The Journal of Animal & Plant Sciences, 29(3): 2019, Page: 657-666
ISSN: 1018-7081

EFFECTS OF OLIVE LEAF EXTRACT (*OLEA EUROPEA L.*) ON GROWTH PERFORMANCE, BLOOD METABOLITES AND ANTIOXIDANT ACTIVITIES IN BROILER CHICKENS UNDER HEAT STRESS

M. J. Agah[1], M. T. Mirakzehi[2] and H. Saleh[2*]

[1]Animal Science Research Department, Fars Agricultural and Natural Resources Research and Education Center, Agricultural Research, Education and Extension Organization (AREEO), Shiraz, Iran
[2]Department of Animal Science, Higher Educational Complex of Saravan. P.O. Box: 99516-34145, Saravan, Sistan and Baluchestan, Iran.
[*]Correspondence Author E-mail: hsaleh.um@gmail.com

ABSTRACT

The aim of the study was to investigate the effects of olive leaf extract (*Olea europea L.*) (OLE) and α-tocopheryl acetate (α-Toc) on growth performance, nutrient digestibility, blood metabolites and antioxidant activities in heat exposed broiler chickens. Total 200 day-old male Ross 308 chicks were distributed in a completely randomized experimental design, with four treatments and five replicates of 10 birds each. Heat stress was applied for 5 h (33°C) from 28 to 42 days. The treatments were: negative control (without α-Toc and OLE; NC); a positive control (with 250 mg kg-1 of α-Toc; PC) and diets with 200 or 400 mg of OLE/kg of diet (OLE1and OLE2, respectively). Heat stress (32± 2°C) was applied daily for all the birds. On day 42, blood samples were taken from two birds per replicate. Chromic oxide was used as inert ileal digestibility markers. The evaluated treatments did not significantly affect body weight gain, feed intake and feed conversion ratio. Ileal digestibility of energy, crude ash, Crude Protein, and phosphorus showed a linear increase with supplementation of OLE and α-toc ($p \leq 0.05$). Cholesterol, triglyceride, Alanine transaminase (ALT) and aspartate aminotransferase (AST) in blood were significantly decreased when chickens were fed diets containing OLE ($P \leq 0.05$). Plasma lipid peroxidation leve land glutathione peroxidase activities were reduced in chickens fed diets supplemented with OLE and α-Toc ($P \leq 0.05$). In conclusion, Supplementation of olive leaf extract to broiler diets as antioxidant components could improve the antioxidant status and reduction in the stressor index of heat stressed broilers.

Key words: Phenolic content, olive leaf, α-tocopheryl acetate, broiler, heat stress.

INTRODUCTION

Heat stress is one of the most important stressors, especially in the hot regions of the world. The birds try to compensate for their reduced ability to dissipate heat during heat distress via increasing their physiological processes (such as, panting, elevated body temperature, and respiratory alkalosis, etc.) and those responsible for decreasing heat production (Teeter and Belay, 1996; Sahin and Kucuk, 2003). Under these metabolic conditions, reduced growth performance and survivabilityoccur in broiler with exposure to high ambient temperature (Donkoh, 1989; Siegel, 1995). Lower plasma concentrations of antioxidant vitamins and minerals increased oxidative damage were observed in stressed poultry (Sahin and Kucuk, 2003). Furthermore, reduced plasma protein concentrations and markedly increased blood glucose and cholesterol concentrations have been reported in poultry subjected to high ambient temperature (Donkoh, 1989; Sahin *et al.* 2002). It also causes increased production of oxygen-derived free radicals, which may give rise to oxidative stress (Sahin and Kucuk, 2003). Oxidation is a very general process affecting lipids, pigments, proteins, DNA, carbohydrates and vitamins (kanner, 1994), however, in excess it can be very harmful. Different indicators were used to evaluate the antioxidative effect (Saleh *et al*., 2017). Contents of isolated malondialdehyde (MDA) and antioxidant enzymes in the blood can generally be used as a biomarker for radical-induced damage and endogenous LPO (Wang *et al.,* 2008; saleh *et al*., 2017). Recent studies have shown that the detrimental effect of heat stress could be partly the result of oxidative stress (Sahin and Kucuk, 2003; Hosseini-Vashan*et al.* 2012). It has been suggested that many of the negative outcome of lipid oxidation and oxidative stressin chicken was diminishedby the use of diets containing such antioxidants as medicinal herb mix and grape pomace and extract peel pomegranate, which are natural antioxidants rich in polyphenols and flavonoids(Sahin and Kucuk, 2003; Goñi *et al.* 2007; Saleh *et al*. 2016). The studies have shown that polyphenols have the capacity to act as powerful antioxidants by scavenging free radicals and terminating oxidative reactions (Yilmaz and Toledo, 2004).The studies have shown that polyphenols from herbal plants have been explored as possible antioxidants (Tuzcu *et al.* 2008; Hosseini-Vashan *et al.* 2012).

The olive tree (*Olea europea* L.) has been widely accepted as one of the species with the highest antioxidant activity via its oil, fruits, and leaves (Bouaziz *et al.* 2004). It is well known that the activity of the olive tree by-product extracts in medicine and food industry is due to the presence of some important antioxidant and phenolic components to prevent oxidative degradations (Allouche *et al.,* 2004).The olive tree has long been recognized as having antioxidant molecules, such as oleuropein, aglycone, oleuropein, hydroxytyrosol, and tyrosol (Jemai *et al.* 2008).Oleuropein is generally the most prominent phenolic compound in olive cultivars and may reach concentrations of up to 140 mg g À1 on a dry matter basis in young olives 60-90 mg/g in dried leaves (Ryan *et al.* 2002). Oleuropein possesses beneficial pharmacological effects such as spasmolytic, immune-stimulant, cardioprotective, hypotensive, anti-inflammatory, antioxidant effects (Visioli *et al*. 1998). Many of these properties have been described as resulting from the antioxidant character of oleuropein (Visioli *et al.* 2002).

