Effects of *Rhus coriaria* on nutrient composition, thiobarbituric acid reactive substances and colour of thigh meat in heat-stressed broilers

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Abstract

Heat stress negatively affects the meat quality in broiler chickens, as indicated by lipid peroxidation. The aim of this study was to investigate the effects of 0.0, 2.5, 5.0 and 10 g sumac fruit powder/kg of the diet, along with 100 mg α-tocopherol acetate (AT)/kg as antioxidants, on meat characteristics of broilers under heat stress conditions. Consumption of 5.0 g sumac/kg decreased the thiobarbituric acid reactive substance (TBARS) concentration in thigh meat. The thigh meat pH increased only as a result of AT consumption. Dietary inclusion of both AT and 10 g sumac/kg decreased the fat content of the meat. None of the meat colour indices, lightness (L*), redness (a*) and yellowness (b*), was affected by dietary treatments. It was concluded that dietary sumac consumption improved the TBARS and pH, and decreased thigh meat fat in broilers under heat stress.

Keywords: Alpha tocopherol acetate, chicken meat quality, intramuscular fat, pH, sumac, lipid oxidation
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Introduction

Heat stress is a major cause of deterioration in performance and even poultry deaths in tropical countries. Reduction of feed intake, liveweight gain and feed efficiency are the most common signs of high ambient temperatures (Siegel, 1995). Environmental stress causes body oxidative stress, resulting in an imbalance of body antioxidant status in poultry (Sahin *et al*., 2001). Environmental stress also induces a decrease in eating quality and functional properties of muscle proteins (Wang *et al*., 2009). Feng *et al*. (2006) reported oxidation stress of the pectoral muscle, denatured muscle protein, lower pH and higher drip loss, lightness and shear force from daily high ambient temperatures. Lipid oxidation is a major cause of muscle quality deterioration and can directly affect quality characteristics such as flavour, colour, texture, nutritive value and safety of the meat (Buckley *et al*., 1995). Normally, pH declines gradually from 7.4 in living muscle to roughly 5.6 - 5.7 within 6 - 8 h post mortem, and then has an ultimate pH at 24 h of about 5.3 - 5.7 (Briskey & Wismer-Pedersen, 1961). A number of studies have demonstrated the effects of heat stress on increased reactive oxygen species (ROS) production and oxidative damage to skeletal muscle of chickens (Mujahid *et al*., 2009). In an experiment, chronic heat exposure (34 ºC) increased the malondialdehyde (MDA) level in skeletal muscle of broiler chickens at four weeks of age (Azad *et al*., 2010). Antioxidants have been shown to inactivate ROS and thus protect the body from oxidative damages. In this regard, dietary supplementation of antioxidants, such as tocopherols, increases this natural antioxidant (vitamin E) in animal food products and prevents the lipid peroxidation in broiler meat (Ajuyah *et al*., 1993). Sahin *et al*. (2002a) reported a reduction in the malondialdehyde (MDA) concentration in serum and tissue of broilers under heat stress at 32 ºC, when supplemented with dietary vitamin E (250 mg dl-α-tocopheryl-acetate/kg diet). Pena *et al*. (2008) reported the lowest cooking loss in meat of broilers fed 250 g ascorbic acid/ton and citric flavonoids under heat stress (32 ºC).

Recently, the use of plant extracts as natural antioxidants has gained interest because of a global trend in reducing the use of synthetic substances (Ahn *et al*., 2002). Nowadays, medicinal plants are receiving lots of attention as feed additives (Abdulkarimi *et al*., 2011; 2012), especially as natural antioxidants. Silybum marianum fruit extract has been reported to reduce the lipid concentration of the thigh muscle and to increase the muscle's resistance to oxidative stress in broilers (Schiavone *et al*., 2007). Ciftci *et al*. (2010) reported that hypocholesterolemic and antioxidant characteristics of cinnamon oil are related to

