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Research Article

Effect of Neem (*Azadirachta indica*) Leaf Powder Supplementation on Some Blood Parameters in Broiler Chickens Exposed to Heat Stress

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SKAA wrote the manuscript. MKIAS, HHAJ and RFAJ conducted the farm experiments and laboratory work.

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Abstract | A total of 320 unsexed broiler chicks were utilized between November 29, 2022, and January 1, 2023, (to investigate the effect of neem leaf powder on some physiological characteristics of broilers exposed to heat stress), these birds were randomly allocated into four treatment groups, The experimental treatments consisted of (T1) a control group without any treatment, and three groups supplemented with neem leaf powder at rates of 200, 400, and 600 mg/kg food, referred to as treatments T2, T3, and T4, respectively. All treatment groups were subjected to heat stress, with a cyclic temperature ranging from 28 to 32 °C. At the age of 14 days, there was a noteworthy reduction (P≤0.05) in the levels of cholesterol T3 and T4 compared to T1. Similarly, at the age of 21 days, there was a decrease in all additional treatments compared to T1. Furthermore, at the age of 35 days, there was a decrease in T2 and T3. At 14 days of age, there was a noteworthy reduction (P≤0.05) in the levels of triglycerides in T1 and T4 compared to T2. Additionally, at 35 days of age, there was a substantial drop in the levels of triglycerides in all supplements compared to T1. At 14 days old, there was a notable reduction (P≤0.05) in glucose levels in all treatment groups compared to T1. Additionally, glucose levels dropped in T3 and T4 at 21 days old. At 14 days of age, there was a statistically significant increase (P≤0.05) in the concentration of glutathione in T3 compared to T1. At the age of 14 days, the addition treatments resulted in a significant decrease (P≤0.05) in the concentration of malondialdehyde compared to T1. Additionally, at the ages of 21 and 35 days, the concentration of malondialdehyde reduced in treatments T3 and T4 compared to T1. At 35 days of age, the concentration of AST showed a substantial rise (P≤0.05) in T3. ALT levels shown a substantial decrease (P≤0.05) across all age groups in the addition treatments when compared to T1. The study demonstrated that the different concentrations of neem leaf powder yielded favorable outcomes, as seen by improvements in both the lipid profile and antioxidant levels.

Novelty Statement | This study is the first one using Neem (*Azadirachta indica*) Leaf Powder in the Iraq/ Babylon to improve broiler growth under heat stress condition.

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Introduction

With the use of intensive breeding techniques, the production of poultry products worldwide is constantly rising (Grzinic *et al.*, 2023). Despite this, the poultry industry is one of the most effective sector of animal production in ensuring food security for a significant portion of the global population. Including heat stress, which is known to have an impact on a variety of bodily tissues in birds, including the integrity of the intestines and the health of the digestive tract, leading to an imbalance in the microbial balance in the intestinal tubule and an imbalance in the digestion and absorption of ingested nutrients (Dokladny *et al.*, 2016), Heat stress induces the production of reactive oxygen species (ROS). The levels of oxygen, hydrogen peroxide, and hydroxyl radicals rise in different chicken tissues, as demonstrated by Khan *et al.* (2012). This leads to the oxidation of lipids, proteins, and DNA, resulting in the impairment of their function, as indicated by Liu *et al.* (1999). Heat is a significant source of stress for hens, leading to various physiological problems, visible alterations, and reductions in development performance and output (Whitehead and Keller, 2003; Zeferino *et al.*, 2016) Neem (*Azadirachta indica*) is a herbal plant with antioxidant, antibacterial, antiviral, antibiotic, and antifungal properties that boost the immune system (Ubua *et al.*, 2019). The neem tree has recently begun to be grown in Iraq, and there are few studies about it and its chemical analysis, the composition of neem leaves includes 15.8% crude protein, 14.6% crude fiber, 8.5% ether extract, 4.5% ash, 13.0% humidity, and 56.6% NFE (Bonsu *et al.*, 2012). Bonsu *et al.* conducted a study in 2012. various components of neem trees have been shown to include over one hundred and thirty-five bioactive compounds, such as azadiechtin, meliacin, gedunin, salanine, nimbin, flacin, sodium nimbolide, cyclic trisulfides, and others (Kharde and Soujanya, 2014). Broiler chicken production involves the rearing of chickens from specific breeds that are known for their large size and meat output, with the aim of obtaining meat of superior quality (Ufele *et al.*, 2020). The use of antibiotics in chicken feed to improve production performance and meet the demand for animal protein is a cause for concern, especially when birds are exposed to heat stress (Lagua and Ampode, 2021). Synthetic antibiotics, like as growth boosters, are expensive and have the potential to harm consumer health by leaving residues in bird tissues (Ezzat *et al.*, 2018). Several herbaceous plants with antibacterial, anti-parasitic, anti-coccal, anti-fungal, anti-tumor, and immune-enhancing qualities can be used as growth stimulants in chicken diets (Subabria and Nagini, 2005). Due to the absence of sweat glands and their elevated body temperatures, chickens are more prone to heat stress compared to other domestic animals (Sahin *et al.*, 2009). Al-Jebory *et al.* (2023) reported that neem leaf powder improved the productive performance of broilers exposed to heat stress, Dayat *et al.* (2023) summarized that adding neem leaf powder to the ration of broilers increased the live body weight and improved the economic efficiency of broilers raised under normal conditions. As a result of the above and because of the harmful effect of heat stress on broilers, the current study examines alleviating the severity of heat stress by adding neem leaf powder to the broiler diet under conditions of heat stress and studying the physiological traits of the broilers.

