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REGULAR ARTICLES

Alleviation of chronic heat stress in broilers by dietary supplementation of betaine and turmeric rhizome powder: dynamics of performance, leukocyte profile, humoral immunity, and antioxidant status

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Abstract Heat stress (HS), one of the most serious climate problems of tropical and subtropical countries, negatively affects the production performance of broilers. Keeping this in view, the current study was aimed at elucidating the effects of supplementing betaine (Bet) and dried turmeric rhizome powder (TRP), either singly or in combination, on growth performance, leukocyte profile, humoral immunity, and antioxidant status in broilers kept under chronic HS. A total of 625 one-day-old Ross male chicks were randomly assigned to five treatment groups (5 replicates of 25 birds per replicate pen). From day 1, the birds were either kept at the thermoneutral zone (TN) or exposed to HS (33±1 °C) to the conclusion of study, day 42. The treatment groups were as follows: thermoneutral control (TN-CON), HS-CON, HS-Bet, HS-TRP, and HS-BT (fed Bet and TRP). The results showed that decreases in body weight gain, feed intake, and increases in feed-to-gain ratio and mortality induced by HS were partially restored by dietary supplementation of Bet and TRP. The heterophil/lymphocyte ratio, total, and IgG antibody titers against sheep red blood cell for secondary responses in the HS-TRP and HS-BT groups were also similar to those of the broilers in the TN-CON group but better ($P<0.05$) than for HS-CON group. An increase ($P<0.05$) in serum concentration of malondialdehyde induced by HS was significantly decreased by dietary supplementations. The serum glutathione peroxidase and superoxide dismutase activities were also higher ($P<0.05$) in the supplemented groups compared to both TN and HS-CON groups. In conclusion, dietary supplementation of either Bet or TRP alone or in combination can partially ameliorate some of the detrimental effects of HS in broilers. Results also suggest that TRP might be better than Bet for improving stress tolerance and immune response in heat-stressed broilers.

Keywords Chronic heat stress · Betaine · Turmeric · Immunity · Antioxidant activity · Broilers

Introduction

In the poultry industry, broilers are genetically selected for a fast growth rate and a high feed efficiency. They are very susceptible to high ambient temperature due to fast metabolic rates and lack of sweat glands (Khan et al. 2012). Heat stress (HS) is characterized by high mortality, decreased feed intake, poor body weight gain, higher feed conversion ratio (Attia et al. 2009; Günal 2013), and immunosuppression (Pamoka et al. 2009) in poultry. Oxidative stress induced by HS has been reported as a primary factor that increases the pathogenesis of several diseases, decreases production results, and results in oxidative deterioration of poultry meat (Huang et al. 2015; Salami et al. 2015). In tropical and subtropical countries like Iran, broilers are raised in an open-house system compared to environmentally controlled housings system in temperate region. However, due to the high cost of cooling poultry houses, dietary manipulations are increasingly used as possible approaches to alleviate the harmful effects of high ambient temperature in poultry (Khan et al. 2012; Renaudeau et al. 2012).

Betaine (Bet) is a common term for trimethylglycine, a substrate for betaine-homocysteine methyl transferase in the

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liver and kidney. It is often found in high concentrations in plants subjected to drought, which is due to the osmoregulatory (water balance) properties of betaine (Attia et al. 2009). Betaine is considered a highly efficient organic osmoprotectant that protects cells from osmotic stress and allows them to continue regular metabolic activities in conditions that would normally inactivate the cell (Alirezaei et al. 2012a; Sakomura et al. 2013). Therefore, it can be hypothesized that adding organic osmolytes, such as Bet, to diets of HS broilers may enhance the birds' ability to tolerate hypertonicity and retain more water in body tissues.

