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The Impact of Dietary *Moringa oleifera* Leaf Supplementation on Stress Markers, Immune Responses, and Productivity in Heat-Stressed Broilers

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ABSTRACT

Chronic stress exposure is detrimental to the modern poultry industry's profitability. Stress exposure induces physiological changes that consequently impair broiler performance. The bioactive compounds found in *Moringa oleifera* (MO) leaf exert beneficial anti-stress and anti-inflammation properties. Thus, we examined the effects of MO supplementation on heat-stressed broilers' performance, immunity, and stress responses. Three hundred 1-day-old Cobb 500 chicks were reared, and at day 21 of age, birds were weighed and symmetrically assigned to three experimental groups (5 replicates × 20 birds). The groups were a control group fed a basal diet under thermoneutral conditions, and two heat-stressed exposed groups received either 0 or 0.2% MO supplementation per kilogram of the basal diet. Growth was monitored during the 3rd to 6th week of age. Moreover, immune responses, stress, and pro-inflammatory markers were quantified in blood samples. In addition, relative weights of the liver and lymphoid organs were measured post-slaughter. Results revealed that heat stress negatively impacted broiler performance and immunity with substantial elevation in corticosterone and pro-inflammatory cytokine levels. However, MO supplementation to heat-stressed broilers mitigated these negative impacts. MO supplementation significantly enhanced broilers' growth performance parameters while reducing stress responses and pro-inflammatory cytokine levels and demonstrated immune modulation effects. These findings confirmed that MO supplementation can improve broiler productivity and alleviate heat stress-leading damages by reducing oxidative stress and inflammation.

Key words: Broilers, *Moringa oleifera*, Heat stress, Growth performance, Immune responses, Stress markers

INTRODUCTION

Globally, there is an exponential rise in the consumption of protein-rich food from animal origin (Wu et al. 2014). Broilers are considered one of the most promising livestock animals to fulfill the protein gap owing to their fast growth and efficient feed utilization (Siegel 2014). Due to the intensive genetic modification, modern broiler chicken is consistently challenged with unfavorable conditions that negatively affect broiler production, physiology, and welfare (Huerta et al. 2023). Global warming poses a considerable challenge to the poultry industry, especially in hot and humid environments. Heat stress induces oxidative damage and inflammatory response (Kamel et al. 2017; Gouda et al. 2020; Ahmad et al. 2022). Chronic exposure to elevated temperatures triggers a cascade of physiological responses, including inhibiting growth, inducing immunosuppression, and impairing meat quality (Zaboli et al. 2019; Awad et al. 2020; Sesay 2022) and rearing broiler chickens under elevated environmental

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temperature-induced immunosuppression with reduction in lymphocyte proliferation and serum antibody titers (Hirakawa et al. 2020). During chronic stress, persistent elevation in corticosterone secretion induces inflammation and immunosuppression, impairing growth and increasing morbidity (Cantet et al. 2021). Due to broilers' susceptibility to heat stress complications, mitigating stress is vital to maintain optimum productivity, health, and well-being (Madkour et al. 2022; Onagbesan et al. 2023).

Several mitigation strategies were proposed for heat stress alleviation. Recently, different natural strategies were suggested to alleviate heat stress effects on broiler performance (Shakeri et al. 2020; Wasti et al. 2020; Nawaz et al. 2021; Sesay 2022). One promising and practical approach is dietary intervention using natural plant-based bioactive phytochemicals (Hosseini-Vashan and Raei-Moghadam 2019; Hu et al. 2019; Alzarah et al. 2021; Moustafa et al. 2021; Ahmad et al. 2022; Kumar et al. 2023). Phytochemicals are secondary metabolites in various plant species with bioactive properties (Achilonu et al. 2018). The bioactive properties exerted by plant phytochemicals include antioxidant, anti-stress, anti-inflammation, antimicrobial, anticoccidial and anti-cancer properties (Zaman et al. 2012; Abbas et al. 2017; Achilonu et al. 2018; Kumar et al. 2023). Several studies have discussed the antioxidant and anti-inflammatory properties of natural phytochemicals in alleviating the adverse effects of stress (Abbas et al. 2012; Saleh et al. 2021; He et al. 2023). Therefore, to counteract the negative consequences of heat stress, identifying potent, dose-effective natural antioxidants and anti-inflammatory agents is crucial for maintaining optimal broiler chicken health, productivity, and welfare (Abdel-Moneim et al. 2020).

