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PAPER

Effects of tannic acid on performance and fatty acid composition of breast muscle in broiler chickens under heat stress

Rohollah Ebrahim,[1] Juan Boo Liang,[1] Mohammad Faseleh Jahromi,[1] Parisa Shokryazdan,[2] Mahdi Ebrahimi,[2] Wei Li Chen,[1] Yong Meng Goh[1,2]

[1]Institute of Tropical Agriculture, Universiti Putra Malaysia, Serdang, Selangor, Malaysia

[2]Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Selangor, Malaysia

Abstract

One hundred twenty day-old broiler chicks (Cobb 500) were randomly assigned into 4 treatment groups to investigate the effects of tannic acid supplementation (TA) on fatty acid composition in breast muscle of broilers under chronic heat exposure conditions. Five pen replicates of 6 chicks each were assigned to each of the following 4 dietary treatments: i) basal diet containing no TA at 25°C (CL); ii) basal diet containing no TA at 35°C (CH); iii) basal diet supplemented with 1% TA at 25°C (TL); and iv) basal diet supplemented with 1% TA at 35°C (TH). At the end of the 5-week experiment, breast muscle samples were collected to examine the fatty acid composition. Results showed that temperature, TA and their interaction effect significantly decreased body weight gain and feed intake. In addition, feed conversion ratio (FCR) significantly increased under high temperature, and addition of TA under high temperature did not improve the FCR. The effects of temperature, TA and their interaction on the saturated and unsaturated fatty acids were not significant (P>0.05). However, monounsaturated fatty acids significantly reduced by adding TA to the diet. Generally, TA improved the fatty acid profile of breast muscle of broilers under heat stress in comparison to the heat stressed chickens, which did not receive TA. Hence, it seems that dietary TA supplementation can be applied as a biological antioxidant for poultry nutrition in hot climatic conditions.

Introduction

Heat stress is considered as a great concern in the poultry industry and could be responsible for stimulating the production of reactive oxygen species (ROS) (Freeman and Crapo, 1982; Laudicina and Marnett, 1990). Feed efficiency, growth rate, mortality and other important traits governing productivity of poultry are adversely affected by severe heat stress. It has been reported that broilers exposed to 32°C showed a 24% decrease in FI by 6 wk of age (Geraert *et al.*, 1996). Fats are the main storage source of energy in animal body (Gunstone *et al.*, 1994; Rustan and Drevon, 2005). Effects of heat exposure on fat deposition have long been the subject of considerable controversy, but most of researchers suggested an increase in fat contents under heat stress conditions (Swain and Farrell, 1975; Howlider and Rose, 1987; Geraert *et al.*, 1996). Shim *et al.* (2006) showed that chronic heat exposure clearly altered the hepatic fatty acid profiles. They investigated lipid metabolism and peroxidation in broiler chicks under chronic heat stress and reported that the heat stress increased peroxidizability index and total saturated fatty acids (ΣSFA), while it significantly decreased monounsaturated fatty acids (MUFA) and total unsaturated fatty acids (ΣUFA).

Tannins are found in many poultry feedstuffs such as sorghum, millet, barley and faba beans. It has been demonstrated that inclusion of feed ingredients containing tannins resulted in undesirable physiological and biochemical effects (Armstrong *et al.*, 1974; Smulikowska *et al.*, 2001) including growth inhibition, negative nitrogen balances, reduced intestinal absorption of sugars and amino acids, reduced immune response, and increased protein catabolism (Santidrian, 1981; Santidrian and Marzo, 1989; Marzo *et al.*, 2002). However, with better understanding of the chemical composition and biological activity of tannins (Mueller-Harvey, 2006), tannin is now known to play a beneficial antioxidant role, preventing lipid peroxidation (Laughton *et al.*, 1991; Morel *et al.*, 1993; Caraceni *et al.*, 1997; Lopes *et al.*, 1999; Rajalakshmi *et al.*, 2001; Glahn *et al.*, 2002) reported that at 0.5% inclusion rate, chestnut tannins had positive effects on carcass characteristics, meat quality, lipid oxidation and fatty acid composition in rabbits.