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better quality of meat. Antioxidative activities of dietary phenolic sources such as oregano, rosemary, sage essential oil and grape pomace have been reported in broiler meat (Simitzis *et al*., 2008). Sumac (*Rhus coriaria* L., family Anacardiaceae) is a medicinal plant with antioxidant properties that grows in a region extending from the Canary Islands over the Mediterranean coastline to Iran and Afghanistan (Nasar-Abbas *et al*., 2004). The fruits of sumac plants contain flavonols, phenolic acids, hydrolysable tannins, anthocyans and organic acids such as malic, citric and tartaric acids (Özcan & Haciseferogullari, 2004). Phenolic compounds are secondary metabolites in the plant material (Kosar *et al*., 2007) responsible for sumac's antioxidant effect. Kosar *et al*. (2007) reported that extract of sumac contains 1.52, 4.14 and 4.13 g gallic acid/kg of methanol extract, ethyl acetate extract and hydrolyzed ethyl acetate, respectively. Although the antioxidant property of sumac has been established, no information is available on its interaction with meat quality, especially under hot climatic conditions. The objective of the current experiment was to investigate the dietary supplementation of sumac on meat thiobarbituric acid reactive substances (TBARS) (as an indicator of lipid peroxidation) content, colour and quality of broiler chickens under conditions of continuous heat stress.

Materials and Methods

The experimental protocols used in the current study were reviewed and approved by the Animal Care Committee of Urmia University. Two hundred and fifty one-day-old male broiler chicks (Ross 308) were purchased from a local hatchery. All the birds were weighed on arrival and randomly divided into five groups. The birds of each group were distributed between five replicate pens with 10 birds each. The same mash starter (from day 1 to day 21) and grower (from day 22 to day 42) diets were used during the experimental period, but the groups were fed different levels of sumac fruit powder: 0.0 (ZS), 2.5 (LS), 5.0 (MS) and 10 g sumac fruit powder (HS)/kg or 100 mg α-tocopherol acetate (AT)/kg (Table 1). The various levels of sumac were replaced by sand in the basal diet (Sharbati Alishah *et al.*, 2013). Fresh sumac fruit powder was purchased from a local market and included in the experimental diets after being ground.

All the birds had free access to feed and water. The house temperature was set at 32 ± 1 ºC during the entire experimental period to induce continuous heat stress. The birds were exposed to 23 h light and 1 h darkness during the experiment. At the end of the experiment (week 6), five birds per treatment were randomly selected and slaughtered. At slaughter, two pieces of the right thigh (upper part) were removed. The first piece was used to determine pH and nutrient composition. The pH of thigh meat was measured with a digital pH meter (TitroLine Easy, Schott Instruments, Mainz, Germany). Dry matter (DM), crude protein (CP), ether extract (EE) and ash contents were determined according to the AOAC methods (AOAC, 1999).

The other piece of thigh meat was used to determine meat colour indices and TBARS. The surface colour of thigh meat was measured through the packaging film, following calibration against a white tile covered with the same film. Individual packages were used for repeated measurements and remained intact during the entire storage period. Colour measurements were reported in terms of lightness (L*), redness (a*) and yellowness (b*). The L*, a*, and b* of breast meat were measured with a Minolta Chroma Meter CR-400 (Minolta Inc., Tokyo, Japan).

To determine TBARS, first 10 g meat samples were weighed into a 50 mL test tube and 1 mL 0.1% BHT was added. Then 35 mL 5% trichloroacetic acid (TCA) were added and the meat sample was homogenized (Ultra-turrax T-25, Janke and Kunkel IKA-Labortechnik, Staufen, Germany) at 13 500 rpm for 30 s. After filtering, 5 mL of the filtrate and 5 mL thiobarbituric acid solution (0.02 mM) were added to the test tube. Tubes were heated in a boiling water bath for 1 h at 100 ºC, then cooled, and absorbance was measured at 532 nm against a blank containing 5 mL TCA and 5 mL TBA solution. TBARS values were expressed as mg per kg of sample (Ulu, 2004).

