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# Dietary *Citrullus colocynthis* Seed Extract Improves Productivity, Antioxidant Status, and Immune Function in Heat-Stressed Laying Hens

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## ABSTRACT

Effective stress management is essential for sustaining optimal poultry performance and ensuring high product quality. This study evaluated the properties of *the Citrullus colocynthis* seed hydroethanol extract *(CCE*) for mitigating laying hen performance, egg quality, immune responses, stress biomarkers, antioxidant activity, and pro-inflammatory mediators under thermoneutral and thermal stress conditions. A group of 360 40-week-old HY-Line Brown laying hens was randomly assigned to four treatment groups (five replicates × 18 hens each). The first and second groups were maintained under thermoneutral conditions (24°C, 50% relative humidity) and fed a control diet (Control) or the control diet with added CCE 1g/kg feed (CCE). The third and fourth groups were subjected to heat stress (35°C from 10:00 to 18:00 daily, 50% relative humidity) and fed the control diet (HS) or the CCE-added diet (HS+CCE). The experiment lasted for eight consecutive weeks. Heat stress significantly impaired egg production, egg quality, bone characteristics, immune responses, and redox balance. CCE supplementation partially alleviated these adverse effects in heat-stressed hens. Under thermoneutral conditions, CCE supplementation also enhanced plasma total antioxidant and enzyme activity as well as T-lymphocyte proliferation relative to the unsupplemented control. These outcomes suggest that dietary CCE supplementation can serve as an innovative strategy to modulate the detrimental impacts of thermal stress on laying hens by enhancing antioxidant defense mechanisms and immune function.

**Keywords:** Laying hen; Heat stress; *Citrullus colocynthis* seed extract; Egg production; Egg quality; Immunity; Stress markers.

## INTRODUCTION

Heat stress (HS) is recently perceived as the most notable environmental stressor affecting laying hens, due to their limited ability to dissipate body heat (Gil et al. 2023). Global warming with recurrent extreme weather events intensifies the negative impacts of HS, which poses continuous challenges for poultry production systems worldwide (Schat and Skinner 2022; Cornescu et al. 2023; Nanto-Hara et al. 2023). Further, HS causes physiological changes in laying hens, such as oxidative stress induction, acid-base imbalance, and immunosuppression (Ribeiro et al. 2025). Consequently, HS has a significant negative impact on egg production, egg quality, feed efficiency, and redox status, especially in tropical and subtropical regions (Ibtisham et al. 2019; Jafari et al. 2021; Tesakul et al. 2025). Thus, under thermal stress circumstances, achieving proper layer productivity with good egg quality requires exploring potent antioxidant agents to mitigate stress and restore homeostasis.

Medicinal plants serve as an abundant source of phytogenic compounds, plant-derived bioactive substances increasingly applied as natural growth enhancers in poultry production. These compounds originate from different plant parts and are commonly provided in the form of oleoresins, flavonoids, or crude extracts (Jambwa et al. 2023; Rodrigues et al. 2025). Phytogenic additives have been shown to stimulate growth, improve feed efficiency, and strengthen immune competence through their antioxidant, anti-inflammation, and antimicrobial activities

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(Ntsongota et al. 2025), ultimately contributing to ameliorating poultry performance and health (Al-Homidan et al. 2020; Rodrigues et al. 2025). Beyond productivity enhancement, phytogenic compounds also present an attractive alternative to antibiotics as growth promoters by supporting animal welfare, avoiding antimicrobial resistance risks, and offering environmentally sustainable solutions for modern poultry systems (Attia et al. 2025; Biswas and Kim 2025). Therefore, in recent years, there has been a continuous effort to identify effective phytogenic compounds that can support poultry production, particularly under unfavourable or challenging rearing conditions.

*Citrullus colocynthis* seeds (CC), an ancient medicinal plant resource, have been widely reported to exhibit potent antioxidant and immunomodulatory properties in poultry exposed to various stressors (Li et al. 2022a; Taschetto et al. 2025). Dietary supplementation with CC seeds has been shown to modulate immune responses in broilers subjected to prolonged cyclic heat stress (Alzarah et al. 2021b). Similarly, integrating CC seeds into laying hen diets mitigated the adverse effects of acute oxidative stress on productivity and immune function, potentially through enhancing antioxidant defences (Abbas et al. 2022a). In addition, (Nassar et al. 2023) reported that CC seed supplementation can alleviate the negative impacts of high stocking density on broiler performance and restore physiological homeostasis. Beyond the seeds, extracts obtained from CC aerial parts have been shown to contain bioactive constituents with strong antioxidant and anti-infectious properties (Altemimi et al. 2023; Thamer and Thamer 2023). CC seed extracts also possess notable *in vitro* antioxidant, anti-inflammatory, and antimicrobial activities, particularly against Gram-positive bacteria (Bourhia et al. 2020; Bourhia et al. 2021; Abdallah et al. 2024). Furthermore, Alzarah et al. (2021a) demonstrated that dietary CC seed extract enhanced production performance and physiological indicators of laying hens reared under thermoneutral conditions. However, despite these promising findings, the comparative effects of dietary CC seed extract on laying hen productivity and physiological responses under varying environmental conditions remain insufficiently explored. This gap highlights the need for further systematic evaluation of CC seed extract as a natural phytogenic additive for laying hens.

Heat stress leads to significant impairments in the productivity and product quality of laying hens. To mitigate these adverse effects and maintain optimal production levels, product quality, and physiological homeostasis, effective dietary adjustment through the inclusion of natural bioactive feed additives represents an innovative strategy. Accordingly, this investigation aimed to evaluate the impact of dietary CC seed ethanol extract (CCE) on laying hens' productivity, egg quality, immune response, stress markers, antioxidant status, and inflammation incidence under thermoneutral and heat stress rearing conditions.