Effect of TA on growth and fatty acid profile in broiler chickens exposure to chronic heat stress has not been reported. Therefore, the aim of the present study was to investigate the potential of TA in preventing the adverse effects of heat stress on the performance and fatty acid profile of breast muscle in broiler chickens.

Corresponding author: Dr. Juan Boo Liang, Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
Tel. +60.38947.2132 - Fax: +60.38947.2213.
E-mail: jbliang@upm.edu.my

Key words: Heat stress; Tannic acid; Performance; Fatty acid profile; Broiler.

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Materials and methods

Experimental design, birds and diets

Animals were cared in accordance to the Animal Care and Use Protocol from the Animal Care and Use Committee of Universiti Putra Malaysia. One hundred and twenty day-old male broiler chicks (Cobb 500), purchased from a commercial hatchery in Malaysia, were weighed and assigned in equal numbers (6 chicks per cage) to 20 battery cages in open-sided poultry house. The chicks were maintained on a 24-h continuous light schedule and allowed *ad libitum* access to corn-soy based starter diet [3000 kcal metabolisable energy (ME)/kg and 217.0 g crude protein (CP)/kg; Table 1] and water for two weeks. After that, half of the birds (10 cages) were randomly selected and transferred to a temperature-controlled chamber set at 25°C, and the remaining 10 cages were transferred to another chamber set at 35°C (H). Birds in randomly selected 5 cages in each chamber were offered basal grower diet (3080 kcal ME/kg and 198.1 g CP/kg; Table 1) and those in the remaining 5 cages were offered basal grower diet supple-

mented with 1% tannic acid (a commercial tannin, Nacalai Tesque, Kyoto, Japan). This resulted in the following 4 treatment groups: i) basal diet at 25°C (CL), ii) basal diet at 35°C (CH), iii) basal diet supplemented with 1% TA at 25°C (TL) and, iv) basal diet supplemented with 1% TA at 35°C (TH). Birds were inspected daily and no mortality was experienced.

Growth performance and sample collections

Birds were weighed on the first day of the experiment and thereafter, weekly for five weeks to calculate body weight gain (BWG). The feed conversion ratio (FCR) was calculated as feed intake (FI)/BWG on per cage basis. At the end of experiment (day 35), blood samples (2 mL per bird) were collected from 6 chickens per treatment for blood parameters determination. Within 1 h of collection, serum was obtained by centrifugation (2500 × *g* for 15 min) for later analyzing of cholesterol, high-density lipoprotein cholesterol (HDL), triglyceride (TG) and low-density lipoprotein cholesterol (LDL).

Feed proximate analysis

The proximate chemical analysis of the feeds was carried out following the standard methods of AOAC (2000). The dry matter (DM) was determined by oven-drying in a forced-air oven for 48 h at 80ºC. The Kjeltec Auto Analyzer (Foss Tecator AB, Høganäs, Sweden) was used to determine nitrogen to calculate the CP (CP = N x 6. 25), while the ether extract (EE) was determined in petroleum ether (40-60ºC) using a 2025 Soxtec Auto Analyzer (Foss Tecator AB, Høganäs, Sweden). Ash content was determined by ashing the samples in a muffle furnace at 550ºC for 4 h.

Determination of fatty acid profiles

The total fatty acids were extracted from feed and meat samples based on the method of Folch *et al.* (1957), as described by Ebrahimi *et al.* (2012), using chloroform/methanol 2:1 (v/v) containing butylated hydroxytoluene to prevent oxidation during sample preparation. One gram experimental diets or breast meat were homogenized in 40 mL chloroform/methanol (2:1 v/v) in a 50-mL stoppered ground-glass extraction tubes. After filtration of the mixture, 10 mL of normal saline solution was added to ease phase separation. Transmethylation of the extracted fatty acids to their fatty acid methyl esters (FAME) was carried out using KOH in methanol and 14% methanolic boron trifluoride (BF_3) according to methods described by AOAC (2000). The FAME were separated by gas chromatography

Table 1. Feed ingredients and composition of the basal diet.

