Journal of Poultry Sciences and Avian Diseases

Journal homepage: www.jpsad.com

Dietary Curcumin, Protexin, and Their Combination Improve Selected Productive, Eggshell, Metabolic, and Endocrine Indicators in Broiler Breeder Hens under Chronic Cyclic Heat Stress

Reza Hassani Farahani[1], Morteza Mehri[1*], Fatemeh Shirmohammad[1], Arash Ghalyanchilangeroudi[2]

1. Department of Animal Science, Shahr-E-Qods Branch, Islamic Azad University, Tehran, Iran
2. Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

*** Corresponding author email address:** mortezamehri@gmail.com

Article Info

Article type:
Original Research

How to cite this article:
Farahani, R. H., Mehri, M., Shirmohammad, F., & Ghalyanchilangeroudi, A. (2026). Dietary Curcumin, Protexin, and Their Combination Improve Selected Productive, Eggshell, Metabolic, and Endocrine Indicators in Broiler Breeder Hens under Chronic Cyclic Heat Stress. *Journal of Poultry Sciences and Avian Diseases, 4(4), 1-8.*
http://dx.doi.org/10.61838/kman.jpsad.220

ABSTRACT

Chronic cyclic heat-stress conditions can challenge productive performance and physiological balance in broiler breeder hens, particularly during the laying period. The present study evaluated dietary curcumin and Protexin, a multi-strain probiotic, alone or in combination, in Arbor Acres broiler breeder hens from 22 to 35 weeks of age. Three hundred hens were assigned to five treatments with four replicate cages per treatment and 15 hens per cage: thermoneutral control, heat-stress control, heat stress+curcumin at 150 mg/kg feed, heat stress+Protexin at 50 g/ton feed, and heat stress+curcumin+Protexin. The thermoneutral group was maintained at 18–24°C, whereas heat-stress groups were subjected to 34°C for 6 h/day. The cage was considered the experimental unit. Egg production and settable egg percentage, defined as externally normal and crack-free eggs, were recorded daily, and egg-quality traits and blood variables were measured at 35 weeks of age. Compared with the heat-stress control, the combined-supplement group increased egg production from 64.95% to 70.87% and settable egg percentage from 63.91% to 68.11%. However, egg weight decreased from 66.98 to 62.89 g. The combined-supplement group also showed greater eggshell thickness, lower serum triglycerides and aspartate aminotransferase activity, higher estradiol and progesterone concentrations, and a lower heterophil-to-lymphocyte ratio. Interleukin-1β was not significantly affected. Overall, dietary curcumin and Protexin improved selected indicators of productivity, eggshell quality, metabolism, endocrine function, and stress response under the present cyclic heat-stress conditions, with the combined treatment showing favorable responses across several endpoints.

Keywords: *Chickens; Curcumin; Eggshell; Heat-Shock Response; Probiotics.*

Article history:
Received 4 March 2026
Revised 28 June 2026
Accepted 15 July 2026
Published online 01 October 2026

1 Introduction

Heat stress is a major environmental challenge for poultry production, particularly in tropical and subtropical regions where high ambient temperature and humidity are common during the summer season (Nawab et al., 2018; Shakeri et al., 2025). Modern poultry strains are particularly vulnerable to thermal challenges due to their high metabolic activity and limited heat-dissipation capacity. Exposure to heat stress can impair thermoregulation, reduce feed intake, alter water consumption, suppress immune responses, and compromise productive and reproductive performance (Jahejo et al., 2016; Varasteh et al., 2015). In breeder hens, these responses are especially important because changes in physiological balance may affect laying performance, egg traits, and reproductive efficiency.

The physiological effects of heat stress are closely linked to endocrine, metabolic, immune, and intestinal responses. Heat stress can increase corticosterone and catecholamine release, promote lipid peroxidation, and alter leukocyte distribution, leading to an increased heterophil-to-lymphocyte ratio, a commonly used indicator of systemic stress in poultry (Attia et al., 2017; Tawfeek et al., 2014). A thermal challenge can also impair intestinal integrity by reducing villus development, weakening mucosal barrier function, and disrupting nutrient absorption. These changes may increase inflammatory pressure and reduce the efficiency of nutrient utilization (Lian et al., 2020). Therefore, nutritional strategies that support antioxidant status, gut function, immune stability, and metabolic balance may help maintain performance under heat-stress conditions (Mohebbifar et al., 2024).