## MATERIALS AND METHODS

### *Citrullus colocynthis* seed extraction and analysis

To produce *Citrullus colocynthis* (CC) seed extraction powder, seeds were manually isolated from fruits, and then dried completely before being finely powdered. A hydroethanolic solvent mixture (70:30 v/v, ethanol: water) was used to extract the CC seed powder (Daradka et al. 2007). After vaporizing solvent residues using a rotary evaporator at 50°C, the extract powder was pooled and stored at 4°C in a dry, dark bottle until use.

### Experimental design

Three hundred sixty 40-week-old HY-Line Brown laying hens, each weighing 1935.2±11.4g on average, were housed in separate cages with three layers each. Four experimental groups consisting of five replicates and eighteen birds were randomly assigned to the hens. The first and second groups were fed a control diet (Control) or a control diet supplemented with 1.0g of CCE/kg food (CCE) while being kept in thermoneutral circumstances (24°C and 50% relative humidity). The third and fourth groups were fed the control diet (HS) or the control diet supplemented with 1.0g of CCE/kg diet (HS+CCE) while being exposed to heat stress conditions (35°C from 10:00 to 18:00 daily with 50% relative humidity). Throughout the research, which lasted until the 48th week of age, the experimental hens were given unlimited access to feed and fresh water. A daily lighting schedule of 17h of light and 7h of darkness at an intensity of 30 Lux was applied. The control diet was designed to provide the nutritional requirements of HY-Line Brown laying hens. Its chemical composition was analyzed following the standard AOAC procedures as adopted by (Latimer Jr. 2023) (Table 1).

**Table 1:** Control diet components and macronutrients composition

| Components | % |
|---|---|
| Yellow corn | 56.65 |
| Soybean meal, 44% CP | 27.5 |
| Wheat bran | 1.00 |
| Limestone | 8.00 |
| Bone meal | 3.00 |
| Soybean oil | 3.00 |
| Sodium chloride | 0.40 |
| Premix[1] | 0.30 |
| DL-Methionine | 0.15 |
| *Diet nutrients* | |
| Metabolizable energy[2] (kcal) | 300.95 |
| Crude protein[3] (CP) (%) | 16.5 |
| Ether extract[3] (%) | 6.60 |
| Crude fibre[3] (%) | 4.70 |
| Calcium[2] (%) | 0.40 |
| Available phosphorus[2] (%) | 0.52 |

[1]Premix (content per kg of the control diet): vitamin A: 8000IU; vitamin E: 15mg; vitamin D: 1500IU; vitamin K: 2mg; vitamin B2: 4mg; vitamin B12:10µg; choline: 500mg; vitamin B3: 25mg; Mn: 60mg; Zn: 50mg. [2]Calculated nutrients. [3]Analysed nutrients.

### Productive efficiency

The performance of the hens was evaluated over an eight-week period, specifically from week 40 to week 48. Key metrics, including the daily count of eggs, the average egg weight (in grams), and the feed consumption per hen per day (g/hen/d), were systematically recorded. Subsequently, the total egg mass was determined by multiplying the number of eggs laid by their mean weight. Daily feed intake per hen was also monitored to calculate the Feed conversion ratio (FCR). The FCR was computed using the formula:

$$FCR = \frac{\text{Feed intake (g)}}{\text{Total egg mass (g) generated during the corresponding time frame.}}$$

**Egg quality**

The evaluation of egg-quality traits was conducted on six eggs per replicate (n=30 per group). Following initial egg weight measurement (EW), eggs internal and external quality parameters including the albumen index (AI), yolk index (YI), yolk color, Hugh unit (HU), shell thickness (ST), and shell strength (SS) were measured following Abbas et al. (2022c). Haugh unit was calculated according to Salehizadeh et al. (2025):

Haugh unit=100×log [Albumin height (mm)+7.6-1.7 EW(g)$^{0.37}$].

**Bone quality**

Upon the termination of the study, a sample of 20 hens per group was humanely euthanized to facilitate bone quality assessment, ensuring that at least one subject from each replicate was included. The left tibia from each selected bird (n=20 per treatment) was collected and weighed using a digital scale accurate to 0.0001g, while A digital electronic pachymeter was used to measure the length of the tibia.

The Seedor Index $SI = \frac{\text{Bone Weight (mg)}}{\text{Bone Length (mm)}}$ was calculated according to Seedor et al. (1991). The corresponding right tibia was reserved for the evaluation of bone resistance. Biomechanical strength was assessed using a TA-XT Plus texture analyzer (Stable Micro Systems, Surrey, UK) equipped with a 50kg load cell. A crosshead speed of 50mm/min was employed. The bone was placed on the Stable Micro Systems Three Point Bend Rig (model HDP/3PB) fixture, which was set up to provide a fixed diaphyseal gap of 3.0cm (Li et al. 2024).

**Stress indicators and antioxidant status**

For each experimental cohort, a set of ten plasma samples was collected via random selection. The concentration of several stress-related biomarkers, namely tumor necrosis factor alpha (TNFα), Interleukin-1 beta (IL-1β), and corticosterone, was determined using specialized chicken ELISA kits (MyBioSource Inc., San Diego, CA, USA; catalog numbers MBS2509660, MBS761055, and MBS701668, in that order). Additionally, the total antioxidant capacity (TAC) was evaluated with a commercially available colorimetric assay kit (MyBioSource Inc., San Diego, CA, USA; MBS2540515). Lastly, the activity of superoxide dismutase (SOD) and the concentration of malondialdehyde (MDA) were quantified utilizing a commercial assay kit supplied by (ab65354 and ab118970, respectively; Abcam Waltham, MA, USA).