Turmeric is the rhizome of *Curcuma longa* L. and is widely cultivated in southern and southeastern tropical Asia. Dried turmeric rhizome powder (TRP) is commonly used as a spice, coloring, flavoring, and traditional medicine (Nouzarian et al. 2011). The curcuminoids (as phenolic compounds) are major antioxidative compounds of TRP and have the ability to inhibit lipid peroxidation and scavenge the superoxide anion and hydroxyl radicals (Ling et al. 2012). Many studies have shown the capacity of curcumin to prevent lipid peroxidation, a key process in the onset and progression of many diseases (Daneshyar et al. 2012). Keeping in view the mentioned pharmaceutical advantages of turmeric, we have hypothesized that the TRP may reduce the negative effects of HS on physiological changes and production of broilers.

Previously, Bet (Attia et al. 2009; He et al. 2015) and several dietary herb additives (Habibi et al. 2014; Akbarian et al. 2015) have been examined for the alleviation of HS in poultry. However, literature describing the effects of Bet and TRP supplementations under chronic HS conditions in broilers is lacking, especially in terms of immunity and antioxidant status. Therefore, the aim of this study was to examine the potential role of Bet and TRP, alone or in combination, on growth performance, leukocyte profile, humoral immunity, and antioxidant status in broilers exposed to chronic HS.

Materials and methods

Birds housing and feeding

A total 625 one-day-old chicks (Ross-308) of mixed sex were used in this study. The broilers were randomly divided into five treatment groups (n=125) with five replicates (n=25) for each group. The chickens were placed in floor pens on fresh pine shavings litter in environmentally controlled houses. For the chickens kept in the thermoneutral zone (TN-CON group), ambient temperature on day 1 was set at 33±1 °C and was decreased 3 °C per week until it reached 24±1 °C; thereafter, the temperature was kept constant for the remaining period of the study. For HS groups, on day 1, the room temperature was maintained at 33±1 °C and was kept constant until day 42. Heat-stressed broilers were fed a basal diet (HS-CON group) or the same basal diet supplemented with 0.1 % betaine (HS-Bet group), 0.2 % turmeric rhizome powder (HS-TRP group), or their combination (HS-BT group) for the entire study period. A three-phase feeding program was used, with a starter diet from d 0 to 10, a grower diet from d 11 to 24, and a finisher diet from d 25 to 42 (Table 1). The relative humidity was kept at 55 % in all groups throughout the trial. Continuous light was provided 24 h for the first 3 days and then 23 L:1D light was adopted for the rest of the trial period. All chickens were fed a maize-soybean meal basal diet and offered water ad libitum. All procedures of this study were performed according to the guidelines for animal experiments at the National Institute of Animal Health.

Betaine (Betafin®, 96 % natural betaine) was obtained from Biochem Company (Brinkstrasse 55, D-49393 Lohne, Germany). Fresh turmeric rhizome (imported from India) was provided from the local market, ground, and mixed with the diets. Estimation of total phenols in the TRP was according to the standard extraction method of Seevers and Daly (1970). A total amount of 16.2 mg/g phenolic compounds was found for the TRP.

Growth performance

Growth performance data were determined on days 21 and 42. To get body weight gain (BWG), initial body weights were subtracted from the final body weights. Feed intake (FI) was determined by subtracting residual feed from the offered feed. Data for FI and BWG were used to calculate the feed-to-gain ratio (FGR). Broiler mortalities were recorded as they occurred daily to correct the FGR per pen and to calculate the mortality rate (%) of the birds at 21 and 42 days of age.

Immune response to sheep red blood cell

In order to assay the primary and secondary immune response, on days 21 and 35, two bird per pens were immunized intramuscularly (breast muscle) with 0.25 mL suspension of 10 % sheep red blood cell (SRBC) in phosphate buffer saline. Blood samples (1.5 mL/bird) were obtained from brachial vein at 7 days after each injection (days 28 and 42). Serum was separated by centrifugation at 3000×g for 10 min and stored at −20 °C for further analysis. Total antibody titers against SRBC were determined by a hemagglutination method using 96-well, U-bottomed microtiter plates according to Wegmann and Smithies (1966). The 2-mercaptoethanol-resistant (MER) antibodies in the serum were measured according to the method described by Bartlett and Smith (2003). The 2-mercaptoethanol-sensitive (MES) antibodies were also estimated as the difference between the total and MER antibody titers. MER antibodies are presumed to be a measure of IgG titers, whereas MES antibodies consist

