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The Protective Role of Ginger *(Zingiber Officinale)* on Alleviation of Heat Stress In Broiler Chicken

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ABSTRACT

Heat stress is one of the most crucial issues in broiler production, particularly in tropical regions. Ginger can serve as an antioxidant. Being part of the nutritional interventions, treatment using ginger has been documented to alleviate the negative impact of heat stress on broiler chickens, in terms of production, physiology, immunology, and antioxidant status of broilers. The present review elucidates the alleviation of heat stress in broiler chicken using ginger based on the most recent literature in order to provide complete information on the impact of ginger powder (GP) on haematology, biochemistry and thyroid hormones of broilers under heat stress (HS) (34 ± 2° C) and thermoneutral temperature (TN) (22 ± 4 °C). An 80 of one-day-old broiler chicks (cobb-500), 20 birds in each group either control (basal diet) or 0.75% ginger. Chronic HS at 42 d of age resulted in significant decrease in PCV, RBCs, total protein, Albumin, serum T3 and T4, whereas increased H/L, triglycerides, cholesterol, LDL-cholesterol, uric acid, AST, ALP, ALT, DBIL, TBIL, and creatinine significantly. Supplementation of GP significantly increased PCV, RBCs, total protein, albumin while significant decrease in AST, uric acid and improvements in lipid profile parameters. Findings of the research study indicated that ginger has improved birds health.

Keywords
Heat stress, Thermoneutral, Broiler chickens, Ginger, *Zingiber officinale*.

1. INTRODUCTION

Poultry industry can provide very high-quality proteins for human nutrition and also it is a source of national income for the community in many countries. In Egypt, the demand for broiler meat is increased rapidly due population growth and the emergence of many recipes containing chicken, loved by all ages. Thus, broiler farming seems to be a part of meat production and consumption in the country so numerous researches worked on broilers developments resulted in breeding programs enhanced feed utilization and growth rate

[1]. In recent years, the frequency of high-temperature days is expected to increase with climate change worldwide, and heat stress (HS) has become one of the most common environmental factors that challenge broiler production [2]. Modern fast-growing broiler genotypes produce more body heat due to their greater metabolic activity, so they are more likely to be sensitive to HS [1]. The retarded growth rate, increased disease outbreaks, and mortality is amongst the problems related to heat stress in broiler production. Birds subjected to high ambient temperatures can induce oxidative stress that decrease growth efficiency and the health of poultry species [3].

[4] reported that medical plant extracts supplementation, as a natural antioxidant against stressors, improved broilers' performance and immune system. Ginger is the rhizome of the plant (*Zingiber officinale*), commonly called gingeris a perennial plant. The efficacy of ginger is purported to be a result of its aromatic, carminative and absorbent properties. Besides, ginger is reported to have detoxification, anti-diabetic, antiemetic potential [5], hepatoprotective, analgesic, radio-protective and anti-inflammatory activities and its extract have been widely demonstrated, and the most important bioactive components responsible for various pharmacological activities of ginger are the gingerols, a group of phenolic compounds including 6-, 8-, 10-gingerol, with the major component being 6-gingerol [6] consumed as a delicacy, medicine, or spice. Preliminary research indicates nine compounds that bind to serotonin receptors which influenced the gastrointestinal function5. These compounds have been reported to have antimicrobial, antioxidative and pharmacological effects [7].

According to [8] *in vitro* tests showed that ginger extract might regulate the quantity of free radicals and the peroxidation of lipids and have anti-diabetic properties. Ginger has useful pharmacological potent chemical substances for use in poultry. [9] also demonstrated that gingerols increased the gastrointestinal motility and had antibacterial properties in laboratory animals. Impairment of endocrine functions involve the biochemical changes caused by excessive heat consumption of poultry. Decreased serum concentrations of T3 and T4, effective animal growth promoters and suppressed function of the immune system at elevated ambient temperatures have been reported [10].