**Immunological response parameters**

Ten blood samples per treatment group were drawn in heparinized tubes and allocated to evaluate various immunological markers. The total white blood cells count (TWBCs) were counted according to Gehad et al. (2008) method. Meanwhile, the heterophil-to-lymphocyte ratio (H/L) was measured according to (Mehaisen et al. 2017) . Another ten blood samples were drawn from each treatment group in heparinized tubes to assess T- and B-lymphocyte proliferation index according to (Alaqil et al. 2020). The immunoglobulin (Ig) assay was performed (n=10) according to the manufacturer's instructions. The IgM and IgG level were measured using commercial ELISA kits designed specifically for hens (MBS706158 and MBS260043, respectively; MyBioSource Inc., San Diego, CA, USA).

**Statistical Analysis**

A one-way ANOVA was conducted to compare experimental group means, using the SPSS software package. When significant differences were noticed, Tukey's post hoc test was performed for statistical significance evaluation at P<0.05.

## RESULTS

**Layer performance**

Table 2 presents the effect of HS and CCE supplementation on laying hens productivity. HS markedly impaired the hens' productivity with observed reduction in feed intake, egg weight, and egg production by 5.5%, 5%, and 21%, respectively, resulting in a higher feed conversion ratio (FCR) and reduced feed efficiency. Supplementation with CCE partially mitigated these adverse effects, leading to a relative improvement in the productivity of heat-stressed layers, although feed intake remained unaffected. Under thermoneutral conditions, CCE supplementation increased egg production by 3% relative to the control, while exerting no significant effects on the other measured production parameters.

**Table 2:** laying hen production performance under thermoneutral (Control) or heat stress (HS) conditions and fed *Citrullus colocynthis* seed extract (CCE)

| Parameters | Control | CCE | HS | HS+CCE | SEM | P-value |
|---|---|---|---|---|---|---|
| Egg production (%) | 92.1$^b$ | 94.8$^a$ | 74.6$^d$ | 87.4$^c$ | 1.53 | <0.001 |
| Egg weight (g) | 64.3$^a$ | 64.9$^a$ | 60.9$^c$ | 62.6$^b$ | 1.13 | <0.001 |
| FI (g/bird/day) | 109$^a$ | 110$^a$ | 103$^b$ | 105$^b$ | 1.24 | 0.005 |
| FCR | 1.82$^c$ | 1.84$^c$ | 2.14$^a$ | 1.96$^b$ | 0.05 | 0.017 |

Means within the same row bearing different superscript letters differ significantly (P<0.05). FI: feed intake; FCR: feed conversion ratio.

**Egg and bone quality parameters**

The effects of HS and dietary CCE addition on tibia and egg quality are presented in Table 3. Exposure to HS significantly impaired internal parameters of egg quality, resulting in reduced Haugh unit, albumen index, and yolk color intensity, while the yolk index remained unaffected. Additionally, HS markedly decreased external egg quality traits, with shell thickness and shell strength reduced by 27% and 9%, respectively. Bone quality indicators were also significantly compromised under HS conditions. Supplementation with CCE partially restored internal egg quality by enhancing the Haugh unit and yolk color and enhanced external shell characteristics, including thickness and strength. Moreover, CCE supplementation to heat-stressed layers successfully restored bone quality parameters to levels similar to the thermoneutral control group.

**Immunity response indicators**

The impact of HS and CCE supplementation on the immune markers of laying hens are presented in Table 4. Exposure to HS significantly reduced the total WBC count, while the H/L ratio increased, indicating a pronounced stress response. Moreover, HS was associated with a marked decline in T- and B-lymphocyte proliferation indexes,

**Table 3:** Egg and bone quality markers of laying hen reared under thermoneutral (Control) or heat stress (HS) conditions and fed *Citrullus colocynthis* seed extract (CCE)

| Parameters | Control | CCE | HS | HS+CCE | SEM | P-value |
|---|---|---|---|---|---|---|
| Haugh unit | 88.37$^{a}$ | 87.91$^{a}$ | 76.85$^{c}$ | 81.45$^{b}$ | 0.82 | <0.001 |
| Albumin index (%) | 10.39$^{a}$ | 10.61$^{a}$ | 8.13$^{b}$ | 9.03$^{b}$ | 0.22 | 0.042 |
| Yolk index (%) | 40.81 | 40.78 | 40.11 | 40.35 | 0.77 | 0.183 |
| Yolk color | 8.17$^{ab}$ | 8.22$^{a}$ | 7.56$^{c}$ | 7.96$^{b}$ | 0.11 | 0.026 |
| Shell thickness (mm) | 0.37$^{a}$ | 0.39$^{a}$ | 0.27$^{c}$ | 0.32$^{b}$ | 0.003 | <0.001 |
| Shell strength (kg/cm$^2$) | 4.16$^{b}$ | 4.34$^{a}$ | 3.79$^{d}$ | 3.98$^{c}$ | 0.06 | <0.001 |
| Tibia resistance (kgf/cm$^2$) | 22.37$^{a}$ | 22.41$^{a}$ | 20.14$^{b}$ | 22.13$^{a}$ | 1.09 | 0.037 |
| Seedor Index | 94.68$^{a}$ | 95.01$^{a}$ | 85.14$^{b}$ | 93.95$^{a}$ | 3.01 | 0.048 |

Means within the same row bearing different superscript letters differ significantly (P<0.05).

