Research Article

Dietary Moringa Leaf Supplementation Affects Heat-Stressed Broilers: II-Blood Metabolites, Antioxidant Activity and Meat Quality

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Abstract | Heat stress exposure negatively influences broilers' productivity and profitability. Stress exposure induces oxidative stress, which affects the general metabolism of heat-stressed birds and impairs carcass yield and meat quality. We investigated the impact of *Moringa oleifera* leaf meal (MLM) supplementation in heat-stressed (HS) broilers on their blood metabolites, antioxidant bio-indicators, carcass yield, and meat quality. We used three hundred one-day-old Cobb chickens and were randomly divided into three groups, each with five replicates of 20 birds. The thermoneutral control group received a basal diet, while the other two groups experienced cyclic thermal stress from day 22 to 42 and were fed the basal diet provided with either 0 or 2 g MLM/kg diet. On day 42, we reported carcass characteristics and evaluated meat yield and quality. Blood samples (n=10) were used for hematological assessment, as well as plasma was employed to measure blood metabolites and antioxidant markers. The results revealed a significant (P<0.05) reduction in red blood cell count (RBC), hemoglobin (HBG) concentration, hematocrit (HCT) percentage, albumin level, and endogenous antioxidant activity. Meanwhile, plasma total protein, cholesterol, triglycerides, creatinine, urea levels, and liver enzymes activity were significantly (P<0.05) increased. The carcass yield and meat quality showed significant (P<0.05) deterioration in response to heat exposure. MLM administration to the basal diet significantly enhanced blood metabolite levels, hematological parameters, antioxidant activities, carcass yield, and meat quality in broiler chickens exposed to HS. Thus, we have confirmed the potentiality of using dietary MLM to adjust the negative effect of HS on broiler metabolism and subsequently improve carcass yield and meat quality.

Keywords | Blood metabolites, Blood hematology, Broiler, Heat stress, Meat quality, *Moringa oleifera*

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INTRODUCTION

Meat produced from broiler farming is recognized as a cost-effective source of high-quality protein on a global scale. However, rearing broilers under stress conditions not only jeopardized their performance but also reduced meat yield and quality. High-temperature-induced stress poses a substantial hindrance to global broiler production, exerting adverse effects on birds' performance and meat quality (Shakeri et al., 2020a, b; Señas-Cuesta et al., 2023). Broilers exposed to high microclimatic temperatures experience metabolic alterations, diminished feed intake (FI), and impaired growth (Shakeri et al., 2019; Kim et al., 2021). These physiological stresses coupled with the induction of oxidative stress are marked by undesirable carcass and meat characteristics, often expressed as the pale, soft, and exudative (PSE) syndrome with post-mortem drop in pH and induction of protein denaturation. PSE meat exhibits poor palatability traits like dry texture, reduced flavor, and increased cooking loss, leading to significant economic losses for producers and hindered consumer satisfaction (Zaboli et al., 2019; Chang et al., 2020). Yang et al. (2023) documented a significant drop in the level of total antioxidant capacity (TAC) and the activities of endogenous antioxidant enzymes in the breast muscle, as well as a detrimental impact of heat stress (HS) on cooking loss, meat pH, and drip loss in Arbor Acres broilers. They also identified 84 differential metabolites mainly related to fatty acids and amino acids. Broilers' dressing and breast muscle % decreased due to cyclic HS exposure (Moustafa et al., 2021). Teyssier et al. (2022) demonstrated that both cyclic and continuous heat exposure had deleterious effects on broiler carcass weight and breast meat yield.