Moringa oleifera is valued for its high nutritive value and abundance of phytochemical and pharmaceutical bioactive compounds (Paikra et al. 2017; Bebas et al. 2023). *M. oleifera* leaves are the most affluent part of several essential bioactive compounds such as phytochemicals, vitamins, minerals, antioxidants, and anti-inflammation agents (Paikra et al. 2017; Srivastava et al. 2023). Dietary MO powder consumption by broiler has been shown to improve body weight gain and feed efficiency (Mahfuz and Piao 2019; Taufek et al. 2022). Furthermore, MO supplementation has been shown to induce immunomodulation, antioxidant, and anti-inflammation response on broiler raised under both normal (Gbore et al. 2021; Khan et al. 2021) or heat-stressed (Jimoh et al. 2022; 2023) conditions. Under heat stress, supplementation with MO leaves has been recognized to recover broiler production performance and antioxidant status and down-regulate pro-inflammatory cytokine gene expression (El-Deep et al. 2019). *M. olifera* supplementation has been reported to be effective as a growth promoter agent, enhancing broilers' growth performance and health status (Kairalla et al. 2023). Similarly, MO leaf extract has exhibited growth-promoting and immunomodulation effects on broilers (Mohamed et al. 2023). Recent reviews by Mnisi et al. (2023) highlight the abundant bioactive compounds in *M. oleifera*, which exhibit antioxidant, anti-inflammatory, and antimicrobial activities. These compounds include vitamins (A, E, B complex, and C), quercetin, kaempferol, myricetin, glycosides, apigenin, luteolin, flavonols, and polyphenols. The beneficial effects and underlying physiological mechanisms of *M. oleifera* leaf meal consumption by heat-stressed broilers remain poorly understood (Khan et al. 2021; Mnisi et al. 2023). Given the current limitations in our understanding of MO supplementation. The current investigation aimed to clarify the effect of MO supplementation on broilers' growth performance, immune function, and physiological stress markers under chronic thermal stress.

MATERIALS AND METHODS

Ethical approval

King Faisal University Research Ethics Committee authorized the experimental procedures and protocols (KFU-REC/2023-08-25) following animal welfare guidelines.

Collection and preparation of *Moringa oleifera* leaf powder

Fresh *Moringa oleifera* leaves were collected, washed with distal water, air-dried in the shade with continuous flipping, and ground to a fine powder. The collected MO was saved at 4°C until use. Leaf meal was included in the basal diet daily at 2g/kg. The gross chemical composition of the supplemented MO was performed (AOAC 2012), while the metabolizable energy (ME) was computed according to Lodhi et al. (2009), and results are shown in Table 1.

Table 1: Chemical analysis of *M. oleifera* leaves

Components	%*
Dry matter	90.11
Crude protein (CP)	29.57
Ether extract (EE)	6.21
Crude fiber (CF	12.18
NFE**	44.93
Ash	7.11
ME kcal/kg***	3383

*The proximate chemical composition is presented on a dry matter basis; **NFE: Nitrogen-free extracts were computed according to the following equation = [100 - (CP%+ EE%+ CF% +Ash%)]; *** Metabolizable energy (ME) was computed by the multiple regression equation of Lodhi et al. (2009).