The purpose of this research was to investigate the effects of dietary olive leaf extract (OLE) as a dietary antioxidant source on growth performance, nutrient digestibility, blood metabolites and antioxidant system of broiler chickens exposed to high ambient temperature.

MATERIALS AND METHODS

Preparation of olive leaf extract: The researchers collected yellow cultivar leaves of olive trees from olive research station of Fars province in Kazeroon, Fars, Iran in February2015 were manually removed air dried under ambient conditions and powdered in a grinder to pass 40-mesh, and then were packed and stored at −20 °C until extraction. Dried powder leaves (2.5 g) were extracted with 40 ml of methanol solvent at a room temperature for 6 h. The extract was filtered through Whatman 47 mm× 0.45 µ filters. The filter paper to be removed with fine particles. After extraction, the solvent was evaporated using a rotary evaporator (Laborota 4000, Heidolph, Germany).The aqueous extract was evaporated at mild temperatures (40-50ºC), in order to avoid the decomposition of the phenolic compounds.

Determination of the DPPH radical cation scavenging activity: 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was estimated a stable and rapid method to measure the antioxidant activity of plant extracts according toSamarth *et al.,*(2007) method with slight modifications. Purified DPPH (Wako Pure Chemical Industries Ltd., Osaka, Japan) was used as a standard in the analysis. Different concentrations of test samples, including OLE and Butylated hydroxytoluene (BHT) (2.5, 5, 10, 20, 40 and 80 µg/ml) were prepared. Briefly, to test tubes containing 1 mL of DPPH (0.006%) in methanol, 1 ml of OLE-methanol solution sample, 1 ml of BHT methanol solution sample, or 1 ml of methanol as the control were added. The absorbance of the solution was measured at 512 nm using a spectrophotometer (UV–vis spectrophotometer SP 8001, Metertech Inc). The inhibition rate of DPPH radical as a percentage (I %) was calculated as follows:

$I\% = (A0 - A1)/A0 \times 100,$

Where A0 is the absorbance of the control reaction (containing all reagents except the test compound) and A1 is the absorbance of the test compound. The amount of sample needed to decrease the initial DPPH concentration by 50%, IC50, was calculated graphically.

Birds, husbandry and treatments: The experimental protocol was approved by the Animal Care Committee of the Fars Research Center for Agriculture and Natural Resources, Shiraz, Iran. A total of 200 male 1-day-old male broiler chickens (Ross 308) were obtained from a commercial hatchery (Larin Amol, Mazandaran, Iran). Birds were maintained under recommended environmental temperature of Ross committee from day 1 to 28. At 28 d of age, the birds were weighed, and randomly allotted to 20 floor pens (1×1 m) with 10 birds each. Broilers were allotted in a completely randomized experimental design, to 4 groups with 5 replicates of 10 birds each and reared from 42 days. Four dietary treatments (Table 1) included negative control diet (without feed additives; NC), positive control diet (mixed with 250 mg · kg^{-1} α-tocopheryl acetate (Razak, Tehran, Iran); PC), and two groups fed with 200 or 400 mg of OLE/kg of diet (OLE1and OLE2, respectively). All diets were isocaloric and isonitrogenous according to ROSS 308 recommendation. During the experimental period, relative humidity and ventilation rate were 44±6% and 0.12 ms^{-1}, respectively. Light with approximately 20 Lux was made available around the clock. Water and mash diets were provided *ad libitum* and exceed the nutritional requirements of chickens as recommended by the broiler management guide (Aviagen, 2014). Heat stress was applied from 9:30 to 15:30 daily for all the birds from day 28 to 42. The temperature was increased gradually within 2h from 21 to 32± 2°C, and was applied for all the birds. The feeding experiment period lasted 14 days (28-42 day age).

Experimental procedures: Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) was measured during the experiment. Feed intake was determined from the difference between supply and residual feed in each pen. Broilers were weighed at the beginning and at the end of the experiment and feed efficiency ratio were calculated at the end of the experimental period.At d 32, birds were fasted for 16 h, then 4 experimental diets containing 0.3 % chromium oxide were assigned until 38 d of age, where excreta was

collected during d 36–38 to determine the apparent digestibility of dry matter (DM), crude protein (CP), and ether extract (EE) and the retention of ash, Ca and P. Feed and excreta samples were stored at -20°C until laboratory analysis. Feed and excreta samples were then freeze-dried prior to analysis. Dry matter, CP, EE, crude fat, ash, calcium, and phosphorus were determined in the feed and excreta according to standard methods (AOAC, 2005), and gross energy content was measured using an adiabatic bomb calorimeter (Model 1281, Parr, Moline, IL) to calculate CP retention and apparent nutrient digestibility. Chromium concentration was measured in dried feed and excreta samples by atomic absorption spectrometry (Varian SpectrAA 50B Atomic Absorption Spectrometer, Varian Ltd, USA) following the procedure established by Williams *et al.* (1962).

Two broiler chickens were randomly selected from each pen at 42 days of life. Blood was collected *via* wing vein puncture using a syringe and about 2 ml of blood was immediately placed in a heparinized tube and kept on ice to obtain the plasma samples and then centrifuged.Total protein, lipids, glucose, uric acid blood enzyme activity, including aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP). The activity of blood enzyme, including AST, ALT and ALP was determined in plasma samples by Auto-analyzer spectrophotometer instrument (BioSystems S.A. Costa Brava 30, 08030 Barcelona, Spain). Thiobarbituric acid reactive substances (TBARs) and the activity of antioxidant enzymes, includingsuperoxide dismutase (SOD) and glutathione peroxidase (GPx) were determined in plasma (Yagi 1984). The GPx and SOD activity were measured using RANSEL and RANSOD kits (Randox Laboratories Limited, Crumlin, UK). The TBARS concentration in plasma was measured according to Draper and Hadley (1990) method. The plasma of TBARS concentration was expressed as nmol ml^{-1}.TBARS was expressed as nanomoles of MDA per milliliter of plasma. Total antioxidant power (TAP) was determined by ferric reducing antioxidant power (FRAP) assay as described by Benzie and Strain (1996). The measurement was conducted at room temperature and a 5-min were the same as that of plasma.