Oxidative stress is one of the central mechanisms through which heat stress affects poultry physiology. Excessive production of reactive oxygen species can damage lipids, proteins, and nucleic acids, particularly when endogenous antioxidant defenses such as superoxide dismutase, catalase, and glutathione peroxidase are insufficient to counteract oxidative load (Li et al., 2020; Song et al., 2014). Curcumin, a bioactive polyphenol derived from Curcuma longa, has been reported to have antioxidant, anti-inflammatory, and immunomodulatory properties in poultry (Attia et al., 2017). Probiotic and direct-fed microbial preparations may also improve gut microbial balance, support intestinal barrier integrity, modulate immune responses, and influence productive performance in poultry (Cramer et al., 2018; Merino-Guzmán et al., 2025). However, information remains limited on the combined use of curcumin and multi-strain probiotics in broiler breeder hens, particularly during early to mid-lay.

Therefore, the present study evaluated the effects of dietary curcumin and Protexin, individually and in combination, on productive performance, egg-quality traits, blood biochemical parameters, hepatic enzyme activity, reproductive hormone profiles, thyroid hormone responses, and selected immune-related indicators in broiler breeder hens exposed to chronic cyclic heat-stress conditions from 22 to 35 weeks of age. In this study, settable eggs were defined as externally normal eggs with intact, crack-free shells; this term does not imply fertility, hatchability, or chick quality.

2 Materials and Methods

2.1 Experimental Design and Animal Management

A total of 300 Arbor Acres broiler breeder hens and 30 roosters, all 22 weeks of age, were used in a completely randomized design with five treatments, four replicate cages per treatment, and 15 hens per cage. Birds were managed according to the Arbor Acres breeder guide (Aviagen, 2016). At 21 weeks of age, birds were transferred to the experimental facility and acclimatized for 10 days. The feeding trial and data collection were conducted from 22 to 35 weeks of age.

The treatments were as follows: thermoneutral control (TN), basal diet under thermoneutral conditions; heat-stress control (HS), basal diet under chronic cyclic heat-stress conditions; HS+curcumin; HS+Protexin; and HS+curcumin+Protexin. Feed and water were provided ad libitum throughout the experiment. Although controlled feeding is commonly used in broiler breeder management, ad libitum feeding was uniformly applied across all treatments to maintain a consistent feeding approach throughout the experimental period.

The TN group was maintained at 18–24°C. The HS control and supplemented HS groups were exposed to 34°C for 6 h/day, from 15:30 to 21:30, from 22 to 35 weeks of age. Outside the heat-stress period, birds remained under thermoneutral conditions. Room temperature was monitored during the exposure period to maintain the target temperature.

Roosters were separately housed under thermoneutral conditions and received the basal diet. Six roosters were assigned to each hen treatment group during the daily mating period from 09:30 to 15:30 and were removed before the

heat-stress period. The same mating schedule was applied to all groups.

2.2 Diets, additives, and feed preparation

The basal diet was formulated for broiler breeder hens during early to mid-lay, in accordance with breeder management recommendations. Briefly, the diet contained 2800 kcal/kg metabolizable energy, 15.0% crude protein, 3.10% calcium, 0.35% available phosphorus, 0.60% digestible lysine, and 0.37% digestible methionine on an as-fed basis.

Curcumin was used as a commercial powdered preparation (Merck, Darmstadt, Germany). According to the supplier specification for a comparable Merck curcumin product, the preparation contained curcumin with an HPLC assay≥75.0%, demethoxycurcumin≤20.0%, and bisdemethoxycurcumin≤5.0%. Curcumin was incorporated into the diet at 150 mg/kg feed, as previously described for heat-stressed laying hens (Liu et al., 2016).

Protexin Concentrate (Kyron Laboratories, South Africa) was used as a multi-strain probiotic powder containing *Lactobacillus plantarum* PXN 47, *Lactobacillus delbrueckii subsp. bulgaricus* PXN 39, Lactobacillus acidophilus PXN 35, *Lactobacillus rhamnosus* PXN 54, *Bifidobacterium bifidum* PXN 23, *Streptococcus thermophilus* PXN 66, and *Enterococcus faecium* PXN 33, with a declared viable count of not less than 2×10^9 CFU/g. Protexin was incorporated at 50 g/ton of feed, equivalent to 0.05 g/kg feed and an expected final concentration of approximately 1×10^5 CFU/g feed, assuming full viability. Probiotic viability after feed mixing and storage was not independently verified (Kyron Laboratories, 2019).