	Starter (1 to 14 days)	Grower (15 to 35 days)
Ingredient, g/kg		
Ground yellow corn	538.9	603.0
Soybean meal (44%)	361.9	305.6
Fish meal	30.0	30.0
Palm oil	37.4	37.4
Choline chloride (60%)	2.5	2.0
Trimix°	1.0	1.0
Common salt (NaCl)	2.0	1.0
DL-methionine	1.8	0.4
Limestone	13.0	13.0
Dicalcium phosphate	11.5	6.5
Composition		
Crude protein, g/kg	217.0	198.1
Crude fat, g/kg	63.6	65.6
Metabolisable energy, kcal/kg	3000	3080
Crude fibre, g/kg	38.0	27.8
Calcium,[#] g/kg[2]	11	9.8
Phosphorus, g/kg	7.1	6

°Trimix (per kg Trimix): iron, 100 g; manganese, 110 g; copper, 20 g; zinc, 100 g; iodine, 2 g; selenite, 0.2 g; cobalt, 0.6 g; santoquin, 0.6 g; folic acid, 0.33 g; thiamin, 0.83 g; pyridoxine, 1.33 g; biotin 2%, 0.03 g; riboflavin, 2 g; cyanocobalamin, 0.03 g; D-calcium pantothenate, 3.75 g; niacin, 23.3 g; retinol, 2000 mg; cholecalciferol, 25 mg; α-tocopherol, 23,000 mg U. [#]Calculated.

Table 2. Fatty acid composition (g/100 g of total identified fatty acids) of the basal diet.

	Starter (1 to 14 days)	Grower (15 to 35 days)
Fatty acid, %		
C14:0	0.59	0.66
C16:0	30.64	27.87
C16:1	0.33	0.32
C17:0	0.21	0.14
C18:0	4.38	3.87
C18:1n-9	36.93	38.75
C18:2n-6	25.06	26.29
C18:3n-6	1.12	1.24
C18:3n-3	0.33	0.32
C20:4n-6	0.20	0.26
C20:5n-3	0.21	0.28
ΣSFA	35.82	32.54
ΣUFA	64.18	67.46
ΣMUFA	37.26	39.07
Σn-6 PUFA	25.26	26.56
Σn-3 PUFA	0.54	0.60
ΣPUFA	25.80	27.16
Σn-6:Σn-3 ratio	46.99	44.55
UFA/SFA	1.79	2.07
PUFA/SFA	0.72	0.83

ΣSFA, sum of C14:0, C16:0, C17:0, C18:0; SFA, saturated fatty acids; ΣUFA, sum of C16:1, C18:1n-9, C18:2n-6, C18:3n-6, C18:3n-3, C20:4n-6, C20:5n-3; ΣMUFA, sum of C16:1, C18:1n-9; MUFA, monounsaturated fatty acids; Σn-3PUFA, sum of C18:3n-3, C20:5n-3; PUFA, polyunsaturated fatty acids; Σn-6PUFA, sum of C18:2n-6, C18:3n-6, C20:4n-6; Σn-6/Σn-3 ratio, Σn-6PUFA (sum of C18:2n-6, C18:3n-6, C20:4n-6)/Σn-3 PUFA (sum of C18:3n-3, C20:5n-3); UFA/SFA, sum of C16:1, C18:1n-9, C18:2n-6, C18:3n-6, C18:3n-3, C20:4n-6, C20:5n-3/sum of C14:0, C16:0, C17:0, C18:0; PUFA/SFA, sum of C18:3n-3, C20:5n-3, C18:2n-6, C18:3n-6, C20:4n-6, C18:1n-9/sum of C14:0, C16:0, C17:0, C18:0. Values are the mean of two replicates.

