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Supplementation of Natural Antioxidants to Reduced Crude Protein Diets for Japanese Quails Exposed to Heat Stress

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ABSTRACT

The study aimed at comparing the effects of the supplementation of natural antioxidant sources to a reduced crude protein diet on the performance, carcass traits, blood parameters, liver antioxidant enzyme activities and meat lipid peroxidation of Japanese quails exposed to heat stress. A total of 640 14-day-old male Japanese quails were exposed to two different temperature treatments (TT): thermoneutral (TN) or heat stress (HS)and were fed five different dietary treatments (DTs). A normal-protein diet (SCP) was formulated according to the National Research Council (24% CP). The reduced crude protein diet (RCP) was formulated to contain 3% less protein than that of the SCP diet. Three additional diets were prepared by supplementing the RCP with200 mg/kg of α-tocopherol acetate (RCP+TA200), pomegranate peel extract (RCP+PPE200) or apple peel extract (RCP+APE200). HS significantly deteriorated the growth performance of quails throughout the experiment. Quails fed the SCP and RCP+PPE200 presented higher hematocrit values. Feeding the RCP+TA200 and RCP+PPE200 diets reduced the blood heterophil/lymphocyte (H/L) ratio compared with the other diets. Quails fed the SCP, RCP+TA200, RCP+PPE200 and RCP+APE200 diets presented higher liver catalase and glutathione peroxidase activities, plasma uric acid level, and lower meat malondialdehyde value at 7days of age compared with those fed the RCP diet. In conclusion, the results show that the extracts of pomegranate peel and apple peel can be used as alternative natural antioxidant sources to vitamin E in the diets of Japanese quails exposed to heat stress and fed a reduced crude protein diet.

INTRODUCTION

Heat stress (HS) caused by increasing industrialization and environmental degradation is one of the most challenging environmental conditions affecting the poultry sector in the world (Ajakaiye *et al.,* 2010). HS leads to the huge economic losses in the poultry industry every year as a result increased mortality and decreased growth performance (Kamboh *et al.,* 2013; Sahin *et al.,* 2013). During HS, birds increase their energy consumption by accelerating their metabolism to maintain optimal body temperature (Ismail *et al.,* 2015). Lipid stores can be mobilized for energy generation to attenuate the stress response during HS. The mobilization of stored body lipids to supply the extra energy required results in lipid peroxidation and oxidative stress (Yang *et al.,* 2010). In conclusion, HS increases malondialdehyde level and decreased the activities of ROS-scavenging enzymes and the levels of natural antioxidants such as vitamin A, C and E in the serum and liver of poultry (Rhoads *et al.,* 2013; Gopi *et al.,* 2015). HS also causes significant increases of blood heterophil to lymphocyte ratio (H/L) and

reduces haematocrit values (Lara & Rostagno 2013; Sahin *et al.,* 2013).

Especially in recent years, diets with low protein level have been recommended to reduce heat production in broilers under HS (Gu *et al.,* 2008) because protein metabolism causes higher heat production compared with that of fats and carbohydrates (Gonzalez-Esquerra & Leeson 2005; Gu *et al.,* 2008). Reduced dietary protein level decreases production of uric acid (UA), which is the main end product of protein metabolism in birds (Sharifi *et al.,* 2015a). The decrease in plasma UA levels in broilers fed a reduced-protein diet increases lipid peroxidation because plasma UA has an antioxidant role (Sharifi *et al.,* 2015b). Plasma UA contributes to scavenge reactive oxygen species (ROS) from tissues, especially superoxide and hydroxyl radicals (Sharifi *et al.,* 2016). Under this condition, dietary antioxidants scavenge ROS and protect the tissues against ROS (Sharifi *et al.,* 2015a).

Moreover, it is also well known that reduced-crude protein diets intensify liver lipogenesis (Behrooj *et al.,* 2012). It was observed that liver lipogenesis increases oxygen demand and forces the right ventricle to pump more blood to the lungs for oxygenation in broilers (Sharifi *et al.,* 2015a,b). Subsequent overwork of the right heart ventricle, in particular, resulted in right ventricular hypertrophy, which is manifested as higher right ventricle: total ventricle ratio (Sharifi *et al.,* 2016).

Several studies show that the dietary supplementation of natural antioxidants, such as vitamin C and E and β-carotene, alleviate the negative effects of lipid peroxidation caused to HS and reduced-protein diets in poultry (Sahin *et al.,* 2009; Abidin & Khatoon, 2013). In addition to vitamin C and E and β-carotene, flavonoids and polyphenols-rich foods, such as fruits, vegetables and beverages including fruit juices, wine, tea, olive leaf, pomegranate and apple peels have received much attention as natural antioxidants (Hayes *et al.,* 2011). Recently, limited attention has been directed to other potential sources of antioxidant phytochemicals such as pomegranate peel (Kanatt *et al.,* 2010; Ahmed *et al.,* 2015).

Pomegranate (*Punicagranatum* L.) is a fruit that belongs to the Punicaceae family and is rich in beneficial phytochemicals (El-Falleh *et al.,* 2012; Ahmed *et al.,* 2015). The peel accounts for approximately 50% of the total fruit weight, and it is a major source of several bioactive compounds including hydrolysable tannins (Kanatt *et al.,* 2010), flavonoids, anthocyanins and other phenolic compounds (Li *et al.,* 2006; Ahmed *et al.,* 2015). It was reported that pomegranate peel has higher antioxidant capacity than the pulp and the seeds due to its higher contents of total phenols, flavonoids and proanthocyanins (Li *et al.,* 2006; Ahmed *et al.,* 2015). The antioxidant activity of pomegranate peel or its extract were demonstrated *in vitro* (Gil *et al.,* 2000; Singh *et al.,* 2002; Li *et al.,* 2006; Naveena *et al.,* 2008b; Kanatt *et al.,* 2010; Sumathy *et al.,* 2013; Malviya *et al.,* 2014; Zaki *et al.,* 2015) and *in vivo* (Shabtay *et al.,* 2008; Moneim 2012; Ahmed *et al.,* 2015).