The colorimetric method and Folin-Ciocalteau reagent were used to determine total phenols (McDonald *et al*., 2001). First, 1 g sumac was crushed in 10 mL (80%) methanol in a pestle and mortar. The extract was filtered and centrifuged at 5000 rpm for 5 min and the supernatant was collected. Then 0.5 mL extract was mixed with Folin-Ciocalteau reagent (1:10 v/v) and Na_2Co_3 (1 mol). After 15 min, this solution was used to determine total phenols using a spectrophotometer (single beam scanning UV/visible spectrophotometer, M 501, Unicosh Co., China) at absorbance of 765 nm. Gallic acid was used as the standard. The amount of 34.57 mg total phenols/g was determined for sumac fruit powder.

The GLM procedure of SAS (2002), based on a completely randomized design with five treatments and five replicates each, was used to analyse the data. When treatment means were significant (P <0.05), Duncan's multiple range test was used to separate the means. Moreover, orthogonal contrasts were constructed in order to compare the mean response variables for birds that received sumac powder with the control birds.

Table 1 Composition of experimental diets (as fed basis)

Ingredients (g/kg)	Starter (0 - 21d)	Grower (22 - 42 d)
Maize	310.8	319.8
Wheat	200.0	250.0
Soybean meal	396.8	339.2
Soybean oil	35.0	39.5
Dicalcium phosphate	21.0	21.5
Limestone	11.0	8.60
L-lysine	2.90	2.20
DL-methionine	3.80	0.8
Vitamin-mineral mix[1]	5.00	5.00
Sodium chloride	3.70	3.40
Sand[2]	10.0	10.0
Total	1000	1000
Calculated analysis		
Dry matter (g/kg)	851.6	854.3
Metabolizable energy (MJ/kg)	12.85	12.95
Crude protein (g/kg)	220	200.0
Crude fat (g/kg)	53.6	58.9
Crude fibre (g/kg)	39.4	36.8
Calcium (g/kg)	10.0	9.0
Available phosphorus (g/kg)	4.50	4.50
Calcium/phosphorus	2.22	2.00
Chloride (g/kg)	3.30	3.00
Sodium (g/kg)	1.60	1.50
Methionine (g/kg)	7.00	3.80
Lysine (g/kg)	14.3	12.4
Arginine (g/kg)	15.4	13.8
Methionine + cysteine (g/kg)	10.7	7.30
Tryptophan (g/kg)	2.90	2.60
Tyrosine (g/kg)	9.80	8.90
Threonine (g/kg)	8.50	7.70

[1] Supplied per kilogram of diet: retinol, 9000 IU; cholecalciferol, 2000 IU; tocopherol, 18 IU; cyanocobalamine, 0 15 mg; riboflavin, 6.6 mg; pantothenate, 10 mg; niacin, 30 mg; choline, 500 mg; biotin, 0.1 mg; thiamine, 1.8 mg; pyridoxine, 3 mg; folic acid, 1 mg; menadione, 2 mg; zinc, 50 mg; manganese, 100 mg; copper, 10 mg; Fe, 50 mg; iodine, 1 mg; Se, 0.2 mg.
[2] Different levels of sumac or vitamin E were replaced by sand in the diets.

Results and Discussion

The effect of dietary sumac on TBARS of thigh meat at week 6 is shown in Figure 1. The ZS and MS birds, respectively, showed the highest and lowest thigh TBARS among the birds in the various treatments (P <0.05). There were no significant differences between the other treatments for thigh TBARS content. Moreover, compared with the control, sumac consumption decreased (P <0.05) the thigh TBARS content. However, no significant differences were indicated between the treatments for meat colour indices, L*, a* and b*, at week 6 (P >0.05) (Table 2). Consistent with these results, Song *et al.* (2008) reported that pigs fed lacquer (*Rhus verniciflua,* a kind of sumac) meal showed linearly lower meat TBARS values. However, the results of the current study regarding the higher meat TBARS content in 10 g/kg of sumac-fed birds conflict with those in Song *et al*'s report, which may be related to a relatively intense oxidative stress in the current experiment owing to continuous heat stress. Daneshyar *et al*. (2012) stated that inclusion of an antioxidant (turmeric rhizome powder) alone to the diets cannot improve the antioxidant defence system during intense oxidative stresses such as T_3-induced ascites.