(Agilent 7890A; Agilent Technologies, Santa Clara, CA, USA), using a Supelco SP 2330 capillary column of 30 m × 0.25 mm ID × 0.2-µm film thickness (Supelco, Bellefonte, PA, USA). The amount of 1 µL of each sample was injected by an auto-sampler (Agilent Auto Analyzer 7683 B series, Agilent Technologies) into the chromatograph, equipped with a split/splitless injector and a flame ionization detector. The carrier gas was nitrogen at a flow rate of 1.2 mL/min. The split ratio was 1:20 after injection of 1 µL of the FAME. The injector temperature was programmed at 250ºC, and the detector temperature was 270ºC. The column temperature program started to run at 150ºC, for 2 min, warmed to 158ºC at 1ºC/min, held for 28 min, warmed to 220ºC at 1ºC/min and then held for 20 min to achieve satisfactory separation. The peaks of samples were identified, and concentrations calculated based on the retention time and peak area of known standards (mix C4-C24 methyl esters; Sigma-Aldrich, Inc., St. Louis, MO, USA). The fatty acid concentrations are expressed as g per 100 g of the sum of identified fatty acids measured in each sample.

Statistical analysis

All data were analyzed by analysis of variance (ANOVA) using the SAS (Statistical Analysis System, 2008) program version 9.2., by a 2×2 factorial arrangement of treatments, in which each pen was considered as the experimental unit. The model utilized included the effects of temperature and TA, as well as the interactive effects. Duncan's test was used for multiple comparisons when a significant interaction was detected. The result considered significant if $P<0.05$.

Results and discussion

Fatty acid compositions of the experimental diets are shown in Table 2. Ether extract, calculated ME, and CP contents of the experimental diets were similar. There was no difference in the total fat contents of the diets, ΣSFA, ΣMUFA, and Σn-6 and Σn-3 polyunsaturated fatty acids (PUFA) contents among the experimental diets.

Growth performance

Effects of temperature and TA supplementation on growth performance of broiler chickens are presented in Table 3. The result showed that high temperature significantly ($P<0.01$) reduced BWG and FI. Feed intake of birds in the CH group (TA-free diet at 35°C, 2951 g) was significantly ($P<0.01$) lower than their counterparts in the CL group (TA-free diet at 25°C, 3290 g). Similarly, supplementation of TA also reduced ($P<0.01$) BWG and FI, but its effect was more drastic than the heat stress (2458 and 2357 g for TL and TH groups, respectively). Within the same temperature (25°C), supplementation of TA depressed FI by 25.3% (3290 *vs* 2458 g). The interaction effect of temperature and TA on BWG and FI was also significant. The FCR significantly increased under high temperature, however, the increase of FCR by adding TA was not significant. Addition of TA at high temperature slightly improved the FCR (Table 3).

Heat stress is a great concern in the poultry industry as productivity is adversely affected by heat stress. Geraert *et al.* (1996) reported that broilers exposed to 32°C showed a 24% decrease in feed intake by 6 wk of age compare to the normal temperature. Mashaly *et al.* (2004) reported that heat stress not only adversely affected the performance, but also inhibited the immune function in chickens. Results of this study showed that high ambient temperature suppressed growth performance through reduction in FI, which is in agreement with the literature (Geraert *et al.*, 1996; Mashaly *et al.*, 2004; Quinteiro-Filho *et al.*, 2012). Results of the present study showed a 10.3% decline in FI of birds kept under 35°C compared to those kept under 25°C. The 10.3% decrease of FI has resulted in 15.3% lower BWG. Furthermore, the result showed that the effects of TA depended on the temperature condition. In high temperature condition,

Table 3. Effect of temperature and tannic acid supplementation on final body weight, body weight gain, feed intake, and feed conversion ratio of broiler chickens.

