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Evaluation of the Protective Activities of Dietary Turmeric Powder on Growth Performance, Biochemical Parameters, Antioxidant Status, and Gene Expression in Heat-stressed Broilers

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Abstract

This study was designed to evaluate the protective effects of turmeric rhizome powder (TRP) against the harmful effects of heat stress (HS) on some biochemical parameters and antioxidant status. Four groups were formed entirely from 72 one-day-old chicks with an initial body weight average of 45.0±3.0 g, including the basal diet: TNC: no supplements were added to the diet (control group); TN-TRP: 300 g TRP /100 kg of feed was supplemented; HS-control: no supplements were added, and the birds were exposed to heat stress; and HS-TRP: 300 g TRP/100 kg of diet was supplemented and the birds were exposed to heat stress) for 35 days. Heat stress groups were subjected to thermal stress (40.0± 5.0˚C) for 8 hours per day from the 21st day to the end of the experiment. At the end of the trial, four healthy birds were randomly selected from each group and slaughtered for sampling and analysis. The serum total protein, albumin, ALT, AST, uric acid, and urea were significantly decreased by the dietary TRP when compared with the HS-control group while creatinine was not affected significantly. The serum total lipid and malondialdehyde (MDA) were significantly dropped but catalase enzyme activity increased. The gene expression levels of peroxisome proliferator-activated receptor (*PPAR-α*), glucagon-like peptide-1 (*GLP-1*), peroxisome proliferator-activated receptor gamma coactivator 1 alpha (*PGC-1α*), and superoxide dismutase-1 (*SOD-1*) were significantly increased by TRP addition. The results suggest that a TRP-supplemented diet affected some biochemical parameters and improved the antioxidant status and expression of studied genes.

KEYWORDS
Heat Stress, Turmeric, Broiler, Blood Metabolites, Antioxidant Status.

INTRODUCTION

Poultry business in Egypt and other developed countries has developed as an agricultural activity, and according to the statistics, poultry industry has a bright future (Shatokhin *et al*., 2017). Generally, one of the challenges that this promising industry faces is high ambient temperatures in tropical and subtropical countries, which can be known as heat stress or thermal stress (Renaudeau *et al*., 2012). Heat stress is defined as a result of continuous shifting in the Earth's normal temperatures, geographical conditions (Reddy, 2015), and increased greenhouse gas concentrations like CO_2, CH4, N_2O, and O_3 in the atmosphere (Olhoff *et al*., 2020).

When compared to mammals, broilers had high relative body temperatures under thermoneutral conditions, so the elevation of ambient temperatures was considered a challenge to the bird (Spiers, 2012). Due to their fast rate of metabolic processes, thick feathered bodies, lack of sweat glands, and elevated body temperatures (Xie *et al*., 2015), broilers may be more responsive to hot weather than other species, especially when exposed to direct solar radiation (Havlicek and Slama, 2011). This may be attributed to the heavy feathering coat (Møller, 2015), which explains why poultry has poor thermoregulation and regulate their body temperatures through evaporation. Anatomically, peripheral nerves called temperature-sensing neurons express thermotransient receptor potentials, which are ionic receptors that respond to temperature by a mechanism that is yet not completely understood (Bohler *et al*., 2021). One theory is that thermosensory transient receptor potential is expressed in the hypothalamus in response to temperature in the brain tissues and surrounding blood vessels, especially in the preoptic area, which is responsible for detecting any differentiation in temperature (Voets *et al*., 2004).

To mitigate the effects of heat stress, birds employ a variety of environmental and behavioral adaptations and mitigation strategies. For example, birds frequently raise their wings and approach cold surfaces (to transfer heat from the higher medium to the lower medium through radiation). Furthermore, one of the main ways to lose heat through panting is the evaporation process (Pawar *et al*., 2016). Birds reduce feed intake, increase water consumption, and spend more time panting and resting (Lara and Rostagno, 2013). The response of birds to heat stress is a decrease in feed intake followed by a decrease in body weight, body weight gain, a high feed conversion ratio, a reduction in meat quality, and a high rate of mortality, all of which have a re-