Apples also are well-known and widespread fruits of the genus *Malus* belonging to the family Rosaceae (Gazalli *et al.,* 2014). Apple peels are a waste product of applesauce and canned apple manufacturing (Wolfe *et al.,* 2003). Apple peels contain high levels of flavonols, anthocyanins, flavon-3-ols, phenolic acids and dihydrochalcones (Sekhon-Loodu *et al.,* 2013; Wang *et al.,* 2014). Previous studies have shown that about 80% polyphenols are concentrated in the apple peel (Leccese *et al.,* 2009). It is reported that the apple peel contains 5-6 times higher total flavonol levels than apple pomace (Rupasinghe & Keon 2008; Sekhon-Loodu *et al.,* 2013). Sekhon-Loodu *et al.* (2013) reported that the fractionated polyphenol from both dried and frozen apple peel presented higher inhibition of lipid oxidation compared with α-tocopherol acetate and buthylated hydroxytoluene (BHT). A study indicated that the ethanolic extracts of the peel and the pulp of apple showed antioxidant activity comparable with ascorbic acid in the DPPH test, and were approximately ten times more active than BHT in the lipoxygenase test (Giomaro *et al.,* 2014).

The present study aimed at comparing the effects of the supplementation of natural antioxidant sources (α-tocopherol acetate, pomegranate – and apple-peel extracts) to reduced-protein diets for Japanese quails exposed to normal or high temperature on their growth performance, carcass traits, blood biochemistry, liver antioxidant enzyme activities and meat lipid peroxidation.

MATERIALS AND METHODS

Birdmanagement and housing

A total of 640 one-day old male Japanese quail (*Coturnix coturnix japonica*) chicks purchased from a commercial hatchery (19 Mayis University Poultry Production and Marketing Plant, Samsun, Turkey) were used in the experiment. The study was conducted in accordance with animal welfare requirements at the Poultry Research Centre of Tokat Gaziosmanpasa University (2016 HADYEK-04).

At arrival, chicks were weighed and randomly distributed into 40 wire cages (16 chicks/cage). Each cage was equipped with nipple drinkers and electrical heating system controlled by thermostats. The cages were located in the experimental poultry house in two identical rooms separated by a door. During the first 14 days of the experiment, the rooms were not separated and standard brooding temperatures were applied to both rooms with temperature gradually decreased from 32ºC to 26ºC by the end of the second week of age. Quails in each cage were randomly assigned to 10 experimental groups, with four replicates of 16 quails each, and according to a 2 (temperature treatments) x 5 (dietary treatments) factorial arrangement. Birds were evaluated from 14 to 35 days of age.

When birds were 14 days of age, the experimental rooms were separated, and each room, with five experimental groups each, was subjected to either thermoneutral temperature or heat stress treatments. The temperatures applied in the rooms were as follows:

1. Thermoneutral temperature room: quails were kept at 24, 22 and 20ºC until 21, 28 and 35 days. Relative humidity in this room ranged from 50 to 60% during the experiment.
2. Heat stress temperature room: quails were exposed to 34ºC for 8 h/d (from 09:00 to 17:00 h) and then (from 17:00 to 09:00 h) to 24, 22, and 20ºC until 21, 28 and 35 days. Relative humidity ranged from 60 to 70% from 14 days until the end of the study. The experiment was conducted during autumn. Temperature and humidity in each room were monitored at two locations using a temperature-humidity recording system. A fluorescent lighting schedule of 23 h light and 1 h dark was used during the study, with an average light intensity of 40 lux/m^2.

Diets

Quails submitted to both temperature regimes were fed one of five different diets in mash form until 35 days of age. The five experimental diets were as follows: standard-crude protein diet (240 g CP/kg; SCP), reduced-CP diet (210 CP g/kg; RCP), reduced CP diet supplemented with 200 mg/kg of α-tocopherol acetate (vitamin E; RCP+TA200), pomegranate peel extract (RCP+PPE200) or apple peel extract (RCP+APE200).

Feed ingredients were ground to 1-mm particle size in preparation for chemical analysis. Prior to formulation, feed ingredients were analyzed for crude protein (CP), ether extract, starch and total sugars, according to the methods of the AOAC (2007). Metabolizable energy (ME) content of the feed ingredients was calculated based on feedstuff energy values of the tables of the WPSA (1989). All diets were formulated to meet minimum nutrient requirements established by the NRC (1994). The ingredients and calculated nutritional composition of the standard and reduced CP diets are given in Table 1. The α-tocopherol acetate (TA) was supplied by Kartal Chemistry Ltd. (Izmit, Turkey). The vitamin and trace mineral premixes were by from Topkim-Topkapı Drug Company Ltd. (Istanbul/Turkey).

Table 1 – Ingredients and calculated nutritional composition of the standard and reduced crude protein (CP) diets (fed on a dry matter basis, g/kg).

Ingredients	Standard CP diet	Reduced CP diet
Corn	534.5	602.2
Fish Meal	35.0	-
Soybean Meal	390.0	350.0
Vegetable Oil	17.0	9.2
Dicalcium Phosphate	4.9	9.5
DL-Methionine	-	0.9
Limestone	11.2	12.0
L-Lysine	-	2.5
L-Threonine	1.0	2.5
Salt	2.9	3.5
Potassium Carbonate	-	4.2
Vitamin Premix[1]	2.5	2.5
Trace Mineral Premix[2]	1.0	1.0
Calculated chemical composition		
Crude Protein, g/kg	240	210
Metabolizable Energy, kcal/kg	2900	2900
Calcium, g/kg	8.0	8.0
Available Phosphorus, g/kg	3.0	3.0
Sodium, g/ kg	1.5	1.5
Methionine+Cystine, g/kg	7.8	7.5
Lysine, g/kg	13.7	10.0
Threonine, g/kg	10.2	10.2

[1]Vitamin premix/kg diet: Vitamin A, 12 000 IU; vitamin D_3 1 500 IU; vitamin E, 50 mg; vitamin K_3 5 mg; vitamin B_1 3 mg; vitamin B_2 6 mg; vitamin B_6 5 mg; vitamin B_{12} 0.03 mg; niacin, 25 mg; Ca-D- pantothenate, 12 mg; folic acid, 1 mg; D-biotin, 0.05 mg; apo-carotenoic acid ester, 2.5 mg; choline chloride, 400 mg.

[2]Trace mineral premix/kg diet: Mn, 80 mg; Fe, 60 mg; Zn, 60 mg; Cu, 5 mg; Co, 0.20 mg; I, 1 mg; Se, 0.15 mg.

Pomegranate and apple peels

Pomegranate fruits (*Punicagranatum* L., Hicaznar) were supplied by the Agricultural Research Institute of West Mediterranean (Antalya, Turkey). Apple fruits (Starking variety) were supplied by a commercial firm in Tokat. The fresh fruits were manually peeled and cut using a shear. The fresh pomegranate and apple peels were immediately treated with 2% $CaCl_2$ in water at 55ºC ± 5 for 10 min to inhibit the oxidation of phenolic compounds and enzyme activities. The excess

of water was drained and the pomegranate and apple peels were collected. The pomegranate or apple peels were dried at 60ºC ± 2 for 48 h in an oven with air circulation, and then ground to 0.25-mm particle size (Sekhon-Loodu *et al.,* 2013).