**Table 4:** Immunity markers of laying hen reared under thermoneutral (Control) or heat stress (HS) conditions and fed *Citrullus colocynthis* seeds extract (CCE)

| Parameters | Control | CCE | HS | HS+CCE | SEM | P-value |
|---|---|---|---|---|---|---|
| Total WBC (×10$^3$/mL) | 61.38$^{a}$ | 65.39$^{a}$ | 39.76$^{c}$ | 51.91$^{b}$ | 2.67 | 0.032 |
| H/L ratio | 0.39$^{c}$ | 0.32$^{c}$ | 0.88$^{a}$ | 0.61$^{b}$ | 0.028 | 0.007 |
| T-cell Proliferation Index | 3.86$^{b}$ | 4.62$^{a}$ | 1.96$^{d}$ | 2.84$^{c}$ | 0.15 | 0.019 |
| B-cell Proliferation Index | 2.96$^{a}$ | 3.12$^{a}$ | 1.13$^{c}$ | 1.98$^{b}$ | 0.14 | 0.043 |
| IgG (mg/ml) | 1.85$^{a}$ | 1.89$^{a}$ | 1.47$^{c}$ | 1.61$^{b}$ | 0.05 | 0.037 |
| IgM (µg/ml) | 489.31$^{a}$ | 516.3$^{a}$ | 343.74$^{c}$ | 423.96$^{b}$ | 12.64 | 0.016 |

Means within the same row bearing different superscript letters differ significantly (P<0.05). WBC: white blood cell; H/L ratio: heterophil and lymphocytes ratio; Ig: immunoglobulin.

**Table 5:** Stress and antioxidant markers of laying hen reared under thermoneutral (Control) or heat stress (HS) conditions and fed *Citrullus colocynthis* seed extract (CCE)

| Parameters | Control | CCE | HS | HS+CCE | SEM | P-value |
|---|---|---|---|---|---|---|
| Corticosteron (pg/mL) | 6.01$^{c}$ | 4.73$^{d}$ | 13.96$^{a}$ | 9.61$^{b}$ | 0.017 | 0.038 |
| TNF-α (pg/mL) | 90.67$^{c}$ | 88.64$^{c}$ | 154.36$^{a}$ | 127.76$^{b}$ | 5.231 | 0.031 |
| IL-1β (ng/mL) | 0.34$^{c}$ | 0.29$^{c}$ | 0.92$^{a}$ | 0.68$^{b}$ | 0.015 | <0.001 |
| MDA (µM/mL) | 2.04$^{c}$ | 1.96$^{c}$ | 3.97$^{a}$ | 2.66$^{b}$ | 0.475 | 0.029 |
| TAC (U/mL) | 4.23$^{b}$ | 6.89$^{a}$ | 2.54$^{d}$ | 3.41$^{c}$ | 0.241 | 0.017 |
| SOD (U/mL) | 294.6$^{b}$ | 369.4$^{a}$ | 210.8$^{d}$ | 253.9$^{c}$ | 18.61 | 0.019 |

Means within the same row bearing different superscript letters differ significantly (P<0.05). TNF-α: tumor necrosis factor-α; IL-1β: interleukin-1β; MDA: malondialdehyde; TAC: total antioxidant capacity; SOD: superoxide dismutase.

as well as a reduction in humoral immunity markers (IgG and IgM). Under HS conditions, dietary CCE supplementation partially restored these immune response parameters, suggesting a mitigating effect. Conversely, under thermoneutral conditions, CCE supplementation did not influence most immune parameters, except for T-lymphocyte proliferation, which significantly elevated compared with the unsupplemented thermoneutral control group.

**Oxidative stress and inflammation markers**

Exposure to HS significantly elevated stress and pro-inflammatory indicators (Table 5). Blood corticosterone, TNF-α, IL-1β, and MDA levels were increased by 2.3, 1.7, 2.7, and 1.9-fold, respectively, relative to the thermoneutral control group. Conversely, the activities of TAC and SOD were significantly decreased by 1.7- and 1.4-fold, respectively, confirming the oxidative stress-inducing effect of HS. Supplementation with CCE effectively attenuated stress and pro-inflammatory responses while enhancing the activity of antioxidant markers. Moreover, under thermoneutral conditions, CCE supplementation significantly elevated TAC and SOD activities compared with the unsupplemented control, further demonstrating the antioxidant potential of CCE.

## DISCUSSION

Chronic exposure to thermal stress led to various productive and physiological disorders. The observed drop in egg production and deterioration in egg quality under HS are primarily attributed to reduced feed intake, which consequently limits the uptake and absorption of essential nutrients. (Ibtisham et al. 2019; Rodríguez et al. 2021; Cornescu et al. 2023; Gil et al. 2023). Heat stress further disrupts intestinal homeostasis, which compromises intestinal barrier integrity, physiology, immunology, and gut microbiome imbalance (Rostagno 2020; Cao et al. 2021; Gao et al. 2025; Liu et al. 2025). The decline in shell quality and egg production observed under HS conditions in the current study aligns with previous findings. Kim et al. (2020) reported that chronic heat stress exposure resulted in reduced laying performance, poorer egg quality (particularly thinner and weaker shells), and notable physiological disturbances. These adverse effects were associated with changes in gut metabolites and disruptions in mineral and lipid uptake (Chen et al. 2021). Meanwhile, CCE supplementation, likely due to its potent antioxidant activity, has been shown to improve laying hen productivity under thermal stress conditions (Alzarah et al. 2021b; Bourhia et al. 2021). In addition, CCE may enhance productive performance by modulating the gut microbiota, strengthening intestinal barrier integrity, and reducing intestinal inflammation induced by heat stress (Kikusato 2021; Latek et al. 2022; Liu et al. 2025). As a phytogenic feed additive, CCE can also stimulate digestive enzyme secretion, improve nutrient utilization, regulate growth-related hormones, and modulate intestinal microbial communities, collectively contributing to better feed efficiency and improved laying performance (Wang et al. 2024). Furthermore, L-citrulline, a non-essential amino acid found in *Citrullus* species, function as a precursor for the synthesis of arginine, which is an essential amino acid for optimum layer production (Uyanga et al. 2020; Chen et al. 2023) thermoregulation (Chowdhury 2023; Morais et al. 2023), immune response (Dao et al. 2021), and bone health (Dao et al. 2023).