Moringa oleifera has emerged as a potential natural supplement for diet additives with the possibility to mitigate HS and improve broiler meat quality. *Moringa* leaf meal (MLM) is rich in nutritive and bioactive compounds, including protein, antioxidants, vitamins, minerals, and other bioactive compounds, which are involved in its protective advantages against oxidation stress as well as inflammation caused by heat exhaustion (Natsir et al., 2019; Islam et al., 2021; Abdoun et al., 2023). Hence, dietary supplementation of *M. oleifera* leaves, owing to their antioxidant potential, would be a promising dietary strategy to recover meat yield and carcass quality during HS (Mahfuz and Piao, 2019). MLM contains essential amino acids and functional proteins that may maintain protein synthesis and muscle development in broiler chickens exposed to HS (Natsir et al., 2019; Islam et al., 2021; Javed et al., 2024). Under normal rearing conditions, feeding broilers with fermented cassava pulp and MLM increased both protein and polyunsaturated fatty acid content (Sugiharto et al., 2020). The broiler's breast muscle contains vital amino acids and unsaturated fatty acids were reported to be dose-increased with MLM supplementation (Jiang et al., 2023). *Moringa* supplementation at 0.5% improved breast amino acid content and enhanced the fatty acid profile (Jiang et al., 2023). Cui et al. (2018) found that adding *Moringa* leaf at a concentration of 1.56% to broiler diets can enhance the polyunsaturated fatty acid levels in meat, increase the stability of oxidation, improve breast muscle color, and reduce fat in the abdomen. *Moringa* leaf extract supplementation to broilers has been stated to enhance breast meat quality and shelf life (Hamada et al., 2021).

The exact mechanism of dietary MLM supplementation on broiler metabolism and performance in response to heat exposure is not fully comprehended. Thus, we aimed to assess the effectiveness of MLM supplementation and to provide profound evidence of its role in enhancing broiler metabolism, oxidative stability, meat yield, and quality during thermal stress exposure.

MATERIALS AND METHODS

Birds management

We confirm that all experimental protocols were approved by the King Faisal University Research Ethics Committee (KFU-REC/2023-08-25). 300 one-day-old Cobb500™ chicks were selected and raised under identical environmental and management circumstances for the initial 21 days of their life. Briefly, for the first three days, all chambers were maintained at 33 °C. The temperature was then decreased to 30 °C for the next four days. Subsequently, all chambers were cooled by 2 °C each week until the chicks reached 21 days old. Initially, all birds were kept under continuous lighting (24 hours) for the first three days of life. From day 4 to day 42, birds were exposed to a constant lighting program of 23 hours light and 1 hour dark each day. Throughout the experiment, birds had unlimited access to feed and water. The ventilation speed was set at 0.3 meters per second during the brooding period, increasing to 3 meters per second after 22 days. From day 21 onwards, birds were weighed and randomly allocated to three groups, each consisting of five replicates with 20 birds each. The study included three experimental groups: a thermoneutral negative control group received a basal diet (Control), a heat-stressed group subjected to cyclic heat stress while fed the basal diet (HS, control positive), and a group supplemented with *Moringa* leaf meal at a level of 2g/kg and subjected to cyclic heat stress (MLM+HS). The thermoneutral state was maintained at 24±1°C and 50% relative humidity. Two groups were exposed to HS at 35±1°C and 50% RH for eight hours, and then to 24±1°C and 50% RH for the remainder of the day from day 22 to day 42 of age. The basal diets were formulated to fulfill the nutritional requirements of broiler chicks according to the National Research Council (NRC, 1994) and the Cobb500 broiler chicken guidance.

Blood biochemical and hematological analysis

Each experimental group had ten blood samples collected, with two samples per replication, using a 3 mL EDTA-coated tube (Lab Use, Carejoy, China). The blood samples were spun in a centrifuge (Sorvall Biofige Pico, Germany) at 1800×g and 4°C for 20 minutes to separate the plasma. Plasma samples were subjected to biochemical analyses to measure concentrations of total protein (TP), albumin (ALB), cholesterol (CHOL), triglyceride (TG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, and urea using specific kits (ab102535, ab235628, ab65390, ab65336, ab241035, ab105135, ab65340, and ab83362, respectively, from Abcam, Waltham, MA, USA). Results were gathered using an automated microplate reader.

Ten more blood samples were obtained in EDTA-coated tubes and diluted 200-fold with physiological saline. Red blood cells (RBC) were quantified using a hemocytometer slide (Marienfeld, Germany) under a light microscope (Olympos BH-2 with Fotoubus, Japan) at 400 × magnification. Blood hemoglobin content (HGB) was determined using the cyanomethemoglobin method developed by Eilers (1967). For hematocrit (HCT) evaluation, blood samples were spun in Wintrobe hematocrit tubes (Cat# 22-362-574; Fisher Scientific, USA) and centrifuged at 1900 ×g for 20 min. at 4°C and the HCT levels were measured using a graduated scale.