Table 1 Ingredients (%) and calculated composition of the basal diet

Ingredient	Starter (days 1–10)	Grower (days 11–24)	Finisher (days 29–42)
Corn	51.89	53.26	57.97
Soybean meal (44 % CP)	38.62	36.52	32.06
Soybean oil	4.59	6.12	6.05
Limestone	1.29	1.07	1.04
Dicalcium phosphate[a]	2.05	1.77	1.64
Sodium chloride	0.27	0.21	0.10
Bicarbonate sodium	0.10	0.19	0.35
Vitamin premix[b]	0.25	0.25	0.25
Mineral premix[c]	0.25	0.25	0.25
DL-Methionin	0.34	0.26	0.22
L-lysin, HCL	0.26	0.10	0.07
L-threonine	0.09	–	–
Calculated analysis			
ME, Kcal/kg	3025	3150	3200
Crude protein, %	22.00	21.00	19.40
Ca, %	1.05	0.90	0.85
Available phosphorous, %	0.50	0.45	0.42
Digestible lysine, %	1.27	1.10	0.97
Digestible methionine+cystine, %	0.94	0.84	0.76
Digestible threonine, %	0.83	0.73	0.68
DEB[d], mEq/kg	250	250	250

[a] Dicalcium phosphate contained 16.8 % phosphorous and 23 % calcium

[b] Vitamin premix per kilogram of diet: vitamin A (retinyl acetate), 9000 IU; vitamin D_3 (cholecalciferol), 2500 IU; vitamin E (alpha-tocopheryl acetate), 18 IU; vitamin K_3 (menadione), 2 mg; thiamine, 1.8 mg; riboflavin, 6.6 mg; niacin (nicotine amid), 30 mg; panthothenic acid, 10 mg; pyridoxine, 3 mg; biotin, 0.1 mg; folic acid, 1 mg; cyanocobalamin, 0.015 mg; choline chloride, 250 mg; antioxidant, 100 mg

[c] Mineral premix per kilogram of diet: Zn (ZnO), 100 mg; Mn ($MnSO_4 \cdot H_2O$), 100 mg; Fe ($FeSO_4 \cdot 7H_2O$), 50 mg; Cu ($CuSO_4 \cdot 5H_2O$), 10 mg; I (KI), 1 mg; Se ($NaSeO_3$), 0.2 mg

[d] DEB=(Na^+, mEq/kg+K^+, mEq/kg)−CL^-, mEq/kg

primarily of IgM titers. Anti-SRBC titers were expressed as the reciprocal of the highest serum dilution resulting in complete agglutination.

Blood parameters

On day 42 of age, two birds per replicate pen were randomly selected for blood sampling. Two blood samples (with and without EDTA) were collected from brachial veins of chickens. Serum used for measurement of antioxidant enzyme activities and whole blood samples were also used to determine leukocyte subsets. Serum samples were collected after centrifugation at 1300×*g* for 10 min at 4 °C and stored (−20 °C) before analysis. Serum glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities were determined colorimetrically (enzymatically), and serum total antioxidant capacity (TAC) and malondialdehyde (MDA) were determined colorimetrically (chemically), using an ELISA microplate reader (Tecan Co., Grodingen, Austria), GPx assay kit (Cayman Chemical Co., Ann Arbor, MI, USA), SOD assay kit (Cayman Chemical Co., Ann Arbor, MI, USA), TAC assay kit (Randox Laboratories Ltd, Crumlin, UK), and MDA assay kit (Cayman Chemical Co., Ann Arbor, MI, USA). All assays were performed according to the manufacturer's instructions without any modification.

To determine blood leucocyte profiles, 100 leucocytes per samples were counted by an optical microscope for heterophil and lymphocyte separation according to the protocol described by Lucas and Jamroz (1961), and then heterophil/lymphocyte (H/L) ratio was calculated.