Extraction of pomegranate and apple peels

The process of ultrasonic extraction of pomegranate and apple peel powder was performed in an ultrasonic bath Elmasonic S100H (Elma Schmidbauer GmbH, Singen, Germany) with a maximum capacity of 1 L (35 kHz, 140 W). In order to obtain the pomegranate and apple peel extract, 100 g of dried pomegranate and apple peel powder were sonicated in 1L 80% ethanol in water three times for 15 min at 10 min intervals between sonications (in triplicate). After the extraction, extracts were filtered using Whatman No. 1 filter papers under vacuum to remove peel debris. The extracts were concentrated to 150-200 mL using a rotary evaporator under reduced pressure at 37ºC at 120 rpm to remove solvents and filtered through Whatman No. 1 filter paper under vacuum. The remaining aqueous solutions were lyophilized at -50ºC and 0.028 mbar, and the crude extracts were kept in vacuum bags at -80ºC until use (Sekhon-Loodu *et al.,* 2013).

The phenolic compounds of pomegranate (PPE) or apple-peel extracts (APE) are given in Table 2.

Table 2 – Analyzed contents of phenolic compounds in APE and PPE (mg/kg extract)

Phenolic compounds	APE	PPE
Fumaric acid	81703	1305
Gentisic acid	262	94
Chlorogenic acid	474	93
Catechin	700	4076
4-hydroxybenzoic acid	448	15
Protocatechuic acid	161	156
Vanillic acid	330	130
Syringic acid	171	235
Rutin	192	101
Ellagic acid	100	10201
Scutellarin	-	110
Quercetin-3-β-D-glucoside	6181	20
Naringin	1	33
Diosmin	315	406
Morin	143	107
Quercetin	360	-

Measurements

Performance parameters

During the experimental period (14 to 35 days of age), the growth performance of quails was evaluated by recording body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) on a weekly basis. Quails were weighed using a scale with a sensitivity of ±0.1 g at hatch and of 1 g thereafter. On the same day, FI was recorded and FCR was calculated weekly as the amount of feed consumed per unit of BWG. Mortality was recorded daily throughout the experiment.

Blood analysis and plasma biochemistry parameters

At 35 days of age, 12 quails from each treatment group were randomly selected and bled from the brachial vein. The bleeding procedure was limited to 1 min or less to minimize the effects of handling stress. Quails were anesthetized with sodium pentobarbital injection (100 mg/kg) before slaughter.

In order to estimate heterophil to lymphocyte ratio (H/L), blood samples were collected in tubes containing EDTA and smeared on a glass slide. After drying, smears were stained with May-Grunwald and Giemsa stains (Gross & Siegel, 1983). One-hundred leukocytes were counted on one slide per quail using a light microscope at x 1,000 magnification. The H/L ratio was calculated by dividing the number of heterophils by the number of lymphocytes.

Blood for hematocrit determination was collected in heparinized capillary tubes and centrifuged in a microhematocrit centrifuge for 7 min at 11500 rpm.

At the end of this trial (35 d), blood samples from the brachial veins of 12 slaughtered quails were collected in tubes containing EDTA. Tubes were centrifuged for 15 min at 3000 rpm to separate the plasma, which was stored in Eppendorf tubes at -20ºC till analyses. Plasma samples were analyzed for total protein and uric acid levels using autoanalyser test kits (Audit Diagnostics, Ireland) and Audit Autoanalyser (Autolab, AMS Srl, The Netherlands), respectively, as described by the manufacturers. These plasma biochemistry parameters were determined at the Biochemistry Department, Faculty of Medicine, Tokat Gaziosmanpasa University.

Carcass parameters

On d 35, 12 quails whose BWs were similar to the group average were selected from each treatment groups and slaughtered by severing the jugular vein to determine preslaughter BW; the relative weights of the heart, liver and lung; hot and cold carcass yields; and breast and thigh meat yields. Carcasses were immediately plucked, processed (removal of head and feet), eviscerated (removal of gastrointestinal tract), weighed, and then chilled overnight in a refrigerator (+4ºC). Measurements included hot and cold carcass yields, breast and thigh meat yields, the relative weight of heart, liver and lung. The relative weight of heart, liver and lung and hot and cold carcass yields

were calculated as a percentage of preslaughter BW. The heart was removed and the right ventricle was dissected from the left ventricle and septum. The right and left ventricles were separately weighed and the ratio of right ventricular (RV) weight to total ventricular (TV) weight (RV/TV) was determined.

Liver antioxidant enzyme activities

The livers of the 12 quails slaughtered per treatment were immediately excised and thoroughly washed in ice-cold potassium phosphate buffer (pH 7.4) to remove the blood on the livers. Approximately 1 g liver was used to prepare the whole liver homogenate (WLH). The liver pieces were diluted 1:10 (wt/vol) with 50 mM an ice-cold Tris-HCl buffer (pH 7.4) and homogenized using an Ultra-Turrax homogenizer (Tekmar Co., Cincinnati, OH, USA). The homogenate was centrifuged for 30 min at 3500 rpm at 4ºC and the supernatant was collected to determine of liver antioxidant enzyme activities.

Liver glutathione peroxidase (GPx) activity was assessed by degradation of hydrogen peroxide (H_2O_2) in the presence of reduced glutathione, according to the method of Paglia & Valentine (1967) and expressed as units/mg protein. Catalase (CAT) activity was measured by the rate of H_2O_2 disappearance according to Aebi (1984) and expressed as units/mg protein. Liver superoxide dismutase (SOD) activity was determined following the xanthine oxidase method described by Sun *et al.* (1988) and expressed as units/mg protein. Protein concentrations of the WLH were determined by the method of Lowry *et al.* (1951).

Malondialdehyde assay

The breast and thigh meats of 12 quails slaughtered per treatment were used to determine the malondialdehyde levels of meats stored in refrigerator for 0 and 7 days. Malondialdehyde, the compound used as an index of lipid peroxidation, was determined by a selective third-order derivative spectrophotometric method (Botsoglou *et al.,* 2002). Samples were thoroughly homogenized (Polytron homogenizer, PCU, Littau/Lucerne, Switzerland) in the presence of 8 mL of aqueous trichloroacetic acid (50 g/L) and 5 mL of butylated hydroxytoluene in hexane (8 g/L), and the mixture was centrifuged. The top layer was discarded and a 2.5 mL aliquot from the bottom layer was mixed with 1.5 mL of aqueous 2-thiobarbituric acid (8 g/L) and further incubated at 70ºC for 30 min. Following incubation, the mixture was cooled to room temperature and submitted to conventional spectrophotometry (Shimadzu, Model UV-160A, Tokyo, Japan) at 530 nm. Malondialdehyde values were calculated by data of the computed least-squares fit of the standard calibration curve prepared using 1,1,3,3- tetraethoxypropane.