Experimental design

A cumulative total number of 300 one-day-old Cobb500™ chicks were obtained. All birds were raised under identical conditions for the first 21 days. Birds were weighed on their 21st day of age and symmetrically assigned to three similar groups applying a randomized complete block design (five replicates × 20 birds). Each group was randomly assigned to receive different conditions: thermoneutral condition (Control), heat stress (HS), and a combination of MO leaf meal supplementation at a level of 0.2% with heat stress exposure (MO+HS). The Control group was raised at 24±1°C and 50% RH, while the other two groups received daily periodic HS from day 22 to 42 at 35±1°C and 50% RH (9 am to 5 pm). Birds were raised on floor pens with 5cm sawdust bedding. All birds were kept at 23hr light: 1hr dark for the entire experiment. The chickens received a corn-soybean basal diet formulated to meet the National Research Council (1994) and Cobb500 broiler chicken guidelines. This diet provided 3150kcal/kg of metabolizable energy (ME) and 20% crude protein.

Growth performance determination

Experimental birds were weighed at 22 and 42 days old, and body weight gain (BWG) was determined for each replicate. Once the experiment was ended, feed residue was weighed. Subtracting the feed residual from the total feed supplied throughout the experiment yielded feed intake (FI) per group replicates. Finally, the feed conversion ratio (FCR) was calculated by dividing FI (g) by BWG (g).

Samples collection

Each experimental group collected 10 blood samples (2 samples per replicate) at day 42 of age in an EDITA-coated tube. Blood samples were collected very slowly from brachial wing venipuncture. Total leukocyte counts (TLCs) and the heterophil-to-lymphocyte ratio (H/L) were evaluated using a few blood drops. Plasma was then collected and stored at -20°C after centrifugation at 1800×g for 20 min and 4°C.

Another fresh blood sample was taken from ten birds from each group. The peripheral blood mononuclear cells were separated, washed, and suspended in a culture medium as Abbas et al. (2017) described. Briefly, a separation medium (Histopaque-1077) was used to isolate PBMCs from blood samples. Then, the isolated PBMCs were washed with RPMI medium. After re-suspending the PBMCs in PBS at a neutral pH (7.2), the cell concentration was adjusted to 1 million cells per milliliter. Next, 1mL of the suspended cells was centrifuged at 1,030 × g for 20 minutes at 4°C. The resulting cell pellets were instantly used for lymphocyte stimulating index evaluation.

Stress markers and pro-inflammatory cytokines levels

Plasma corticosterone concentration was evaluated (n=10) using an ELISA kit (MyBioSource, CA, USA; MBS701668). Using chicken-specific ELISA assays, the concentrations of tumor necrosis factor-alpha (TNF-α), malondialdehyde (MDA), and interleukin 1β (IL-1β) in the plasma were determined (MyBioSource, CA, USA; MBS761055, MBS2509660, and MBS260816, respectively).

Total and differentiation Leukocytic counts

According to Gehad et al. (2008), a few drops of fresh blood were used to count TLCs (n=10) using a hemocytometer slide. In addition, the H/L ratio was determined according to Mehaisen et al. (2017). The differential leukocytes were counted with a 1000× oil-immersion light microscope on two slides.

Evaluation of immune responses

Antibody titers specific for sheep red blood cell antigens (SRBC-Ab) were quantified to assess broilers' humoral immune response. Ten birds were allocated to each group, and an intravenous injection of 0.2mL was administered to a 5% suspension of SRBCs on day 35 of age. Following one week of vaccination, wing vein blood samples were collected, sera were separated, and antibody titers were determined using the micro-hemagglutination technique, according to Alzarah et al. (2021). Meanwhile, the proliferation index of different lymphocyte cells was evaluated to assess the cell-mediated and humoral immune responses. The collected PBMCs were instantly processed to measure T- and B-lymphocyte proliferation index (SI) as Abbas et al. (2020) described.