Statistical analysis

Data were analyzed in a completely randomized design using the general linear models procedures of SAS (SAS Institute Inc. 2001). Data on growth performance parameters were analyzed on a pen basis, whereas data on leukocyte profile, immune response, and oxidant/antioxidant parameters were

based on individual broilers. The percentage data on mortality was transformed to $\sqrt{n+1}$ for statistical analyses (Mead et al. 1993). The transformed data followed a normal distribution. Log2 transformations were performed on antibody titers prior to statistical analysis. When the differences were significant ($P<0.05$), mean values between treatments were compared using Tukey's test.

Results

The effects of HS and dietary supplements on performance parameters are presented in Table 2. The TN-CON group had higher ($P<0.05$) BWG, FI, and lower FGR compared with all HS groups. The HS-CON group had 17.2 % less BWG on day 21 and 27.0 % less on day 42 as compared with the TN-CON group. The HS-CON birds also consumed 14.7 % less feed compared with the TN-CON group on day 42. The BWG of all supplemented group was higher than for HS-CON group but lower than for TN-CON group. Although there were no significant differences among the supplemented groups in FI at 21 days of age, the HS-TRP and HS-BT groups had significantly higher FI than broilers in the HS-CON group at 42 days of age. The results also indicated that at 21 days of age, the HS-Bet group experienced significantly lower FGR compared to the HS-CON group. On day 42, all supplemented HS groups had lower FGR compared with the HS-CON group. The mortality rate was non-significant ($P>0.05$) among experimental groups, on day 21. In contrast, TN-CON and HS-BT groups had lower ($P<0.05$) mortality rate compared with the HS-CON group on day 42.

Data for leukocyte profiles are presented in Table 3. On day 42, the TN-CON group had higher ($P<0.05$) lymphocyte count and lower heterophil count and H/L ratio compared with the HS-CON group. The H/L ratio in the HS-TRP and HS-BT groups was also lower than in broilers in HS-CON group ($P<0.05$), but similar to the value in TN-CON group ($P>0.05$). However, the dietary supplements in the current study failed to induce any significant impact on other leukocyte subsets.

As Table 4 shows, the primary anti-SRBC titers of total and IgM antibodies were lower in the HS-CON group compared with the TN-CON group. In the case of secondary response, there was also an increase ($P<0.05$) in total and IgG anti-SRBC titers in the TN-CON group compared to the HS-CON group. The additives used in this study failed to induce any significant effect on the primary antibody response at 35 days of age. In contrast, on day 42, serum secondary total and IgG anti-SRBC titers were significantly higher ($P<0.05$) in HS-TRP and HS-BT groups than in HS-CON group but similar ($P>0.05$) to the values in TN-CON group (Table 4).

Serum TAC and MDA concentrations and SOD and GPx activities were higher ($P<0.05$) in the HS-CON group compared with the TN-CON group (Table 5). Furthermore, the serum GPx and SOD activities were increased ($P<0.05$) in supplemented groups compared to the both the TN and HS-CON groups. The serum MDA concentration in the HS-Bet, HS-TRP, and HS-BT groups was also lower ($P<0.05$) than in the HS-CON group, where the HS-BT group had the lowest MDA level. However, serum TAC level was not different when the supplemented groups were compared with the HS-CON group.

Table 2 Body weight gain (BWG), feed intake (FI), feed-to-gain ratio (FGR), and mortality rate in broilers reared under normal temperature or heat stress conditions

	Treatment group						
Parameter	TN-CON	HS-CON	HS-Bet	HS-TRP	HS-BT	SEM	*P* value
BWG, g							
21 days	848.9^{a}	703.2^{c}	775.0^{b}	754.3^{b}	762.6^{b}	19.6	0.048
42 days	2513^{a}	1835^{c}	2110^{b}	2047^{b}	2094^{b}	43.7	<0.001
FI, g							
21 days	1205	1188	1225	1230	1228	30.2	0.244
42 days	4347^{a}	3707^{c}	3819^{bc}	3848^{bc}	3895^{b}	57.1	0.008
FGR, g:g							
21 days	1.42^{c}	1.69^{a}	1.58^{b}	1.63^{ab}	1.61^{ab}	0.031	0.019
42 days	1.73^{c}	2.02^{a}	1.81^{bc}	1.88^{b}	1.86^{b}	0.043	0.034
Mortality rate (%)							
21 days	3.2	4.8	4.0	3.2	2.4	0.82	0.181
42 days	4.8^{b}	9.6^{a}	5.6^{ab}	6.4^{ab}	3.2^{b}	1.63	0.025

