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The Protective Role of *Curcuma Longa* (Turmeric) on Broilers Exposed to Chronic Heat Stress

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ABSTRACT

Heat stress (HS) is one of the most significant stressors in the broiler industry in hot regions. In order to provide complete information on the impact of turmeric powder (TP) on hamartiology, biochemistry and thyroid hormones of broilers under thermoneutral conditions (TN) (22 ± 4 °C) and heat stress (HS) (34 ± 2° C), the present research was carried out. A 60 of one-day-old (Cobb) broiler chicks, 15 birds in each replicate either control (basal diet) or 0.5% turmeric. Chronic HS at 42 d of age resulted in significant decrease in HGB, PCV, RBCs, total protein, Albumin, serum T3 and T4, whereas increased H/L, triglycerides, cholesterol, LDL-cholesterol, uric acid, AST, ALT, ALP, TBIL, DBIL and creatinine significantly. Supplementation of 0.5% TP significantly increased HGB, PCV, total protein, while significant decrease in AST, ALT, ALP, uric acid, total cholesterol and triglycerides. Results indicated that groups receiving turmeric have improved lipid profile.

Keywords:
Heat stress, Thermoneutral, Broiler chickens, Curcuma, Turmeric.

1. INTRODUCTION

Poultry, because of its low-fat content, is one of the key sources of animal protein for high quality meat. Temperature is one of the most significant factors that can have a negative impact on poultry as a rise in temperature above the thermo neutral zone (18-24 ° C) due to the climate or other factors can cause cascading effects on thermoregulation and could be deadly for birds as birds are more susceptible to high temperatures [1]. When exposed to high temperatures in the environment, will break the redox body balance and result in oxidative stress that affects health of poultry species [2]. Chronic HS, which is a cycle of heat exposure followed by a comfortable temperature for the rest of the day [3]. Heat stress raises cholesterol concentrations, decreases serum protein concentrations and increases blood products. Notably, high ambient

temperature often contributes to impaired status of antioxidants and causes poultry oxidative stress. Additionally, parameters of red blood cells (RBCs) and WBCs serve as references of animal's immune system [4].

In conjunction, physiological and metabolic responses such as changes in blood parameters, concentrations of plasma hormones, oxidative stress, and meat acidity have been identified a consequence of high temperature [5]. Valuable information about animal health and immune status is given in the serum biochemical profile [6]. In broiler chicks, elevated temperature raises serum aspartate aminotransferase (AST) and alanine aminotransaminase (ALT), cholesterol, triglycerides and low-density lipoproteins (LDL-C) [7]. Chronic heat stress in warm-blooded animals causes various hormonal responses, including impairment of endocrine functions. Thyroid hormones are of particular concern: triiodothyronine (T3) and thyroxine (T4). Decreased serum concentrations of T3 and T4, substantial animal growth promoters and suppressed function of the immune system at high ambient temperatures have been reported [8]. Turmeric (Curcuma longa) is a rhizomatous herbaceous perennial plant widely known in other countries, used as a food additive, preservative and colouring agent as a dried yellow powder. Turmeric is anti-inflammatory, antiprotozoal, hypotensive, antibacterial, antioxidant, anti-diabetic, antifungal, antiviral, antifibrotic and hypercholesteraemic [9]. In chicken, turmeric powder (TP) has been shown to increase haemoglobin (Hb) whereas reducing RBC and albumin concentrations [10]. Turmeric has decreased the activity of some enzymes AST, ALT and alkaline phosphatase (ALP), which have typically been increased by heat stress to enhance broiler liver function [11, 12] also, by improving the activities of antioxidant enzymes, it prevents lipid peroxidation [13]. The purpose of this research was to explore broiler haematological, biochemical and thyroid hormones attributable to the addition of TP plants to the broilers diets while they exposed to cyclic heat stress at 34 ± 2 ℃ for 8 hours per day.

2. MATERIALS AND METHODS

I. MATERIALS

I.1 Diets: Dried turmeric rhizomes were obtained from Crop Department, Agriculture Research Center, Giza, Egypt. At 21 days of age, it is included into the basal diets at 5 gm/kg diet [45]. Basal diets met the NRC [14].