Parameter	Temperature, °C		Tannic acid, %		CL	TL	CH	TH	SEM	P		
	25	35	0	1						Temperature	TA	Temperature ×TA
FBW, g	1616.5	1440.4	1730.7	1326.2	1870.9^{a}	1362.2^{c}	1590.5^{b}	1290.3^{d}	73.532	**	**	**
BWG, g/day	44.91	39.91	48.19	36.63	52.2^{a}	37.60^{c}	44.20^{b}	35.6^{c}	1.499	**	**	**
FI, g	2873	2653	3120	2407	3290^{a}	2458^{c}	2951^{b}	2357^{d}	86.631	**	**	**
FCR	1.83	1.90	1.86	1.88	1.80^{b}	1.87^{a}	1.91^{a}	1.89^{a}	0.013	**	ns	*

CL, basal diet at low temperature; TL, basal diet supplemented with 1% TA at low temperature; TA, tannic acid; CH, basal diet at high temperature; TH, basal diet supplemented with 1% TA at high temperature; FBW, final body weight; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio. Means within a row with no common superscripts differ significantly. **Significant at 1% level; *significant at 5% level; ns, not significant. Values are mean ± SE of 5 replicate cages, each with 6 chickens.

Table 4. Effect of temperature and tannic acid on blood cholesterol, high and low density lipoprotein, and triglyceride (mg/dL).

Parameter	Temperature, °C		Tannic acid, %		CL	TL	CH	TH	SEM	P		
	25	35	0	1						Temperature	TA	Temperature ×TA
Cholesterol	3.05	2.82	2.98	2.89	3.07^{a}	3.04^{a}	2.90^{b}	2.74^{c}	0.028	**	ns	ns
HDL	2.01	1.78	1.95	1.84	1.99^{a}	2.02^{a}	1.90^{b}	1.66^{c}	0.102	**	ns	ns
LDL	0.80	0.79	0.82	0.78	0.86	0.74	0.78	0.81	0.036	ns	ns	ns
TG	0.41	0.50	0.45	0.46	0.48	0.35	0.43	0.58	0.029	ns	ns	ns

CL, basal diet at low temperature; TL, basal diet supplemented with 1% TA at low temperature; TA, tannic acid; CH, basal diet at high temperature; TH, basal diet supplemented with 1% TA at high temperature; HDL, high density lipoprotein; LDL, low density lipoprotein; TG, triglyceride. Means within a row with no common superscripts differ significantly. **Significant at 1% level; Significant at 5% level; ns, not significant. Values are mean ± SE of 5 replicate cages, each with 6 chickens.

addition of TA caused lower BWG and FI, but addition of TA in low temperature slightly improved BWG and FI.

Tannins are usually considered as anti-nutritive substances because of their ability to form stable complexes with dietary nutrients, thereby they decrease the feed digestibility (McSweeney *et al.*, 2001; Smulikowska *et al.*, 2001). The biological effect of tannins in poultry nutrition is related to their adverse effects on feed intake (Armstrong *et al.*, 1974) and nutrient utilization (Smulikowska *et al.*, 2001). Armstrong *et al.* (1974) showed that addition of tannic acids with varying molecular weights to a non-resistant sorghum grain diet resulted in significant depressions in chicken performance. Also, Chang and Fuller (1964) presented evidence that when grain sorghums containing relatively high levels of tannin were fed to young chicks, the growth rate was retarded and liver lipids slightly elevated. Similar results were obtained by feeding 1% of the diet tannic acid, which is equal to that occurring in the high tannin grain sorghum. However, in contrast, some researchers indicate that tannins can improve growth performance at normal conditions (Maertens and Struklec, 2006; Kermauner and Lavren i , 2008; Dalle Zotte and Cossu, 2010). Liu *et al.* (2009) showed natural extracts of chestnut wood, with high tannin contents, had no significant effect on live weight, productive traits, hot carcass weight, dressing percentage, skin weight, pH, cooking losses, shear force and color.