2. MATERIALS AND METHODS

2.1 Formulation of Ginger Powder: Ginger was acquired from the Crop Department, ARC, Dokki, Giza, Egypt, then was dried, cut into small slices for simple and adequate drying. Ginger was ground using an electric grinder and packed in paper bottles, then processed at (21-24°C) and then mixed at 7.5 gm/kg of diet [11] at 21-42 days of age.

2.2 Experimental design and Birds:

An (80) seemingly stable, one-day aged broiler chicks (Cobb-500) from El-K ahera Poultry Agency, 10th of Ramadan District, Al-Sharkia provincial capital, Egypt. They were kept in hygienic environment on the base of the pens. All pens were coated with 8 cm deep fresh wood shaving and equipped with hogs and watered in 10 birds per m2 environmental rooms in the research poultry unit in Animal Health Research Institute, Egypt. The birds had unrestricted access to the purified water (DW) and eat for the whole experimental era, the experimental period (42 days of age). Basal diet (Table 1) has been formulated to satisfy the recommended criteria of the NRC National Research Council [12] for all nutrients. The initial house temperature was set at 32°C and decreased steadily by 2°C every week for 3 weeks.

The trial consisted of four care groups with two foods (basal diet or 7.5 g/kg ginger diet) and two temperatures (thermoneutral or heat stress). Birds were arbitrarily classified into two classes: thermo-neutral treatment class A: birds reared at ambient temperatures of 22 ± 4 °C daily and relative humidity (55 ± 3, RH)[13]. Class B heat treatment: birds grown in air temperature (34 ± 2 °C) everyday from 9:00 a.m. to 5:00 p.m. A relative humidity of 65 per cent until the conclusion of the study [14]. Each party is classified into

two groups: 1: Thermoneutral Ginger Group (TNG), 2: Thermoneutral Control Group (TNC), 3: Heat Stress Ginger Group (HSG), 4: Heat Stress Control Group (HSC).

2.3 Haematological parameters and blood leucocyte profiles

Until collection of blood samples, feed was removed from all birds for a duration of 12 hours. 1 mL Blood samples were obtained from five birds randomly chosen for each procedure in EDTA anticoagulant treated vials. Haematological indices were haematocrit (HCT), haemoglobin (Hb), erythrocytes (RBCs), mean corpuscular haemoglobin (MCH), Mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), White Blood Cell (WBCs), the number of lymphocytes, monocytes, heterophile, basophils and eosinophil. They were assayed by the Sysmex XT 2000 IV Automatic Haematology Analyzer (Japan) was in conjunction with the techniques and measurements mentioned in [15]. Wright-Giemsa complex staining was done to prepare blood samples for a microscopy study of the WBC profile, 100 leukocytes/sample were counted by an optical microscope using the Neubauer haemocytometer following the protocol of [16] Calculation of H/L values utilize blood clotting after staining by Giemsa staining technique.

2.4 Biochemistry

At 35 days of the trial, 2 ml blood was obtained from 5 birds by the wing vein to get serum by centrifugation at 3000 rpm for 15m. Serum was assayed for estimation of albumin, total protein, , aspartate aminotransferase activity (AST), ALP, alanine aminotransferase (ALT), TBIL (total bilirubin), DBIL (direct bilirubin), creatinine, uric acid, Total cholesterol (TC), HDL-C, Triacylglycerol (TAG) and LDL-C using commercial test kits (Spinreact, S pain) by spectrophotometer. Globulin was calculated by the difference of total protein and albumin.

2.5 Thyroid hormones analysis

Thyroxine (T4) and Triiodothyronine (T3) hormones were determined by enzyme-linked immunosorbent assay (ELISA) kits validated for use in poultry (MyBioSource, Inc. San Diego, California, U.S.A) determined using Biochrom Asys Expert Plus Microplate Reader (Biochrom Ltd., Cambridge, United Kingdom).

Table 1: Composition of the broiler diet (for 100 kg feed)

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

*Per kg of diet, vitamin and mineral combinations given.