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flection on the financial return (Ranjan *et al*., 2019). By extension, there are many physiological, immunological, hormonal (Lara and Rostagno, 2013), metabolic (Bonnet *et al*., 1997), and biochemical (Khan *et al*., 2002; Jaiswal *et al*., 2017) impairments as a result of heat stress. Heat stress impairs blood chemistry, it could result in increasing total protein and liver function parameters (Huang *et al*., 2018a). The hypothalamus-pituitary-adrenal axis became stimulated through heat stress, which elevated serum corticosteroids (Quinteiro-Filho *et al*., 2012). High doses of corticosteroids impair lipid metabolism (Mujahid *et al*., 2007). The role of oxidative reactions in many metabolic pathways is well understood, but under uncontrolled conditions, such as high temperatures, these reactions rate accelerate and cause damage to tissues and organs due to disruptions in the equilibrium of these reactions' oxidant products and cellular antioxidants, which is known as oxidative stress (Lin *et al*., 2006; Estévez, 2015). Diabetes, cancer, atherosclerosis, COPD, and chronic kidney disease have all been linked to oxidative stress (Liguori *et al*., 2018).

Numerous publications offer a variety of strategies for the alleviation of the negative consequences of heat stress, including environmental and dietary changes (Pawar *et al*., 2016; Ranjan *et al*., 2019). One of the nutritional modification methods depends on the creation of a balance between antioxidants and oxidation by-products to face the oxidative threats by adding vitamins, antioxidants, and phytochemicals (Sahin *et al*., 2001; Diarra and Tabuaciri, 2014).

In Southeast Asian countries, turmeric powder is frequently used as a spice, food preservative, and coloring agent. *Curcuma longa*, or Curcuma domestica, is a perennial herb that is grown anywhere between sea level and 1200 meters above it. It was used in conventional medicine for coughs, anorexia, diabetic wounds, rheumatism, and sinusitis (Bejar, 2018). The key element of turmeric is called curcumin (diferuloylmethane), which has the molecular formula $C_{21}H_{20}O_2$ (NCBI, 2023). It was reported that it has several pharmacological effects, including anti-inflammatory, antioxidant, antibacterial, antiviral, antifungal, and anti-carcinogenic (Chattopadhyay *et al*., 2004). The effectiveness of turmeric is affected by the percentage of the active substance in it, which is affected by the type of plant, the soil, the method of harvesting, and the method of extracting both the powder and the oil (Li *et al*., 2011). Turmeric and curcumin improve liver functions (Ramadan *et al*., 2021), all growth parameters (Almayali *et al*., 2021), regulate lipid metabolism (Xie *et al*., 2019), and enhance the activity of the antioxidant enzymes (Hosseini-Vashan *et al*., 2012).

Therefore, the goal of this study was to investigate the effects of dietary turmeric powder on blood metabolites, antioxidant status, and gene expression, which might help to improve the general health of broilers under heat stress.

MATERIALS AND METHODS

The Study's Ethical Statement

The use of animals in this investigation conforms to all applicable rules, moral standards, and other requirements. The scientific committee of the Faculty of Veterinary Medicine at Zagazig University in Egypt has examined the research protocol (ZU-IACUC/2/F/242/2022). The scientific panel ensured that the animals were kept in suitable conditions and had access to veterinarian services. All workers involved in animal care were adequately skilled in both the experimental process and the ethical treatment of the animals. Every researcher involved in this experiment was also educated in the handling and management of avian species.

Animals' housing, experimental design, and diet

This investigation was carried out at the experimental unit of the Faculty of Veterinary Medicine at Zagazig University, Egypt. A local hatchery provided a total of 72 one-day-old birds (cobb 500). Birds were allocated to four treatments, with two replicates of nine chickens based on a completely randomized design. The thermoneutral control (TNC) birds were fed only the basal diet. The thermoneutral turmeric rhizome powder group (TN-TRP) received a basal diet supplemented with 300 gm of TRP per 100 kg diet (Chattopadhyay *et al*., 2004). In the heat stress control group (HSC), birds in this group were fed only the basal diet and subjected to HS. In the heat stress turmeric rhizome powder (HS-TRP) group, birds in this group were fed a basal diet supplemented with 300 gm of TRP /100 kg diet and subjected to HS. The birds were reared in floor pens littered with sawdust for five weeks. The lighting program is a full-time program. During the first three weeks of the experiment, the pen temperature was set at 22.0±5.0°C. For the remainder of the experiment, pens from the heat-stressed groups were exposed to a temperature of 40.0±5.0°C for eight hours from 7 a.m. to 3 p.m. For all groups, food and water were supplied *ad libitum*. All birds were fed a starter diet during the first 21 days of age and a finisher diet from 22 to 35 days. Starter and finisher rations are obtained from Al-Eman Company for poultry and livestock rations. The ingredients and composition of the broiler diet for all broilers are reported in Table.1.