For feed preparation, each additive was first premixed with a small amount of basal feed, then blended into the complete diet using a mechanical mixer. Diets were prepared at regular intervals and stored in sealed containers in a cool, dry place to minimize moisture exposure and maintain additive stability.

2.3 Blood Sampling and Biochemical Analysis

At 35 weeks of age, two hens per replicate cage were selected for blood sampling, giving eight sampled hens per treatment. Selected hens had body weights close to the corresponding cage mean. Blood was collected from the wing vein between 08:00 and 10:00 after an 8-h feed withdrawal, with water remaining available, to reduce short-term postprandial variation in circulating metabolites (Cramer et al., 2018). The same withdrawal period and sampling window were applied to all treatment groups.

Approximately 6 mL of blood was collected from each bird using sterile syringes. For each sample, 3 mL was transferred into heparinized tubes for plasma separation and into plain tubes for serum preparation. Samples were centrifuged at 3000 rpm for 15 min, and serum and plasma were stored at −20°C until analysis.

Serum glucose, cholesterol, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) were measured using a Hitachi 912 autoanalyzer (Hitachi Ltd., Tokyo, Japan) with commercial diagnostic kits (Pars Azmoon, Tehran, Iran; licensed by DiaSys Diagnostic Systems, Germany), according to the manufacturer's instructions. Plasma non-esterified fatty acids (NEFA) were measured using an enzymatic colorimetric kit (FUJIFILM Wako, Japan). Serum total T3 and total T4 were determined using commercial ELISA kits (Monobind Inc., USA). All samples were analyzed in duplicate, and the mean value was used for statistical analysis.

2.4 Egg Production and Egg-Related Parameters

Egg production was recorded daily for each replicate cage throughout the experiment. Hen-day egg production was calculated as: number of eggs produced per cage per day/number of hens present in that cage×100. Settable eggs were defined as eggs with normal external shape and intact, crack-free shells. Settable egg percentage was calculated as: number of settable eggs/total eggs produced×100.

Egg-quality traits were evaluated at 35 weeks of age using three randomly selected eggs per replicate cage. Egg weight was measured using a digital scale. Eggs were broken onto a flat surface, and the yolk diameter was measured using a digital caliper. Yolk weight was measured after separation from the albumen and removal of adhering albumen. For shell weight, emptied shells were rinsed with distilled water, shell membranes were manually removed, and shells were dried in a forced-air oven at 105°C for 24 h before weighing. Eggshell thickness, without membranes, was measured at the air cell, equator, and sharp end using a micrometer, and the mean of the three measurements was used for analysis. Egg-quality measurements were performed by trained personnel using calibrated equipment, and samples were coded before assessment.

2.5 Sex steroid hormones and immune indicators

Plasma concentrations of 17β-estradiol and progesterone were quantified using commercial competitive ELISA kits for chicken estradiol and progesterone (CUSABIO Technology LLC, Wuhan, China; CSB-E12013C and CSB-E12012C, respectively), according to the manufacturer's instructions. Plasma interleukin-1β (IL-1β) was measured using a chicken IL-1β ELISA kit (CUSABIO Technology LLC; CSB-E11230Ch). Samples and standards were assayed in duplicate. Absorbance was read at 450 nm using a BioTek ELx800 microplate reader (BioTek Instruments, Winooski, VT, USA), and concentrations were calculated from the standard curve generated for each plate.

2.6 Leukocyte differential count and heterophil to lymphocyte ratio

Immediately after blood collection, thin blood smears were prepared from fresh whole blood on clean glass slides. Smears were air-dried, fixed in methanol, and stained with Wright–Giemsa stain. Differential leukocyte counts were performed under oil immersion at 1000× magnification. A total of 100 leukocytes per smear were classified as heterophils, lymphocytes, monocytes, eosinophils, or basophils. The heterophil-to-lymphocyte ratio was calculated for each bird by dividing the percentage of heterophils by the percentage of lymphocytes.