Statistical analysis: Data were collected and analyzed as a completely randomized design using the general linear model procedure of SAS (Version 9.1, SAS Institute, 2003). Chicken data from every pen were averaged before further analysis and the pen was the independent experimental unit.Significant differences among treatments were determined at $P < 0.05$ by Duncan's multiple range tests. The statistical model of the experiment is shown below.

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:

Y_{ij}: being any observation
μ: is the general mean
T_i: effect of the i-th treatment
e_{ij}: effect of random error

RESULTS

Antioxidant activity of OLE: Inhibition of DPPH radicals due to the scavenging ability of OLE and BHT is shown in Figure 1. The scavenging effect of OLE and BHT on DPPH radicals increased in a dose-dependent manner. An IC50 concentration of OLE and BHT was calculated 28.02 and 18.51 micrograms per milliliter respectively.

Growth Performance: In general, no differences in BWG, FI, or FCR was observed in male broilers fed with different diets (Table 2) (P>0.05). The birds fed diet containing OLE1 had a marginally higher body weight in comparison with the other treatments (98.54 g/day, P=0.078).

Apparent Digestibility: The results of the apparent digestibility of energy, CP, Ash, EE, Ca and P are shown in Table 3. In general, the supplementation of α-tocopheryl acetate and plant extract improved all items of digestibility except for the EE and Ca compared to NC treatment. Apparent digestibility of energy, CP, ash and P was greater (P<0.01) in the PC group compared with the NC and OLE2 groups. However, no effect of supplementation was detected for EE digestibility (P > 0.05). For Ca, the highest value of fecal apparent digestibility was detected in chickens fed diet supplemented with OLE1 and significantly was different compared to those fed NC and OLE2 diets. Results showed that birds fed OLE2 had significantly lower CP apparent digestibility compared to those fed PC (70.12 vs. 81.98).

Blood metabolites: Tables 4 and 5 summarize data obtained on the effect of experimental treatments on blood metabolites and some serum enzymes, respectively. No significant influence of experimental diets on blood glucose and total protein was observed (P>0.05). Data showed that the lowest levels of blood cholesterol and triglyceride were obtained in OLE1 and OLE2 groups, respectively, and were significantly different compared to the NC group. For the blood concentration of uric acid, birds fed OLE2 diet had significantly lower levels compared to the other treatments (P<0.01). Results in Table 5 showed that supplementation of OLE at the level of 400 mg/kg of diet affected ALT and ALP activity by the same pattern of changes. The birds fed OLE2 had a significantly lower activity of ALT and ALP, compared to those fed NC diets (P<0.05). Dietary treatments did not affect AST activity (P>0.05).

Antioxidant status: Results in Table 6 showed that the plasma level of FRAP was significantly increased in PC, OLE1, and OLE2 groups compared to NC group respectively (P<0.05). The highest level of FRAP was obtained in PC group, but the differences were not significant compared to OLE1 group (949.5 vs. 856.5 nmol/ml, P>0.05). Results showed that dietary OLE supplementation at 400 mg/kg seemed to have no favorable effect on plasma antioxidant power as reflected by FRAP. The Dietary treatments had no influence on the plasma SOD activity (P>0.05). Based on plasma GPx activity, experimental groups could be arranged into the following order: OLE1>OLE2>PC>NC, with a statistically significant difference between NC and other groups (P<0.05). Compared to the other groups, chickens fed OLE2 diet had significantly lower plasma concentration of MDA at heat stress conditions (P<0.01). MDA was not significantly different among birds fed PC and OLE1 diets (P>0.05).

Table 1. Composition of basal diets (g/kg) and nutrient levels.[1]

Ingredient	Starter (1-10d)	Grower (11-23d)	Finisher (24-42d)
Maize	525.5	530.5	541.2
Soybean meal	340	346.7	348.6
Gluten meal	56.3	30	15.0
Limestone	13.2	10.8	10.4
Dicalcium phosphate	17.6	15.5	14.0
Salt	3.6	4.7	4.2
DL-methionine	3.2	0.6	0
L-lysine	4.2	2.0	0
L-threonine	1.0	2.5	1.9
Corn oil [2]	30.4	51.7	59.7
Vitamin premix[3]	2.5	2.5	2.5
Mineral premix[4]	2.5	2.5	2.5
Calculated nutrients and energy			
AME, (MJ/kg)	12.6	13.1	13.3
Crude protein (g/kg)	235.2	220	210
Lysine (g/kg)	14.4	12.5	10.9
Met +Cys (g/kg)	10.7	9.5	8.6
Calcium (g/kg)	10.5	9.0	8.5
Available phosphorous (g/kg)	5.0	4.5	4.2

[1]The basal pretreatment and treatment diet were the same. Treatment diets were supplemented with α -tocopheryl acetate (250 mg/kg) or olive leaf extract (200 and 400 mg/kg) to the basal diet as part of the premix.
[2]Without antioxidant additives in the production stage.
[3]Vitamin premix provided per kilogram of diet: retinyl acetate, 11,000 IU; cholecalciferol, 1,800 IU; DL-α-tocopheryl acetate, 11 mg; menadione sodium bisulphate, 2 mg; riboflavin, 5.7 mg; pyridoxine hydrochloride, 2 mg; cyanocobalamin, 0.024 mg; nicotinic acid, 28 mg; folic acid, 0.5 mg; pantothenic acid, 12 mg; choline chloride, 250 mg.
[4]Mineral premix provided per kilogram of diet: Mn, 100 mg; Zn, 65 mg; Cu, 5 mg; Se, 0.22 mg; I, 0.5 mg; and Co, 0.5 mg.