Table 1. Composition of diet for starter (0-21 days) and finisher (21-35 days) period in broiler chickens under thermoneutral and heat stress situations.

Ingredients	Starter Diet (0-21 days).	Finisher Diet (22-35 days).
Corn, Grain	48.2	58.7
Wheat	8	7.5
Soybean meal (40% CP)	28.5	20.5
Protein Conc.	10	10
Vegetable Oil	4	2.5
Salt	1	0.5
Vitamin + Mineral	0.3	0.3
Composition:		
AME(kcal/kg)	3079	3102.6
Crude Protein	22.06	19.37
Lysine	1.21	1.03
Methionine + Cystine	0.82	0.75
Calcium	1.2	0.95
Phosphorus (%)	0.44	0.42

Supplied the following per kilogram of diet: Vit. A, 25000 IU; Vit.D, 5000 IU; Vit. E,12.5 IU; Vit. K,2.5 IU; Vit.B1,1mg; Vit.B2, 8 mg; Vit.B6, 3 mg; Vit.B12, 0.015 mg; Folic acid, 0.025 mg; Nicotinic acid, 17.5 mg; Calcium pantothenate, 12.5 mg; Fe, 80 mg; Cu, 10 mg; Mn, 80 mg; Se, 0.15 mg; I, 0.35 mg.

Chemicals and Powder Used

Medications or supplements

The naturally dried turmeric root powder was purchased from one of the traditional and commercial perfumery shops in Zagazig, Ash Sharqiyah, Egypt.

Chemicals for qRT-PCR

Qiazol (Qiagen; Germany), Chloroform HPLC grade, Isopropanol HPLC grade, and 70 % Ethanol HPLC grade (Sigma Aldrich),

High-Capacity cDNA Reverse Transcription Kit cDNA Kit; (Applied Bio systems™, USA), and TOP real™ qPCR 2X PreMIX (SYBR Green with low ROX) (Cat.P725 or P750) (Enzynomics, Korea).

Inclusion and Exclusion Criteria

The animals used in our investigation were healthy and lacked any observable diseases. To reduce the possibility of inter-individual variation because of the variability, each group of individuals selected had neighboring weights.

Growth Performance

On days 7, 14, 21, 28, and 35, all birds were weighed. By subtracting the body weight between two subsequent weights, the weekly body weight gain was determined. Every week, the amount of feed still in each pen was weighed, and the amount of feed added to each pen was noted. Subtracting the leftover feed from the supplied feed yielded the feed intake. Calculating the feed conversion ratio involved dividing the weekly feed intake by the weekly body weight gain.

Blood and Tissue Samples

At the end of the experiment, at 35 days of age for Cobb 500, four birds were chosen at random from each group. Blood was drawn from the wing vein, put into a tube for blood serum isolation, and allowed to clot. Serum isolation after blood sampling by centrifugation at 3,000 rpm for 15 minutes then, the serum is stored in Eppendorf tubes (1.5 ml) at -20°C until analysis. As soon as tissue samples (hypothalamus, liver, and intestine) were taken, they were coated with 50 mg/1 Qiazol and kept at -8°C for total RNA extraction.

Biochemical Analysis

Colorimetric methods were used for the analysis of total proteins, albumin, ALT, AST, uric acid, and creatinine as described by Young (1990). Total lipid concentration was determined by a method described by Chabrol and Charonnat (1973). Urea was determined by the methods of Fawcett and Scott, (1960). Catalase enzyme analysis is also determined by a colorimetric method as described by Aebi (1984). Malondialdehyde analysis is based on the method of Hiroshi *et al*. (1979).