Statistical analysis

Data were analyzed according to a 2x5 factorial arrangement by two-way analysis of variance using the General Linear Model procedure of SPSS statistic package (SPSSWIN 2007). Dietary and temperature treatments and their interactions were evaluated. Significant differences among treatment means were separated using Duncan's multiple range test (Duncan 1955). Data were assumed to be statistically significant when $p<0.05$. Chi-square analysis was performed for mortality rates.

RESULTS AND DISCUSSION

Performance Parameters

Average BW of quails at 14 days of age was 54±0.306 g and did not differ statistically among the treatment groups. Weekly average body weight (BWs), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) of 14- to 35-d-old quails are shown in Table 3.

Table 3 shows that the supplementation of natural antioxidant sources (α-tocopherol acetate and pomegranate – and apple-peel extracts) to the reduced CP diet did not significantly affect quail BW, BWG, FI and FCR. As shown in Table 3, quail BWs at 21 ($p<0.05$), 28 ($p<0.01$) and 35 ($p<0.05$) days of age were reduced only when birds were maintained in HS. This result is in agreement with the findings of Bonnet *et al.* (1997) who reported that the BW of male broilers from 4 to 6 wks of age was decreased by HS compared with those maintained at TN temperatures. The reduced BW due to HS may be due lower amounts of nutrients available for growth because HS reduces nutrient digestibility (Hai *et al.,* 2000).

HS reduced quail BWG during the period of 14 to 35 days ($p<0.05$) compared with that of quails reared at TN. This result is consistent with those of Gonzalez-Esquerra & Leeson (2005), Faria Filho *et al.* (2007) and Gu *et al.* (2008), who reported that HS significantly decreased the BWG of broilers from 21 to 42 days of age. This may be explained by the reduction of the digestibility of protein, lipid and starch as result of HS (Zuprizal *et al.,* 1993; Bonnet *et al.,* 1997; Faria Filho *et al.,* 2007). Moreover, Hai *et al.* (2000) showed that the passage of the digesta from the crop or small intestine was suppressed by HS (32ºC) and that the activities

Table 3 – Effects of the experimental treatments on the body weight, body weight gain, feed intake and feed conversion ratio of quails reared under thermoneutral(TN) or heat stress (HS) conditions

DT	TT	BW (g)				BWG (g)	FI (g)	FCR (g:g)
		14 d	21 d	28 d	35 d	14-35 d	14-35 d	14-35 d
SCP	TN	54	107	156	192	138	540	3.90
	HS	55	105	153	192	137	507	3.70
RCP	TN	54	106	155	194	141	537	3.82
	HS	54	105	149	188	134	551	4.13
RCP+TA200	TN	54	107	156	194	140	531	3.78
	HS	54	103	150	190	136	579	4.27
RCP+PPE200	TN	53	107	157	196	143	541	3.80
	HS	54	105	148	187	133	553	4.17
RCP+APE200	TN	54	106	153	195	141	511	3.63
	HS	54	105	148	188	134	549	4.10
DTs	SCP	54	106	154	192	138	523	3.80
	RCP	54	106	152	191	137	544	3.97
	RCP+TA200	54	105	153	192	138	555	4.02
	RCP+PPE200	54	106	152	192	138	547	3.99
	RCP+APE200	54	105	151	191	137	530	3.87
SEM		0.306	1.356	2.008	2.948	2.871	17.907	0.111
TTs	TN[1]	53	106[a]	155[a]	194[a]	141[a]	532[b]	3.79[b]
	HS[2]	54	104[b]	150[b]	189[b]	135[b]	548[a]	4.07[a]
SEM		0.194	0.857	1.270	1.864	1.816	6.395	0.070
p-value								
DT			NS	NS	NS	NS	NS	NS
TT			*	**	*	*	*	**
DT x TT			NS	NS	NS	NS	NS	NS

[a-b] Values in the same column not sharing a common superscript significantly differ (*p<0.05;**p<0.01).

DT: dietary treatments; TT: temperature treatments; [1]TN: thermoneutral temperature; [2]HS: heat stress.

SCP: Diet with standard crude protein level (24% crude protein, 2900 kcalME/kg); RCP: Reduced crude protein diet (21% crude protein, 2900 kcalME/kg); RCP+TA200:RCP supplemented with 200 mg α-tocopherol acetate/kg; RCP+PPE200:RCP supplemented with 200 mg pomegranate peel extract/kg; RCP+APE200:RCP supplemented with 200 mg apple peel extract/kg

of three digestive enzymes (trypsin, chymotrypsin and amylase) and nutrient digestibility were reduced at HS.

Furthermore, in the present study, no significant BWG differences were determined in quails fed the SCP and RCP diets at both temperatures (TN and HS). This finding is in agreement with the result of Aydilek *et al.* (2012), who reported that there are no significant differences in the BWG of broilers fed diets with 18%or 23%CP and maintained at 22ºC or 30ºC. The results suggest that the reduced CP diet can support the same BWG as the standard CP diet when highly-digestible ingredients are used and digestible amino acids levels were well balanced in the diets (Widyaratne & Drew, 2011). On the other hand, our BWG results are different from the finding of Temim *et al.* (1999), who reported that feeding a high CP diet (20%vs. 25%) significantly increased the BWG of broilers from 28 to 42 days of age.

Quails submitted to HS presented higher FI during the period of 14 to 35 days (*p<0.05)* with those at TN. This may be due to the higher energy requirement for maintenance of broilers fed the low CP diets (Nieto *et al.,* 1997; Furlan *et al.,* 2004). The lack of influence of CP on FI observed in the present study is in agreement with the results of Gonzalez-Esquerra & Leeson (2005) and Aydilek *et al.,* 2012), who reported that broiler FI was not influenced by dietary CP content (18% and 23%).