Materials and Methods

This study was conducted at Al-Anwar Poultry Company/ Babylon Governorate. During the study period, a total of 320 unsexed broiler chicks were utilized. These birds were randomly allocated into four treatments, with each treatment consisting of 80 chickens and four repetitions.

T1: control group without addition.
T2: Add neem leaf powder 200 mg/kg diet.
T3: Add neem leaf powder 400 mg/kg diet.
T4: Add neem leaf powder 600 mg/kg diet.

All treatments were subjected to thermal stress by exposing them to a cyclic temperature range of 28–32 degrees celsius, with other environmental conditions constant. All treatment groups were administered neem leaf powder from the beginning to the conclusion of the trial, in addition to being provided with unrestricted access to food and water throughout the entire duration of the experiment. The National Research Council (NRC, 1994) selected meal compositions containing protein at levels of 23.14% in the initiator diet, 21.6% in the growth diet, and 20.17% in the final diet, the metabolic energy each diet were 3100.00, 3099.18, and 3204.89 kcal/kg.

Physiological traits

Blood samples obtained from avian specimens at the ages of 14, 21, and 35 days. We quantified the levels of cholesterol, glucose, glutathione, malondialdehyde, and the enzymes ALT and AST in the avian blood serum. We utilized a measuring apparatus (Kit) manufactured by the German company Roche to approximate the concentration of cholesterol, employing the methodology established by Franney and Elias (1968), and the methodology outlined by Grundy *et al.* (2004) for triglycerides. The glucose concentration was determined using a measuring equipment (Kit) manufactured by the German company Roche, following the method described by Coles in 1986. The glutathione enzyme and malondialdehyde were quantified using a measurement kit (Kit) provided by Roche Company, following the protocols outlined by Rotrack *et al.* (1973) and Buege and Aust (1978) specifically for malondialdehyde. The content of both ALT (Alanin amino transferase) and AST (Aspartate amino transferase) enzymes was determined using the measurement instrument (Kit) manufactured by the German company Roche, following the method developed by Ritman and Frankel (1957).

Statistical analysis

A study was conducted using a completely randomized design (CRD) to examine the impact of various treatments on all attributes. To determine significant differences between means, multiple range tests were employed. The data were analyzed using the Statistical Analysis System (SAS, 2012), according to model: Yij = μ + Ti + eij.

Results and Discussion

Table 1 displays the effect of including neem leaf powder into the food of broilers on the content of cholesterol. There was a statistically significant reduction (P≤0.05) in treatment T3 and T4 as compared to T1. Simultaneously, treatment T2 exhibited no notable disparities in comparison to the other treatments at the age of 14 days, and the concentration experienced a considerable decline. At 21 days of age, all additional treatments showed higher cholesterol levels compared to T1. At 35 days of age, treatments T2 and T3 exhibited a substantial drop in cholesterol levels compared to T1, whereas treatment T4 did not show any significant difference compared to the other treatments except T2.

Table 1: The effect of Supplements neem leaf powder (*Azadirachta indica*) on cholesterol concentration at the age of 14, 21 and 35 days in broiler chickens under heat stress conditions (mean ± Standard error).

Mean± stander error			Treatments
35 days	**21 days**	**14 days**	
201.77±3.52a	171.99±14.33a	166.96±12.27a	T1
166.22±4.15c	160.05±4.83b	159.56±16.99ab	T2
176.81±2.34b	163.21±3.93b	155.29±5.71b	T3
192.11±7.75ab	157.42±2.42c	155.15±4.83b	T4
*	*	*	Significant

The means with distinct letters within the same column exhibit significant differences among them (P<0.05) *, NS, not significant. The control treatments, T1, did not receive any treatment. The treatments were supplemented with neem leaf powder T2, T3, and T4, at rates of 200, 400, and 600 mg/kg diet , respectively.