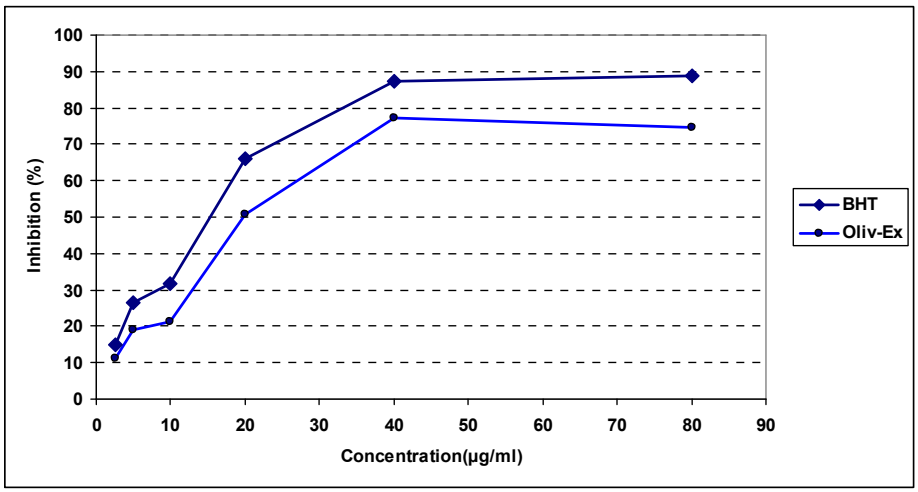

Figure 1. 1,1-Diphenyl-2-picryl-hydrazyl scavenging activities of olive leaf extract and Butylated hydroxytoluene

Table 2. Effects of dietary olive leaf extract (OLE) supplementation on the growth performance of broiler chickens reared under heat stress during 28-42 d of age.

Item	Treatment[1]				SEM	P-value
	NC	PC	OLE1	OLE2		
Average daily gain, g	90.77	96.46	98.54	90.24	1.27	0.078
Average daily feed intake, g	161.66	169.89	170.64	160.89	2.02	0.211
Feed conversion, feed/gain	1.78	1.76	1.74	1.78	0.02	0.721

[a-b] Means in the same row with no common superscripts differ significantly (P < 0.05).
[1]NC = negative control: basal diet without α-tocopheryl acetate and OLE; PC = positive control: basal diet + α-tocopheryl acetate (250 mg/kg); OLE1 = basal diet + OLE (200 mg/kg); OLE2 = basal diet + OLE (400 mg/kg).

Table 3. Effects of dietary olive leaf extract (OLE) supplemenationt on the apparent digestibility (%) of energy, ether extract, Ash, calcium, phosphorus, and CP retention in broiler chickens reared under heat stress during 28-42 d of age.

Item	Treatment[1]				SEM	P-value
	NC	PC	OLE1	OLE2		
Energy	79.27^{c}	88.98^{a}	88.07^{a}	81.42^{b}	0.33	0.001
CP	69.38^{b}	81.98^{a}	76.35^{ab}	70.12^{b}	1.15	0.003
Ash	39.99^{b}	63.31^{a}	61.29^{a}	48.95^{b}	1.77	0.004
Ether extract (EE)	94.47	96.14	95.53	95.35	0.29	0.261
Calcium	46.44^{c}	65.13^{ab}	67.25^{a}	53.15^{bc}	2.09	0.006
Phosphorus	61.67^{b}	75.97^{a}	73.06^{a}	64.08^{b}	0.83	0.001

[a-b] Means in the same row with no common superscripts differ significantly (P < 0.05).
[1]NC = negative control: basal diet without α-tocopheryl acetate and OLE; PC = positive control: basal diet + α-tocopheryl acetate (250 mg/kg); OLE1 = basal diet + OLE (200 mg/kg); OLE2 = basal diet + OLE (400 mg/kg).

Table 4. The effects of dietary olive leaf extract (OLE) supplementation on the some blood metabolites of broiler chickens reared under heat stress.

Item	Treatment[1]				SEM	P-value
	NC	PC	OLE1	OLE2		
Glucose (mg/dl)	327.7	306.2	279.8	313.8	6.40	0.091
Cholesterol (mg/dl)	126.7^{a}	110.8^{b}	107.0^{b}	114.7^{b}	1.98	0.013
Triglyceride (mg/dl)	51.8^{a}	41.7^{b}	40.7^{b}	34.2^{b}	1.69	0.013
Uric acid (mg/dl)	8.11^{a}	6.08^{b}	5.84^{b}	4.24^{c}	0.20	0.001
Total protein (g/dl)	2.96	3.03	3.00	3.12	0.074	0.892

[a-b] Means in the same row with no common superscripts differ significantly (P < 0.05).
[1]NC = negative control: basal diet without α-tocopheryl acetate and OLE; PC = positive control: basal diet + α-tocopheryl acetate (250 mg/kg); OLE1 = basal diet + OLE (200 mg/kg); OLE2 = basal diet + OLE (400 mg/kg).

Table 5. Effects of dietary olive leaf extract (OLE) supplementation on the some serum enzymes of broiler chickens reared under heat stress.

Item	Treatment[1]				SEM	P-value
	NC	PC	OLE1	OLE2		
ALT (U/L)	25.83^{a}	19.17^{b}	22.50^{ab}	21.17^{b}	0.67	0.017
AST (U/L)	271.7	236.5	249.8	259.2	6.99	0.361
ALP (U/L)	3254.2^{a}	3072.3^{ab}	2720.3^{bc}	2588.7^{c}	72.62	0.015

[a-b] Means in the same row with no common superscripts differ significantly (P < 0.05).
[1]NC = negative control: basal diet without α-tocopheryl acetate and OLE; PC = positive control: basal diet + α-tocopheryl acetate (250 mg/kg); OLE1 = basal diet + OLE (200 mg/kg); OLE2 = basal diet + OLE (400 mg/kg).

Table 6. Effects of dietary olive leaf extract extract (OLE) supplementation on ferric reducing antioxidant power (FRAP), superoxide dismutase (SOD), glutathione peroxidase (GPx), and malondialdehyde (MDA) concentrations of plasma in broiler chickens reared under heat stress.