Means within the same raw without a common superscript letter differ significantly ($P<0.05$)

TN-CON thermoneutral control group, *HS-CON* heat stress control group, *HS-Bet* betaine-supplemented heat stress group, *HS-TRP* turmeric rhizome powder-supplemented heat stress group, *HS-BT* betaine and turmeric rhizome powder-supplemented heat stress group

Table 3 Blood leukocyte profile in broilers reared under normal temperature or heat stress conditions

Parameter	Treatment group					SEM	*P* value
	TN-CON	HS-CON	HS-Bet	HS-TRP	HS-BT		
Monocytes	3.33	4.66	3.26	2.50	2.67	0.79	0.257
Eosinophils	0.80	2.67	2.37	1.27	0.83	0.68	0.296
Basophils	0.67	1.67	1.87	0.83	1.30	0.57	0.521
Heterophil (H)	27.5^{b}	39.5^{a}	35.0^{ab}	30.2^{ab}	31.7^{ab}	3.06	0.020
Lymphocyte (L)	67.7^{a}	51.5^{b}	57.5^{ab}	65.2^{a}	63.5^{a}	3.80	0.044
H:L	0.41^{b}	0.76^{a}	0.61^{ab}	0.46^{b}	0.49^{b}	0.07	<0.001

Means within the same raw without a common superscript letter differ significantly ($P<0.05$)

TN-CON thermoneutral control group, *HS-CON* heat stress control group, *HS-Bet* betaine-supplemented heat stress group, *HS-TRP* turmeric rhizome powder-supplemented heat stress group, *HS-BT* betaine and turmeric rhizome powder-supplemented heat stress group

Discussion

The study was planned to investigate the effects of Bet and TRP supplementations on broiler performance and physiological traits when reared under chronic HS. Results revealed that broilers exposed to HS had poor growth performance. Deteriorated performance of HS group can be attributed to a higher expenditure of energy for physiological adaptation (acclimation) to changing environmental temperatures instead of growth enhancement (Renaudeau et al. 2012). Alternatively, it is believed that less BWG in the HS broilers is due to a lower appetite and FI, as it may be a crucial defense mechanism to help reduce heat production. It is also reported that HS disturbs the balance of intestinal flora and, hence, decreases nutrient digestibility and absorption (Feng et al. 2012), resulting in a substantially lower BWG and FI.

Heat stress-induced decrease in growth performance was partially alleviated by dietary supplementation of Bet and TRP. Attia et al. (2009) observed that HS significantly suppressed growth, feed intake, and impaired FGR of slow-growing broilers during 21–84 days of age, whereas feeding Bet can improve BWG and lower FGR. Similarly, the Bet-supplemented broilers showed higher feed consumption, BWG, and lower FGR compared with the HS group (He et al. 2015). The rise in BWG in Bet-supplemented broilers can be explained by the findings that during period of osmotic disturbance, caused by HS in broilers, Bet is involved in the improvements in the morphological characteristics of intestinal epithelia, resulting in an improved growth rate and feed efficiency (Sakomura et al. 2013).

To our knowledge, no study has monitored the effect of TRP supplement on growth performance in HS broilers, but there are reports that addition of turmeric powder to broiler diets improved feed efficiency over the entire experimental period (Nouzarian et al. 2011; Abou-Elkhair et al. 2014). Improvement in BWG and FGR of HS broilers with the inclusion of TRP could be attributed to its favorable effects on intestinal microflora. The ethanol turmeric extract has been also reported to exhibit high potential to inhibit some pathogenic bacteria of chicken (Nouzarian et al. 2011), which could

Table 4 Antibody titers (log2) against sheep red blood cell (SRBC) in broilers reared under normal temperature or heat stress conditions