Moreover, FCR was not influenced by the dietary treatments; however, quails submitted to HS presented significantly higher FCR from 14 to 35 days compared with those reared at TN (p<0.01). Faria Filho *et al.* (2007) also reported that HS significantly deteriorated the FCR of broilers between 21 and 42 days of age compared with those reared at TN. This may be due to the reduction of trypsin, chymotrypsin and amylase activities caused by HS, resulting in reduced digestibility and absorption of nutrients, such as protein, carbohydrate and lipids. In the present experiment, broilers fed the low CP diet supplemented with synthetic amino presented similar FCR as those fed the standard CP protein diet, independently of HS. Aydilek *et al.* (2012) also reported that broilers fed diets with two CP levels and reared under TN and HS

presented the same FCR. Moreover, our FCR results are consistent with the studies of Sahin *et al.* (2002) and Habibian *et al.* (2016), who did not find any influence of vitamin E supplementation on the FCR of broilers reared at TN or HS. On the other hand, Sahin & Kucuk (2001) demonstrated improvements in FCR in heat-stressed Japanese quail fed a diet supplemented with 250 mg/kg vitamin E.

Hematocrit value and heterophil/lymphocyte ratio

The effects of experimental treatments on hematocrit value and blood heterophil/lymphocyte (H/L) ratio of quails reared under TN and HS were summarized in Table 4.

As shown in Table 4, quails exposed to HS presented lower hematocrit value compared with those reared at TN (p<0.01).In addition, the SCP and RCP+PPE200 diets increased the hematocrit value of quails (p<0.05).The heterophil/lymphocyte ratio on day 35 of quails exposed to HS was increased compared with those reared in TN (p<0.05). Feeding the RCP+TA200 and RCP+PPE200 diets decreased the heterophil/ lymphocyte ratio of blood in quails compared to those of quails fed the SCP, RCP and RCP+APE200 diets (p<0.05). This finding is in agreement with the results of Ipek *et al.* (2007), who reported that dietary supplementation of vitamin C and E alone or in combination reduced the H/L ratio of heat-stressed Japanese quails. In addition, Prieto and Campo (2010) indicated that dietary allicin or capsaicin supplementation to heat-stressed white leghorn chickens decreased their H/L ratio compared with those fed a diet with no additives, suggesting that antioxidants compounds, such as allicin and capsaicin, may alleviate the oxidative deterioration caused by HS. Both Ipek *et al.* (2007) and Mahmoud *et al.* (2014) reported that dietary ascorbic acid (at 0.5 g/kg) reduced H/L ratio and plasma corticosterone level of Japanese quails and broilers under HS, respectively.

The reduced H/L ratio obtained in quails fed the diets supplemented with vitamin E and pomegranate extract in our study may be due to their inhibitory effect on glucocorticoid synthesis in birds (Sharifi *et al.,* 2016), which reduces plasma corticosterone level and consequently increasing lymphocyte numbers and reducing H/L ratio (Noyan, 1993).

Liver antioxidant enzyme activities

The effects of experimental treatments on the liver antioxidant enzyme activities of quails reared under TN and HS are summarized in Table 5.

Table 4 – Effects of experimental treatments on hematocrit value and heterophil/lymphocyte (H/L) ratio of quails reared under thermoneutral (TN) or heat stress (HS) conditions.

DTs	TTs	Hematocrit value	H/L ratio
SCP	TN	51.3	0.350
	HS	48.7	0.460
RCP	TN	49.9	0.450
	HS	46.1	0.600
RCP+TA200	TN	49.2	0.340
	HS	47.4	0.390
RCP+PPE200	TN	51.7	0.200
	HS	49.0	0.440
RCP+APE200	TN	49.0	0.520
	HS	48.1	0.510
DTs	SCP	50.0^{a}	0.410^{b}
	RCP	48.0^{b}	0.530^{a}
	RCP+TA200	48.4^{b}	0.370^{c}
	RCP+PPE200	50.4^{a}	0.320^{c}
	RCP+APE200	48.6^{b}	0.520^{a}
SEM		1.045	0.060
TTs	TN[1]	50.2^{a}	0.370^{b}
	HS[2]	47.9^{b}	0.480^{a}
SEM		0.661	0.038
p value			
DTs		*	*
TTs		**	*
DTs x TTs		NS	NS

$^{a-b}$ Values in the same column not sharing a common superscript significantly differ (*p<0.05; **p<0.01).

DT: dietary treatments; TT: temperature treatments; [1]TN: thermoneutral temperature; [2]HS: heat stress.

SCP: Diet with standard crude protein level (24% crude protein, 2900 kcal ME/kg); RCP: Reduced crude protein diet (21% crude protein, 2900 kcal ME/kg); RCP+TA200: RCP supplemented with 200 mg α-tocopherol acetate/kg; RCP+PPE200: RCP supplemented with 200 mg pomegranate peel extract/kg; RCP+APE200: RCP supplemented with 200 mg apple peel extract/kg.

As indicated in Table 5, quails under HS presented lower liver catalase activity compared with those reared under TN (p<0.05). This result is consistent with the findings of Jena *et al.* (2013) and Ayazi (2014), who reported that HS reduced liver catalase activity of broilers. The superoxide radicals formed during HS prevent the activity of catalase (Halici *et al.,* 2012). The reduced catalase activity observed in quails submitted to HS indicates that the exposure to HS impaired the birds' ability to detoxify H_2O_2 via catalase and hydrogen peroxide (H_2O_2) accumulation (Halici *et al.,*2012). Catalase reacts with the generated H_2O_2 resulting in molecular oxygen and water, protecting cells against hydrogen peroxide toxicity and lipid peroxidation (Jena *et al.,* 2013).

Higher liver catalase activity was observed especially when the RCP+TA200 and PRD+PPE200 diets were fed compared with the other diets (p<0.05). This is in agreement with the findings of Jena *et al.* (2013), who

Table 5 – The effects of experimental treatments on the liver antioxidant enzyme activities of quails reared under thermoneutral (TN) or heat stress (HS) conditions.

DTs	TTs	Catalase	SOD	GPX
SCP	TN	0.426	0.107	0.983
	HS	0.164	0.105	1.09
RCP	TN	0.281	0.109	1.19
	HS	0.210	0.073	0.398
RCP+TA200	TN	0.424	0.117	1.94
	HS	0.418	0.113	0.681
RCP+PPS200	TN	0.395	0.111	1.36
	HS	0.286	0.096	1.06
RCP+APE200	TN	0.291	0.111	0.692
	HS	0.223	0.074	1.00
DTs	SCP	0.295^{c}	0.106	1.05^{a}
	RCP	0.246^{e}	0.091	0.793^{c}
	RCP+TA200	0.421^{a}	0.115	1.31^{a}
	RCP+PPE200	0.341^{b}	0.104	1.17^{a}
	RCP+APE200	0.257^{d}	0.093	0.875^{b}
SEM		0.066	0.008	0.188
TTs	TN[1]	0.363^{a}	0.111	1.330^{a}
	HS[2]	0.260^{b}	0.092	0.751^{b}
SEM		0.042	0.005	0.119
p value				
DTs		*	NS	*
TTs		*	NS	*
DTs x TTs		NS	NS	NS

$^{a-c}$ Values in the same column not sharing a common superscript significantly differ (*p<0.05).