Optimum egg quality parameters are crucial to ensure breeder profitability. The egg quality parameters were impaired in response to HS. We observed a reduction in the Haugh unit, albumin index, and yolk color, as well as a reduction in shell strength and thickness associated with HS exposure. Kim et al. (2024) reported that severe HS can cause a decline in Haugh unit and light egg yolk color, which are indicators of poor egg quality. They indicated that the alteration in heat-stressed layers' physiology and metabolism is the main cause of poor egg quality and production reduction. Besides, HS alter gut metabolites and disturb mineral and lipid metabolism, which likely impaired calcium uptake and utilization in shell formation under thermal stress (Kim et al. 2020). A reduction in plasma calcium and phosphorus levels, which is critical for egg production and shell quality, was associated with HS exposure (Nanto-Hara et al. 2023; Ribeiro et al. 2025). Furthermore, chronic HS reported to negatively affect bone strength in the heat-stressed chickens probably due to the decreased feed intake (Kim et al. 2020). In addition, HS can cause disarray in essential nutrient metabolism, resulting in impaired performance (Li et al. 2022b). The CCE supplementation partially alleviated these negative impacts, probably through its abundance of multifunctional bioactive compounds (Bourhia et al. 2021; Abbas et al. 2022a).

Heat stress triggers a number of physiological disturbances, including the activation of the hypothalamic-pituitary-adrenal cortical system, leading to elevated corticosterone levels, which induce immunosuppression and negatively affect metabolic hormones and endocrine systems (He et al. 2018; Kim and Lee 2023). Moreover, other stress indicator parameters, such as the increase in H/L ratio, support earlier observations that heat stress negatively impacts poultry performance and physiology (Gil et al. 2023). Also, Abbas et al. (2022b) reported that HS induces immunosuppression and inflammation in laying hens. Wang et al. (2023) confirmed that HS ultimately impairs poultry immune function by inducing oxidative stress and inflammation in immune organs. Immunity is compromised under HS conditions, leading to redox imbalance, immune organ damage, reduced IgG and IgM levels, and impaired humoral and cell-mediated responses (Nayak et al. 2024; Kumar et al. 2025). However, CCE, as a potent source of various bioactive phytochemicals, including flavonoids, saponins, coumarins, and tannins, can mitigate these adverse effects and enhance the immune response of heat-stressed hens (Alzarah et al. 2021a; Alzarah et al. 2021b). These phytochemical constituents underpin the seed extract's antioxidant, antimicrobial, and anti-inflammatory activities, making it a promising candidate for mitigating stress-related disturbances in poultry (Bourhia et al. 2020; Khan et al. 2023; Abdallah et al. 2024; Khalid et al. 2024). The present study examined CCE *in vivo* using the laying hen as animal model and demonstrated its antioxidant, immunomodulation and anti-inflammatory activities. Further research is recommended to explore different *Citrullus colocynthis* seed extracts and its full potential and safety profiles for dietary poultry supplementation.

## Conclusion

The present results provide valuable understandings on the influence of CCE supplementation on laying hen productivity, product quality, immunomodulation, oxidative stress and inflammation status under different environmental conditions. Supplementing heat-stressed layers with CCE proved to enhance layers production and physiological performances. It can be concluded that CCE dietary supplementation is useful natural mitigation practice to mitigate the physiological stress driven by HS and consequently improve layers productivity and product quality. More researches are needed to reveal the mode of action and physiological benefits of incorporating different *Citrullus colocynthis* seed extracts into poultry diets.

## DECLARATIONS

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**Conflict of Interest:** The authors declare no conflict of interest.

**Data Availability:** All relevant data supporting the findings of this study are provided within the article.

**Ethics Statement:** The Research Ethics Committee of King Faisal University, Saudi Arabia, revised and permitted all animal-conducted investigational processes (Approval No. KFU-REC-2025-July-ETHICS3329).

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## REFERENCES


Abbas AO, Alaqil AA, Kamel NN and Moustafa ES, 2022a. *Citrullus colocynthis* seed ameliorates layer performance and immune response under acute oxidative stress induced by paraquat injection. Animals 12(8): 945. https://doi.org/10.3390/ani12080945

Abbas AO, Alaqil AA, Mehaisen GMK and El Sabry MI, 2022b. Effect of organic selenium-enriched yeast on relieving the deterioration of layer performance, immune function, and physiological indicators induced by heat stress. Frontiers in Veterinary Science 9: 880790. https://doi.org/10.3389/fvets.2022.880790

Abbas AO, Alaqil AA, Mehaisen GMK and Kamel NN, 2022c. Effect of dietary blue-green microalgae inclusion as a replacement to soybean meal on laying hens' performance, egg quality, plasma metabolites, and hematology. Animals 12(20): 2816. https://doi.org/10.3390/ani12202816

Abdallah EM, Ali AMH, Hashem HF, Mujawah AA and Elsharkawy ER, 2024. Chemical profiling and pharmaceutical and biological activities of methanolic extract of *Citrullus colocynthis* L. seeds collected from the arid zone of Qassim, Saudi Arabia against *Aphis craccivora*. Advancements in Life Sciences 11(4): 833-842. https://doi.org/10.62940/als.v11i4.3135

Alaqil AA, Abbas AO, El-Beltagi HS, El-Atty HKA, Mehaisen GMK and Moustafa ES, 2020. Dietary supplementation of probiotic lactobacillus acidophilus modulates cholesterol levels, immune response, and productive performance of laying hens. Animals 10(9): 1588. https://doi.org/10.3390/ani10091588

Al-Homidan IH, Ebeid TA, Al-Muzaini A, Abou-Emera OK, Mostafa MM and Fathi MM, 2020. Impact of dietary fenugreek, mung bean, and garden cress on growth performance, carcass traits, blood measurements, and immune response in broiler chickens. Livestock Science 242: 104318. https://doi.org/10.1016/j.livsci.2020.104318