Item	Treatment[1]				SEM	P-value
	NC	PC	OLE1	OLE2		
FRAP (nmol/mL)	607.7 ^{c}	949.5^{a}	856.5 ^{ab}	768.5 ^{b}	26.54	0.002
SOD (U/ml)	337.0	359.7	340.7	349.2	6.84	0.659
GPx (U/ml)	757.33 ^{b}	831.5 ^{ab}	961.8 ^{a}	917.8 ^{a}	22.94	0.023
MDA (nmol/mL)	2.06 ^{a}	1.78 ^{b}	1.79 ^{b}	1.48 ^{c}	0.04	0.001

$^{a-b}$ Means in the same row with no common superscripts differ significantly ($P < 0.05$).
[1]NC = negative control: basal diet without α-tocopheryl acetate and OLE; PC = positive control: basal diet + α-tocopheryl acetate (250 mg/kg); OLE1 = basal diet + OLE (200 mg/kg); OLE2 = basal diet + OLE (400 mg/kg).

DISCUSSION

Several studies have shown that the supplementation of antioxidant containing polyphenols can improve productivity in broilers, despite inconsistencies in the results obtained. For instance, the results obtained for pomegranate pomace and pomegranate pomace extract, grape and olive pomace, olive leaves, and rosemary extracts, as well as different mixtures of polyphenol sources, can either increase (Eid *et al*. 2003;Tavarez *et al*., 2011; Bravo *et al*., 2014), decrease, or have no effect on productivity (Goni *et al*., 2007; Gurbuz *et al*., 2010; Brenes *et al*., 2012). Benavente-García *et al.* (2000) found that olive leaves had an antioxidant activity higher than that of vitamins C and E, and attributed this effect to synergy between flavonoids, oleuropeosides, and substituted phenols. In contrast to the results of the present study, Lee *et al.,* (2009) reported that olive leaf extracts exhibited an antioxidant activity equal to that of α-tocopherol but markedly higher than that of BHT. This discrepancy is most likely to reflect a difference in the storage period between olive leaf harvest and their distribution to chemical reactions and the effect of storage on the chemical stability of total polyphenols of olive leaves. Paiva-Martins *et al.* (2009) reported that the dry olive leaf powder was chemically stable after 30 d of storage at room temperature. The stability of olive leaves might be attributed to the fact that the drying process could deactivate the action of oxidative and hydrolytic enzymes that would destroy the antioxidant compounds in the product. In this experiment, the time period between leaf harvesting and used in the trial lasted about two months. Therefore, the storage time may be affected by the functional value of leaves total phenolic contents.

Many researchers have reported that, olive leaf extract supplementation into the diets regulate animal digestion activity, increases the digestive juices that stimulate the appetite and food consumption of the animals, shows antibacterial and antifungal effects, and prevents against diseases, and result in improvements in the performances of the animals. In the current experiment, the data on chicken's performance for each experimental group showed that OLE exerted no growth-promoting effect when administered up to 400 mg/kg. Birds in our trial showed comparable FI, BWD, and FCR in all groups a with tendency of α-tocoforol and 200 mg/kg OLE supplement to impair the growth parameters of broilers compared to broilers fed control diets and 400 mg/kg OLE. The findings of the current study are in line with a previous study (Shafey *et al.,* 2013) who reported that dietary supplementation of OLE did not influence the performance (BWG, FI, and FCR) of chickens during the starter, finisher and, the whole experimental periods. Also, it is in accordance with Cayan *et al.,* (2015) who reported that the dietary olive leaf powder did not affect feed intake and the feed conversion ratio of laying hens. Erener *et al.* (2009), on the other hand, reported that the supplementation of olive leaf extract in broiler diets led to a greater increase in body weight, and also to an improvement in the feed conversion ratio. It seems that the daily heat stress negatively affects the performance of broiler chickens. Geraert *et al.* (1996) reported that when broilers exposed to an environmental temperature of 32°C, showed 14% decrease in feed intake by 4 weeks of age and a 24% reduction by 6 weeks of age, lower growth rate, and a reduction in feed efficiency. Therefore, the dietary supplementation of OLE, which contained natural antioxidant, may improve feed efficiency by reducing the effects of thermal stress in broilers. Further research is required to elucidate the effects of different supplements rich in polyphenols on the performance broiler..

Negative effects on the digestion and absorption of protein, starch, sugar, amino acids, and fat have been found (Surai, 2014), which could be due to the inhibition of enzyms, amylases, proteases, and/or lipases in the small intestine, the binding of bile acids, and/or a negative effect of polyphenols on the histological structure of the intestine (Surai, 2014). On the other hand, High ambient temperature is shown to decrease nutrient digestibility (Mahmoud and Edens, 2003) possibly because of excessive ROS that resulted to oxidation and destruction of cellular biological molecules, and finally caused a variety of impairments to intestinal tissues

(Zhao and Shen, 2005). Impaired intestinal function can, therefore, cause reduced nutrient digestibility and, in turn, reduced growth performance. The problems associated with intestinal impairment from high ambient temperature may be solved by supplementation of antioxidants with effective free-radical scavenging properties. In the current experiment, the results indicated that supplementation of OLE at a level of 400 mg/kg in birds which are exposed to regular daily high ambient temperature may cause reduced nutrient digestibility compared to those fed lower level of OLE or PC. As shown in table 3 the differences between OLE1 group and NC or PC groups were not significant (P>0.05). It seems that the lack of differences could be attributed to the lower content of polyphenols in the OLE1 compared to OLE2 group to cause detrimental effects. Rawel *et al.* (2002) reported that phenolic compounds of plant extracts combine and form precipitates with proteins and consequently block the amino acid residues. Finally, proteins bound in this form decreased the digestibility and biological value of protein (Rawel *et al.,* 2002). Moreover, this negative effect on nutrient digestibility could also be related to the capacity of oleuropein in OLE extract to affect endogenous enzyme activities. Oleuropein is able to activate pepsin on the one hand but has inhibitory effects toward other digestive and metabolic enzymes, namely trypsin, glycerol dehydrogenase, glycerol-phosphate dehydrogenase, glycerokinase, and lipase on the other hand (Polzonetti *et al.,* 2004). The inhibition of mineral absorption was attributed to the polyphenols compound present in grape seed and pomegranate extracts (Goni *et al*., 2007).These results suggested that supplementation of broiler diets with either OLE at 200 mg/kg or α-tocopheryl acetate offers the potential to increase nutrients apparent digestibility.