2.7 Statistical Analysis

Data were analyzed using SPSS software (IBM SPSS Statistics, version 21.0). The replicate cage was considered the experimental unit for all variables, with four cages per treatment. Daily egg production and settable egg percentage were summarized as cage-level means for the whole experimental period. End-point egg-quality, biochemical, endocrine, and immune variables were averaged within each replicate cage before analysis.

Treatment effects were evaluated using one-way ANOVA with treatment as the fixed factor. When significant treatment effects were detected, means were compared using Tukey's post hoc test. Normality and homogeneity of variance were checked using Shapiro–Wilk and Levene's tests, respectively. Results are presented as Mean±pooled SEM, and significance was declared at $p \leq 0.05$. Because the analysis was based on the planned five-treatment comparison, responses in the combined curcumin+Protexin group were not interpreted as evidence of additive or synergistic effects.

3 Results

All performance and egg-related variables were analyzed at the cage level, with four replicate cages per treatment. For blood, endocrine, biochemical, and immune variables, two hens were sampled per replicate cage, and values were averaged within the cage before analysis.

3.1 Reproductive performance and egg quality

Reproductive performance and egg-quality traits are presented in Table 1. Compared with the HS control, dietary supplementation increased egg production and the percentage of settable eggs ($p<0.05$). The combined-supplement group showed the highest egg production value (70.87%) compared with the HS control (64.95%). Settable egg percentage also increased from 63.91% in the HS control to 68.11% in the combined-supplement group. However, egg weight was lower in supplemented HS groups than in the HS control, decreasing from 66.98 g in the HS control to 62.89 g in the combined-supplement group ($p<0.05$). Eggshell thickness was higher in all supplemented HS groups than in the TN and HS controls ($p<0.05$), whereas shell weight did not differ among treatments ($p>0.05$). Yolk diameter was reduced in supplemented HS groups, with the lowest value observed in the combined-supplement group.

Table 1. Curcumin and Protexin effects on egg traits in thermoneutral and heat-stressed breeders*

Parameters	TN control	HS control	HS+Curcumin	HS+Protexin	HS+Curcumin +Protexin	SEM
Egg production (%)	65.57^{c}	64.95^{c}	68.91^{b}	67.23^{b}	70.87^{a}	0.75
Settable eggs (%)	63.35^{c}	63.91^{c}	65.22^{b}	65.01^{b}	68.11^{a}	1.40
Egg weight (g)	67.83^{a}	66.98^{a}	63.74^{b}	63.14^{b}	62.89^{b}	1.20
Yolk weight (g)	18.95^{a}	18.84^{a}	18.90^{a}	18.74^{b}	17.74^{c}	0.23
Shell thickness (mm)	0.39^{b}	0.39^{b}	0.40^{a}	0.40^{a}	0.40^{a}	0.001
Shell weight (g)	5.61	5.54	5.62	5.61	5.61	0.11
Yolk diameter (mm)	32.08^{a}	32.00^{a}	30.34^{b}	30.36^{b}	29.44^{c}	0.26

*Values are presented as Mean±SEM; n=4 replicate cages per treatment. TN control=basal diet under thermoneutral conditions. HS control=basal diet under cyclic heat exposure. Means within a row with the heterogenous superscript letters differ significantly based on Tukey's test ($p \leq 0.05$). Rows without superscript letters indicate no significant difference among treatments ($p>0.05$).

3.2 Metabolic indices and hepatic enzyme activities

Serum metabolites and enzyme activities are shown in Table 2. Compared with the HS control, supplemented HS groups showed lower triglyceride, cholesterol, and NEFA concentrations (p<0.05). The combined-supplement group had the lowest triglyceride and NEFA values among the HS treatments. AST and ALT activities were also lower in supplemented HS groups than in the HS control (p<0.05), with the lowest values observed in the combined-supplement group. LDH differed among treatments (p<0.05), although the combined-supplement group was not the lowest. Glucose was also lower in supplemented HS groups than in the HS control (p<0.05).