RNA Extraction, cDNA Synthesis, and qRTC-PCR

Total RNA was isolated from hypothalamic peroxisome proliferator-activated receptor (*PPAR-α*), glucagon-like peptide-1 (*GLP-1*), peroxisome proliferator-activated receptor gamma coactivator 1 alpha (*PGC-1α*), and liver superoxide dismutase-1 (*SOD-1*) samples using the Qiazol (Qiagen, Germany), and concentration was assessed by measuring absorbance at 230- 260 nm. The ratio accepted for RNA quality fell in the range of 1.8-2, and values outside the range were excluded. Total RNA was reverse-transcripted with high-capacity reverse transcriptase cDNA synthesis kits (Applied Biosystem, USA) as described by Khamis *et al*. (2019) and Khamis *et al*. (2020). According to Livak and Schmittgen, (2001), the mRNA expression was evaluated using qPCR and the $^{2-\Delta\Delta C}$t method. Then, according to the manufacturer's instructions, qPCR mixes were prepared on ice. They took place with the use of rotor genes (Khamis *et al*., 2021). The primers were produced by Sangon Biotech using the Primer 5.0 software and are shown in Table 2.

Statistical Analysis

Results were analyzed as mean ± SEM (standard error of the mean). To assess the influence of the four treatment groups under thermoneutral and heat stress on the different biochemical parameters, one-way and two-way analyses of variance (ANOVA) with repeated measures followed by Tukey's Honestly Significant Difference (Tukey's HSD) test as a post hoc test were used. The statistical significance level was set at $P < 0.05$. All analyses and charts were done using the Statistical Package for Social Sciences version 24.0 (SPSS, IBM Corp., Armonk, NY) and GraphPad Prism 8.0.2 (GraphPad Software, Inc.).

RESULTS

Growth Performance

The effects of turmeric powder on birds' growth performance parameters are displayed in Table 3. All growth performance parameters were negatively influenced by heat stress. The addition of TRP to the diet had no significant effects on the birds' body weight during the first three weeks of the experiment. The addition of TRP to the diet under either thermoneutral or heat stress conditions did not significantly increase body weight when compared to the control group in the fourth and fifth weeks of the experiment. TRP supplementation did not affect body weight gain during the first three weeks of the experiment. In the 4th and 5th weeks of the experiment, heat stress decreased the weight gain, and the addition of turmeric powder increased it. During the first three weeks of the experiment, TRP addition significantly increased the feed intake when compared with the control groups. As a result of heat stress, birds reduced their feed consumption in the 4th and 5th weeks. On the other hand, groups that were supplemented with turmeric powder showed a significant augmentation in feed intake in the same period. The FCR was not significantly affected by turmeric supplementation in the first two weeks of the experiment. FCR was significantly lower in the third, fourth, and fifth weeks when compared to a control group. Statistically, the groups supplemented with TRP showed the best FCR.

Table 2. The primers sequence and parameters

Primers	Primers Forward	Primers Reverse	Product Length	Accession No.
PPAR-α	AGTAAGCTCTCAGAAACTTTGTTG	AAGGTTGAAACAGAAGCCGC	162	NM_001001464.1
GLP-1	GGCTGAAGAAATGGGCCGAA	TTGGCAGCCATATCATCCAGG	81	NM_001190165.5
PGC-1α	AGTAAGCTCTCAGAAACTTTGTTG	AAGGTTGAAACAGAAGCCGC	144	NM_001006457.2
SOD-1	TGATGACCTGGGTAGAGGGG	ACAACGGTTAGCACTTGGCT	104	NM_205064.2
actin-b	GTGGATCAGCAAGCAGGAGT	ATCCTGAGTCAAGCGCCAAA	182	NM_205518.2

Note, the abbreviations of the primer names are as the following: *PPAR-α*: peroxisome proliferator-activated receptor alpha; *GLP-1*: glucagon-like peptide-1; *PGC-1α*: peroxisome proliferator-activated receptor gamma coactivator 1-alpha; *SOD-1*: superoxide dismutase one.

Table 3. Effects of dietary TRP on body weight (g), weight gain (g), feed intake (g), and feed conversion ratio (g/g) during the experiment in thermoneutral and heat stress groups.