Liver and lymphoid organs relative weight

At the end of the experiment, ten birds from each group were slaughtered, and the liver, spleen, bursa of Fabricius, and thymus were thoroughly dissected and weighed using a digital balance (iGene Labserve Pvt Ltd, New Delhi, India). The relative organ weight was calculated by dividing organ weight (g) by live body weight (g).

Statistical analysis

We analyzed the data using one-way ANOVA in SPSS software (IBM Corp, NY, USA). To compare treatment means, we carried out Duncan's multiple-range test with a significance level of $P<0.05$.

RESULTS

Growth performance

The results revealed significant variations in growth performance, as measured by final BW, BWG, FI, and FCR, among the experimental groups (Table 2). The Control chickens had the highest final body weight, followed by MO+HS and HS groups, demonstrating a substantial positive impact of MO supplementation on overall growth under heat stress. The BWG during the last three weeks followed a similar pattern, with the Control group surpassing both HS and MO+HS groups. However, MO supplementation significantly enhanced final BW by 18% and BWG by 34% compared with the HS treatment. An enormous reduction in FI was noticed in the HS group.

Table 2: Broiler production performance under heat stress and *M. oleifera* supplementation

Parameters	Control	HS	MO+HS	SEM	P value
Initial BW (21 day), g	742	751	735	24.72	0.621
Final BW (42 day), g	2375^{a}	1690^{c}	1995^{b}	78.16	0.0001
BWG (21-42 day), g	1633^{a}	939^{c}	1260^{b}	76.43	0.0001
FI, g	3031^{a}	2264^{c}	2537^{b}	129.27	0.0001
FCR	1.86^{c}	2.41^{a}	2.01^{b}	0.031	0.0001

Means with different superscripts in the same row differ significantly ($P<0.05$); BW: body weight; BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio.

In contrast, MO supplementation significantly increased FI by 12%. Notably, FCR was influenced, with the Control group having the lowest value compared to the HS and MO+HS groups. Under heat stress, MO supplementation improved broiler FCR, indicating enhanced feed utilization in the MO group. Our findings suggest that MO supplementation can promote growth in broilers experiencing heat stress.

Stress biomarkers and pro-inflammatory cytokines levels

Blood stress marker levels demonstrated significant changes among the experimental groups (Table 3). Plasma corticosterone level, a stress marker hormone, was considerably elevated by 2.5-fold in the HS group compared to the Control group, indicating a marked stress response. Lipid peroxidation marker malondialdehyde (MDA) displayed a similar trend, with the highest level

observed in the HS group. Similarly, the IL-1β and TNF-α, pro-inflammatory markers, substantially increased the HS group. Conversely, the MO supplementation significantly reduced corticosterone, MDA, and pro-inflammatory cytokines levels compared to the HS group, implying a potential stress relief effect. These findings underline the negative impact of HS on physiological stress markers with oxidative stress and inflammation induction, suggesting a potential ameliorative effect with the dietary OM intervention.

Table 3: Stress biomarkers of broiler reared under heat stress and *M. oleifera* supplementation

Parameters	Units	Control	HS	MO+HS	SEM	P value
Corticosterone	pg/mL	5.16^{c}	12.91^{a}	8.93^{b}	0.48	0.001
MDA	μM/mL	1.21^{c}	3.27^{a}	2.17^{b}	0.13	0.001
IL-1β	pg/mL	232.7^{c}	544.6^{a}	379.4^{b}	29.9	0.001
TNF-α	pg/mL	87.3^{c}	156.5^{a}	127.4^{b}	4.81	0.001

Means with different superscripts in the same row differ significantly (P<0.05); MDA: malondialdehyde; IL-1β: interleukin 1β; TNF-α: tumor necrosis factor-alpha.