Altemimi AB, Mohammed MJ, Alkanan ZT, Abdulrahman SH, Allaitha SA, Abedelmaksoud TG, Alkaisy QH, Najm MAA, Cacciola F and Srinivasan PV, 2023. Bioactive compounds of *Citrullus colocynthis* aerial parts: characterization and biological properties. Plant Biosystems 157(6): 1151-1160. https://doi.org/10.1080/11263504.2023.2257720

Alzarah MI, Alaqil AA, Abbas AO, Nassar FS, Mehaisen GMK, Gouda GF, Abd El-Atty HK and Moustafa ES, 2021a. Inclusion of *Citrullus colocynthis* seed extract into diets induced a hypolipidemic effect and improved layer performance. Agriculture 11(9): 808. https://doi.org/10.3390/agriculture11090808

Alzarah MI, Althobiati F, Abbas AO, Mehaisen GMK and Kamel NN, 2021b. *Citrullus colocynthis* seeds: A potential natural immune modulator source for broiler reared under chronic heat stress. Animals 11(7): 1951. https://doi.org/10.3390/ani11071951

Attia YA, Addeo NF, Bovera F, Abd Al-Hack ME, AlBanoby MA, Alhotan RA, Khafaga AF, Hafez HM and de Oliveira MC, 2025. Phytogenic substances as novel feed supplements and their application in livestock nutrition. In: Phytogenic and Phytochemical as Alternative Feed Additives for Animal Production. Attia YA, El-Hack MEA, Alagawany MM, Elnaggar AS (eds). Bentham Science Publishers Pte. Ltd., Singapore, pp 4-18. https://doi.org/10.2174/9789815322767l250101

Biswas S and Kim IH, 2025. A thorough review of phytogenic feed additives in non-ruminant nutrition: production, gut health, and environmental concerns. Journal of Animal Science and Technology 67(3): 497-519. https://doi.org/10.5187/jast.2025.e26

Bourhia M, Bouothmany K, Bakrim H, Hadrach S, Salamatullah AM, Alzahrani A, Alyahya HK, Albadr NA, Gmouh S, Laglaoui A, El Mzibri M and Benbacer L, 2021. Chemical profiling, antioxidant, antiproliferative, and antibacterial potentials of chemically characterized extract of *Citrullus colocynthis* L. Seeds. Separations 8(8): 114. https://doi.org/10.3390/separations8080114

Bourhia M, Messaoudi M, Bakrim H, Mothana RA, Sddiqui NA, Almarfadi OM, El Mzibri M, Gmouh S, Laglaoui A and Benbacer L, 2020. *Citrullus colocynthis* (L.) Schrad: Chemical characterization, scavenging and cytotoxic activities. Open Chemistry 18(1): 986-994. https://doi.org/10.1515/chem-2020-0124

Cao C, Chowdhury VS, Cline MA and Gilbert ER, 2021. The microbiota-gut-brain axis during heat stress in chickens: A review. Frontiers in Physiology 12: 752265. https://doi.org/10.3389/fphys.2021.752265

Chen S, Yong Y and Ju X, 2021. Effect of heat stress on growth and production performance of livestock and poultry: Mechanism to prevention. Journal of Thermal Biology 99: 103019. https://doi.org/10.1016/j.jtherbio.2021.103019

Chen Y, Zhang B, Wang B, Zhang M, Fan W and Li W, 2023. Effects of dietary arginine supplementation on production performance, serum biochemicals, antioxidant capacity, and immunity of laying Wulong geese. Poultry Science 102(7): 102727. https://doi.org/10.1016/j.psj.2023.102727

Chowdhury VS, 2023. L-Citrulline: A novel hypothermic amino acid promoting thermotolerance in heat-exposed chickens. Animal Science Journal 94(1): e13826. https://doi.org/10.1111/asj.13826

Cornescu GM, Panaite TD, Untea AE, Varzaru I, Saracila M, Dumitru M, Vlaicu PA and Gavris T, 2023. Mitigation of heat stress effects on laying hens' performances, egg quality, and some blood parameters by adding dietary zinc-enriched yeasts, parsley, and their combination. Frontiers in Veterinary Science 10: 1202058. https://doi.org/10.3389/fvets.2023.1202058

Dao HT, Moss AF, Bradbury EJ and Swick RA, 2023. Effects of L-arginine, guanidinoacetic acid and L-citrulline supplementation in reduced-protein diets on bone morphology and mineralization of laying hens. Animal Nutrition 14: 225-234. https://doi.org/10.1016/j.aninu.2023.03.012

Dao HT, Sharma NK, Bradbury EJ and Swick RA, 2021. Response of laying hens to L-arginine, L-citrulline and guanidinoacetic acid supplementation in reduced protein diet. Animal Nutrition 7(2): 460-471. https://doi.org/10.1016/j.aninu.2020.09.004

Daradka H, Almasad MM, Qazan W, El-Banna NM and Samara OH, 2007. Hypolipidaemic effects of *Citrullus colocynthis* L. in rabbits. Pakistan Journal of Biological Sciences 10(16): 2768-2771. https://doi.org/10.3923/pjbs.2007.2768.2771

Gao J, Ren H, Wu X, Zou C, He B and Ma W, 2025. Dietary glycerol monolaurate mitigates heat stress-induced disruption of intestinal homeostasis and hepatic lipid metabolism in laying hens. Stress Biology 5(1): 49. https://doi.org/10.1007/s44154-025-00243-8

Gehad A, Mehaisen G, Abbas A and Mashaly M, 2008. The role of light program and melatonin on alleviation of inflammation induced by lipopolysaccharide injection in broiler chickens. International Journal of Poultry Science 7(2): 193-201. https://doi.org/10.3923/ijps.2008.193.201

Gil MG, Gomez-Raya L, Torres O, Cigarroa-Vazquez FA, Davila SG and Rauw WM, 2023. Heterophil/lymphocyte response of local Spanish breeds of laying hens to cold stress, heat stress, and water restriction. Journal of Thermal Biology 113: 103542. https://doi.org/10.1016/j.jtherbio.2023.103542