Antioxidant markers

Blood plasma total antioxidant capacity (TAC) was measured with a colorimetric testing kit (ab65329; Abcam, Waltham, MA, USA). Superoxide dismutase (SOD) and catalase activity were measured in plasma using colorimetric tests (ab65354 and ab83464, respectively; Abcam, Waltham, MA, USA). The ceruloplasmin assay was conducted utilizing an ELISA kit (MBS2099661; MyBioSource Inc., San Diego, CA, USA).

Carcass yield and meat quality determination

Ten birds were chosen at random from each group at the end of the trial duration (two birds per group replication). Each bird was weighed, assassinated according to Islamic slaughtering practices (Regenstein and Chaudry, 2001), and had its feathers removed. The dressing % was determined relative to the live body weight. The breast muscles, thigh muscles, and abdominal fat were promptly removed from the warm corpse, weighed, and expressed as a proportion of the carcass weight. The pH_{24} of the meat samples was measured in three different places using a Hanna portable pH meter (HI 99163, Hanna Instruments, Romania), and the average value was determined. Meat color was determined using two-centimeter-thick deboned meat samples. A Chroma Meter (CR-400 Minolta Co., Osaka, Japan) was used to assess the color of the skinless breast and thigh muscles on their rear surface. 24 hours after death, 50 g of meat samples were put in a plastic bag and fried at 80°C for 20 minutes. Once the cooked samples reached room temperature, they were weighed to determine the cooking loss, which was determined as the percentage of weight lost after cooking. Shear force was assessed with the Warner-Bratzler shear force method utilizing a texture analyzer (TA.XTplus, Stable Microsystems, UK). The meat samples were cooked at 70°C and subsequently cooled to 4°C. The cooked samples were sliced into smaller samples and subjected to shearing force in a direction perpendicular to the fibers' longitudinal axis. This was done using a load cell with a maximum capacity of 50 N and a crosshead speed of 1 mm/s. The shear force sample was calculated as the mean of the subsample values.

Statistical analysis

The SPSS statistical program (IBM Corp, 2011) was used to do a one-way analysis of variance to the data following the model equation below:

$$Y_{ij}=\mu+\alpha_i+\varepsilon_{ij}$$

Where: Y_{ij} is the observed value of the dependent variable for the j^{th} observation in the i^{th} group. μ is the overall mean of the dependent variable. α_i is the treatment effect of the i^{th} group. ε_{ij} is the random error term.

Afterwared, a post hoc test was conducted using Duncan's test to distinguish between means, with a significance level of $P<0.05$.

RESULTS AND DISCUSSION

Blood hematology and metabolites

Hematological and biochemical parameters of blood were affected by heat exposure (Table 1). Significant ($P<0.05$) reductions in RBC count, blood HGB level, and HCT% were observed. However, MLM significantly ($P<0.05$) increased the blood HGB concentration compared with the Control and HS groups. Furthermore, MLM supplementation restored the hematocrit percentage to the normal control level indicating a positive effect of MLM on heat-stressed broiler blood hematology. Furthermore, blood biochemical parameters data revealed significant ($P<0.05$) changes in all the investigated measurements. When compared to the Control, heat exposure induced a significant ($P<0.05$) increase in blood TP, CHOL, and TG levels. Contrarily, plasma ALB levels, a negative acute phase protein, showed a significant ($P<0.05$) reduction of 30% under stress exposure. A substantial ($P<0.05$) increase in blood liver enzyme activity and kidney function indicators were also noticed. Under stress conditions, MLM supplementation significantly ($P<0.05$) altered these biochemical changes restoring partial balance. Blood CHOL and TG levels decreased significantly ($P<0.05$) in response to MLM supplementation reaching the values of the Contol group or lower.

Table 1: Blood hematological and biochemical measurements of broilers subjected to heat stress and received 0 (HS) or 2g/kg diet of *Moringa oleifera* leaf meal (MLM+HS).