The results of blood cholesterol, HDL, LDL and TG analysis of the broilers are summarized in Table 4. Results show that cholesterol and HDL contents were significantly ($P<0.01$) reduced by high temperature (from 3.05 to 2.82 mg/dL for cholesterol, and from 2.02 to 1.78 mg/dL for HDL). However, heat stress had no effect on LDL and TG contents of serum. Supplementation of TA alone or in combination with high temperature had no effect on the four measured parameters.

There are few reports on the effects of TA on lipid metabolism (Yugarani *et al.*, 1992; Levrat *et al.*, 1993; Osada *et al.*, 2006). Yugarani *et al.* (1992, 1993) reported that TA significantly reduced serum and hepatic lipid concentrations in rats fed high-fat diets. However, they administered low doses of TA (less than 100 mg/kg). Furthermore, Osada *et al.*, (2006) showed that dietary polyphenol tended to reduce fatty acid synthesis and promote fatty acid β-oxidation as compared with a high fat diet alone. Levrat *et al.* (1993) indicated pre-fermented condensed tannin (quebracho) increased the activity of liver 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase), a rate limiting enzyme of cholesterogenesis. In the present study, lower plasma cholesterol (especially HDL) in chickens under heat stress indicated that endogenous cholesterol metabolism was probably disturbed.

Among the measured fatty acids and their ratios, high temperature had significant effects only on C16:1, C17:0 and Σn-6/n-3 ratio, however, the TA showed significant effects on C14:1, C16:1, C17:0, C18:0, C18:1n-9, C20:4n-6, C20:5n-3, ΣMUFA, Σ n-6 PUFA, Σ n-3 PUFA, ΣPUFA and PUFA/SFA (Table 5). High temperature significantly ($P<0.01$) increased C16:1, but reduced C17:0. However, supplementation of TA significantly ($P<0.01$) increased the C17:0, but reduced the C16:1 content. The TA also had increasing effects on C14:1, C20:4n-6, C20:5n-3, Σ n-6 PUFA, Σ n-3 PUFA, ΣPUFA and

Table 5. Effect of dietary supplementation of temperature and tannic acid on the fatty acid composition (g/100 g total fatty acids) of breast muscle of broilers.