He SP, Arowolo MA, Medrano RF, Li S, Yu QF, Chen JY and He JH, 2018. Impact of heat stress and nutritional interventions on poultry production. World's Poultry Science Journal 74(4): 647-664.

https://doi.org/10.1017/S0043933918000727

Ibtisham F, Nawab A, Niu Y, Wang Z, Wu J, Xiao M and An L, 2019. The effect of ginger powder and Chinese herbal medicine on production performance, serum metabolites and antioxidant status of laying hens under heat-stress condition. Journal of Thermal Biology 81: 20-24. https://doi.org/10.1016/j.jtherbio.2019.02.002

Jafari MJ, Iranpour S, Gravandi S, Tehrani BJ, Askari M, Omidi A and Nasori M, 2021. The effects of heat stress exposure on free amino acid concentrations within the plasma and the brain of heat-exposed chicks: A systematic review and meta-analysis. Journal of Thermal Biology 97: 102872. https://doi.org/10.1016/j.jtherbio.2021.102872

Jambwa P, Nkadimeng SM, Mudimba TN, Matope G and McGaw LJ, 2023. Antibacterial and anti-inflammatory activity of plant species used in traditional poultry ethnomedicine in Zimbabwe: A first step to developing alternatives to antibiotic poultry feed additives. Journal of Ethnopharmacology 300: 115687. https://doi.org/10.1016/j.jep.2022.115687

Khalid A, Zahra N, Mushtaq I, Zeeshan B, Sarwar A, Binsaleh AY, Al-Hoshani N, Alwethaynani MS, Alhhazmi AA, Al-Joufi FA and Aziz T, 2024. In-vivo and in-silico analysis of anti-inflammatory, antipyretic and analgesic activity of methanolic seed extract of *Citrus colocynthis*. Journal of the Chilean Chemical Society 69(4): 6220-6227. https://doi.org/10.4067/s0717-97072024000406220

Khan M, Khan M, Al-hamoud K, Adil SF, Shaik MR and Alkhathlan HZ, 2023. Diversity of *Citrullus colocynthis* (L.) schrad seeds extracts: Detailed chemical profiling and evaluation of their medicinal properties. Plants 12(3): 567. https://doi.org/10.3390/plants12030567

Kikusato M, 2021. Phytobiotics to improve health and production of broiler chickens: Functions beyond the antioxidant activity. Animal Bioscience 34(3): 345-353. https://doi.org/10.5713/ab.20.0842

Kim DH and Lee KW, 2023. An update on heat stress in laying hens. World's Poultry Science Journal 79(4): 689-712. https://doi.org/10.1080/00439339.2023.2239769

Kim DH, Lee YK, Lee SD, Kim SH, Lee SR, Lee HG and Lee KW, 2020. Changes in production parameters, egg qualities, fecal volatile fatty acids, nutrient digestibility, and plasma parameters in laying hens exposed to ambient temperature. Frontiers in Veterinary Science 7: 412. https://doi.org/10.3389/fvets.2020.00412

Kim HR, Ryu C, Lee SD, Cho JH and Kang H, 2024. Effects of heat stress on the laying performance, egg quality, and physiological response of laying hens. Animals 14(7): 1076. https://doi.org/10.3390/ani14071076

Kumar H, Bhardwaj I, Nepovimova E, Dhanjal DS, Shaikh SS, Knop R, Atuahene D, Shaikh AM and Béla K, 2025. Revolutionising broiler nutrition: The role of probiotics, fermented products, and paraprobiotics in functional feeds. Journal of Agriculture and Food Research 21: 101859. https://doi.org/10.1016/j.jafr.2025.101859

Latek U, Chłopecka M, Karlik W and Mendel M, 2022. Phytogenic compounds for enhancing intestinal barrier function in poultry-A Review. Planta Medica 88(3-4): 218-236. https://doi.org/10.1055/a-1524-0358

Latimer Jr. GW, 2023. Official Methods of Analysis of AOAC International, 22nd edn. Oxford University Press, New York, USA.

Li QY, Munawar M, Saeed M, Shen JQ, Khan MS, Noreen S, Alagawany M, Naveed M, Madni A and Li CX, 2022a. *Citrullus colocynthis* (L.) Schrad (bitter apple fruit): promising traditional uses, pharmacological effects, aspects, and potential applications. Frontiers in Pharmacology 12: 791049. https://doi.org/10.3389/fphar.2021.791049

Li X, Uyanga VA, Jiao H, Wang X, Zhao J, Zhou Y, Li H and Lin H, 2024. Effects of low dietary calcium and lipopolysaccharide challenges on production performance, eggshell quality, and bone metabolism of laying hens. Frontiers in Physiology 15: 1396301. https://doi.org/10.3389/fphys.2024.1396301

Li Z, Zhao Y, Zhuang Y, Xu Z, Wu C, Liu P, Hu G, Li G, Chen W, Gao X and Guo X, 2022b. Effects of N-acetyl-L-cysteine on serum indices and hypothalamic AMPK-related gene expression under chronic heat stress. Frontiers in Veterinary Science 9: 936250. https://doi.org/10.3389/fvets.2022.936250

Liu H, Luo Y, Qu M and Xu L, 2025. Research progress on effects of heat stress on intestinal Immune function and nutritional regulation in livestock and poultry. Chinese Journal of Animal Nutrition 37(1): 160-168. https://doi.org/10.12418/CJAN2025.014

Mehaisen GM, Eshak MG, Elkaiaty AM, Atta AM, Mashaly MM and Abass AO, 2017. Comprehensive growth performance, immune function, plasma biochemistry, gene expressions and cell death morphology responses to a daily corticosterone injection course in broiler chickens. PloS One 12(2): e0172684. https://doi.org/10.1371/journal.pone.0172684