Table 2 indicates a statistically significant reduction (P≤0.05) in the level of triglycerides in T4 when compared to T2, T1, and T3. The concentration exhibited a decline in the T1 treatment in comparison to T2, and T3 shown no significant variation from T1 and T2 at the 14-day mark. At 35 days old, there was a notable reduction (P≤0.05) in all further treatments compared to T1. However, there were no significant variations across the treatments at 21 days old.

Table 3 demonstrates a statistically significant reduction (P≤0.05) in glucose concentration in all treatment groups compared to T1 at the 14-day mark. Similarly, at the 21-day mark, there was a significant decrease (P≤0.05) in T3 and T4 compared to T1 and T2. However, no significant differences in glucose concentration were observed at the 35-day mark of the experiment.

Table 2: The effect of Supplements neem leaf powder (*Azadirachta indica*) on Tri triglyceride concentration at the age of 14, 21 and 35 days in broiler chickens under heat stress conditions (mean ± Standard error).

Mean± stander error			Treatments
35 days	**21 days**	**14 days**	
97.05±5.51 a	78.20±1.65	92.78±5.47 b	T1
79.18±2.16 c	77.81±3.55	100.22±8.86 a	T2
83.37±10.02 b	79.81±5.53	99.08±3.21 ab	T3
80.60±4.04 b	76.98±1.36	85.24±3.96 c	T4
*	N.S	*	Significant

The means with distinct letters within the same column exhibit significant differences among them (P<0.05) *, NS = not significant. The control treatments, T1, ,did not receive any treatment. The treatments were supplemented with neem leaf powder T2, T3, and T4 at rates of 200, 400, and 600 mg/kg diet respectively.

Table 3: The effect of supplements neem leaf powder (*Azadirachta indica*) on Glucose concentration at the age of 14, 21 and 35 days in broiler chickens under heat stress conditions (mean ± Standard error).

Mean± stander error			Treatments
35 days	**21 days**	**14 days**	
210.40±5.93	230.07±5.86 a	266.79±9.56 a	T1
214.95±1.71	229.18±2.82 a	243.16±6.53 b	T2
208.61±4.39	201.32±2.99 b	241.80±2.55 b	T3
209.35±6.06	213.61±3.47 b	213.06±2.08 c	T4
N.S	*	*	Significant

The means with distinct letters within the same column exhibit significant differences among them (P<0.05) *, NS, not significant. The control treatments, T1, did not receive any treatment. The treatments were supplemented with neem leaf powder T2, T3, and T4 at rates of 200, 400, and 600 mg/kg diet , respectively.

Table 4 displays a statistically significant rise (P≤0.05) in the concentration of the enzyme glutathione in all treatments involving additions, when compared to T1. T3 had the greatest value. There were no significant variations observed between the treatments for the same characteristic at 21 and 35 days of age.

Table 5 displays the impact of incorporating neem powder into the diets of broilers at various ages on the concentration of almondhyde. The results indicate a significant reduction (P ≤ 0.05) in treatments T3 and T4 compared to T1. Additionally, there is a significant decrease in concentration in T2 compared to T1 at 14 days of age, as well as at 14 and 35 days of age. There was a statistically significant reduction (P ≤ 0.05) in the same characteristic observed in T3 and T4 when compared to T1 and T2.

Table 4: The effect of Supplements neem leaf powder (*Azadirachta indica*) on Glutathione enzyme concentration at the age of 14, 21 and 35 days in broiler chickens under heat stress conditions (mean ± Standard error).

Mean± stander error			Treatments
35 days	**21 days**	**14 days**	
201.03±3.67	187.14±9.58	193.58±6.74 c	T1
198.79±9.51	186.14±2.51	203.49±13.84 b	T2
203.04±7.44	190.01±2.14	226.96±8.43 a	T3
200.55±5.69	189.91±12.93	205.53±4.13 b	T4
N.S	N.S	*	Significant

The means with distinct letters within the same column exhibit significant differences among them (P<0.05) *, NS, not significant. The control treatments, T1, did not receive any treatment. The treatments were supplemented with neem leaf powder T2, T3, and T4, at rates of 200, 400, and 600 mg/kg diet , respectively.

Table 5: The effect of supplements neem leaf powder (*Azadirachta indica*) on Malondehyde concentration at the age of 14 , 21 and 35 days in broiler chickens under heat stress conditions (mean ± Standard error).

Mean± stander error			Treatments
35 days	**21 days**	**14 days**	
8.58±3.69 a	10.32±1.24 a	10.51±0.88 a	T1
8.39±0.06 a	10.58±0.56 a	8.81±0.14 bc	T2
6.18±0.21 c	8.90±0.67 b	7.19±0.04 c	T3
7.43±0.13 b	8.34±0.81 b	9.01±0.25 b	T4
*	*	*	Significant

The means with distinct letters within the same column exhibit significant differences among them (P<0.05) *, NS = not significant. The control treatments, T1, did not receive any treatment. The treatments were supplemented with neem leaf powder T2, T3, and T4, at rates of 200, 400, and 600 mg/kg diet, respectively.

