 2^\circ\text{C}$ temperature and $60 \pm 5\%$ humidity level constantly.

Herbal extract supplemented (Digestarom[®] Poultry) at the level of 0 and 100 mg/kg of diet

^{a,b}: Means bearing different superscripts within the same column are statistically significant ($P < 0.05$).

Dietary herbal extract supplementation did not affect carcass yield, pH values, WHC and, CL at 0 and 2 days after slaughter. Similarly, nor herbal extract supplementation neither different ambient temperature had significant effects on TMAB in serum of birds at day 0 and 2 of postmortem.

However, dietary herbal extract supplementation had decreased effect on TMAB numbers on the 5th day after slaughtering ($P < 0.05$). Moreover, a significant interaction was observed for the b* color index of meat at the 24th and 72nd hours after slaughtering ($P < 0.05$). While heat stress lowering

the value, dietary herbal extract supplementation increased the meat b* color levels in birds (Table 8). No interaction was noted between dietary herbal

extract supplementation and heat stress for serum MDA levels and H/L ratio at day 42 of the experiment (Table 9).

Table 8. The color indexes of meat of quails subjected to normal or high ambient temperature fed a basal diet supplemented with the herbal extract.

Applications		Colour Index of Meat								
Heat Stress	Herbal Extract	L* _{24th} h	L* _{72nd} h	L* ₁₂₀ h	a* _{24th} h	a* _{72nd} h	a* ₁₂₀ h	b* _{24th} h	b* _{72nd} h	b* ₁₂₀ h
-	-	48.75	47.78	49.25 ^{ab}	9.34	10.37	9.43	8.49 ^a	10.39 ^a	9.51
-	+	49.05	47.87	51.77 ^a	8.13	8.80	8.93	7.20 ^b	8.08 ^b	10.62
+	-	49.06	48.75	51.30 ^a	9.01	9.28	9.86	7.98 ^{ab}	7.18 ^b	9.45
+	+	49.06	48.17	48.82 ^b	8.91	9.13	9.96	8.69 ^a	9.11 ^{ab}	9.97
SEM		0.55	1.30	1.04	0.36	0.72	0.63	0.36	0.69	1.16
Heat Stress										
-		48.90	47.82	50.51	8.73	9.59	9.18	7.84	9.24	10.06
+		49.06	48.46	50.05	8.96	9.20	9.91	8.34	8.15	9.71
Herbal Extract										
-		48.91	48.26	50.27	9.17	9.83	9.64	8.24	8.78	9.48
+		49.06	48.02	50.30	8.52	8.96	9.44	7.94	8.60	10.29
ANOVA		P								
Heat Stress		0.78	0.63	0.26	0.51	0.60	0.26	0.18	0.12	0.76
Herbal Extract		0.79	0.86	0.98	0.06	0.24	0.76	0.42	0.79	0.49
Heat Stress × Herbal Extract		0.78	0.94	0.02	0.11	0.33	0.64	0.01	0.004	0.80

Heat Stress has been applied, 35 ± 2°C temperature and 60 ± 5% humidity level constantly.

Herbal extract supplemented (Digestarom® Poultry) at the level of 0 and 100 mg/kg of diet

^{a,b}: Means bearing different superscripts within the same column are statistically significant (P<0.05).

Table 9. Serum Heterophil/Lymphocyte Ratio (H/L) and malondialdehyde (MDA) levels of quails subjected to normal or high ambient temperature fed a basal diet supplemented with the herbal extract.

Applications		Stress Indicators	
Heat Stress	Herbal Extract	H/Lratio	MDA (nmol/mL)
-	-	0.41	0.56
-	+	0.44	0.57
+	-	0.58	0.57
+	+	0.48	0.56
SEM		0.08	0.05
Heat Stress			
-		0.43	0.56
+		0.53	0.57
Herbal Extract			
-		0.50	0.57
+		0.46	0.56
ANOVA		P	
Heat Stress		0.21	0.90
Herbal Extract		0.65	0.97
Heat Stress × Herbal Extract		0.40	0.83

Heat Stress has been applied, 35 ± 2°C temperature and 60 ± 5% humidity level constantly.