Morais MVM, Lima HJDA, Silva FNA and Gomes MVFC, 2023. Indicators of thermal comfort and nitrogen digestibility as a function of digestible arginine: lysine ratios in the diet of laying Japanese quails raised in hot weather. Journal of Thermal Biology 115: 103597. https://doi.org/10.1016/j.jtherbio.2023.103597

Nanto-Hara F, Yamazaki M, Murakami H and Ohtsu H, 2023. Chronic heat stress induces renal fibrosis and mitochondrial dysfunction in laying hens. Journal of Animal Science and Biotechnology 14(1): 81. https://doi.org/10.1186/s40104-023-00878-5

Nassar FS, Abbas AO, Alaqil AA and Kamel NN, 2023. Sustainable broilers production performance under high stocking condition through colocynth Seed supplementation. Sustainability 15(6): 5102. https://doi.org/10.3390/su15065102

Nayak N, Bhanja SK, Chakurkar EB and Sahu AR, 2024. Adaptive capability of slow-growing backyard poultry as indicated by physiological and molecular responses in a hot and humid coastal climate. Journal of Thermal Biology 125: 103985. https://doi.org/10.1016/j.jtherbio.2024.103985

Ntsongota Z, Ikusika O and Jaja IF, 2025. The role of phytogenic feed additives in growth and immune response in livestock production: a global systematic review. Frontiers in Animal Science 6: 1703112. https://doi.org/10.3389/fanim.2025.1703112

Ribeiro AG, Silva RdS, Alves CVBdV, Campos DB, da Silva DA, Nascimento JCdS, da Silva EG, Saraiva EP, Costa FGP, Pereira WE, Carvalho LRRA and Guerra RR, 2025. Gene expression of calcium transporters Calbindin-D28K and TRPV6 in Japanese quails (*Coturnix japonica*) subjected to phytase super-dosing and under different temperatures. Poultry Science 104(4): 104937. https://doi.org/10.1016/j.psj.2025.104937

Rodrigues HLdS, Kolososki IMM, Benevides VP, Saraiva MMS and Berchieri Junior A, 2025. Essential oils used in the poultry industry: Would it be an effective green alternative against *Salmonella* spp. dissemination and antimicrobial resistance? The Microbe 6: 100248. https://doi.org/10.1016/j.microb.2025.100248

Rodríguez M, Montalvo G, Morales J, Jiménez-Martín M, Aparicio M and Piñeiro C, 2021. Environmental monitoring and disease prediction. In: Advancements and Technologies in Pig and Poultry Bacterial Disease Control. Foster N, Kyriazakis I, Barrow P (eds). Academic Press, NY, USA, pp 145-169. https://doi.org/10.1016/B978-0-12-818030-3.00011-8

Rostagno MH, 2020. Effects of heat stress on the gut health of poultry. Journal of Animal Science 98(4): skaa090. https://doi.org/10.1093/jas/skaa090

Salehizadeh A, Torki M, Darbemamieh M and Sharifi SD, 2025. Diet inclusion of housefly larvae and frass supplemented by Rayabold (enzyme and probiotic) on performance of laying hens and egg quality. Poultry Science 104(10): 105544. https://doi.org/10.1016/j.psj.2025.105544

Schat KA and Skinner MA, 2022. Chapter 14 - Avian immunosuppressive diseases and immune evasion. In: Avian Immunology. Kaspers B, Schat KA, Göbel TW, Vervelde L (eds), 3rd edn. Academic Press, Boston, USA, pp: 387-417. https://doi.org/10.1016/B978-0-12-818708-1.00018-X

Seedor JG, Quartuccio HA and Thompson DD, 1991. The bisphosphonate alendronate (MK-217) inhibits bone loss due to ovariectomy in rats. Journal of Bone and Mineral Research 6(4): 339-346. https://doi.org/10.1002/jbmr.5650060405

Taschetto EA, Godoy GL, Albigo EL, Raddatz GA, Dalmoro YK, Alencar JA, Borges SA and Stefanello C, 2025. Bioequivalence of a commercial polyherbal choline for replacement of choline chloride for broiler chickens. Journal of Applied Poultry Research 34(4): 100571. https://doi.org/10.1016/j.japr.2025.100571

Tesakul S, Mitsuwan W, Morita Y and Kitpipit W, 2025. Effects of heat stress on egg performance in laying hens under hot and humid conditions. Veterinary World 18(4): 851-858. https://doi.org/10.14202/vetworld.2025.851-858

Thamer FH and Thamer N, 2023. Gas chromatography – Mass spectrometry (GC-MS) profiling reveals newly described bioactive compounds in *Citrullus colocynthis* (L.) seeds oil extracts. Heliyon 9(6): e16861. https://doi.org/10.1016/j.heliyon.2023.e16861

Uyanga VA, Jiao H, Zhao J, Wang X and Lin H, 2020. Dietary L-citrulline supplementation modulates nitric oxide synthesis and anti-oxidant status of laying hens during summer season. Journal of Animal Science and Biotechnology 11(1): 103. https://doi.org/10.1186/s40104-020-00507-5

Wang J, Deng L, Chen M, Che Y, Li L, Zhu L, Chen G and Feng T, 2024. Phytogenic feed additives as natural antibiotic alternatives in animal health and production: A review of the literature of the last decade. Animal Nutrition 17: 244-264. https://doi.org/10.1016/j.aninu.2024.01.012

Wang Y, Yang X, Li S, Wu Q, Guo H, Wang H, Su P and Wang J, 2023. Research Note: Heat stress affects immune and oxidative stress indices of the immune organs of broilers by changing the expressions of adenosine triphosphate-binding cassette subfamily G member 2, sodium-dependent vitamin C transporter-2, and mitochondrial calcium uniporter. Poultry Science 102(8): 102814. https://doi.org/10.1016/j.psj.2023.102814