I.2 Birds and experimental design: A total of (60) apparently healthy, One-day old, unsexed Cobb broiler chicks obtained from El-Kahera Poultry Company 10th of Ramadan City in Al-Sharkia governorate, Egypt and carried out in summer season (August). They were housed in floor pens bedded with fresh wood shavings litter of 8 cm depth and provided with 10 birds / m^2 in the experimental poultry unit in Animal Health Research Institute (AHRI), Egypt and fed on a basal diet till the end of experiment (42 days). From day 1-20 of age, all chickens raised at 32°C then decreased by 2°C/ week.

II. METHODS

2.1 Experimental design

All chickens allocated in to 4 equal groups at day 21, 15 chickens each: **Class I:** Thermoneutral condition (TN), with ambient temperature of (22± 4°C) daily and relative humidity (55±3) divided into two groups, thermoneutral control group (TNC) supplemented with basal diet only and thermoneutral turmeric group (TNT) supplemented with turmeric powder (5 gm/kg diet). **Class II**: Heat stress condition (HS), an ambient temperature (34 ± 2 °C) daily from 9:00 to 17:00 h. and a relative humidity 65% divided in to two groups, heat stress control (HSC) group received basal diet only and heat stressed turmeric (HST) group supplemented with turmeric powder (5 gm/kg diet).

2.2 Haematological parameters and blood leucocyte profiles

On the morning of 42th day, five birds per treatment selected and venous blood collected from the wing vein into EDTA anticoagulant treated vials. The haematological indices were, haemoglobin concentration (Hb), haematocrit percentage (HCT), the number of erythrocytes (RBCs), Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), White

Blood Cell (WBCs), the number of lymphocytes, heterophile, monocytes, eosinophil, basophils were assayed by Sysmex XT 2000 IV Automated Haematology Analyzer (Japan) described by [15]. For microscopic examination of the WBC with Wright-Giemsa complex staining, one hundred WBCs per samples counted using the Neubauer haemocytometer following procedure of [16] then Calculating H/L Values.

2.3 Serum biochemistry parameters

Before blood sample collections, feed was removed from all birds for a period of 12 h. At 42 days of the experiment, 4 ml of blood collected from the wing vein allowed to clot then centrifuged at 3000 rpm for 15 minutes so serum separated and transferred to Eppendorf tubes (1.5 mL) stored at -20°C for estimation of total protein, albumin, AST, ALT, ALP, total and direct bilirubin, uric acid, creatinine, total cholesterol (TC), Triacylglycerol (TAG), High-density lipoprotein (HDL-C) and LDL-C were measured by colorimetric methods using the procedure described by Spinreact Co., Spain and according to the manufacturer's instructions [17]. All of these assays were performed spectrophotometrically by Turner 690 Chemistry Analyzer in chemistry department, AHRI, Egypt. The concentration of serum globulin was determined by the difference between the concentration of total protein and albumin.

2.4 Serum thyroid hormones analysis

The enzyme-linked immunosorbent assay (ELISA) kits validated for use in chickens (MyBioSource, Inc. San Diego, California, U.S.A) is used to evaluate triiodothyronine (T3) hormone and thyroxine (T4) hormone, as calculated by Biochrom Asys Expert Plus Microplate Reader (Biochrom Ltd., Cambridge, United Kingdom).

2.5 Statistical Analysis

The data obtained was analysed using the mathematical computer program SPSS 16.00 Software using a one-way ANOVA process (SPSS Inc., Chicago, IL, USA).

Table 1: The broiler diet structure (for 100 kg feed)

	Starter (0-21 days of age)	Finisher (22-42 days)
Components		
Corn	48.20	58.70
Wheat	8.00	7.50
Soybean meal (40%)	28.50	20.50
Protein concentration (50%)	10.00	10.00
Vegetable oil	4.00	2.50
Salt	1.00	0.50
Vit + Min mix*	0.30	0.30
Total	100.00%	100.00%
Composition calculated**		
ME (kcal/kg)	3079.00	3102.60
Crude protein	22.06	19.37
Lys.	1.21	1.03
Meth + Cyc.	0.82	0.75
Ca (%)	1.2	0.95
P (%)	0.44	0.42

*Per kg of diet, the vitamin and mineral combination provided.