Group	Body Weight				
	7 days	14 days	21 days	28 days	35 days
Thermoneutral Control	202.50±9.68	481.25±9.66	913.75±31.32	1288.75±38.2^{abc}	2012.50±42.70^{abc}
Thermoneutral Turmeric	207.50±2.50	492.50±4.79	977.50±13.15	1450.00±35.36^{a}	2200.00±20.41^{a}
Heat Stress Control	203.75±3.75	482.50±4.79	912.50±55.43	1212.50±62.50^{d}	1950.00±84.16 ^{c}
Heat Stress Turmeric	206.25±2.39	491.25±7.18	975.00±26.30	1265.00±11.90^{cd}	2075.00±14.43 ^{abc}
	Body Weight Gain				
	7-14 days	14-21 days	21-28 days	28-35 days	
Thermoneutral Control	283.75±8.26	471.25±20.85	627.50±15.48^{b}	742.50±32.56^{c}	
Thermoneutral Turmeric	288.75±9.65	555.00±21.02	722.50±12.50^{a}	833.75±13.75^{a}	
Heat Stress Control	282.50±5.95	467.50±21.26	508.75±15.60^{c}	621.25±11.97^{b}	
Heat Stress Turmeric	288.75±9.66	553.75±21.15	596.25±16.75^{b}	712.50±4.33^{c}	
	Feed Intake				
Thermoneutral Control	331.50±1.44 ^{b}	640.75±0.75 ^{b}	907.50±1.66^{b}	1071.75±1.38 ^{b}	
Thermoneutral Turmeric	391.25±1.38 ^{a}	703.25±2.69 ^{a}	986.25±1.65^{a}	1149.50±2.60 ^{a}	
Heat Stress Control	331.75±2.25 ^{b}	641.50±1.71^{b}	883.25±1.03^{c}	1022.25±1.97 ^{d}	
Heat Stress Turmeric	392.25±1.38 ^{a}	703.75±1.03^{a}	942.75±1.31^{d}	1096.75±1.93 ^{c}	
	Feed Conversion Ratio				
Thermoneutral Control	1.17±0.04	1.61±0.04 ^{a}	1.72±0.10 ^{a}	1.75±0.04 ^{abc}	
Thermoneutral Turmeric	1.12±0.08	1.27±0.04 ^{b}	1.37±0.03 ^{b}	1.49±0.03 ^{c}	
Heat Stress Control	1.17±0.04	1.62±0.02 ^{a}	1.81±0.02 ^{a}	1.97±0.05 ^{a}	
Heat Stress Turmeric	1.12±0.03	1.27±0.03 ^{b}	1.50±0.04 ^{c}	1.77±0.24 ^{abc}	

$^{a, b, c}$ Means within the same column carrying different superscripts are significantly different at (P value < 0.05).

Table 4. Effects of dietary turmeric rhizome powder on liver functions and total lipid on 35 days of the experiment in thermoneutral and heat stress groups.

Group	Parameters							
	Total protein (g/dl)	Albumin (g/dl)	ALT (U/L)	AST (U/L)	Uric acid (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Total lipid (mg/dl)
Thermoneutral Control	3.71±0.052^{c}	1.41±0.032 ^{c}	15.38±0.262^{c}	281.67±1.453 ^{b}	5.71±0.24^{c}	17.68±0.174 ^{c}	0.55±0.086	210.33±1.452 ^{d}
Thermoneutral TRP	2,42±0.079^{b}	0.70±0.038 ^{b}	11.01±0.266^{b}	164.00±1.528 ^{c}	4.61±0.15^{b}	17.17±0.186 ^{c}	0.57±0.088	199.33±1.450 ^{a}
Heat Stress Control	4.56±.051^{a}	1.74±0.027^{a}	22.27±0.260 ^{a}	376.33±1.856 ^{a}	9.05±0.12^{a}	20.49±0.194 ^{a}	0.057±0.089	221.67±1.667 ^{b}
Heat Stress TRP	3.67±0.078 ^{c}	0.97±0.026 ^{d}	12.90±0.265^{d}	184.67±1,453 ^{d}	6.60±0.13^{c}	18.93±0.176 ^{b}	0.65±0.086	215.00±1.527 ^{c}

$^{a-d}$ Means with different superscript letters within the same column are significantly different at P<0.05, in the determination of the interaction between the two factors (heat stress and treatments). ALT: alanine aminotransferase; AST: aspartate aminotransferase.