Table 2 Meat colour, lightness (L), redness (a) and yellowness (b)[1], at 42 days old of broiler chickens fed 0.0 (ZS), 2.5 (LS), 5.0 (MS) and 10 g (HS) sumac fruit powder /kg diet or 100 mg α-tochopherol acetate (AT))/kg diet under continuous heat stress of 32 ºC

Treatment	L	a	b
Zero sumac	56.60	2.68	11.10
Low sumac	49.22	4.14	8.54
Medium sumac	54.96	2.84	7.95
High sumac	54.68	2.84	8.80
α-tocopherol	49.40	3.01	7.50
Pooled SEM[2]	1.41	0.34	0.51
P-value	0.36	0.62	0.41
Contrast			
Zero sumac vs. sumac	0.44	0.67	0.62

[1] Each value represents the mean of five cages with two chicks per cage.
[2] Pooled standard errors of the mean.

The lower TBARS content of thigh meat in AT-fed birds of the current experiment is connected to the antioxidant ability of vitamin E. It is known that vitamin E is the first line of defence against lipid peroxidation. By its free radical quenching activity, it breaks chain propagation and thus terminates free radical attack at an early stage (McDowell, 1989). Sahin *et al*. (2002a) reported that supplemental vitamin E during heat stress (32 ºC) decreases liver MDA in broiler chickens. In another experiment on chickens, Morrissey *et al.* (1997) reported that dietary supplementation of diets with α-tocopherol increased tissue α-tocopherol concentrations, and simultaneously decreased the MDA concentration. Sahin *et al.* (2002c) reported that dietary supplementation of Japanese quails reared under heat stress (34 ºC) diets with vitamin E (250 mg/kg) decreased serum MDA concentrations.

Based on changes in thigh meat TBARS, the researchers expected improvements in meat colour indices in MS birds, but no treatment effect was indicated for meat lightness (L), redness (a) and yellowness (b). Colour is a major criterion that may be used by consumers to judge meat quality. The rate of discoloration is closely related to the rate of myoglobin oxidation induced by lipid oxidation (Yin & Faustman, 1993). Lawrie (1991) attributed the pale colour of meat to the denaturation of sarcoplasmic proteins, which causes increased light scattering in the muscle.

No treatment effect was observed for DM, ash and CP levels of thigh meat at week 6 (P >0.05) (Table 3), though both the pH and EE from thigh meat were affected by dietary treatments (P <0.05). The pH of AT birds was higher than that of LS- and HS-fed birds (P <0.05) whereas there were no significant differences between the meat pH of other birds (P >0.05). Consumption of either the HS or AT decreased the meat EE level at week 6 (P <0.05). In orthogonal comparisons, sumac consumption decreased the pH of meat compared with the control (P <0.05).

The decreasing effect of vitamin E on EE of poultry products has been reported. Sahin *et al*. (2002b) showed that dietary supplementation of 125 mg vitamin E (dl- α-tocopheryl acetate)/kg decreased the EE content of egg yolk in Japanese quails under heat stress (34 ºC). There is no information about the effect of sumac *(Rhus coriaria)* on meat nutrient composition and the current investigation is the first to be reported. The reduced meat EE level in HS fed birds in the present study could be owing to the flavonoid content of sumac. Song *et al*. (2008) indicated that feeding different levels of lacquer (*Rhus verniciflua* stokes) meal to finishing pigs resulted in linearly lowered total saturated fatty acid concentrations in meat.