DT: dietary treatments; TT: temperature treatments; [1]TN: thermoneutral temperature; [2]HS: heat stress. SOD: Superoxide dismutase; GPX: Glutathione peroxidase

SCP: Diet with standard crude protein level (24% crude protein, 2900 kcal ME/kg); RCP: Reduced crude protein diet (21% crude protein, 2900 kcal ME/kg); RCP+TA200: RCP supplemented with 200 mg α-tocopherol acetate/kg; RCP+PPE200: RCP supplemented with 200 mg pomegranate peel extract/kg; RCP+APE200: RCP supplemented with 200 mg apple peel extract/kg.

observed that catalase activity in the erythrocytes of broiler breeders at the 8th week of their experiment was significantly higher with a diet supplemented with vitamin E than with the control diet. In addition, the obtained result indicates that vitamin E and pomegranate peel extract exert their antioxidant effect in quails under HS by neutralizing hydrogen peroxide radicals donating one electron to free radical chains (Avanzo *et al.,* 2001). On the other hand, Halici *et al.* (2012) found that dietary vitamin E supplementation reduced the activity of catalase in the muscle tissue in heat-stressed Japanese quails.

No effects of DT or TT on liver superoxide dismutase activity of liver in quails was detected (p>0.05).

Lower glutathione liver peroxidase (GPx) activity was observed in HS quails compared with those reared under TN (p<0.05). Moreover, higher GPx activity of liver was detected in quails fed the SCP, RCP+TA200 and RCP+PPE200 diets compared to the RCP and RCP+APE200 diets (p<0.05), and those fed the RCP+APE200 diet presented significantly higher GPx activity compared to those fed the RCP diet (p<0.05). This finding is in agreement with the results of Halici *et al.* (2012) and Ayazi (2014), who found that vitamin E supplementation to the diet of heat-stressed quails increased their liver GPx activity.

Genena & Agamy (2017) reported fed pomegranate peel promoted a significant increase in plasma catalase (CAT) and GPx activities in hyperlipidemic rats. The antioxidant properties of pomegranate peel are possibly due to its content of potent tannins and anthocyanins that scavenge a wide spectrum of free radicals (Gil *et al.,* 2000; Aviram *et al.,* 2000). Pomegranate peel extract may be an important factor in protecting the tissue against oxidative injury by increasing the free-radical scavenging activity of CAT and GPx (Moneim, 2012). Moneim (2012) showed that pomegranate peel extract significantly increased brain CAT activity in rats. Pomegranate can counteract oxidative stress effects through its antioxidant properties (Dkhil *et al.,* 2013). The results obtained in the present study with pomegranate peel extract may be attributed to its phenolic compounds and linolenic acid content. The antioxidant activity of phenolic compounds and linolenic acid are mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Wei *et al.,* 2015). Wei *et al.* (2015) reported that pomegranate administration to rats induced by carbon tetrachloride significantly enhanced their liver GPx activity.

Plasma total protein and uric acid levels

The effects of experimental treatments on plasma total protein and uric acid levels in quails reared under TN and HS were given in Table 6.

As shown in Table 6, both DT and TT influenced plasma uric acid level (p<0.05). Quails exposed to HS presented lower plasma uric acid levels compared to those reared at TN (p<0.05). According to Imik *et al.* (2013), increasing concentrations of plasma corticosterone during HS increased the rate of uric acid excretion. The uric acid level results of the present study are in agreement with the findings of Adams (1968) and Siegel & Van Kampen (1984), who showed that uric acid levels were reduced in broilers submitted to HS.

The SCP, RCP+TA200, RCP+PPE200 and RCP+APE200 diets increased plasma uric acid levels

Table 6 – Effects of experimental treatments on plasma total protein and uric acid levels in quails reared under TN and HS.

DTs	TTs	Uric Acid	Total Protein
SCP	TN	5.79	2.73
	HS	5.37	2.93
RCP	TN	4.09	2.67
	HS	3.11	2.20
RCP+TA200	TN	5.61	2.68
	HS	4.36	2.64
RCP+PPE200	TN	4.88	2.96
	HS	4.56	2.29
RCP+APE200	TN	4.76	2.62
	HS	3.52	2.49
DTs	SCP	5.58[a]	2.83
	RCP	3.60[c]	2.43
	RCP+TA200	4.98[ab]	2.66
	RCP+PPE200	4.72[ab]	2.63
	RCP+APE200	4.14[b]	2.56
SEM		0.363	0.139
TTs	TN[1]	5.02[a]	2.73
	HS[2]	4.18[b]	2.51
SEM		0.230	0.089
p value			
DTs		*	NS
TTs		*	NS
DTs x TTs		NS	NS

[a-c] Values in the same column not sharing a common superscript significantly differ (*p<0.05).

DT: dietary treatments; TT: temperature treatments; [1]TN: thermoneutral temperature; [2]HS: heat stress.

SCP: Diet with standard crude protein level (24% crude protein, 2900 kcal ME/kg); RCP: Reduced crude protein diet (21% crude protein, 2900 kcal ME/kg); RCP+TA200: RCP supplemented with 200 mg α-tocopherol acetate/kg; RCP+PPE200: RCP supplemented with 200 mg pomegranate peel extract/kg; RCP+APE200: RCP supplemented with 200 mg apple peel extract/kg.

of quail compared with the RCP diet (*p<0.05).* This result is consistent with the findings of Imik *et al.* (2013), who reported that the plasma uric acid levels of heat-stressed broilers were enhanced by the dietary supplementation of vitamin C or α-lipoic acid. On the other hand, Sahin *et al.* (2002) showed that, during HS, increasing concentrations of ACTH enhanced serum uric acid levels, whereas vitamin E supplementation reduced uric acid levels. Likewise, Yassein *et al.* (2015) showed that the dietary supplementation of pomegranate peel powder to quail diets significantly decreased plasma uric acid level.

Plasma total protein levels of quails in the present study were not affected neither by DTs nor TTs. This is consistent with the findings of Sahin *et al.* (2002) and Imik *et al.* (2013), who demonstrated that dietary supplementation of different antioxidants (vitamin C, α-lipoic acid or vitamin E) did influence plasma total protein levels.