Biochemical Parameters

Effects of Turmeric Powder on Liver Functions

Table 4 shows the serum total protein (g/dl), albumin (g/dl), ALT (U/L), and AST (U/L) levels in broiler chicks fed TRP under thermoneutral and heat-stress conditions. Heat stress significantly elevated the serum total protein, albumin, ALT, and AST, while TRP addition significantly reduced the serum total protein, albumin, ALT, and AST. It is important to note that in the HS-TRP group, the dropping of serum total protein reached that of the TNC group level, while the reduction level of ALT and AST is still higher than that of the TNC group.

Effects of Turmeric Powder on Kidney Functions

Table 4. shows the effects of TRP dietary supplementation effects on serum uric acid, urea, and creatinine under normal and heat stress conditions. Heat stress raised serum uric acid and urea levels but did not considerably affect the creatinine levels, and treatment of stressed broilers with turmeric significantly declined both uric acid and urea levels while the creatinine level was not changed.

Effects of Turmeric Powder on Total Lipids

The effects of TRP additions to the broiler diet on total lipids (mg/dl) are presented in Table 4. Total lipid levels in broilers exposed to HS augmented significantly, while in the group that was treated with turmeric powder, total lipid levels were lowered.

Antioxidant Status

The effects of TRP additions to the broiler diet on catalase (U/ml) and MDA (nmol/ml) are presented in Table 5. In the HS groups, the catalase enzyme showed a drop in its activity while the MDA concentration was significantly raised. TRP enhanced catalase enzyme activity but did not reach the level of the ther-

moneutral control group and lowered the concentration of serum malondialdehyde. And it is worth mentioning that MDA in the supplemented heat stress group is still lower than in the TNC group.

Table 5. Effects of dietary turmeric rhizome powder on some serum antioxidant status on 35 days of the experiment in thermoneutral and heat stress groups.

Group	Parameter	
	Catalase (U/L)	MDA (nmol/ml)
Thermoneutral Control	61.00±00.29 [a]	4.67±0.040 [c]
Thermoneutral TRP	61.43±0.29 [a]	4.09±0.041 [d]
Heat Stress Control	56.40±0.29 [c]	5.23±0.04 [a]
Heat Stress TRP	58.65±0.29 [b]	4.90±0.043 [b]

[a-c] Columns with no common superscript differ significantly (P<0.05). MDA: malondialdehyde.

The Gene Expression Levels

The effects of TRP additions to the broiler diet on levels of gene expression are presented in Table 6. When compared with the TNC group, the expression levels of *PPAR-α*, *GLP-1*, *PGC-1α*, and *SOD-1* were dropped by heat stress, but either under thermoneutral or heat stress conditions, the addition of turmeric powder enhanced the gene expression level.

DISCUSSION

This work was conducted to evaluate the effects of turmeric rhizome powder supplementation on broiler performance and some biochemical parameters when subjected to heat stress. The obtained results showed that broilers exposed to HS had a bad growth performance (Lara and Rostagno, 2013). Deterioration in growth performance can be explained as systemic responses where the energy consumed to incorporate with elevated high ambient temperature was increased rather than that used to promote growth performance (Renaudeau *et al*., 2012). Also, one of the adverse effects of heat stress was decreased appetite to minimize body heat production which is followed by low weight gain and feed intake, and a high feed conversion ratio (Feng *et al*., 2012). One of the reasons that can explain the decline in weight gain and feed intake is that chronic heat stress negatively affected feed digestibility and reduced gastrointestinal tract size (Bonnet *et al*., 1997). In this work, the addition of TRP to the heat-stressed group partially improved the growth performance parameters. Improvement in body weight and body weight gain was not significant, and these results were consistent with those obtained by Hosseini-Vashan *et al*. (2012). In contrast results from the present study, TRP supplementation either to thermoneutral or heat stress conditions caused a significant elevation in body weight and weight gain (Akhavan-Salamat and Ghasemi, 2016). In this work, the addition of turmeric powder enhanced the feed intake. These results agree with those of Mustafa *et al*. (2021a), and differ from those of Hosseini-Vashan *et al*. (2012). It is believed that this improvement in food intake was caused due to the antibacterial action of turmeric and its key element curcumin which improve the intestinal microflora status and is followed by the better utilization of feed (Ling *et al*., 2012). It is believed that one of the reasons that led to the increase in the rate of food consumption was the aromatic scent of turmeric (Gumus *et al*., 2018). Results from this study included a relative improvement in feed conversion rate after turmeric dietary treatment. These results agree with the results of El-Maaty *et al*. (2014) and Sadeghi and Moghaddam (2018), and disagree with those of Gumus *et al*. (2018).