Immune response parameters

The immunological parameters indicated significant alterations under different environmental and dietary conditions (Table 4). Heat stress exposure has a notable impact, reflected in the decreased TLC and the increased H/L ratio, indicating the onset of stress. Additionally, the specific humoral immune response (indicated by SRBC-Ab and B-lymphocyte SI) and cell-mediated immune response (indicated by T-lymphocyte SI) were both significantly reduced in the HS treatment compared to the other two treatments, suggesting suppression of both arms of acquired immune responses under HS conditions. However, MO treatments significantly attenuated the negative effect of HS on broilers' immune responses. When it comes to MO supplementation, the TLC, SRBC-Ab titer, T-lymphocyte SI, and B-lymphocyte SI were all significantly increased by 1.1, 1.3, 1.2, and 1.3-fold, respectively, compared to HS treatment.MO supplement significantly reduced the H/L ratio by 1.2-fold. These findings emphasize heat stress's immunosuppressive effect and dietary MO intervention's immunomodulation potential.

Table 4: Immunological measurements of broiler reared under heat stress and *M. oleifera* supplementation

Parameters	Control	HS	MO+HS	SEM	P value
TLC, $\times 10^3$/mL	85.22^{a}	45.61^{c}	50.26^{b}	1.89	0.025
H/L ratio	0.36^{c}	0.71^{a}	0.57^{b}	0.02	0.014
SRBC-Ab titer, $\log_2$	8.87^{a}	5.67^{c}	7.28^{b}	0.27	0.031
T-lymphocyte SI	3.11^{a}	1.91^{c}	2.33^{b}	0.14	0.021
B-lymphocyte SI	2.43^{a}	1.57^{c}	1.98^{b}	0.08	0.035

Means with different superscripts in the same row differ significantly (P<0.05); TLC: Total Leukocytic counts; H/L: ratio heterophil/lymphocyte ratio; SRBC-Ab: Sheep red blood cell antibody; SI: stimulating index.

Organ relative weight

According to the results presented in Table 5, the relative weight of the liver and primary lymphoid organs significantly altered in the experimental groups. A notable decrease in liver % was detected in the HS treatment compared to the other two treatments. Similarly, the thymus and bursa, primary lymphoid organs of the avian immune system, showed reduced percentages in the HS group, suggesting an immunosuppressive effect of heat stress. Conversely, the MO+HS group displayed intermediate values, implying a potential protective effect of MO dietary intervention. Meanwhile, the spleen did not exhibit significant changes among the experimental groups. These findings featured the diverse and organ-specific effects of HS and the potential mitigating role of the MO dietary intervention.

Table 5: Liver and lymphoid organs relative weight (%) of broiler reared under heat stress and *M. oleifera* supplementation

Organ	Control	HS	MO+HS	SEM	P value
Liver	4.21^{a}	3.31^{c}	3.62^{b}	0.116	0.033
Thymus	0.53^{a}	0.28^{c}	0.39^{b}	0.018	0.013
Bursa of Fabricius	0.18^{a}	0.086^{c}	0.12^{b}	0.014	0.001
Spleen	0.97	0.81	0.85	0.011	0.650

Means with different superscripts in the same row differ significantly (P<0.05).

DISCUSSION

Heat stress is detrimental to broiler growth (Awad et al. 2020; Ahmad et al. 2022). Heat stress exposure primarily reduced FI, adversely impacting BW, BWG, and FCR. Heat stress was reported to reduce FI, BW, and FCR in broilers (Quinteiro-Filho et al. 2010; Jahanian and Rasouli 2015; Beckford et al. 2020). Quinteiro-Filho et al. (2010) attributed the negative impacts observed on heat-stressed broilers' production performance, immunity, and intestinal mucosa changes to the activation of the physiological stress responses, evidenced by persistently elevated serum corticosterone levels. Interestingly, Mohamed et al. (2023) observed improved weight gain in broilers supplemented with MO leaf extract alongside cyclophosphamide (an immunosuppression agent). Our study aligns with these findings, demonstrating the positive effects of MO supplementation on broiler production parameters under heat stress. The observed improvement can be attributed to the documented benefits of MO on intestinal architecture, which potentially enhances bird nutrient absorption (El-Badawi et al. 2018; Moreno-Mendoza et al. 2021; Jiang et al. 2023). Furthermore, Abdelsalam and Fathi (2023) suggest that MO supplementation can enhance rabbit performance due to its high concentration of phytochemicals, potentially acting as a growth promoter and probiotic. Supporting this notion, Ullah et al. (2022) reported improved growth, carcass quality, and nutrient digestibility in *E. coli*-infected broilers supplemented with 100 ml/L of MO leaf extract. Kanwal et al. (2022) also reported a substantial increase in BWG and low FCR in broiler-fed moringa leaf powder. Therefore, MO supplementation to heat-stressed birds can be a beneficial management practice for broiler breeders to improve flock productivity under stressful conditions.