Parameter	Treatment group					SEM	*P* value
	TN-CON	HS-CON	HS-Bet	HS-TRP	HS-BT		
Primary anti-SRBC titer at 28 days of age							
Total antibody	5.75^{a}	4.42^{b}	4.34^{b}	4.80^{ab}	5.08^{ab}	0.38	0.004
IgM	3.24^{a}	2.48^{b}	2.43^{b}	2.88^{ab}	2.95^{ab}	0.29	0.029
IgG	2.51	1.94	1.91	1.92	2.13	0.27	0.091
Secondary anti-SRBC titer at 42 days of age							
Total antibody	6.58^{a}	5.10^{b}	5.88^{ab}	6.14^{a}	6.30^{a}	0.30	<0.001
IgM	2.95	2.44	2.86	2.76	2.80	0.19	0.117
IgG	3.63^{a}	2.66^{b}	3.02^{ab}	3.38^{a}	3.50^{a}	0.21	0.013

Means within the same raw without a common superscript letter differ significantly ($P<0.05$)

TN-CON thermoneutral control group, *HS-CON* heat stress control group, *HS-Bet* betaine-supplemented heat stress group, *HS-TRP* turmeric rhizome powder-supplemented heat stress group, *HS-BT* betaine and turmeric rhizome powder-supplemented heat stress group

Table 5 Serum total antioxidant capacity (TAC) and malondialdehyde (MDA) contents and glutathione peroxidase (Gpx) and superoxide dismutase (SOD) activities in broilers reared under normal temperature or heat stress conditions

Parameter	Treatment group					SEM	*P* value
	TN-CON	HS-CON	HS-Bet	HS-TRP	HS-BT		
TAC (mmol/L)	0.51^{b}	0.67^{a}	0.69^{a}	0.65^{a}	0.72^{a}	0.04	0.027
MDA (μmol/L)	3.10^{b}	4.64^{a}	2.50^{bc}	2.63^{bc}	2.07^{c}	0.35	0.013
GPx (nmol/min/mL)	124.0^{c}	145.6^{b}	168.7^{a}	166.3^{a}	165.3^{a}	7.52	0.008
SOD (U/mL)	102.3^{c}	129.5^{b}	149.0^{a}	146.2^{a}	151.7^{a}	6.23	0.044

Means within the same raw without a common superscript letter differ significantly ($P<0.05$)

TN-CON thermoneutral control group, *HS-CON* heat stress control group, *HS-Bet* betaine-supplemented heat stress group, *HS-TRP* turmeric rhizome powder-supplemented heat stress group, *HS-BT* betaine and turmeric rhizome powder-supplemented heat stress group

lead to better growth and feed utilization. It is also possible that antioxidant compounds, especially curcumin, in the turmeric (Ling et al. 2012) could alleviate the detrimental effects of HS on birds' performance. Our results also showed that a combination of Bet and TRP gives no more benefits than each additive alone in terms of BWG and FGR.

Heat stress at 33 °C throughout the experimental trial increased mortality rate in the HS-CON group compared with the TN-CON group. This may be due to a fall in immunity and resistance against diseases by HS. In a study on broilers, HS is reported to enhance pathogen colonization and impact food safety risk (Song et al. 2013). In the current study, the mortality rate in the HS-BT group was similar to that in the TN-CON group. The decrease in mortality in HS-BT group could be attributed to a cumulative effect of Bet and TRP, which serve to promote intestinal function, beneficial bacteria, and disease resistance (Nouzarian et al. 2011; Sakomura et al. 2013).