** Estimates calculated in conjunction with [12].

2.6 Statistical Analysis

All values are viewed as a mean ± standard error (SE). Statistical analysis was conducted using Spss Software version 16.00 Program (SPSS Inc., Chicago, IL, USA) using the One-way ANOVA multi-group comparative test. Differences between the means of the two time points were evaluated using the t-test, $P<0.05$ found to be important.

3. RESULTS

3.1 Effect of HS and GP on Haematology of broilers in 42 day

Chronic heat stress (HSC group) resulted in a substantial drop in RBC (Red Blood Cell), PCV and monocyte values relative to the TNC group and a substantial rise in the H/L ratio owing to a decline in lymphocytes and a significant increase in heterophils. It was observed that daily consumption of GP showed improvement in certain haematological indices relative to the HSC community (Table 2).

3.2 Effect of ginger powder on serum metabolites measurements

In the TNG community, there was a substantial increase in total protein and globulin levels. Chronic heat-stressed broilers had a significant decrease ($P<0.05$) in total protein and albumin levels, while the sig raise in AST, ALP, ALT, T. bilirubin, D. bilirubin, uric acid and creatinine when compared to TNC group. GP induced a substantial increase in total protein and albumin, while AST, uric acid, decreased dramatically relative to the HSC group. It was also found that persistent heat stress (HSC group) caused a substantial rise in cholesterol, triglyceride and LDL, although the concentration of HDL-C was not substantially decreased relative to broilers in the thermoneutral group (TNC). It was noticed that the dietary supplementation of GP shows a substantial decrease ($P<0.05$) in LDL, triglycerides and total cholesterol with a significant rise in HDL-C (Table 3).

Table 2: Effect of ginger Powder on haematological indices.

Parameters[1]	Treatments			
	Thermoneutral condition		Heat stress conditions	
	TNC (n=5)	TNG (0.5%) (n=5)	HSC (n=5)	HSG (0.5%) (n=5)
PCV %	31±1.39	32.06±2.12	24.8±0.71#	30.76±1.08*
Hb (g/dl)	11.74±0.4	12.16±0.31	9±0.23	11.326±0.22
RBCs ($x10^6$/mm3)	3.73±0.27	3.95±0.21	2.454±0.05#	3.668±0.2*
WBCs ($x10^3$/mm3)	4.46±0.15	4.29±0.11	5.768±0.24#	4.768±0.18
Platelets	5.55±0.43	5.684±0.3	4.876±0.18	5.052±0.27
Lymphocyte%	73.1±2.49	75.2±1.65	67.06±1.8	73.06±1.82
Heterophils%	22.5±1.37	21.71±1.05	28.83±0.67#	22.244±1.16*
H/L ratio	0.307±0.01	0.289±0.01	0.432±0.02#	0.31±0.02*
Monocyte%	2±0.1	1.9±0.19	1.41±0.03#	1.616±0.07
Eosinophil%	2.1±0.45	0.9±0.24	1.1±0.15	1.8±0.11
Basophils%	0.3±0.06	0.29±0.09	1.6±0.52	1.28±0.08
MCV (fl)	85.447±8.62	81.720±5.69	101.333±4.22	85.157±6.5
MCH (pg)	31.945±1.8	31.148±1.9	36.723±1.08	31.27±1.82

MCHC (g/dL)	38.318±2.75	38.562±2.56	36.436±1.54	37.035±1.69

[1]* P<0.05 when compared with heat stress control group, # P<0.05 when compared with thermoneutral control group. Data are presented as mean ±SE (n=5).

3.3 Effect of HS and GP on thyroid hormones

The impact of dietary treatments on broilers T3 and T4 declined substantially due to chronic heat stress relative to TNC, while dietary supplementation of ginger increased significantly (P<0,05) T3 and T4 compared to HSC (Table 3).

Table 3: Effect of Ginger Powder (GP) on biochemical variables and thyroid hormones.