** calculations determined according to NRC (1994).

3. RESULTS

3.1. Effect of HS and TP on Haematology of broilers in 42 day

Chronic heat stress (HSC group) induced a significant decrease in RBC, PCV and monocytes counts compared to TNC population. Also, a significant increase in H/L ratio due to reduction in Lymphocytes and significant increase in Heterophils. Supplementation of TP improved haematological parameters than HSC (Table 2).

3.2. Effect of turmeric on serum metabolites measurements

HSC broilers had a significant decrease (P< 0.05) in total protein and albumin levels while significant increase in AST, ALT, ALP, T. bilirubin, D. bilirubin, Uric acid and creatinine when compared with TNC group. TP caused significant increase in total protein with non-significant increase (p > 0.05) in albumin and globulin while AST, ALT, ALP significantly decreased, uric acid with non-significant decrease in total and direct bilirubin than HSC group. It was also observed that, cholesterol, triglyceride and LDL HS significantly increased with non-significant decrease in HDL-C concentration in HSC group as compared with broilers in the thermoneutral group (TNC). It is interesting to note that, dietary supplementation of TP showed significant decrease (P< 0.05) in total cholesterol and triglycerides with non-significant decrease in LDL contents and non-significant increase in HDL-C (Table 3).

Table 2: Effect of Turmeric Powder (TP) on haematological indices of broilers at 42 d of age under thermoneutral and heat stress conditions (34±2°C) (Mean±SE).

Parameters[1,2]	Treatments[3]			
	Thermoneutral condition		Heat stress conditions	
	TNC (n=5)	TNT (0.5%) (n=5)	HSC (n=5)	HST (0.5%) (n=5)
PCV %	31±1.39	32.944±1.81	24.8±0.71*	30.92±0.86*
Hb (g/dl)	11.74±0.4	11.526±0.59	9±0.23*	11.452±0.57*
RBCs (x10^6/mm3)	3.73±0.27	3.684±0.31	2.454±0.05*	2.975±0.09*
WBCs (x10^3/mm3)	4.46±0.15	4.35±0.22	5.768±0.24*	4.98±0.14
Platelets	5.55±0.43	5.02±0.28	4.876±0.18	5.154±0.28
Lymphocyte%	73.1±2.49	71.974±1.7	67.06±1.8	71.34±1.45
Heterophils%	22.5±1.37	23.666±1.29	28.83±0.67*	24.54±0.8*
Monocyte%	2±0.1	2.4±0.12	1.41±0.03*	1.9±0.12
Eosinophil%	2.1±0.45	1.7±0.14	1.1±0.15	1.68±0.12*
Basophils%	0.3±0.06	0.26±0.04	1.6±0.52	0.54±0.15
H/L ratio	0.31±0.01	0.33±0.02	0.43±0.02*	0.34±0.01*
MCV (fl)	85.4±8.62	90.8±5.47	101.3±4.22	104.3±4.26
MCH (pg)	31.9±1.8	32.2±3.07	36.7±1.08	38.8±2.87
MCHC (g/dL)	38.33±2.75	35.26±1.82	36.4±1.54	37.2±2.14

[1]Abbreviations: PCV; Haematocrit; Hb: Haemoglobin; RBCs: Erythrocytes; WBCs: Leukocytes; H/L ratio: Heterophils / Lymphocyte ratio.

[2]*asterisks* indicate values that are significantly different between TNC and TNT; TNC and HSC; HSC and HST. (**p*<0.05); Values are means ±SEM (Standard Error of Mean).

[3]TNC: Thermoneutral control, TNT: Thermoneutral turmeric, HST: Heat stress turmeric, HSC: Heat stress control.