In the present study, HS induced increased blood H/L ratio and lowered antibody responses to SRBC which were reversed when TRP was supplemented alone or in combination with Bet in the diet. The rise in the blood heterophil and H/L ratio (as a stress indicator) is considered as an indication of overactivation of the hypothalamic-pituitary-adrenal (HPA) axis (Schmidt et al. 2012). Certainly, the increased sensitivity of the HPA axis is deleterious, given that it impairs growth rate and compromises body immune system (Sohail et al. 2010; Haldar et al. 2011). As TRP is believed to influence the gut health and microbiota (Faghani et al. 2014), it can be hypothesized that a healthy and balanced microbial community may have helped normalize stress-induced symptoms. The findings of the present study are also in agreement with Bartlett and Smith (2003), who found lower humoral response against SRBC when chickens were exposed to environmental temperatures ranging from 32.2 to 43.0 °C for short intermittent periods. A recent study by Kim et al. (2013) showed that the chickens fed turmeric-supplemented diets had enhanced systemic humoral immune responses compared with control birds. In support of this, Lee et al. (2011) observed that the broilers immunized with an *Eimeria profilin* protein and fed diets supplemented with turmeric oleoresin had higher antibody levels compared with immunized and infected chickens fed a non-supplemented diet. The improvement in the immune response may be due to the phenolic components, especially curcumin, present in the TRP that are able to induce pharmacological properties, including anti-inflammatory, antimicrobial, and antioxidant activities (Trujillo et al. 2013).

Heat stress has been reported to induce oxidative stress and production of free radicals, which induces lipid peroxidation and oxidative damage to cellular membranes (Salami et al. 2015). In the current study, a significant increase in the accumulation of TAC and GPx in HS broilers was considered a mechanism of protection against oxidative stress, as reported earlier (Sohail et al. 2011). The content of MDA, the product of lipid peroxidation, was also elevated in the HS group. Similarly, Tan et al. (2010) demonstrated an increase in the production of free radicals, in concordance with an increase in the activity and concentration of antioxidant parameters during HS. The oxidative stress has been related not only to the increased production of free radicals but also to changes to the scavenging capacity of antioxidant systems. To adapt to oxidative stress, the antioxidant systems in the body contain antioxidant enzymes such as SOD and GPx which are employed to protect the body from oxidative stress (Huang et al. 2015).

Dietary supplementation of Bet and TRP, particularly in combination, either partially or markedly ameliorated oxidative damage induced by HS, indicating that these supplements could improve the antioxidant status in broilers under high ambient temperature. In accordance with the increased serum GPx and SOD activities, MDA concentration in the serum is decreased by inclusion of Bet and TRP in broiler diets. The present study demonstrated for the first time that Bet can act as an antioxidant agent in HS-induced oxidative stress for broilers. Supplemented Bet has been shown to increase the activity of the main antioxidant enzyme (GPx) and decrease lipid peroxidation in the breast muscle tissue of broilers reared under thermoneutral conditions (Alirezaei et al. 2012a). Betaine is a methylating agent like *S*-adenosyl methionine (SAM) and it also spares methionine via the betaine-homocysteine methyl transferase pathway (Deminice et al. 2015). Therefore, Bet may have antioxidant effects against

oxidative damage by restorating SAM, which contributes to an enhancement in the supply of substrate needed for the synthesis of glutathione that protects the cell from reactive oxygen species (ROS) and reactive metabolites (Alirezaei et al. 2012b). Akbarian et al. (2015) also reported that dietary supplementation with *Curcuma xanthorrhiza* essential oil at 200 or 400 mg/kg significantly increased erythrocyte GPx and SOD activities in chickens at 38 days of age. The beneficial effect on the antioxidant system of the chicken by TRP is most likely attributed to the phenolic compounds, mainly curcumin and xanthorrhizol (Rukayadi and Hwang 2013). In this regard, it has also been suggested that phenolic compounds can support antioxidant system possibly through the following: direct scavenging of ROS produced after oxidative stress and/or prevent the formation of ROS by inhibiting enzymes (Thring et al. 2011).

In conclusion, results of the present study suggest that dietary supplementation of either Bet or TRP, alone or in combination, partially restored the HS-induced impairment in growth performance. Our results also indicate that TRP might be better than Bet for improving stress tolerance and humoral immunity in heat-stressed broilers. Moreover, it appears that both supplements, used in this study, exert antioxidant protection through their ability to activate the antioxidant enzymes and also to scavenge hydroxyl radical. This effect may act as a spare mechanism for living cells when they are challenging with HS for a long time, for example, the condition of the current study.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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