Parameters	Control	HS	MLM+HS	SEM	P value
RBC, $\times10^6$/mm^3	2.27[b]	2.04[c]	2.33[a]	0.02	0.0328
HGB, g/dL	9.67[b]	9.11[c]	10.13[a]	0.02	0.0062
HCT, %	34.12[a]	29.34[b]	33.28[a]	0.29	0.0334
TP g/dL	3.61[c]	5.46[a]	4.43[b]	0.21	0.0017
Albumin, g/dL	1.86[a]	1.31[c]	1.61[b]	0.51	0.0178
CHOL, mg/dL	195.67[b]	230.42[a]	184.64[c]	6.24	0.0322
TG, mg/dL	172.61[b]	196.21[a]	176.57[b]	4.82	0.0394
AST, U/mL	71.35[c]	121.41[a]	107.78[b]	4.11	0.0274
ALT, U/mL	12.78[c]	23.75[a]	16.45[b]	1.52	0.0046
Creatinine, mg/dL	0.33[c]	0.56[a]	0.41[b]	0.04	0.0381
Urea, mg/dL	4.63[c]	6.35[a]	5.19[b]	0.37	0.0195

[a,b,c] Superscript letters represent statistically significant differences between means within the same raw ($P < 0.05$). RBC: red blood cells; HGB: hemoglobin; HCT: hematocrit; TP: total protein; CHOL: cholesterol; TG: triglycerides; AST: aspartate aminotransferase; ALT: alanine aminotransferase.

Blood hematology reflects the number and volume of blood cells and the HGB content, which is the main carrier of oxygen. Under heat stress, there was a serious reduction reported in the number and volume of RBCs with a decrease in hemoglobin content in broilers (Moustafa et al., 2021). Meanwhile, MLM supplementation significantly (P<0.05) improved hematological parameters and restored normal homeostasis. Javed et al. (2024) reported an increase in RBC, HGB, and HCT% in rats administered *Moringa* leaf protein. Moreover, Majidi et al. (2023) indicated a significant increase in blood HGB in broilers fed *Moringa* leaf meal under thermoneutral rearing conditions. Iron supplementation has been reported to improve the hematological parameters in Pekin ducks (Xie et al., 2019), turkey poults (Agashe et al., 2024), and broiler chickens (Behroozlak et al., 2020). The high abundance of macro- and microminerals reported in *Moringa* leaves, especially iron, a mineral responsible for preventing anemia, may be accountable for the observed hematological modulation (Su and Chen, 2020; Islam et al., 2021; Peñalver et al., 2022).

Our earlier research showed the harmful impact of heat on both performance and immunological response. HS poses a significant challenge for the broiler industry since it adversely affects bird performance and meat quality. This study examines the impact of HS on broiler blood biochemical, hematological, and antioxidant parameters as physiological indicators of undesirable alteration in metabolism and their subsequent influence on carcass yield and meat quality parameters. Blood biochemical parameters are good indicators of birds' metabolism and general physiology. The present results detected significant changes in several blood parameters with increasing TP, CHOL, and TG levels. Heat stress has been reported to disturb protein and lipid metabolism in broiler muscle and plasma (Zampiga et al., 2021).

Our previous investigation detected a significant decrease in liver relative weight and an augment in hepatic cell DNA damage. The current study verified liver injury induced by heat stress with high liver enzyme activities (AST and ALT). Ma et al. (2022) reported serious liver damage and hepatic cell apoptosis induced by chronic heat stress. Several investigations have indicated a significant elevation in liver enzyme activity in the reaction to heat exposure (Farag and Alagawany, 2018; Ma et al., 2021). Moreover, the increasing levels of creatinine and urea reflect the negative impact of HS on kidney function markers, as well as an increase in protein breakdown (Qaid and Al-Garadi, 2021). Moreover, Xie et al. (2015) indicated a close relationship between plasma total cholesterol and environmental temperature which increases in response to thermal stress. MLM supplementation reduced plasma CHOL, TG, AST, ALT, creatinine, and urea. Mohamed et al. (2022) noted an enhanced liver and renal function with a substantial reduction in serum AST, ALT, creatinine, and uric acid levels with MLM supplementation in the heat-stressed broilers. *Moringa* leaf extract exerted hepatoprotective effects and reduced AST and ALT activities in rats with antitubercular drug-induced liver damage (Pari and Kumar, 2002). The positive effects of MLM on alleviating changes in blood metabolic markers reflect its antioxidant, anti-inflammatory, and hypocholesterolemic potentials (Anwar et al., 2007; Sreelatha and Padma, 2009; Peñalver et al., 2022). Moreover, albumin is classified as a negative acute phase reactant protein (APP) that diminishes in response to stress exposure to conserve amino acids for positive acute phase proteins (Gulhar et al., 2024). Ceruloplasmin (Cp), a positive APP that exhibits strong antioxidant activity, has been shown to enhance in response to heat exposure (Hall et al., 2001). Accordingly, the observed reduction in plasma albumin and the elevation in Cp levels indicate the onset of thermal stress response and inducing oxidative damage and inflammation in the HS group.