Parameters[1]	Treatments			
	Thermoneutral condition		Heat stress conditions	
	TNC (n=5)	TNG (0.5%) (n=5)	HSC (n=5)	HSG (0.5%) (n=5)
Albumin (g/dL)	2.244±0.22	1.548±0.06	1.212±0.06#	1.61±0.06*
Total protein (g/dL)	3.948±0.22	5.018±0.07#	2.712±0.13#	3.776±0.21*
Globulin (g/dL)	1.704±0.03	3.47±0.05#	1.5±0.11	2.166±0.25
ALT (U/L)	8.67±0.34	8.57±0.25	11.546±1.14	8.75±0.25
ALP (IU/dL)	1483.4±100.1	1478.8±13.97	2466.6±40.32#	2237.8±48.18
AST (U/L)	237.2±2.22	230.1±2.09	287.88±3.85#	259.72±3.25*
DBIL (mg/dL)	0.158±0.01	0.1516±0	0.326±0.04#	0.2106±0.04
TBIL (mg/dL)	0.946±0.05	0.934±0.02#	1.6686±0.09#	1.498±0.05
Triglyceride (mg/dL)	96±1.7	90.4±1.63	137.8±2.96#	119±1*
T. Cholesterol (mg/dL)	124.6±1.57	108±1.92#	150±2.7#	129±3*
LDL-Cholesterol (mg/dL)	48.6±1.67	26.72±2.01#	74.24±3.72#	44.4±4.69*
HDL-Cholesterol (mg/dL)	56.8±1.77	63.2±2.08	48.2±2.4	60.8±1.88*
Creatinine (mg/dL)	0.352±0.04	0.298±0.03	0.538±0.01#	0.422±0.03
Uric acid (mg/dL)	5.508±0.28	5.144±0.23	8.558±0.48#	5.422±0.27*
T4 (ng/ml)	1.9±0.08	2.076±0.34	1.028±0.07#	1.724±0.09*
T3 (ng/ml)	154.6632±3.38	158.102±1.58	139.3606±1.5#	166.3826±2.72*

[1]* P<0.05 when compared with heat stress control group, # P<0.05 when compared with thermoneutral control group. Data are presented as mean ±SE (n=5).

4.DISCUSSION

The same findings were observed by [17] who reported that RBCs and PCV values for HS birds were substantially poor at the 6th week of age. If heat stress is prolonged, in vivo endotoxin can directly affects erythrocytes negatively and cause liver and kidney damage by inhibiting erythropoietin production, leading to a decrease in the number of RBCs. [18]. The present findings are similar with [17] that has shown that the quantity of WBCs in the HS group of birds reared on a diet without any supplementation compared to the normal temperature group can be due to a stress elevated cortisol concentration that is responsible for increased leukocyte output. The current study showed that the H and H/L values of broilers under heat stress were substantially higher with a substantial decrease in monocytes than in the TNC population due to elevation of plasma corticosterone that in accordance with [19].

Our analysis showed improvement in certain hematological parameters relative to the control control group in HS groups in accordance with [19] who demonstrated that the addition of ginger (0.5 per cent) to broiler diets increased Hb, RBCs, WBCs and Lymphocytes (L) and decreased Heterophile (H) of chicks significantly compared to the control group (35-41°C). Similarly, [20] found that, A significantly lower heterophil value was recorded in the vitamin E- supplemented accompanied by ginger in broiler strains subjected to chronic heat stress. Heat stress may contribute to changes in some of the body's biochemical indexes, especially the enzyme content, which can generally be investigated using markers such as clinical actions and biochemical blood parameters that may suggest tissue damage [21]. Our findings showed that there was a major improvement in total protein and globulin in the TNG community in accordance with [22] that supplemented ginger powder (6 g/kg).