3.3 Effect of HS and TP on thyroid hormones

The effect of the experimental diets on broilers T3 and T4 have a significant decrease due to chronic heat stress compared with TNC while the addition of TP has non-significant increase T3 and T4 than HSC (Table4).

Table 3: Effect of Turmeric Powder (TP) on serum metabolites of broilers at 42 d of age under thermoneutral and heat stress conditions (34±2°C) (Mean ±SE).

Parameters[1,2]	Treatments[3]			
	Thermoneutral condition		Heat stress conditions	
	TNC (n=5)	TNT (0.5%) (n=5)	HSC (n=5)	HST (0.5%) (n=5)
Total protein (g/dL)	3.948±0.22	4.268±0.28	2.712±0.13*	3.876±0.18*
Albumin (g/dL)	2.244±0.22	2.554±0.31	1.212±0.06*	1.652±0.18
Globulin (g/dL)	1.704±0.03	1.714±0.03	1.5±0.11	2.224±0.34
AST (U/L)	237.2±2.22	233.84±2.26	287.88±3.85*	244.28±9.73*
ALT (U/L)	8.67±0.34	7.78±0.27	12.146±0.64	8.848±0.08*
ALP (IU/dL)	1483.4±100.1	1499.8±26.06	2466.6±40.32*	1782.2±18.22*
TBIL (mg/dL)	0.946±0.05	0.624±0.02	1.6686±0.09*	0.9502±0.01
DBIL (mg/dL)	0.158±0.01	0.1878±0	0.326±0.04*	0.176±0.01
T. Cholesterol (mg/dL)	124.6±1.57	122.6±2.6	150±2.7*	127±3.62*
Triglyceride (mg/dL)	96±1.7	95±2.59	137.8±2.96*	112.4±2.01*
HDL-Cholesterol (mg/dL)	56.8±1.77	59±3.99	48.2±2.4	53.2±4.16
LDL-Cholesterol (mg/dL)	48.6±1.67	44.6±6.07	74.24±3.72*	51.32±6.11
Uric acid (mg/dL)	5.508±0.28	4.864±0.21	8.558±0.48*	4.882±0.09*
Creatinine (mg/dL)	0.352±0.04	0.318±0.03	0.538±0.01*	0.484±0.04

[1]Abbreviations: ALT: Alanine transaminase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; TBIL: total bilirubin; DBIL: direct bilirubin; U/L=unit of enzyme activity/L.

[2]*asterisks* indicate values that are significantly different between TNC and TNT; TNC and HSC; HSC and HST. (*p<0.05); Means ±SEM Values (Standard Error of Mean).

[3]TNC: Thermoneutral control, TNT: Thermoneutral turmeric, HST: Heat stress turmeric, HSC: Heat stress control.

Table 4: Effect of Turmeric Powder (TP) on serum thyroid hormones of broilers at 42 d of age under thermoneutral and heat stress conditions (34±2°C) (Mean ± SE).

Parameters[1,2]	Treatments[3]			
	Thermoneutral condition		Heat stress conditions	
	TNC (n=5)	TNT (0.5%) (n=5)	HSC (n=5)	HST (0.5%) (n=5)
T3 (ng/ml)	154.66±3.38	157.58±2.91	139.36±1.5*	145.343±1.66
T4 (ng/ml)	1.9±0.08	1.87±0.02	1.028±0.07*	1.384±0.06

[1]Abbreviations: T3: Triiodothyronine; T4: Thyroxine.

[2]*asterisks* indicate values that are significantly different between TNC and TNT; TNC and HSC; HSC and HST. (*p<0.05); Values are means ± SEM (Standard Error of Mean).

[3]TNC: Thermoneutral control, TNT: Thermoneutral turmeric, HST: Heat stress turmeric, HSC: Heat stress control.