The serum glucose, cholesterol and triglyceride levels were expected to be lower in the groups with OLE addition. According to Krzeminski *et al*. (2003) Hypocholesterolemic property of OLE may be related to the reduction of intestinal absorption of cholesterol, or decrease its synthesis by the liver. Also, Prasad and Kalra (1993) reported that OLE stimulates the biliary secretion of cholesterol and its excretion in feces. Sung *et al.* (2004) reported that isoflavone contents of OLE havean inhibitory effect on catalytic domain of 3-hydroxy-3-methyl glutaryl (HMG) CoA reductase for cholesterol synthesis. As shown in table 4 the birds fed OLE2 diet significantly had the lowest levels of triglyceride and uric acid. In agreement with results of the current experiment, Sung *et al.*(2004) reported that flavonoids can decrease the availability of substrates for the synthesis of triglyceride. Atlan *et al.* (2003) suggested that increase cortisol level in heat stress broiler chickens lead to an increase of the catabolism rate of proteins to produce uric acid. It was reported that Uric acid is one potent scavenger of free radicals in birds. Moreover, Uric acid is one part of the nonenzymatic defense system, as indicated by significantly increased plasma level in chickens subjected to the heat stress (Simoyi *et al.,* 2002). However, the results may be affected by many factors in animal studies such as the species of the animals, the properties of the ingredients and the physiological situation of the animals.

Enhanced levels of serum ALT, AST and ALP are used as indicators of liver damage (Ozaki *et al.,* 1995). Data showed that the activity of ALT and ALP was suppressed by the supplementation of OLE at the level of 400 mg/kg to heat-stressed broiler diets. Results showed that supplementation of OLE preventing free radical generation and stopping the resulting damage occurred in the hepatocytes. The phenolic structure of OLE helps to reduce liver damage occurred by free radicals in heat stressed chickens. It seems that the hepatoprotective effect of OLE might be related to its antioxidant property.

Several enzymes such as SOD and GPx can be scavenging formed reactive oxygen species (ROS) which act as antioxidants. Endogenous protection against oxidative stress is achieved by enzymes that catalytically remove free radicals and other reactive species. Mice fed with pomegranate juice revealed decreased biomolecule damage by declining Glutathione (GSH) and Glutathione disulphide (GSSG) levels without changing the GSH/GSSG ratio and by decreasing hepatic antioxidant endogenous enzymes (GPx, catalase, SOD and GST), most probably in relation with less ROS production (Faria *et al*. 2007). These compounds have shown biological activities such as radical scavenging (Fares *et al*., 2011). It seemed that administration of OLE in the current experiment elevated oxidative stress, as evident from the low plasma level of uric acid. In contrast, significant elevated plasma FRAP levels in PC and OLE1 chickens should be ascribed to the increased plasma concentration of uric acid (Table 5, Simoyi *et al*., 2003), which is the main excretory end product of protein metabolism in poultry. Lin *et al*. (2006) reported that the plasma concentration of uric acid was significantly increased by the proteolysis induced by stress. In principle, data showed that antioxidant activity in PC and OLE1 diets exhibited significantly higher scavenging free radical capacity using FRAP method. Antioxidant enzymes, such cytosolic GPx, are involved in the conversion of hydrogen peroxide, a powerful and potentially harmful oxidizing agent, into the water and molecular oxygen. Hydrogen peroxide is unstable, and its breakdown forms hydroxyl free radicals which are highly detrimental (Liska, 1998). The results suggested that the addition of OLE to the diet improved overall antioxidant protection in birds under heat stress conditions, and could enhance their ability to convert highly reactive hydrogen peroxide to water.

The reduced serum MDA level in antioxidant supplemented birds in comparison to those in control group indicated that lipid peroxidation was reduced by OLE and a-Toc via enhancing antioxidant action. Based on the results, plasma MDA concentrations decreased with increasing levels of dietary OLE in broiler chickens under heat stress. It seems that the decrease of serum MDA level using supplementary OLE is due to its antioxidant characteristic in reducing both ROS and lipid peroxidation. The antioxidant characteristic of OLE in decreasing the MDA was previously reported in other studies (Ismail *et al.,* 2012; Tavafi *et al.,* 2012). Our results justify further research in this area to determine the mode of action of these plant products as an organic feed additive in broiler diets.

Conclusion: In conclusion, Supplementation of olive leaf extract to broiler diets as antioxidant components could improve the antioxidant status, liver function via reduction in the activity of dehydrogenases enzyme, and reduction in the stressor index of heat stressed broilers. The findings of the current study showed that OLE supplementation at a level of 200 mg/kg over the course of 14 days under daily heat stress exhibited positive effects on the apparent digestibility (%) of energy, Ash, phosphorus, and CP retention. Moreover, olive leaf extract addition into diets improved the performance slightly when broiler chickens are reared in heat stress condition. Our study illustrates a plausible approach to decreased negative effect high ambient temperature supplementation 200 mg/kg.

REFERENCES

Allouche, N., I. Fekiand, and S. Sayadi (2004).Toward a high yield recovery of antioxidants and purified hydroxytyrosol from olive mill w aste waters. J. Agric. Food Chem. 52:267-273.

AOAC (2005). Official Methods of Analysis. 18th ed. Association of Analytical Chemists, AOAC International, Arlington VA.