Herbal extract supplemented (Digestarom® Poultry) at the level of 0 and 100 mg/kg of diet.

Discussion

Growth performance

The results are partly consistent with the finding of several researchers who have reported that HS is associated with lower growth performance of quails in terms of BW gain, FI, and FCR (Sahin *et al.*, 2004; 2005; Onderic *et al.*, 2005; Bonfim *et al.*, 2016). In

the actual, HS significantly affects the physiology of quails, by changing the hormonal status of the bird. (Habibu *et al.*, 2016). The birds under HS also tend to reduce heat production by limiting feed intake. Consequently, the birds subjected to HS represent worsened growth performance. In the other words, HS mainly affects quails especially during the later

growing phase of the raising period because higher metabolic activity results in higher heat production but has less ability to dissipate heat from the body (Vale *et al.*, 2010). Parallel to literature, FI of quails subjected to HS suppressed almost 37 g/bird than others in the second part of the growing phase of the present study. On the other hand parameters for growing performance including BW gain and FI only affected by HS, numerically. This might be due to the high temperature applied in the study that was insufficient for quails, which can tolerate tropical regions. Another reason for the obtained result is that the animal welfare in the cage might prevent the chronically applied HS effects. Similar results were observed for FCR in birds subjected to HS and TN conditions. This result contradicts earlier findings (Sahin *et al.*, 2005; Onderic *et al.*, 2005). Contrary to our result Onderic *et al.* (2005) reported that the Japanese quails subjected to HS have a significant increase in FCR by the level of 4.3% than the birds under TN conditions. Suppression of the FCR might due to modification in the metabolic nutrient utilization for the birds (Geraert *et al.*, 1996). On the other hand, some researchers (Bonfim *et al.*, 2016; Habibian *et al.*, 2016) found only a numerical decrease in FCR when the quails were exposed to higher temperatures.

In the present study, herbal extract addition had improvement effects on BW and BWG. Similarly, some studies have reported that dietary herbal extract enhanced the growth performance of quails (Parlat *et al.*, 2005; Biricik *et al.*, 2012). Dalkılıç *et al.* (2015) found out herbal extract supplementation to the diet had positive effects on growth performance in quails during heat stress. In contrast, some other studies (Bülbül *et al.*, 2015; Özcan, 2016; Çetin *et al.*, 2017) show that dietary herbal extract supplementation does not affect growth performance in broiler chickens. Aromatic plants have been used in human nutrition for many years, both for their protective properties against diseases and flavor-enhancing effects (Christaki *et al.*, 2012). These additives both increase feed consumption and stimulate the secretion of digestive enzymes by enhancing the flavor of the feed. They also prevent the retention of pathogenic microorganisms to the digestive microbiota. (Jamroz *et al.*, 2003; Karasu and Öztürk, 2014). On the other hand, it has been reported in many studies (Biricik *et al.*, 2012; Özdemir and Azman, 2013; Bülbül *et al.*, 2015; Özcan, 2016; Çetin *et al.*, 2017) that herbal extract addition to poultry diets had also no significant effect on FI or FCR same as the present study. But the results on FI might be commented as while the HS suppressed the FI of birds in the growing period, the herbal extract addition to diets buffered the negative effect. However, Biricik *et al.* (2012) reported that the addition of myrtle oil to quail rations in increasing doses (0, 500, 1000, 2000, and

5000 mg/kg) significantly improved the FCR. These conflicting results from different studies might be related to the composition of herb substances; variations of extraction method, and additional levels or the differences in HS conditions.