In the current research, serum total protein and albumin concentrations are significantly reduced by HS, which, in agreement with [23]. Decreased overall serum proteins may be due to elevated oxidative stress caused by elevated ambient temperatures. Oxidative stress enhances the development of reactive oxygen species [24] that destroy biomolecules such as nucleic acids, proteins and lipids. [20] found that heat stress significantly decreased overall serum protein while ginger supplementation significantly increased TP in broiler chicks. GP caused a substantial increase in albumin while uric acid decreased dramatically relative to the HSC group. The addition of GP to the diet has relieved the HS effect that, in accordance with [25], who stated that, addition of GP (10 g) in the diet of laying hens under HS has raised the amount of total protein. New study has shown that dietary supplementation of ginger has declined by AST and ALT relative to HSC (Table 3). This is in accordance with [20].
Similarly, [26] observed that mean serum AST activity was slightly lower in birds fed ginger (2%) with supplementation at high temperature (28-36°C) on days 15-42 compared to control ($P < 0.05$).

The beneficial impact of ginger on liver function may be attributed to its antimicrobial and antioxidant compounds [27]. Uric acid is a potent scavenger of free radicals in birds, and high concentrations of ACTH have been linked with elevated rates of uric acid. This may be attributed to higher plasma corticosterone levels caused by heat stress, which in turn raises the blood uric acid content [28]. Chronic heat stress produced a large rise in uric acid as compared to TNC group. Chronic heat stress caused a significant increase in uric acid compared to the TNC population, while GP enrichment in the diet resulted in a significant decrease that was consistent with the results of [29] that used aqueous ginger extract (0.4 and 0.6 per cent) to Broiler's drinking water.

The present study showed that supplementation of GP to thermoneutral control group (TNC group) broilers led to a substantial decrease in total cholesterol and LDL-C although HDL-C was higher relative to thermoneutral control group broilers (TNC) that in line with the results of [30] who supplemented GP (5 g/kg) to broilers at 42 d age. The present study showed that persistent heat stress (HSC community) resulted in a substantial rise in total cholesterol, triglyceride and LDL-C relative to broilers in the thermoneutral group (TNC) that in accordance with [20]. It is important to notice that the dietary supplementation of GP shows a substantial decrease ($P<0,05$) in total cholesterol, triglycerides, LDL-C and cholesterol with a significant rise in HDL-C (Table 3). Elevated serum cholesterol levels at high temperatures may be triggered by hyperstimulation of the adrenal gland producing adrenocorticotropin hormones [31] for which cholesterol serves as a precursor.

β–hydroxy-b-methyleglutaryl coenzyme A (HMG-CoA) is active in cholesterol synthesis and ginger is a strong inhibitor of HMGR and thus inhibits cholesterol synthesis [27]. In addition, [25] observed that GP had antihypercholesterolemia activity in the laying hen, which suggests that certain additives had the potential to alleviate stress. It is well known that high temperature conditions increase the activation of the hypothalamic-pituitary-adrenal (HPA) axis and thus increase the level of corticosterone [1, 32] with a

concomitant decrease in T3 and T4 concentrations in poultry [33]. These endocrinological changes induced by chronic heat stress could reduce growth efficiency and redistribute nutrient metabolism to lipogenesis and proteolysis [34]. [35] It has been observed that elevated temperatures influence the levels of thyroid hormones. New study has demonstrated that GP dietary supplementation has sig. Increase ($P<0.05$) T3 and T4 relative to HSC (Table 3). In accordance with the findings of [36], Ginger powder (0.5% of diets) was observed to increase serum concentrations of T3 and T4 in thermal-stressed broilers by 32-34 ℃ for 4 h.

5. CONCULSION

This research carried out the protection administration of ginger in broiler diet as it did not have acute toxic side effects as recorded during the experimental period. It can be used as a source of phytobiotic feed additive to substitute the chemical growth promoter to render broiler farming profitable according to healthier profiles of broilers receiving experimental diets. It can also be inferred that dietary supplementation of ginger shows an increase in the immunity of broilers, possibly due to the high antioxidant activity of ginger.

It also showed ginger rhizomes can be locally cheaper and easy to use with no harmful effects compared to synthetic antibiotics.

List of abbreviations

ACTH: Adrenocorticotropic hormone
NRC: National Research Council
ARC: Agriculture Research Centre

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