Table 3 Nutrient composition[1] of thigh meat from chickens fed 0.0 (ZS), 2.5 (LS), 5.0 (MS) and 10 g sumac fruit powder (HS)/kg diet or 100 mg α-tocopherol acetate (AT)/kg diet at day 42 of age under continuous heat stress conditions (32 ºC)

Treatment	Dry matter (g/kg)	Ether extract (g/kg)	Ash (g/kg)	pH	Crude protein (g/kg)
Zero sumac	272.6	299.8^{a}	12.3	6.33^{ab}	190.5
Low sumac	268.6	285.4^{ab}	14.5	6.30^{bc}	197.8
Medium sumac	267.0	285.3^{ab}	12.8	6.36^{ab}	191.7
High sumac	264.5	248.6^{b}	13.7	6.22^{c}	197.5
α-tocopherol	276.6	208.7^{c}	14.2	6.40^{a}	201.9
Pooled SEM[2]	2.40	8.80	0.60	0.02	1.70
P-value	0.62	0.002	0.79	0.008	0.23
Contrast					
Zero sumac vs. sumac	0.37	0.45	0.95	0.002	0.90

[ab] Means with no common superscript for each mineral and column are different significantly (P <0.05).
[1] Each value represents the mean of 5 cages with two chicks per cage.
[2] Pooled standard errors of the mean.

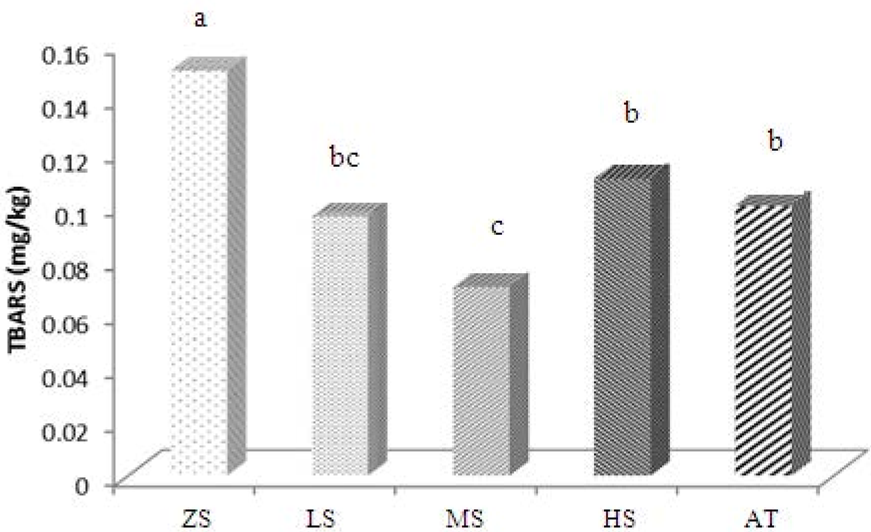

Figure 1 Thiobarbituric acid reactive (TBARS) in meat of broiler chickens fed 0.0 (ZS), 2.5 (LS), 5.0 (MS) and 10 g sumac fruit powder (HS)/kg diet or 100 mg α-tochopherol acetate (AT)/kg diet under continuous heat stress conditions (32 ºC) at day 42 of age.

Muscle pH variation is also related to glycogenolysis, and increased catecholamine secretion in response to an acute stressor just prior to slaughter. Increased glycogen breakdown lowers the pH post slaughter (Briskey & Wismer-Pedersen, 1961). The pH decline of meat after slaughter is higher during heat stress conditions (Feng *et al*., 2006). High meat pH values were observed for α-tocopherol fed birds and the researchers expected the same results for the sumac-fed ones, but feeding the sumac, especially at the highest level, decreased the pH of the meat in the current study. This is the first investigation on the effect of sumac on meat pH during heat stress and no other information is available. However, consistent with these results, Song *et al*. (2008) observed a reduced pH value of meat in finishing pigs consuming a diet containing 2 g lacquer meal/kg in the diet on days 6, 8 and 10 of refrigerated storage.

Conclusions

In the present study it was observed that TBARS values of thigh meat decreased in MS-fed birds. It was concluded that dietary supplementation of 5.0 g sumac/kg in the diet can decrease thigh lipid peroxidation in broiler chickens under heat stress, and hence improve meat quality. At a higher sumac level (10 g/kg), both pH and EE level of thigh meat decreased, and consequently worsened meat quality.

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