4. DISCUSSION

The present study found that, Broilers in HSC showed significantly lower RBC, Hb and PCV in agreement with [18] who reported that, HS bird's RBC, Hb and PCV values were significantly low in comparison with stress free group at 6th week of age. Also, data for WBCs profiles showed higher lymphocyte number with less heterophil no. in thermoneutral control group relative to the heat stressed control group on day 42 [19]. Turmeric extract capsules administered orally to broilers just 3/week in (15- 35) day under thermoneutral

conditions, erythrocyte concentrations were not influenced by turmeric ($p>0.05$) [21]. Similarly, the PCV of broilers in different groups were no significant difference [22]. Stress may affect haematological counts, inducing ACTH and release epinephrine and norepinephrine culminating in heterophils that constitute the defence first line with an effective chemotactic reaction against florigens [23].

The present findings are in accordance with [18] which showed that WBC numbers increased in the HS community may be attributable to stress-related rise in cortisol concentration that is responsible for increased leukocyte production while turmeric supplementation (0.4 percent) at the week no. 4 resulted in a substantial decrease in WBC counts when exposed to heat stress so it enhanced many haematological parameters [18]. The results in the present study showed improvement of haematological parameters TP in diet than HSC group that were in agreement with similar study [24] who demonstrated that, a substantial increase in the levels of RBCs, WBCs, Hb and PCV % was observed due to the different feed additives of TP relative to the control group. By stimulating the production of bile, curcumin in turmeric will speed up fat emulsification to make fat digestion more optimal. Optimized fat digestion can indirectly increase the availability of ß-oxidation or fat metabolism substrates that ultimately improve succinyl-CoA production through the metabolic cycle of Krebs required for the synthesis of haemoglobin [21].

Consequently, increased succinyl-CoA production was most likely accompanied by an increase in haemoglobin synthesis. On reverse, the effect of turmeric on haematocrit in broiler chickens at day 42 of age under heat stress (32±1°C) have no changes [25]. Similarly, there are no changes in haemoglobin and haematocrit content in broilers fed 8g TP/kg diet [9, 26]. Glucocorticoid is released in stressful conditions, leading to increased development of heterophils from the bone marrow, thereby increasing the number of heterophils in circulation [27]. In accordance with [18, 28], the current study found that the H and H/L values of broilers under heat stress were significantly higher with a significant decrease in monocytes than those in the TNC community as a consequence of elevation of plasma corticosterone.

The present study found that, the supplementation of TP in diet increased lymphocytes while significant decrease in heterophils and H/L ratio that in agreement with the results of [29, 30] who demonstrated that, the dietary supplementation of curcum essential oil reduced the Heterophile / Lymphocyte ratio. The small rise in the chicks blood components due to increased turmeric addition in diet that attributed to the antioxidant status improvement of broilers due to bioactive compounds. In the HST group, the H/L ratio was also lower than that of broilers in the HSC group, but close to the TNC value. Heat stress may lead to changes in some of the body's biochemical indexes, especially the enzyme content, and can generally be examined using indicators such as clinical behaviour and biochemical parameters of the blood that can indicate tissue damage [31].

Different tropical hot conditions obviously influenced biochemical parameters in broiler [32]. The current study found, serum total protein and albumin concentrations are significantly decreased by HS which in agreement with [7] who demonstrated that mean serum protein in broilers reared HS zones was significantly affected by temperature area. Oxidative stress enhances the production of ROS, which results in biomolecules such as nucleic acid, protein, and enzymes being desaturated. Increased oxidative stress caused by HS can be related to the reduction in serum protein consumption, partially due to reduced protein intake and scarcity of necessary amino acids. Low serum protein levels can also be responsible for reduced protein digestibility at HS [33]. The 38˚C program for 10 h., an important reduction in total protein and albumin amounts at HS relative to control levels were observed in broiler chickens [34].

The current study showed that dietary TP supplementation induced high significance in total protein with non-significant increase in albumin that was consistent with [35] who demonstrated that, total protein contents in groups fed 200g and 400g TP in diets have increased than the basal group. A substantial rise in

the average serum protein concentration of broiler chickens receiving a feed containing 0.5 percent turmeric powder [36]. Changes in the production of serum enzymes arise under hot stress conditions [37]. Serum ALT level is clinically assessed as a diagnostic liver function study [38, 39]. The present study resulted in significant increase in AST, ALT, ALP in HSC group than TNC group which in agreement with [40] who reported that heat stress-induced chicken liver injury caused an extremely significant elevation in the activity of AST and ALT liver damage enzymes. The biochemical parameters increase could theoretically be related to higher cellular production (ROS). Free radicals affect the metabolites of the blood serum [41]. Increased serum ALT levels could be attributable to decreased cell-level antioxidant enzymes [42].