Fatty	Temperature, °C		Tannic acid, %		CL	TL	CH	TH	SEM	P		
acid/ratio	25	35	0	1						Temperature	TA	Temperature ×TA
C14:0	1.47	1.68	1.24	1.98	1.34	1.64	1.15	2.32	0.217	ns	ns	ns
C14:1	0.56	0.43	0.29^{b}	0.74^{a}	0.33^{bc}	0.85^{a}	0.25^{c}	0.64^{a}	0.092	ns	*	*
C16:0	24.28	24.65	25.24	23.53	24.86	23.60	25.63	23.46	0.516	ns	ns	ns
C16:1	3.49^{b}	4.92^{a}	4.80^{a}	3.49^{b}	3.84	3.07	5.77	3.92	0.326	*	*	ns
C17:0	2.67^{a}	1.77^{b}	1.50^{b}	3.09^{a}	1.85	3.65	1.15	2.53	0.229	**	**	ns
C18:0	14.93	10.69	15.00^{a}	10.17^{b}	16.43	13.13	13.58	7.22	1.195	ns	*	ns
C18:1n-9	26.30	29.18	31.18^{a}	23.60^{b}	28.84	23.25	33.53	23.96	1.550	ns	*	ns
C18:2n-6	11.07^{a}	14.39^{b}	11.05	14.75	10.99^{b}	11.16^{b}	11.11^{b}	18.33^{a}	1.057	*	ns	*
C18:3n-6	0.65	1.79	0.66	1.90	0.67	0.63	0.64	3.17	0.535	ns	ns	ns
C18:3n-3	0.71	0.78	0.66	0.84	0.67	0.76	0.66	0.92	0.048	ns	ns	ns
C20:4n-6	2.64	2.16	1.76^{b}	3.17^{a}	2.32	3.02	1.19	3.33	0.329	ns	*	ns
C20:5n-3	2.15	1.69	1.49^{b}	2.43^{a}	1.84	2.51	1.15	2.35	0.171	ns	**	ns
C22:5n-3	4.41	1.62	1.16	5.25	1.37	8.06	0.94	2.43	1.354	ns	ns	ns
C22:6n-3	4.60	4.20	3.90	5.007	4.59^{ab}	4.63^{ab}	3.21^{b}	5.38^{a}	0.332	ns	ns	*
ΣSFA	43.00	38.79	43.00	38.77	44.49	42.02	41.51	35.53	1.393	ns	ns	ns
ΣUFA	56.63	61.20	56.99	61.22	55.50	57.97	58.48	64.46	1.393	ns	ns	ns
ΣMUFA	30.36	34.54	36.28^{a}	27.85^{b}	33.01	27.18	39.55	28.52	1.701	ns	**	ns
Σ n-6 PUFA	13.72	16.56	12.81^{b}	17.92^{a}	13.32^{b}	14.18^{b}	12.30b	21.66^{a}	1.157	ns	*	*
Σ n-3 PUFA	11.88	8.30	7.23^{b}	13.53^{a}	8.48	15.97	5.97	11.10	1.451	ns	*	ns
ΣPUFA	25.61	24.87	20.05^{b}	31.46^{a}	21.81	30.16	18.28	32.70	1.869	ns	**	ns
Σn-6/Σn-3	1.45^{b}	2.19^{a}	1.90	1.72	1.65	1.21	2.15	2.24	0.168	*	ns	ns
UFA/SFA	1.38	1.61	1.35	1.67	1.27	1.51	1.43	1.83	0.093	ns	ns	ns
PUFA/SFA	0.64	0.67	0.47^{b}	0.88^{a}	0.50	0.81	0.44	0.94	0.077	ns	**	ns

CL, basal diet at low temperature; TL, basal diet supplemented with 1% TA at low temperature; TA, tannic acid; CH, basal diet at high temperature; TH, basal diet supplemented with 1% TA at high temperature; ΣSFA, sum of C14:0, C16:0, C17:0, C18:0; SFA, saturated fatty acids; ΣUFA, sum of C16:1, C18:1n-9, C18:2n-6, C18:3n-6, C18:3n-3, C20:4n-6, C20:5n-3; ΣMUFA, sum of C16:1, C18:1n-9; MUFA, monounsaturated fatty acids; Σn-6PUFA, sum of C18:2n-6, C18:3n-6, C20:4n-6; PUFA, polyunsaturated fatty acids; Σn-3PUFA, sum of C18:3n-3, C20:5n-3; Σn-6/Σn-3 ratio, Σn-6PUFA (sum of C18:2n-6, C18:3n-6, C20:4n-6)/Σn-3 PUFA (sum of C18:3n-3, C20:5n-3); UFA/SFA, sum of C16:1, C18:1n-9, C18:2n-6, C18:3n-6, C18:3n-3, C20:4n-6, C20:5n-3/sum of C14:0, C16:0, C17:0, C18:0; PUFA/SFA, sum of C18:3n-3, C20:5n-3, C18:2n-6, C18:3n-6, C20:4n-6, C18:1n-9/sum of C14:0, C16:0, C17:0, C18:0. For temperature, tannic acid or their interactions, means within a row with no common superscripts differ significantly. **Significant at 1% level; *significant at 5% level; ns, not significant. Values are mean ± SE of 5 replicate cages, each with 6 chickenst.