Table 2. Curcumin and Protexin effects on serum metabolites and enzymes in thermoneutral and heat-stressed breeders*

Parameters	TN control	HS control	HS+Curcumin	HS+Protexin	HS+Curcumin+Protexin	SEM
Glucose (mg/dl)	194.87^{a}	195.01^{a}	190.5^{b}	190.25^{b}	180.02^{c}	0.94
Triglyceride (mg/dl)	1859.75^{a}	1877.36^{a}	1790^{b}	1800.5^{b}	1732.37^{c}	21.85
Cholesterol (mg/dl)	192^{a}	194^{a}	183^{b}	185^{b}	183^{b}	2.00
NEFA (mg/dL)	19.74^{a}	16.92^{a}	11.56^{b}	12.13^{b}	10.43^{b}	0.28
LDH (U/L)	58.55^{a}	57.37^{a}	51.00^{c}	51.25^{c}	54.65^{b}	1.30
AST (U/L)	249.00^{a}	251.00^{a}	231.12^{b}	234.20^{b}	211.85^{c}	5.20
ALT (U/L)	26.85^{a}	27.11^{a}	22.00^{b}	23.60^{b}	19.15^{c}	0.79
T3 (ng/dL)	3.11	3.10	3.13	3.14	3.11	0.05
T4 (ng/dL)	5.01^{b}	4.98^{b}	5.25^{a}	5.25^{a}	5.45^{a}	0.08
T3/T4 ratio (unitless)	0.62^{a}	0.62^{a}	0.60^{b}	0.60^{b}	0.57^{c}	0.01

*Values are presented as Mean±SEM; n=4 replicate cages per treatment. TN control=basal diet under thermoneutral conditions. HS control=basal diet under cyclic heat exposure. Means within a row with the heterogenous superscript letters differ significantly based on Tukey's test (p≤0.05). Rows without superscript letters indicate no significant difference among treatments (p>0.05).

3.3 Sex steroid hormones and thyroid hormones

Hormone concentrations are summarized in Table 3. Estradiol and progesterone differed among treatments (p<0.05). Estradiol was higher in the curcumin and combined-supplement groups than in the HS control, while the Protexin group showed an intermediate response. The combined-supplement group did not differ from the curcumin-alone group for estradiol. Progesterone was higher in the curcumin and combined-supplement groups than in the HS control, whereas the Protexin group did not differ from the HS control. T4 was higher in the supplemented HS groups than in the HS control group (p<0.05), whereas T3 did not differ among treatments. Consequently, the T3/T4 ratio was lower in supplemented HS groups, with the lowest value observed in the combined-supplement group (p<0.05).

Table 3. Effects of dietary treatments on plasma estradiol and progesterone in TN and HS broiler breeder hens*

Parameters	TN control	HS control	HS+Curcumin	HS+Protexin	HS+Curcumin+Protexin	SEM
17β-estradiol (pg/mL)	321.85^{c}	319.75^{c}	357.01^{a}	342.03^{b}	355.54^{a}	3.11
Progesterone (ng/mL)	4.52^{b}	4.33^{b}	4.98^{a}	4.53^{b}	5.00^{a}	0.05

*Values are presented as Mean±SEM; n=4 replicate cages per treatment. TN control=basal diet under thermoneutral conditions. HS control=basal diet under cyclic heat exposure. Means within a row with the heterogenous superscript letters differ significantly based on Tukey's test (p≤0.05). Rows without superscript letters indicate no significant difference among treatments (p>0.05).

3.4 Immune-related indices

Immune-related indices are presented in Table 4. Interleukin-1β did not differ among treatments (p>0.05). The heterophil-to-lymphocyte ratio was higher in the HS control than in the TN control and was lower in all supplemented HS groups than in the HS control (p<0.05). Although the combined-supplement group showed the lowest numerical H/L ratio among the HS treatments, the supplemented HS groups did not differ statistically from one another.

Table 4. Effects of dietary curcumin and Protexin supplementation on immune-related parameters in TN and HS broiler breeder hens*

Parameters	TN control	HS control	HS+Curcumin	HS+Protexin	HS+Curcumin +Protexin	SEM
Interleukin-1β (pg/mL)	183.25	184.05	182.50	183.12	182.18	7.15
Heterophil-to-lymphocyte ratio	0.22^{c}	0.63^{a}	0.52^{b}	0.57^{b}	0.51^{b}	0.05

*Values are presented as Mean±SEM; n=4 replicate cages per treatment. TN control=basal diet under thermoneutral conditions. HS control=basal diet under cyclic heat exposure. Means within a row with the heterogenous superscript letters differ significantly based on Tukey's test (p≤0.05). Rows without superscript letters indicate no significant difference among treatments (p>0.05).