In this trial, heat stress elevated measured liver and kidney functions. Dietary treatment with turmeric powder considerably decreased total protein, albumin, ALT, and AST. In a study by Emadi *et al*. (2007a), adding different levels of turmeric powder not affected total protein and notably dropped albumin. In an investigation of six weeks, the serum total protein showed no significant changes due to the use of various levels of turmeric powder, except for the 4th week, when serum total protein recorded a significant elevation while serum albumin recorded lower values in the second and fourth weeks. In the same work, different levels of TRP significantly lowered ALT and had no significant changes in the serum AST (Qasem *et al*., 2016). Treatment of heat-stressed broilers by TRP showed a significant elevation in total protein but albumin was not significantly elevated. Also, considerably dropped ALT and AST (Ramadan *et al*., 2021). The use of different levels of TRP and curcumin increased the total protein either under normal or HS conditions (Mustafa *et al*., 2021b). Treating the heat-stressed broiler rabbits with a 300 mg/100 kg diet did not affect serum total protein, albumin, ALT, and AST (Basavaraj *et al*., 2011). Different levels of TRP which were added to the broiler diet caused a considerable rise in AST while ALT activity was lowered as reported by Emadi *et al*. (2007b). Clinically, liver tissue damage is determined by the high activity of serum aminotransferases (Dwivedi *et al*., 1991). Reduction of both means that turmeric may exert hepatoprotective and antioxidant effects in response to heat stress and oxidative stress (Sugiharto, 2020).

Various doses of TRP were used for six weeks, showing no significant effect on serum creatinine (Qasem *et al*., 2016). In agreement with the obtained findings, exposure to chronic heat stress elevated serum uric acid and creatinine in birds, treatment with dietary turmeric powder ameliorates uric acid and creatinine serum levels (Ramadan *et al*., 2021). Treatment of heat-stressed broilers with TRP had no significant effect on serum urea or creatinine but significantly dropped the uric acid levels (Baghban Kanani *et al*., 2016). One of the theories that could clarify this apparent abnormality in kidney function under high ambient temperature is that HS caused necrotizing and degenerative changes that lead to renal failure (Huang *et al*., 2018b). The improvement in kidney functions in the heat-stressed group which was treated with turmeric powder may be attributed to nephroprotective effect exerted by turmeric (Sundararajan *et al*., 2014).

The current study revealed that TRP can ameliorate the disturbance in total lipids due to HS. The addition of turmeric to the diet of heat-stressed broilers significantly reduced triglyceride, cholesterol, LDL, and VLDL levels while increased HDL levels (El-

Table 6. Effects of dietary turmeric rhizome powder on levels of gene expression on 35 days of the experiment in thermoneutral and heat stress groups.

Group	Parameters			
	PPAR-α.	*GLP-1.*	*PGC-1α.*	*SOD-1*
Thermoneutral Control	1.01±0.08 [b]	1.02±0.11 [a]	1.08±0.23 [ab]	1.00±0.05 [ab]
Thermoneutral TRP	1.26±0.03 c	1.66±.08 [c]	1.20±.03 [a]	1.22±0.06 [a]
Heat Stress Control	0.23±0.02 [a]	0.30±0.01 [b]	0.29±0.05 [c]	0.33±0.02 [c]
Heat Stress TRP	0.90±0.04 [b]	0.86±0.03 [a]	0.83±0.02 [b]	0.90±0.01 [b]

[a-c] Means with different superscript letters within the same column are significantly different at (P<0.05), in the determination of the interaction between the two factors (heat stress and treatments). *PPAR-α*: peroxisome proliferator-activated receptor alpha; *GLP-1*: glucagon-like peptide-1; *PGC-1α*: peroxisome proliferator-activated receptor gamma coactivator 1-alpha; *SOD-1*: superoxide dismutase one.

Maaty *et al*., 2014; Ramadan *et al*., 2021). On the other hand, different levels of turmeric powder had no significant effects on LDL, HDL, and total cholesterol but suppressed the total triglyceride level (Nouzarian *et al*., 2011). Ahmad-Raus *et al*. (2001) attributed the hypercholesteremic action of turmeric due to its ability to promote bile fluid secretion, biliary cholesterol secretion, and bile acid and cholesterol elimination in feces. Xie *et al*. (2019) suggested that curcumin improved lipid profile parameters due to its influence on the expression levels of genes responsible for lipogenesis and lipolysis.