Antioxidant markers

Plasma endogenous antioxidant markers are presented in Table 2. In the HS-exposed group, the TAC, SOD, and catalase activities were significantly (P<0.05) reduced by 1.7, 1.2, and 1.3-fold, respectively, while the ceruloplasmin levels showed a 1.7-fold rise in comparison to the Control group. Remarkably, MLM supplementation to heat-stressed birds significantly (P<0.05) boosted the levels and activities of the studied antioxidant markers and reduced the ceruloplasmin levels compared to those in the heat-stressed group that did not receive feed additives.

Table 2: Antioxidant markers of broilers subjected to heat stress and received 0 (HS) or 2g/kg diet of *Moringa oleifera* leaf meal (MLM+HS).

Parameters	Control	HS	MLM+ HS	SEM	P value
TAC, nmol/mL	4.91[a]	2.84[c]	3.65[b]	0.26	0.0158
SOD, U/mL	310.6[a]	257.8[c]	290.4[b]	8.54	0.0391
Catalase, U/mL	0.84[a]	0.63[c]	0.71[b]	0.02	0.0433
Cp, pg/mL	1.21[c]	2.06[a]	1.84[b]	0.02	0.0012

[a,b,c] Superscript letters represent statistically significant differences between means within the same raw (P < 0.05). TAC: total antioxidant capacity; SOD: superoxide dismutase; Cp: ceruloplasmin.

A state of oxidative stress was identified by several factors. In our previous study, corticosterone circulation, lipid peroxidation, proinflammatory cytokines, and heterophil-to-lymphocyte ratio were remarkably increased. In the present study, endogenous antioxidant enzymes and TAC were decreased with serious alteration in blood biochemical and hematological parameters. The sum of these harmful reactions in response to thermal stress exposure induced production loss, impaired carcass yield, and hindered meat quality (Chen et al., 2021). HS significantly influences blood antioxidant markers (Mohamed et al., 2022). In the current study, plasma total antioxidant capacity, SOD, and catalase activities decreased whereas ceruloplasmin concentration increased. The harmful changes in blood antioxidant activities reflect the induction of oxidative stress mediated by heat exposure (Xue et al., 2017; Sumanu et al., 2023). *Moringa* leaf supplementation elevated antioxidant enzyme activity and TAC, which illustrates an oxidative stress relief property (Nandave et al., 2009; Cui et al., 2018; Mohamed et al., 2022).

Carcass yield

Carcass composition relative to the live body weight of broilers exposed to HS and fed with MLM is presented in Table 3. HS drastically lowered (P<0.05) dressing, breast muscle, and abdominal fat percentages. Meanwhile, no change in leg percentage was found among the experimental groups. Remarkably, under thermal stress, MLM supplementation improved (P<0.05) dressing percentage resulting in the highest breast yield.

Meat is the end product of the broiler industry. Broilers reared under hyperthermal conditions produce less meat with low quality. The shift in protein and lipid metabolism under stress conditions is the mediator of such phenomena (Qaid and Al-Garadi, 2021). Moreover, the observed elevation in corticosterone circulation during chronic heat exposure directly modulates metabolism, causing lipolysis and proteolysis (Ma et al., 2021). Continuing secretion of corticosterone, in response to stress exposure, has also been reported to upset growth by reducing insulin-like growth factor, a growth-mediated hormone, which subsequently impairs skeleton muscle growth (Nawaz et al., 2021). Şenay et al. (2019) reported that heat stress significantly reduced both hot and cold carcass yield in heat-stressed quails.