4 Discussion

In the present study, the TN and HS control groups were similar across several core outcomes, indicating that chronic cyclic heat stress resulted in limited impairment in some variables. Therefore, the findings are best interpreted as treatment-associated improvements in selected productive and physiological indicators under the present heat-stress protocol, rather than definitive evidence of reversal of heat-stress damage. Dietary curcumin, Protexin, and their combination increased egg production and the percentage of settable eggs compared with the HS control. However, this improvement occurred alongside a reduction in egg weight, suggesting that egg number and egg size responded differently to supplementation. The increase in eggshell thickness, together with unchanged shell weight, may partly reflect changes in egg size and shell distribution. Eggshell quality and egg size are important traits in poultry production, and previous studies have linked shell characteristics, calcium metabolism, and egg-quality traits with productive and reproductive outcomes (Kiggundu et al., 2025).

The supplemented groups also showed favorable changes in lipid-related metabolites and hepatic enzyme activities. Lower triglyceride, cholesterol, and NEFA concentrations in the supplemented HS groups suggest improved lipid status under chronic cyclic heat stress. Curcumin has been reported to influence lipid metabolism through pathways related to lipogenesis and fatty acid oxidation, including the regulation of acetyl-CoA carboxylase and carnitine palmitoyltransferase (Xie et al., 2019). Probiotics and direct-fed microbials may also support nutrient utilization and metabolic stability by improving gut microbial balance, intestinal barrier function, and immune responses (Shokrinejad Gerdin et al., 2025). In the present study, the combined-supplement group showed the most favorable numerical profile for several metabolic and hepatic enzyme endpoints; however, this should be interpreted as a treatment-group response rather than evidence of synergy.

Reproductive hormone responses were also affected by supplementation. Estradiol and progesterone were higher in the curcumin and combined-supplement groups than in the HS control, which may help explain the higher egg production observed in these groups. Previous studies have linked lipid dysregulation in breeder hens with impaired ovarian function and altered steroidogenesis (Liu et al., 2016; Pan et al., 2014). Therefore, the improved lipid-related profile observed in supplemented groups may have contributed to more favorable endocrine responses. Curcumin has also been reported to improve endocrine and productive responses in heat-stressed laying hens (Liu et al., 2016; Saraswati et al., 2014). For thyroid hormones, T4 increased while T3 remained largely unchanged, resulting in a lower T3/T4 ratio. This pattern may reflect altered peripheral conversion of T4 to the metabolically active T3 and could represent metabolic adaptation to heat-stress conditions rather than a direct improvement in thyroid function.

The immune-related results showed that supplementation reduced the heterophil-to-lymphocyte ratio relative to the HS control, whereas IL-1β was not significantly affected. The H/L ratio is widely used as an indicator of physiological stress in poultry, and lower values in the supplemented groups suggest a more favorable stress-related profile (Onagbesan et al., 2023). These findings are consistent with the reported anti-inflammatory and immunomodulatory properties of curcumin and the gut-supporting effects of probiotic supplementation (Attia et al., 2017; Mostafaie et al., 2024). The absence of a significant IL-1β response suggests that the observed immune-related effect was more evident in leukocyte distribution than in this single cytokine marker.

Overall, dietary curcumin and Protexin improved selected productive, eggshell, metabolic, endocrine, and stress-related indicators in broiler breeder hens under chronic cyclic heat-stress conditions. The combined-supplement group showed favorable responses for several endpoints, but these responses should not be interpreted as additive or synergistic without formal interaction testing. The findings support the potential value of curcumin and Protexin as dietary strategies for broiler breeder hens exposed to cyclic heat-stress conditions, while further studies are needed to clarify the mechanisms and reproductive outcomes associated with these responses.