High environmental temperatures stimulate a variety of stress responses, including substantial elevation in corticosterone hormone and lipid peroxidation levels. Moreover, the significant elevation of pro-inflammatory cytokines indicates a sharp stress response to heat stress exposure. Studies have shown that exposure to thermal stress increases corticosterone levels in different broiler breeds (Xu et al. 2018; Beckford et al. 2020). Furthermore,

HS stimulates the secretion of pro-inflammatory cytokines (Cantet et al. 2021). He et al. (2019) found an up-regulation of IL-1β and TNF-α mRNA in the spleen of heat-stressed broilers. Under heat stress conditions, our study demonstrated that the supplementation of MO significantly alleviated stress responses with a reduction in stress markers and pro-inflammatory cytokines. MO leaves were reported to possess antioxidant and anti-inflammation potentials (Haroen et al. 2022). The bioactive compounds responsible for the MO antioxidant and anti-inflammation properties included vitamins, phenolic acids, and flavonoids were summarized by Vergara-Jimenez et al. (2017). Heat stress induces oxidative stress in broilers, but MO supplementation was found to reduce lipid peroxidation and support antioxidant pathways, counteracting this adverse effect (Jimoh et al. 2022). Consistent with the current data, Jimoh et al. (2023) reported that MO leaf supplementation at 5% significantly reduced serum corticosterone, IL-1β, and TNF-α levels in severe heat-stressed broilers. Several mechanisms have been reported as potential for MO antioxidant and anti-inflammatory activities (Abu-Zeid et al. 2021; Chiș et al. 2024). The proposed mechanisms included regulating cytokine production by inhibiting pro-inflammatory enzymes and cytokine production levels (e.g., IL-1β and TNF-α). Chen et al. (2021) demonstrated the anti-inflammation properties of *M. oleifera* leaf *in vitro* with significant inhibition on COX-2 and pro-inflammatory cytokines levels. The substantial reduction in corticosterone and lipid peroxidation and pro-inflammation cytokine levels observed in the current study suggests a potential mitigating effect of MO supplementation on oxidative stress responses induced by heat exposure.

The immune system's primary function is protecting individuals against infections and toxins. Several studies have reported that exposure to heat stress impairs broilers' immune response. Chronic stress can depress the immune system through increased corticosterone secretion levels (Cantet et al. 2021). Our previous investigation demonstrated the immunosuppressive effect of corticosterone injection in broilers (Mehaisen et al. 2017). Corticosterone injection resulted in several immunosuppressive effects, including reduced primary lymphoid organs relative weights, decreased TLC counts, an elevated H/L ratio, and low T- and B-lymphocyte stimulation indexes. Consistently, the HS group exhibited a 2.5-fold increase in corticosterone level with significant suppression in specific antibody response to SRBC and both humoral and cell-mediated proliferation index. The immune suppression in this study could be due to the negative impact of HS on primary lymphoid organ relative weight, which is reported to be related to loss of functional structure and reducing lymphocyte number (Jahanian and Rasouli 2015; Hirakawa et al. 2020). The relative weight of the thymus and bursa showed a significant reduction in response to HS. Hirakawa et al. (2020) found that HS exposure induces primary lymphoid organ atrophy. Also, Jahanian and Rasouli (2015) reported a significant decrease in primary lymphoid organs' relative weights with no effect on spleen relative weight in broilers introduced to HS at 35°C from day 15 to 42 of age. Heat stress was reported to induce histopathological alterations of lymphoid tissues, which impaired their immune function (Rebez et al. 2023). Cyclic heat exposure at 37°C for 8h/day reduced lymphoid organ growth index in broilers (He et al. 2019). These results confirmed the intricate relationship between heat stress and organ proportions and function.