3.5. Carcass traits

The effects of the experimental treatments on the carcass traits of quails are summarized in Table 7.

As shown in Table 7, heat-stressed quails presented significantly lower liver relative weight compared to those reared under TN (p<0.05). Tawfeek *et al.* (2014) also showed lower liver yield in broilers submitted to HS, and Konca *et al.* (2009) and Tawfeek *et al.* (2014), who reported that the dietary antioxidant supplementation did not have any significant effect on the liver relative weight of broilers.

A significant interaction between DTs and TTs was determined for lung relative weight of the evaluated quails (p<0.05). The lung relative weights of the quails fed the SCP, RCP and RCP+APE diets were not significantly affected by TTs; however, the quails fed the RCP+TA200 and RCP+PPE200 diets presented significantly lower lung relative weights when submitted to HS compared with TN (p<0.05).The relative weights of lung of quails exposed to TN were not significantly influenced by DTs, but in those submitted to HS, lung relative weights were significantly reduced by all the evaluated diets compared with the SCP diet (p<0.05).

HS significantly decreased the hot and cold carcass yields of quails compared to those of quails exposed to TN (p<0.05). This result is in agreement with the findings of Tawfeek *et al.* (2014), who reported reduced carcass yield in heat-stressed broilers compared with those in TN conditions. This reduction may be due to impaired protein synthesis by HS (Temim *et al.,* 2000). Conversely, Habibian *et al.* (2016) reported that the carcass yield of broilers was not influenced by environmental temperature.

There is a significant interaction between DTs and TTs for hot and cold carcass yields in the present experiment (p<0.05). The hot and cold carcass yields of quails exposed to TN were significantly increased by the SCP, RCP+TA200 and RCP+PPE200 diets compared to those of quails fed the RCP and RCP+APE200 diets (p<0.05). On the other hand, the hot and cold carcass yields of quails exposed to HS were not significantly affected by the DTs.

The ratio of the right ventricular weight to the total ventricular weight (RV/TV ratio) was significantly higher in HS compared with TN (p<0.05). It is evident that quails exposed to HS are in a pre-ascitic condition. In contrast to our finding, some studies indicated that the observed significant increase of the RV/TV ratio was due to a reduction in dietary CP (Behrooj *et al.,* 2012; Sharifi *et al.,* 2015a,b). In addition, Sharifi *et al.* (2015a,b) also reported that the dietary

Table 7 – Effects of experimental treatments on carcass traits of quails reared under thermoneutral (TN) or heat stress (HS) conditions.

DTs	TTs	PSBW, g	Liver, %	Lung, %	Hot Carcass Yield,%	Cold Carcass Yield,%	RV/TV	Breast Meat Yield,%	Thigh Meat Yield,%
SCP	TN	192	2.34	$^{A}1.11^{a}$	$^{A}73.0^{a}$	$^{A}72.7^{a}$	0.240	38.7	24.9
	HS	192	2.30	$^{A}1.12^{a}$	$^{A}70.6^{b}$	$^{A}70.2^{b}$	0.243	38.6	25.3
RCP	TN	191	2.38	$^{A}1.09^{a}$	$^{B}70.0^{a}$	$^{B}69.7^{a}$	0.249	38.4	25.0
	HS	189	2.25	$^{B}1.00^{a}$	$^{A}69.5^{a}$	$^{A}69.2^{a}$	0.272	38.8	23.3
RCP+TA200	TN	192	2.47	$^{A}1.04^{a}$	$^{A}74.0^{a}$	$^{A}73.6^{a}$	0.246	38.6	25.1
	HS	189	1.86	$^{C}0.80^{b}$	$^{A}69.9^{b}$	$^{A}69.5^{b}$	0.252	38.3	24.5
RCP+PPE200	TN	196	2.51	$^{A}1.12^{a}$	$^{A}72.1^{a}$	$^{A}71.8^{a}$	0.246	38.3	24.3
	HS	186	1.92	$^{C}0.77^{b}$	$^{A}69.0^{b}$	$^{A}68.7^{b}$	0.262	38.8	24.5
RCP+APE200	TN	194	2.51	$^{A}1.04^{a}$	$^{B}71.1^{a}$	$^{B}70.8^{a}$	0.250	38.3	24.8
	HS	185	1.99	$^{B}0.98^{a}$	$^{A}68.6^{b}$	$^{A}68.3^{b}$	0.267	38.7	23.7
	SCP	192	2.32	1.11	71.8	71.4	0.241	38.7	25.1
	RCP	190	2.31	1.05	69.8	69.4	0.260	38.6	24.2
	RCP+TA200	191	2.16	0.92	71.9	71.6	0.249	38.4	24.8
	RCP+PPE200	191	2.21	0.94	70.6	70.2	0.254	38.6	24.4
	RCP+APE200	189	2.25	1.01	69.9	69.5	0.259	38.5	24.2
SEM		1.500	0.111	0.055	0.354	0.352	0.007	0.362	0.238
TTs	TN	193	2.44^{a}	1.08	72.1^{a}	71.7^{a}	0.246^{b}	38.4	24.8
	HS	188	2.06^{b}	0.93	69.5^{b}	69.2^{b}	0.259^{a}	38.6	24.3
SEM		0.949	0.070	0.035	0.224	0.223	0.004	0.229	0.150
p value									
DTs		NS	NS	NS	NS	NS	NS	NS	NS
TTs		NS	*	NS	*	*	*	NS	NS
DTs xTTs		NS	NS	*	*	*	NS	NS	NS

$^{a-c}$ Values in the same column not sharing a common lowercase superscript indicate significant differences between temperature treatments (*p<0.05).

$^{A-C}$ Values in the same column not sharing a common uppercase superscript indicate significant differences among dietary treatments (*p<0.05)

DT: dietary treatments; TT: temperature treatments; [1]TN: thermoneutral temperature; [2]HS: heat stress. PSBW: preslaughter body weight; RT/TV: right ventricle weight to total ventricle weight ratio.

SCP: Diet with standard crude protein level (24% crude protein, 2900 kcal ME/kg); RCP: Reduced crude protein diet (21% crude protein, 2900 kcal ME/kg); RCP+TA200: RCP supplemented with 200 mg α-tocopherol acetate/kg; RCP+PPE200: RCP supplemented with 200 mg pomegranate peel extract/kg; RCP+APE200: RCP supplemented with 200 mg apple peel extract/kg to RCP diet

supplementation of an antioxidant to reduced-CP diet of broilers reduced the RV/TV ratio.