Table 3: Carcass yield of broilers subjected to heat stress and received 0 (HS) or 2g/kg diet of *Moringa oleifera* leaf meal (MLM+HS).

Parameters	Control	HS	MLM+ HS	SEM	P value
Dressing (%)	68.92[a]	66.43[b]	68.56[a]	0.187	0.0310
Breast (%)	35.69[b]	34.31[c]	36.92[a]	0.119	0.0211
Leg (%)	30.54	29.69	29.86	0.129	0.0752
Abdominal fat (%)	2.45[a]	1.98[b]	2.41[a]	0.026	0.0427

[a,b] Superscript letters represent statistically significant differences between means within the same raw (P < 0.05).

Table 4: Meat quality parameters of broilers subjected to heat stress and received 0 (HS) or 2g/kg diet of *Moringa oleifera* leaf meal (MLM+HS).

Parameters	Control	HS	MLM+ HS	SEM	P value
pH 24 h	6.08[a]	5.89[b]	6.01[a]	0.02	0.0146
Lightness (L*)	50.32	49.82	48.69	1.43	0.5243
Yellowness (b*)	10.35	10.18	10.85	1.25	0.4374
Redness (a*)	4.67	5.22	5.44	0.83	0.0834
Cooking loss %	12.41[c]	16.31[a]	14.63[b]	0.52	0.0263
Shear force, kg	2.89	3.05	3.31	0.31	0.1362

[a,b] Superscript letters represent statistically significant differences between means within the same raw (P < 0.05).

Meat quality

Meat quality measurements illustrated different responses to heat exposure (Table 4). In response to HS exposure, the ultimate meat pH (pH_{24}) decreased whereas cooking loss increased significantly (P<0.05). Nevertheless, meat color and sharer force did not differ among the experimental groups (P>0.05). Markedly, MLM supplementation to chronic heat-stressed birds maintained a meat pH_{24} in comparison to the Control group and lessened cooking loss. The current study's findings suggest that MLM supplementation may have a beneficial impact on adjusting carcass yield and enhancing meat quality in heat-stressed broilers.

Simultaneously, the quality of broiler meat produced under thermal stress conditions is negatively affected (Zaboli et al., 2019; Nawaz et al., 2021). Meat pH is correlated with other meat quality parameters such as protein denaturation, meat color, drip loss, and cooking loss (Zaboli et al., 2019). We observed a substantial drop in pH_{24} and an increase in cooking loss in the heat-stressed group with no effect on meat color. These findings are consistent with other reports demonstrating a significant reduction in pH and an increase in cooking loss with no effect on meat color in case of chronic HS exposure (Cramer et al., 2018; Shakeri et al., 2020a). Majidi et al. (2023) found a significant increase in broiler production performance and carcass yield with *Moringa* leaf meal supplementation under thermoneutral conditions. The positive effect of MLM supplementation on meat yield and quality can be attributed, on the one hand, to its antioxidant potential that reduces oxidative stress damage induced by HS (Mohamed et al., 2022). On the other hand, the reported improvement in growth rate, feed intake, and feed efficiency in heat-stressed birds by MLM supplementation may be the reason for the observed high dressing and breast meat percentages.

CONCLUSION AND RECOMMENDATION

Heat stress exposure to modern broilers deteriorated meat quality, impaired antioxidant balance, and negatively altered blood biochemical and hematological parameters. Meanwhile, *Moringa* leaf meal (MLM) supplementation to thermal-stressed broilers partially restored the homeostatic balance and improved meat yield and quality. Further research is required to verify the influence of MLM on meat quality and the optimum dose required for the full restoration of homeostatic balance under heat stress exposure.

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NOVELTY STATEMENT

Our present and previous studies provides new insights into the beneficial effects of *Moringa oleifera* leaf supplementation on different physiological aspects in heat-stressed broilers. Our results indicate that *M. oleifera* leaf meal can be safely added to broiler diet as a stress relief agent to improve broiler redox status, carcass yields, and meat quality with a positive alteration in hematological and metabolic parameters.

AUTHOR'S CONTRIBUTION

All authors contributed equally to the manuscript.

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Conflicts of interest

The authors have declared no conflict of interest.

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