The present study found that, the activities of blood enzymes like ALT, AST and ALP were substantially decreased by the supplementation of TP in the heat stressed broiler diet in accordance with [9] that recorded a substantial decrease in the activity of ALT, AST and ALP enzymes in broiler chickens fed 0.4% turmeric powder under heat stress, resulting in enhanced digestion, increased metabolism of nutrients and an increase in turmeric powder. The current findings showed that globulin was suppressed by HS but increased in heat stressed broilers by supplementation of TP in diet in accordance with [43] who stated that globulin increased significantly compared to the positive and negative control groups of the experiment in the treatment groups with turmeric powder. Serum globulin levels indicated that there was a potential for improved humoral immune (HI) levels for chickens in treated classes. The rate of bilirubin conjugation and excretion is affected by interference with the normal functions of the liver. High bilirubin levels are thus used as measures of liver function and bile excretion status [44]. The latest findings have shown that, the substantial rise in TBIL and DBIL is significant in accordance with [34].

In the present study, supplementation of TP in diet caused decrease in blood uric acid that was significantly increased due to HS. Moreover, Blood uric acid at day 42 of age subjected to heat stress 32°C decreased in broilers that had diets supplemented with 0.5% turmeric. Elevated plasma corticosterone concentrations may increase serum uric acid level. Uric acid is a potent scavenger of free radicals in poultry, and increasing levels of ACTH have been associated with increased concentrations of uric acid. That may be due to elevated levels of plasma corticosterone due to heat stress, that in turn increases the contents of blood uric acid [25]. The decreased levels of uric acid in birds fed phytogenic supplements may be due to their ability to depress the production of ACTH and uric acid. Turmeric was stated to be able to reduce the blood ACTH concentration [45]. Chronic heat stressed HSC group broilers had significant increase in creatinine when compared with TNC group while blood creatinine was not affected by supplementation of TP in diet that in agreement with the results of [25] who found that at day 42 of age the dietary treatments of 0.5% turmeric subjected to heat stress has no significant effect on creatinine content.

The present study revealed that, chronic heat stress (HSC group) resulted in significant increase in total cholesterol, triglyceride and LDL-C while as compared with broilers in the thermoneutral group (TNC). It is interesting to note that, dietary supplementation of TP caused significant reduction ($P< 0.05$) in cholesterol and triglycerides with non-significant decrease in LDL-C contents with an increase in HDL-C (Table 3). The present results are in agreement with who demonstrated that, on day 42 in high ambient temperature Chronic heat stress (HSC group) induced a significant increase in cholesterol, triglyceride and LDL-C than the TNC group. The HSC group had an elevated serum total cholesterol level, presumably attributed to a rise in corticosterone concentrations due to hypothalamic-pituitary-adrenal stimulation [46]. Similarly, the addition of 400 mg/kg TP to diets dramatically decreased serum concentrations of LDL-C and TC in broiler chickens exposed to heat [47]. Current research showed that, the effect of chronic heat stress on broilers T3 and T4 have significant decreased compared with TNC while the dietary supplementation of TP has increased ($p > 0.05$) T3 and T4 than HSC (Table 4). Also, High temperature affects the levels of thyroid hormones. The results of a study showed that daily average of T3 decreased significantly compared to control group and T4 response was relatively less when broiler chickens were exposed to HS [8].

5. CONCLUSION

The safe administration of TP and the potential use of TP as a source of phytobiotic feed additive were recognized in this study to offset chemical antibiotics and other growth promoters in order to increase the productivity of broiler growth and make broiler farming more efficient.

List of abbreviations

ACTH: Adrenocorticotropic hormone
ROS: Reactive oxygen species
NRC: National Research Council
EDTA: Ethylene diamine tetra acetic acid

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