As indicated in Table 7, neither DTs nor TTs significantly influenced quail breast and thigh meat yields. These results are in agreement with the findings of Faria Filho *et al.* (2005) and Habibian *et al.* (2016), who showed that the breast and thigh meat yields of 49-day-old broilers were not affected by environmental temperature. On the other hand, Ain Baziz *et al.* (1996) and Azad *et al.* (2010) demonstrated that broilers chronically reared under HS presented a significant decrease in breast meat yield. Our results concur with the findings of Behrooj *et al.* (2012) and Sharifi *et al.* (2015a,b), who reported no significant breast and thigh meat yield differences between broilers fed a reduced CP diet and a standard CP diet. In addition, Sharifi *et al.* (2015b) did not find any significant breast meat yield differences among broilers fed SCP, RCP and RCP+L-carnitine diets. Habibian *et al.* (2016) also reported that the breast meat yield of TN and heat-stressed broilers was not influenced by vitamin E supplementation.

Malondialdehyde values of breast and thigh meat

The effects of the experimental treatments on the malondialdehyde (MDA) values of the breast and thigh meats of quails are summarized in Table 8.

As indicated in Table 8, higher breast meat MDA values were determined in HS quails than those reared at TN both on days 0(p<0.01) and 7 (p<0.05). This is in agreement with the results of Gu *et al.* (2008) and of Habibian *et al.* (2016), who reported that the MDA levels in the breast meat was significantly increased when broilers were submitted to HS. Oxidative stress is considered as part of the stress response of broilers to heat exposure. Birds are not able to eliminate the free radicals caused by HS from the body and the level of lipid oxidation is increased (Gu *et al.,* 2008; Habibian *et al.,* 2016). Aoyagi *et al.* (1997) found that broilers exposed to HS had lower antioxidant defense capacity and higher lipid peroxidation level and MDA values in the plasma and the liver. In the present study, the increase of MDA values in the breast meat of HS quails indicates cell damage by HS.

Table 8 – The effects of experimental treatments on malondialdehyde values in the breast and thigh of quails reared under TN and HS conditions.

DTs	TTs	Day 0	Day 7
		Breast Thigh	Breast Thigh
SCP	TN	0.356 0.385	1.60 1.50
	HS	0.924 1.000	1.67 2.20
RCP	TN	0.635 0.369	1.89 2.48
	HS	0.880 1.130	2.17 3.11
RCP+TA200	TN	0.312 0.231	0.80 1.19
	HS	0.705 0.776	1.48 1.46
RCP+PPE200	TN	0.265 0.255	1.18 1.13
	HS	0.716 0.911	1.26 1.46
RCP+APE200	TN	0.234 0.364	1.58 1.43
	HS	0.778 1.080	2.08 2.34
DTs	SCP	0.640^{b} 0.692^{b}	1.64^{c} 1.85^{b}
	RCP	0.757^{a} 0.749^{a}	2.03^{a} 2.79^{a}
	RCP+TA200	0.509^{c} 0.503^{d}	1.14^{d} 1.32^{c}
	RCP+PPE200	0.490^{c} 0.583^{c}	1.22^{d} 1.29^{c}
	RCP+APE200	0.506^{c} 0.721^{a}	1.83^{b} 1.89^{b}
SEM		0.074 0.063	0.200 0.281
TTs	TN[1]	0.360^{b} 0.321^{b}	1.41^{b} 1.55^{b}
	HS[2]	0.801^{a} 0.979^{a}	1.73^{a} 2.11^{a}
SEM		0.047 0.040	0.127 0.178
p value			
DTs		* *	* **
TTs		** **	* **
DTs x TTs		NS NS	NS NS

[a-d] Values in the same column not sharing a common superscript significantly differ (*p<0.05).

DT: dietary treatments; TT: temperature treatments; [1]TN: thermoneutral temperature; [2]HS: heat stress.

SCP: Diet with standard crude protein level (24% crude protein, 2900 kcal ME/kg); RCP: Reduced crude protein diet (21% crude protein, 2900 kcal ME/kg); RCP+TA200: RCP supplemented with 200 mg α-tocopherol acetate/kg; RCP+PPE200: RCP supplemented with 200 mg pomegranate peel extract/kg; RCP+APE200: RCP supplemented with 200 mg apple peel extract/kg.

The dietary treatments also significantly influenced breast meat MDA values both at 0 and 7 days of storage (p<0.05). The lowest breast meat MDA values on day 0 were obtained when the RCP+TA200, RCP+PPE200 and RCP+APE200, and with the RCP+TA200 and RCP+PPE200 diets on day 7 of storage (p<0.05). These results are consistent with those of Habibian *et al.* (2016), who showed that the dietary supplementation of vitamin E reduced the level of lipid oxidation (MDA value) of the breast meat of broilers under HS, and with those of Saleh *et al.* (2015, 2017), who reported that dietary pomegranate peel extract significantly delayed the lipid oxidation of broiler meat. These findings may be attributed to polyphenolic compounds with the antioxidant activity present in PPE (Saleh *et al.,* 2017), in addition to the synergism among phenolic compounds (Devatkal *et al.,* 2010). These polyphenolic compounds enter the circulatory system, distributed, and retained in breast meat of broilers, where they remain functional (Saleh *et al.,* 2017). The antioxidant activities of these polyphenols are a result of their ability to donate hydrogen molecules to block free radical chain reactions during the oxidation process, converting them into stable end products (Qin *et al.,* 2013). In addition, polyphenols act as antioxidants by scavenging free radicals and binding metals, as well as to their reducing power (Naveena *et al.,* 2008 a,b; Selani *et al.,* 2011; Mahmmod 2014).

Higher thigh meat MDA values were detected in HS quails compared with those maintained at TN both on days 0 (p<0.05) and 7 (p<0.01)of storage. This finding does not agree with the results of Gu *et al*. (2008), who did not find any significant effect of HS on thigh meat MDA values of broilers. The lowest thigh meat MDA value on day 0 of storage was obtained in the quails fed the RCP+TA200 diet (p<0.05), and on day 7 with the RCP+TA200 and RCP+PPE200 diets (p<0.01).

CONCLUSION

In conclusion, pomegranate peel and apple peel extracts can be used as alternative natural antioxidant sources to vitamin E in the diets of quails exposed to heat stress and fed a reduced crude protein diet, as shown by the growth performance, blood hematocrit and H/L ratio, plasma uric acid level, liver catalase and glutathione peroxidase activities, and meat lipid peroxidation results.

DISCLOSURE STATEMENT

No potential conflict of interest is reported by the authors.

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