Atlan. O., A. Pabuccuoglu., A. Atlan., S. Konyalioglu, and H. Bayrakta (2003). Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. Br. Poult. Sci. 44: 545-550.

Aviagen (2014). Ross 308 broiler management guide. Available in http://en.aviagen.com/ross-308/.

Benavente-García, O., J. Castillo., J. Lorente., A.Ortuno, and JA. Del Rio (2000). Antioxidant activity of phenolics from *Olea europaea L.* leaves. Food Chem. 68: 457-462.

Benzie, IF, and J.J. Strain (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Biochem. 239: 70-76.

Birben, E., U.M Sahiner., C, Sackesen., S, Erzurum, and O. Kalayci (2012). Oxidative stress and antioxidant defense. World Allergy Organ. J. 5:9–19

Bouaziz, M., M. Chamkha, and S. Sayadi (2004). Comparative study on phenolic content and antioxidant activity during maturation of the olive cultivar Chemlali from Tunisia. J. Agric. Food Chem. 52: 5476-5481.

Bravo , D., V. Pirgozliev, and S.P Rose (2014) . A mixture of carvacrol, cinnam aldehyde, and capsicum oleoresin improves energy utilization and growth performance of broiler chicken fed maize based diet. J. Ani. Sci., 92: 1531-1536.

Cayan, H, and G. Erener (2015). Effect of olive leaf (*Olea europaea*) powder on laying hens performance, egg quality and egg yolk cholesterol levels. Asian Australas. J. Anim. Sci.28: 538-543.

Donkoh, A (1989). Ambient temperature: a factor affecting performance and physiological response of broiler chickens. Int. J. Biometeorol. 33: 259-265.

Draper, H.H, and M. Hadley (1990). MDA determination as index of lipid peroxidation. Methods Enzymol. 186: 421- 430.

Eid, Y.Z., A. Ohtsuka, and Hayashi K. Tea polyphenols reduce glucocorticoid- induced growth inhibition and oxidative stress in broiler chickens. Brit Poult Sci, 44: 127-132. 2003.

Erener, G., N. Ocak., E. Ozturk., S. Cankaya, and R. Ozkanca (2009). The effects of olive leaf extract on performance, some blood parameters and cecal microflora of broilers. The Scientific and Technological Research Council of Turkey, Agriculture, Forestry and Veterinary Research Group, AFVRG-Project No: 1070820 report of final results.

Fares, R., S. Bazzi., S.E Baydoun, and R.M Abdel-Massih (2011). The antioxidant and anti-proliferative activity of the lebanese *Olea europaea* extract. Plant Foods Hum. Nutr. 66: 58-63.

Faria, A., R. Monteiro., N. Mateus., I. Azevedo, and C. Calhau (2007). Effect of pomegranate (Punica granatum) juice intake on hepatic oxidative stress. Eur J Nutr. 46:271–278.

Goñi, I., A. Brenes., C. Centeno., A. Viveros., F. Saura-Calixto., A. Rebolé., I. Arija, and R. Estevez (2007). Effect of dietary grape pomace and vitamin E on growth performance, nutrient digestibility, and susceptibility to meat lipid oxidation in chickens. Poultry Sci. 86: 508–516.

Gurbuz, E., T. Balevi., V. Kurtoglu., B. Coskun., Y. Oznurlu., Y. Kan and M. Kartal (2010). Effects of Echinacea extract on the performance,

antibody titres, and intestinal histology of layer chicks. Brit Poult Sci, 51: 805-810.

Geraert, P.A., J.C.F Padilha, and S. Guillaumin (1996). Metabolic and endocrine changes induced by chronic heat exposure in broiler chickens: Growth performance, body composition and energy retention. Br. J. Nutr. 75:195-204.

Hosseini-Vashan, S.J., A. Golian., A. Yaghobfar., A. Zarban., N. Afzali, and P. Esmaeilinasab (2012). Antioxidant status, immune system, blood metabolites and carcass characteristic of broiler chickens fed turmeric rhizome powder under heat stress. Afr. J. Biotechnol. 94: 16118-16125.

Ismail, I., A. Ghanema, and K.M Sadek (2012). Olive leaves extract restored the antioxidant perturbations in red blood cells hemolysate in Streptozotocin induce diabetic rats. World Acad. Sci. Eng. Technol. 64: 159-165.

Jemai, H., I. Fki., M. Bouaziz., Z. Bouallagui., A. El Feki., H. Isoda, and S. Sayadi (2008). Lipid-lowering and antioxidant effects of hydroxytyrosol and its triacetylated derivative recovered from olive tree leaves in cholesterol-fed rats. J. Agric. Food Chem. 56: 2630-2636.

Kanner, J (1994). Oxidative processes in meat and meat products: Quality implications. Meat Sci. 36:169–189.

Krzeminski, R., S. Gorinstein., H. Leontowicz., M. Leontowicz., M. Gralak., J. Czerwinski., A. Lojek., M. Ciz., O. Martin-Belloso., N. Gligelmo-Miguel, and Trakhtenberg S (2003). Effect of different olive oils on bile excretion in rats fed cholesterol-containing and cholesterol-free diets. J Agric Food Chem. 51: 5774-5779.

Lee, O.H., B.Y Lee., J. Lee., H.B Lee., J.Y Son., C.S Park., K. Shetty, and Y.C Kim (2009). Assessment of phenolics-enriched extract and fractions of olive leaves and their antioxidant activities. Bioresour. Technol. 100: 6107-6113.

Lin, H., S.J Sui., H.C Jiao, J. Buyse, and E. Decuypere (2006). Impaired development of broiler chickens by stress mimicked by corticosterone exposure. Comp. Biochem. Physiol.143:400-405.

Liska, D.J (1998). The detoxification enzyme system. Altern. Med. Rev. 3:187-198.

Mahmoud, K.Z, and Edens F.W (2003). Influence of selenium sources on age-related and mild heat stress-related changes of blood and liver glutathione redox cycle in broiler chickens (Gallus domesticus). Comp. Biochem. Physiol. 136:921-934.