This study showed a considerable improvement in antioxidant status after TRP dietary treatment either under normal or heat stress conditions. Malondialdehyde is used as an indicator of oxidative stress and lipid peroxidation (Najeeb *et al*., 2012). Many studies have found that turmeric and its active ingredients boost the activity of antioxidant enzymes (Glutathione peroxidase, SOD, and catalase); on the other hand, they decrease the level of MDA, and so reduce the peroxidation of lipids (Akhavan-Salamat and Ghasemi, 2016). Furthermore, addition of turmeric powder to the diet of heat-stressed broilers did not affect catalase activity or MDA concentration (Sadeghi and Moghaddam, 2018). Turmeric and its key element curcumin were reported to inhibit the production of reactive oxygen species, improve the antioxidant capacity, decrease MDA levels, and thus inhibit lipid peroxidation (Wang *et al*., 2015).

The peroxisome proliferator-activated receptor alpha (*PPAR-α*) is a nuclear hormone receptor that controls how fatty acids are transported and oxidized (Brunt *et al*., 2011), so it is characterized as one of the main regulators of lipid metabolism (Chiazza and Collino, 2016). The addition of curcumin increased the expression level of PPAR-alpha, showed a reduction in plasma LDL, plasma and hepatic triglycerides, and abdominal fat deposition (Xie *et al*., 2019). Based on the previous data, we can now explain why total lipids in the present study were reduced. Turmeric powder and its active ingredient curcumin affect some genes which are responsible for the regulation of lipid metabolism. This result confirms the molecular hypolipidemic effect of turmeric as mentioned by (Zingg *et al*., 2013).

Glucagon-like peptide-1 (*GLP-1*) is a hypoglycemic peptide secreted by enteroendocrine L cells (Shim and Yu, 2020). After a high-content carbohydrate diet, *GLP-1* rapidly works to enhance the production of insulin and glucose-dependent insulin, inhibit appetite, and delay the emptying of the stomach (Appleyard, 2003). Curcumin has a considerable effect that enhances the *GLP-1* signal (Planes-Muñoz *et al*., 2018; Tsuda, 2018). In this study, we did not evaluate the serum glucose level, but based on the previous studies, heat stress significantly increased glucose levels (Khan *et al*., 2002). The addition of various levels of turmeric powder and curcumin, either under thermoneutral or heat-stress conditions, significantly normalizes serum glucose (Mustafa *et al*., 2021b).

PGC-1 (peroxisome proliferator-activated receptor gamma coactivator-1 alpha) is a transcription factor. This gene controls the metabolism of cellular energy and takes part in lipid and carbohydrate metabolism (Liang and Ward, 2006). *PGC-1α* plays a key role in managing mitochondrial activities, notably the expression of genes responsible for antioxidant status (Rius-Perez *et al*., 2020). The obtained results agree with the findings of Zhai *et al*., (2015). They confirmed that the curcumin increased the PGC-1alpha expression level and noted that the increase in expression level depended on curcumin doses. In this study, the effect of TRP on this gene was present in total lipid, MDA concentration, and catalase enzyme activity.

Superoxide dismutase-1 (*SOD-1*) is a major antioxidant defense enzyme (Trancikova *et al*., 2011). In the present study, heat stress decreased the expression level of *SOD-1*. The supplementation with TRP improved the *SOD-1* expression level either under heat stress or thermoneutral situations. In vitro, bile acids reduced the expression level of *SOD-1* and curcumin promoted its expression level (Bower *et al*., 2010). Also, the current results were consistent with that of Yarru *et al*. (2009).

CONCLUSION

The addition of turmeric rhizome powder to the diet of heat-stressed broilers decrease serum total protein, albumin, ALT, AST, urea, and total lipids. increased catalase activity and decrease MDA concentration. Turmeric and its key element curcumin improve the expression level of *PPAR-α*, *GLP-1*, *PGC-1α*, and *SOD-1*. Turmeric powder added to the diet can ameliorate the bad effects of heat stress in broilers.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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