5 Conclusion

Dietary curcumin, Protexin, and their combination improved selected indicators of productivity, eggshell quality, metabolism, hepatic enzymes, endocrine function, and stress response in broiler breeder hens exposed to chronic cyclic heat stress from 22 to 35 weeks of age. The combined-supplement group showed favorable responses for several endpoints, including egg production, settable egg percentage, triglycerides, AST, estradiol, progesterone, and heterophil-to-lymphocyte ratio. However, because formal

interaction testing was not performed, these responses should not be interpreted as evidence of additive or synergistic effects. Overall, the findings support the potential use of curcumin and Protexin as dietary strategies to improve selected physiological and productive responses in broiler breeder hens under cyclic heat-stress conditions.

Acknowledgements

The authors would like to thank all individuals who supported the execution of this research and contributed to its successful completion.

Use of Artificial Intelligence

During manuscript preparation, the authors used Grammarly to improve English grammar and readability. The authors reviewed and edited all content and take full responsibility for the final manuscript. No AI or AI-assisted tools were used to generate or modify tables.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

R.H.F: Conceptualization; Investigation; Data curation; Formal analysis; Visualization; Writing - original draft. M.M: Conceptualization; Methodology; Supervision; Project administration; Writing - review and editing. F.S: Investigation; Data curation; Validation; Writing - review and editing. A.G.L: Methodology; Resources; Validation; Writing - review and editing.

Data Availability Statement

Data are available from the corresponding author upon reasonable request.

Ethical Considerations

This study was conducted in accordance with the ethical principles and research regulations of Islamic Azad University, Shahr-e-Qods Branch. Ethical approval was obtained from the relevant ethics committee of Islamic Azad University, Shahr-e-Qods Branch, under the approval code IAU/QB-285348509408610119012162603997.

Funding

The authors declare that they received no external financial support for this study.

References

Attia, Y. A., Al-Harthi, M. A., & Hassan, S. S. (2017). Turmeric (Curcuma Longa Linn.) As A Phytogenic Growth Promoter Alternative For Antibiotic And Comparable To Mannan Oligosaccharides For Broiler Chicks. *Revista Mexicana De Ciencias Pecuarias*, *8*(1), 11-21. [DOI]

Aviagen. (2016). Arbor Acres® Plus Parent Stock Performance Objectives And Management Supplement Fast Feathering.

Cramer, T., Kim, H., Chao, Y., Wang, W., Cheng, H., & Kim, Y. (2018). Effects Of Probiotic (Bacillus Subtilis) Supplementation On Meat Quality Characteristics Of Breast Muscle From Broilers Exposed To Chronic Heat Stress. *Poultry Science*, *97*(9), 3358-3368. [PMID: 30137545] [PMCID: PMC7009280] [DOI]

Jahejo, A. R., Rajput, N., Rajput, N. M., Leghari, I. H., Kaleri, R. R., Mangi, R. A., & Et Al. (2016). Effects Of Heat Stress On The Performance Of Hubbard Broiler Chicken. *Cells, Animal and Therapeutics*, *2*(1), 1-5.

Kiggundu, M., Odaru, Z., Nampijja, Z., Mulindwa, H., Nangonzi, R., Kamatara, K., & Et Al. (2025). Variation In Egg Quality Traits Among Indigenous Chicken Ecotypes Of Uganda. *Journal of Poultry Sciences and Avian Diseases*, *3*(4), 22-31. [DOI]

Kyron Laboratories. (2019). Protexin Concentrate: Product Information Leaflet.

Li, Q., Wan, G., Peng, C., Xu, L., Yu, Y., Li, L., & Et Al. (2020). Effect Of Probiotic Supplementation On Growth Performance, Intestinal Morphology, Barrier Integrity, And Inflammatory Response In Broilers Subjected To Cyclic Heat Stress. *Animal Science Journal*, *91*(1), e13433. [PMID: 32671948] [DOI]

Lian, P., Braber, S., Garssen, J., Wichers, H. J., Folkerts, G., Fink-Gremmels, J., & Varasteh, S. (2020). Beyond Heat Stress: Intestinal Integrity Disruption And Mechanism-Based Intervention Strategies. *Nutrients*, *12*(3), 734. [PMID: 32168808] [PMCID: PMC7146479] [DOI]

Liu, Z. C., Su, C. M., Xie, Y. L., Chang, C. J., Chen, J. Y., Wu, S. W., & Et Al. (2016). Intracellular Lipid Dysregulation Interferes With Leukocyte Function In The Ovaries Of Meat-Type Hens Under Unrestricted Feed Intake. *Animal Reproduction Science*, *167*, 40-50. [PMID: 26874430] [DOI]