Ozaki, M., S .Fuchinoue., S. Teraoda, and K. Ota (1995). The in vivo cytoprotection of ascorbic acid against ischemia/reoxygenation injury of rat liver. Arch. Biochem. Biophys. 318:439-445.

Paiva-Martins, F., S. Barbosa., V. Pinheiro., J.L Mourão, and Outor-Monteiro, D (2009). The effect of olive leaves supplementation on the feed digestibility, growth performances of pigs and quality of pork meat. Meat Sci. 82: 438–443.

Polzonetti, V., D. Egidi., A. Vita, S.Vincenzetti, and P. Natalini (2004). Involvement of oleuropein in (some) digestive metabolic pathways. Food Chem. 88: 11-15.

Prasad, K, and J. Kalra (1993). Oxygen free radicals and hypercholesterolemic atherosclerosis: effect of vitamin E. Am. Heart J.125: 958-971.

Rawel, H.M., D.Czajka, and J. RohnS,Kroll (2002). Interactions of different phenolic acid and flavonoids with soy proteins. Int. J. Biol. Macromolec. 30:137-150.

Ryan, D., M. Antolovich., P.renzler., K. Robards, and S. Lavee (2002). Biotransformations of phenolic compounds in *Olea europea L*. Sci. Hortic. 92:147-176.

Sahin K, Kucuk O (2003). Heat stress and dietary vitamin supplementation of poultry diets. Nutr. Abstr. Rev.73: 41R-50R.

Sahin, K., N.Sahin, and S. Yaralioglu (2002). Effects of vitamin C and vitamin E on lipid peroxidation, blood serum metabolites and mineral concentrations of laying hens reared at high ambient temperature. Biol. Trace Elem. Res. 85: 35-45.

Saleh, H., A. Golian., H. Kermanshahi, and M.T Mirakzehi (2017). Effects of dietary α-tocopherol acetate,pomegranate peel, and pomegranate peel extracton phenolic content, fatty acid composition, and meat quality of broiler chickens. J Appl Anim Res. 45: 629–636

Saleh, H., A. Golian., H. Kermanshahi, and M.T Mirakzehi (2016). Antioxidant status and thigh meat quality of broiler chickens fed diet supplemented with α-tocopherolacetate, pomegranate pomace and pomegranate pomace extract. Ital J Anim Sci.17: 1-10

Samarth, R.M., M. Panwar., M. Kumar., A. Soni., M. Kumar, and A.Kumar (2007). Evaluation of antioxidant and radical scavenging activities of certain radioprotective plant extracts. Food Chem. 106: 868-873.

SAS (2008). SAS 9.2 (Cary, NC, SAS Institute Inc.).

Shafey, TM, Al-Ruqaei IM, Almufarij SI (2013). Effect of Feeding Olive Leaves Extract (Oleuropein) on the Performance, Nutrient Utilization, Small Intestine and Carcass Characteristics of Broiler Chickens. J. Anim. Vet. Adv. 12: 740-746.

Siegel, H.S (1995). Stress, strains and resistance. Br. Poult. Sci. 36: 3-12.

Simoyi, M.F., E. Falkenstein., K.V Dyke., K.P Blemings and H. Klandorf (2003). Allantoin, the oxidation

production of uric acid is present in chicken and turkey plasma. Comp. Biochem. Physiol. 135:325-335.

Simoyi, M.F., K. Van Dyke, and H. Klandorf (2002). Manipulatioin of plasma uric acid in broiler chicks and its effect on leukocyte oxidative activity. Am. J. Physiol. Regul. Integr. Comp. Physiol. 282:791-796.

Sung, J.H., S.J Choi., S.W Lee., K.H Park , and T.W Moon (2004). Isoflavones found in Korean soybean paste as 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. Biosci. Biotechnol. Biochem. 68:1051-1058.

Surai, P. F (2014). Polyphenol compounds in the chicken/animal diet: from the past to the future. J .Anim Physi and Anim Nut, 98: 19-31.

Tavafi, M., H. Ahmadvand, and P. Toolabi (2012). Inhibitory effect of olive leaf extract on gentamicin-induced nephrotoxicity in rat. Iran J. Kidney Dis. 6: 25-32.

Tavarez, M.A., D.D Boler., K. N Bess., J. Zhao., F. Yan., A.C Dilger., F.K Mckeith and J. Killefer (2011). Effect of antioxidant inclusion and oil quality on broiler performance, meat quality, and lipid oxidation. Poultry Sci, 90: 922-930.

Teeter, RG, and T. Belay. (1996). Broiler management during acute heat stress. Anim. Feed Sci. Technol. 58: 127-142.

Tuzcu, M., N. Sahin, M. Karatepe., G. Cikim., U. Kilinc, and K. Sahin (2008). Epigallocatechin-3-gallate supplemention can improve antioxdant status in stressed quial. Br. Poult. Sci. 49: 643-348.

Visioli, F., A. Poli, and C. Galli. (2002). Antioxidant and other biological activities of phenols from olives and olive oil. Med. Res. Rev. 22: 65-75.

Visioli, F., S. Bellasta, and C. Galli (1998). Oleuropein, the bitter principle of olives, enhances nitric oxide production by mouse macrophages. Life Sci. 62: 541-546.

Williams, C.H., D.J David, and O. Iismaa (1962). The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. J Agri Sci; 59:381–385

Wang, Y.Z., C.L Xu., Z.H An, J.X Liu, and J. Feng (2008). Effect of dietary bovine lactoferrin on performance and antioxidant status of piglets. Anim. Feed Sci. Technol. 140: 326-336.

Yilmaz, Y, and R.T Toledo (2004). Major flavonoids in grapeseeds and skins: antioxidant capacity of catechin, epi-catechin and gallic acid. J Agr Food Chem. 52:255–260.

Zhao, R, and G.X Shen (2005). Functional modulation of antioxidant enzymes in vascular endothelial cells by glycated LDL. Atherosclerosis.179:277-284.