Nevertheless, the MO supplementation showed an intermediate improvement in vital lymphoid organs relative to weight, suggesting potential protective and immunomodulation effects. Kumar et al. (2021) found an improvement in serum antioxidant and immune status in broilers supplemented with MO leaf extract. The significant improvement in immune response markers in the supplemented group can be attributed to bioactive substances with antioxidant and immunomodulation properties (Chhikara et al. 2021; Abd El-Hack et al. 2022; Xu et al. 2024). Additionally, MO leaf alcoholic extract was reported to increase total leukocyte count in cyclophosphamide-induced immunosuppression in broilers (Mohamed et al. 2023). This study highlighted the complex interplay between heat stress, inflammation, and oxidative stress.

In all vertebrates, including birds, the liver is a vital organ, orchestrating vital biochemical processes for survival. This multi-functional organ is responsible for a vast range of biological roles including digestion, metabolism, production, and detoxification (Zaefarian et al. 2019). Some liver protein products are blood proteins, enzymes, hormones, and immune factors. Zaefarian et al. (2019) concluded that the greater liver mass is considered a good sign and related to higher nutrient metabolic activity. Cyclic heat stress was reported to induce liver injury and decreased liver weight of broiler chickens (Tang et al. 2022). Ma et al. (2022) demonstrated that chronic HS exposure at 32°C for 14 days induced apoptosis in the broiler liver. The reduction in liver relative weight suggests the induction of liver injury and loss of hepatocyte function by heat stress. MO supplementation to heat-stressed broilers showed an increase in liver relative weight. This finding aligns with the reported improvement of liver function in heat-stressed rabbits given MO, suggesting MO's potential to mitigate heat-induced liver damage via anti-apoptotic mechanisms (Yasoob et al. 2022). The abundant bioactive compounds found in MO leaves have been reported to protect against liver damage, hepatic fibrosis, and liver injury (Vergara-Jimenez et al. 2017; Chhikara et al. 2021; Xu et al. 2024). Srivastava et al. (2023) stated that MO quercetin is responsible for the hepatoprotective effect observed in MO-supplemented animals. Finally, MO supplementation offers a multi-pronged strategy to combat heat stress in broilers. The current data partially explained MO's potential growth-promoting and stress-relief effect, but further research is crucial to fully understand MO bioactive interactions with broiler physiology under heat stress.

Conclusion

Under the current experimental conditions, heat stress impaired broiler performance and immunological response and induced an acute stress response. However, due to its rich bioactive compounds, MO nutritional intervention improved heat-stressed broiler productivity, reduced stress responses and exhibited an immunomodulation effect. These comprehensive findings highlight the positive influence of MO dietary intervention on growth

performance and stress mitigation in broiler chickens reared under heat-stress conditions. Further research is needed to understand the accessibility and availability of MO bioactive compounds in broilers and the optimum MO dosage for different breeds, ages, and environment breeding conditions.

Author's Contribution: Conceptualization: AOA and NKA; methodology: AOA, NKA, FSN and GFG; validation: FSN, GFG; formal analysis: NNK and GFG; investigation: NKA, AOA and GFG; resources: HMS; data curation, HMS; writing—original draft preparation, NNK; writing—review and editing, AOA and HMS; visualization: NNK and HMS; supervision: AOA.

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