Merino-Guzmán, R., Luna-Cardoso, M., Tellez-Isaías, G., Latorre, J. D., & Hargis, B. M. (2025). Effect Of Glucose Oxidase And A Commercial Bacillus-Based Direct Feed Microbial Supplementation On The Productive Performance, Intestinal Iga, Gut Permeability, And Cecal Microbiota Of Broiler Chickens. *Journal of Poultry Sciences and Avian Diseases*, *3*(4), 1-11. [DOI]

Mohebbifar, A., Kamyab, A., Sedgh-Gooya, S., & Torki, M. (2024). The Effect Of Protein And Selenium On Broiler Performance And Blood Parameters In Summer Conditions. *Journal of Poultry Sciences and Avian Diseases*, *2*(3), 40-52. [DOI]

Mostafaie, Z., Torki, M., Chaghamirza, K., & Sharifi, R. (2024). Effects Of Direct-Fed Microbials On Productive Performance, Qualitative Traits Of Eggs, Blood Biochemicals, and Ileal Microflora Of Laying Hens Fed Wheat-Soybean-Based Diet. *Journal of Poultry Sciences and Avian Diseases*, *2*(1), 43-51. [DOI]

Nawab, A., Ibtisham, F., Li, G., Kieser, B., Wu, J., Liu, W., & Et Al. (2018). Heat Stress In Poultry Production: Mitigation Strategies To Overcome The Future Challenges Facing The Global Poultry

Industry. *Journal of Thermal Biology*, *78*, 131-139. [PMID: 30509629] [DOI]

Onagbesan, O. M., Uyanga, V. A., Oso, O., Tona, K., & Oke, O. E. (2023). Alleviating Heat Stress Effects In Poultry: Updates On Methods And Mechanisms Of Action. *Frontiers in Veterinary Science*, *10*, 1255520. [PMID: 37841463] [PMCID: PMC10569619] [DOI]

Pan, Y. E., Liu, Z. C., Chang, C. J., Huang, Y. F., Lai, C. Y., Walzem, R. L., & Et Al. (2014). Feed Restriction Ameliorates Metabolic Dysregulation And Improves Reproductive Performance Of Meat-Type Country Chickens. *Animal Reproduction Science*, *151*(3-4), 229-236. [PMID: 25458320] [DOI]

Saraswati, T. R., Manalu, W., Ekastuti, D. R., & Kusumorini, N. (2014). Effect Of Turmeric Powder To Estriol And Progesterone Hormone Profile Of Laying Hens During One Cycle Of Ovulation. *International Journal of Poultry Science*, *13*(9), 504. [DOI]

Shakeri, M., Khezri, A., & Le, H. H. (2025). Innovative Strategies To Mitigate Heat Stress In Broiler Chickens. *Journal of Poultry Sciences and Avian Diseases*, *3*(3), 57-69. [DOI]

Song, J., Xiao, K., Ke, Y., Jiao, L., Hu, C., Diao, Q., & Et Al. (2014). Effect Of A Probiotic Mixture On Intestinal Microflora, Morphology, And Barrier Integrity Of Broilers Subjected To Heat Stress. *Poultry Science*, *93*(3), 581-588. [PMID: 24604851] [DOI]

Tawfeek, S. S., Hassanin, K. M. A., & Youssef, I. M. I. (2014). The Effect Of Dietary Supplementation Of Some Antioxidants On Performance, Oxidative Stress, And Blood Parameters In Broilers Under Natural Summer Conditions. *Journal of World's Poultry Research*, *4*(1), 10-19. [DOI]

Varasteh, S., Braber, S., Garssen, J., & Fink-Gremmels, J. (2015). Galacto-Oligosaccharides Exert A Protective Effect Against Heat Stress In A Caco-2 Cell Model. *Journal of Functional Foods*, *16*, 265-277. [DOI]

Xie, Z., Shen, G., Wang, Y., & Wu, C. (2019). Curcumin Supplementation Regulates Lipid Metabolism In Broiler Chickens. *Poultry Science*, *98*(1), 422-429. [PMID: 30053224] [DOI]