PUFA/SFA ratio, but decreasing effect on C18:0, C18:1n-9 and ΣMUFA. The interaction of temperature and TA had significant effects on C14:1, C18:2n-6, C22:6n-3 and Σn-6 PUFA. For C14:1, increasing of the temperature reduced the protective effects of TA. The contents of C18:2n-6 and Σn-6 PUFA in the breast muscle of chickens were in their highest amount in the TH group (treatment including TA at 35°C). For C22:6n-3, treatments with high temperature without TA and with TA (CH and TH) showed the least and the most amounts of C22:6n-3, respectively.

The essential fatty acids play an important role in immunity, inflammation and blood clotting. Since essential fatty acids are not synthesized in the chicken's body, their presence in the body depends on both their presence in the diet, and their rate of oxidation in the tissues (Fisher, 1984). Therefore, with supplementations of the TA as an antioxidant, the breast muscle essential fatty acid profile can be protected under high temperature. Trebble *et al.* (2004) showed that diet supplementation with antioxidant, inhibits lipid peroxidation and the production of free radicals that can result from increased PUFA in the breast muscle. In this study, addition of TA improved the Σn-6/n-3 ratio, Σn-6 PUFA, UFA/SFA and PUFA/SFA under high temperature, which is favoured by the health conscious consumers of meat.

Omega-3 fatty acids are important fatty acids for normal metabolism and incorporated in nearly all biological compartments of poultries, humans and animals, but some of their potential health benefits are controversial. In the present study, addition of TA caused to increase the n-3 fatty acids such as eicosapentaenoic acid (C20:5n-3) significantly. Among the Σn-6 PUFA, the linoleic acid (C18:2 n-6) is an important fatty acid, which is precursor of arachidonic acid. Arachidonic acid contributes to the production of eicosanoids, which are a group of biologically important lipids, including prostaglandins, thromboxanes, lipoxins and leukotrienes (Bourre *et al.*, 1993).

Based on the results of the present study, TA decreased the amounts of MUFA, but increased n-3, n-6 and total PUFA in the breast muscle of broilers. However, it does not have any effect on the SFA contents of the muscles. These results were to some extend comparable with the report of Cherian *et al.* (2002), who investigated the muscle fatty acid composition of broilers fed sorghum containing high amount of tannin. However, they reported that the total SFA, MUFA, and n-3 and n-6 PUFA were not different among the experimental groups. In this study, the interaction effect of temperature and TA on the fatty acid composition of breast muscle of broilers was significant only for C14:1, C18:2n-6, C22:6n-3 and Σn-6 PUFA. These results were in contrast with the results of Shim *et al.* (2006) who exposed broiler chickens to chronic heat stress with diets supplemented by taurin as an antioxidant. They reported that by adding taurin to the diet the total levels of SFA decreased, but MUFA and UFA levels increased, as compared to chicks fed the control diet under heat stress condition. Khokhar and Apenten (2003) reported that tannic acid antioxidant efficiency depends on the number of the relative positions of the hydroxyl groups bound to the aromatic ring and the site of binding. Some researchers showed that tannic acid antioxidant/pro-oxidant behaviour is a dose-dependent manner because an increase in non-toxic concentrations of tannic acid caused a slight non-significant increase of O_2^-, NO and malondialdehyde (MDA). It seems that low concentrations of tannic acid are beneficial for cells, as scavengers for ROS intermediates, causing prevention of forming ROS and the concomitant enhancement of lipid peroxidation (Salminen *et al.*, 2001; Bertram *et al.*, 2003; Perron and Brumaghim, 2009).

Conclusions

In conclusion, although in the present experiment supplementation of TA did not alleviate adverse effect of chronic heat stress on growth performance, it improved the breast muscle fatty acid profile of the broilers. These results are important in terms of increasing the meat quality, which is linked to human health, and suggest that the TA could be potentially applied as a biological antioxidant for poultry nutrition in hot climatic condition.

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