Carcass yields and meat traits

In the present study, nor dietary herbal extract supplementation neither HS had a significant effect on carcass yields in quails. These results are in agreement with those of Köksal and Küçükersan (2012) and Karadağoğlu *et al.*, (2016), who reported no effect of the dietary herbal extract on hot and cold carcass yield of quails. In addition, Buğdaycı and Ergün (2011) also declared that rosemary essential oil addition to broiler diets had no effects on hot and cold carcass characteristics. Similarly, Bonfim *et al.* (2016) reported that the carcass yield of quail was not influenced by environmental temperature. However, other studies have reported HS can affect some carcass characteristics negatively (Habibian *et al.*, 2016; Zeferino *et al.*, 2016). Onderic *et al.* (2005) reported that the cold carcass percentage of quail was decreased by 8% under heat stress.

Meat quality is a major concern for consumers, and it can be defined as the set of parameters and characteristics of meat (Elmasry *et al.*, 2012; Melo *et al.*, 2016). Some studies show that herbal extract addition has a significant effect on meat quality parameters in birds (Jang *et al.*, 2008; Biricik *et al.*, 2012) and affects pH value (Gümüş *et al.*, 2017), WHC and CL (Aminzade *et al.*, 2012; Elmalı *et al.*, 2014), and meat color characteristics (Gümüş *et al.*, 2017) in quails. In the present study, HS significantly affects pH value at the 15th minute postmortem. As the environmental temperature rises, the poultry increases their breathing rate. Depending on the increasing number of respiration, the amount of CO₂ in the blood decreases, and therefore acid-base balance changes develop rapidly. This change in blood pH with the loss of bicarbonate ions can affect the pH level of meat (Kaplan *et al.*, 2006; Lara and Rostagno, 2013). Similarly, Feng *et al.* (2008) reported that HS significantly decreased the pH level in breast meat of chickens. However, HS did not affect pH value at 24 h postmortem. Normal pH value is between 5.4 - 6.0 24 h postmortem according to Terlouw and Rybarczyk (2008). The pH in the present study was on average of 5.7, which can be considered as a normal pH. In addition, Tavaniello *et al.* (2014) found similar meat pH values for quails under heat stress conditions. On the other hand, HS had no significant effect on WHC and CL at 0 and 2 d postmortems in the present study. Moreover, the thermal environment had no significant effect on color characteristics at 24 (L*, a*), 72 (L*, a*), and 120 h (L*, a*, b*) postmortem in quails. However, there was an interaction between herbal extract

addition and HS about yellowness (b^*) of meat at 24 and 72 h postmortem. The results indicated that the addition of herbal extract to diets showed similar yellowness of the meat between TN and HS conditions in quails. Also, TMAB numbers, except 5th

day of postmortem, was affected neither by herbal extract supplementation nor HS in the present study. The result is in agreement with Gümüş *et al.* (2017) who found that the addition of thyme essential oil into quail diets had no effect on physicochemical and microbiological properties of breast meat including TMAB numbers. In contrast, Tekeli (2007) determined that ginger and propolis extracts addition had significant effects on TMAB numbers in the jejunum of broilers. Even though the demonstration of the conflict results is difficult, this might be due to the differences in study procedure, birds' age, diet composition, and nutritive value of feeds.

Blood parameters

No treatment differences were observed in any of the blood parameters measured in the present study. Herbal extract addition and thermal environment had no effects on H/L ratio and MDA values on 42 d of the trial. Although our data is in accordance with Alipour *et al.* (2015) study, it contradicts some other studies (Konca *et al.*, 2015; Çetin *et al.*, 2017) that observed altered blood H/L ratio or MDA levels. The difference in response of results in published studies might be the prediction value of ambient temperature to induce HS in quails that were insufficient because experimental birds could tolerate

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higher temperature values.

Conclusion

The present study showed that although HS decreased FI at the second term of the growth phase, dietary herbal extract supplementation had no strong effect on growth performance, meat quality, and blood parameters in quails subjected to TN or HS. Meanwhile, herbal extract supplementation to some extent helped to buffer the negative effects of HS on FI, FCR, carcass yield, and meat quality parameters. Even though herbal extract addition to diets seems a useful approach for quails under HS, this could be a misleading result in the case of birds that were not under stress. Therefore, it may be beneficial to confirm the results of the study with various herbal extracts in addition to diets of quail raised at higher temperatures.

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