Table 6: The effect of supplements neem leaf powder (*Azadirachta indica*) on AST enzyme concentration at the age of 14 , 21 and 35 days in broiler chickens under heat stress conditions (mean ± Standard error).

Mean± stander error			Treatments
35 days	**21 days**	**14 days**	
70.35±0.85 b	77.37±6.30	57.45±5.35	T1
66.10±1.25 c	74.51±7.44	56.01±0.46	T2
77.90±1.91 a	77.31±4.46	54.12±1.19	T3
71.28±2.03 b	79.70±4.60	55.06±1.25	T4
*	N.S	N.S	Significant

The means with distinct letters within the same column exhibit significant differences among them (P<0.05) *, NS, not significant. The control treatments, T1, did not receive any treatment. The treatments were supplemented with neem leaf powder T2, T3, and T4, at rates of 200, 400, and 600 mg/kg diet , respectively.

Table 6 shows that at 35 days old, there was a significant decrease (P≤0.05) in the concentration of the AST enzyme in T2, T1, and T4 compared to T3. However, there were no significant differences between the treatments at 14 and 21 days old.

Table 7 demonstrates a statistically significant reduction (P≤0.05) in the concentration of the ALT enzyme at the ages of 14 and 21 days in the addition treatments compared to T1. Additionally, at the age of 35, there was also a significant decrease (P≤0.05) in treatments T3 and T4 compared to T1. Treatment T2 did not show a significant difference compared to the other treatments.

Table 7: The effect of Supplements neem leaf powder (*Azadirachta indica*) on ALT enzyme concentration at the age of 14, 21 and 35 days in broiler chickens under heat stress conditions (mean ± Standard error)

Mean± stander error			Treatments
35 days	**21 days**	**14 days**	
7.14±0..88 a	8.47±0.93 a	6.42±0.12 a	T1
7.05±0.06 ab	4.52±0.67 d	4.55±0.33 c	T2
6.98±0.09 b	7.22±0.61 b	5.87±0.35 b	T3
6.73±0.11 b	6.67±0.02 c	5.71±0.08 b	T4
*	*	*	Significant

The means with distinct letters within the same column exhibit significant differences among them (P<0.05) *, NS, not significant. The control treatments, T1, did not receive any treatment. The treatments were supplemented with neem leaf powder T2, T3, and T4, at rates of 200, 400, and 600 mg/kg diet, respectively.

Monitoring and measuring an animal's physiological, nutritional, and pathological states can be facilitated by examining changes in blood hematological and biochemical metabolites (Webb *et al*., 2022). Research has indicated that birds raised in high temperatures experience a decrease in their physiological and productive abilities (Ohtsu *et al*., 2015). This drop is likely caused by the thermoregulation process triggered by heat stress. Multiple organs and tissues, including the liver, experience both behavioral and physiological harm (Jastrebski *et al*., 2017). The liver plays a crucial role in regulating the body's metabolic equilibrium and serves as a significant hub for antioxidant generation, which consequently reduces resistance to heat stress (Santana *et al*., 2021). Additionally, it leads to an elevation in the secretion of the hormone corticosterone during periods of stress. As a consequence, the growth hormone decreases, while the corticosterone hormone directs the body's energy stores to cope with stress, leading to a decrease in growth (Jastrebski *et al*., 2017).

Heat stress leads to alterations in cellular function due to exposure to elevated temperatures, resulting in heightened mitochondrial activity and subsequent elevation in reactive oxygen species generation. Acute stress causes a rise in the activity of the electron transport chain and the creation of superoxide, resulting in excessive superoxide production that harms the body's proteins. Prolonged heat

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stress can result in cellular damage and the degradation of mitochondria (Akbarian *et al*., 2016). Antioxidants, both in the form of enzymes and non-enzymes, function within a healthy organism to eliminate free radicals and maintain a stable oxidative equilibrium (Akbarian *et al*., 2016).

The elevated levels of cholesterol, triglycerides, observed in the blood of birds treated with control may be attributed to heat stress. The elevated room temperature causes an increase in blood serum cholesterol concentration beyond normal levels, potentially due to a reduction in secretion rate. Birds subjected to elevated temperatures have increased concentrations of thyroid hormones, including thyronine, triiodine, T3, thyroxine, and T4. Reduced thyroid gland function often results in elevated blood cholesterol levels due to a decrease in the rates of both cholesterol production and bile excretion (Al-Jebory *et al*., 2023). Research has shown that heat stress negatively impacts birds physiology by promoting the secretion of catecholamines from the adrenal medulla. These substances elevate blood glucose levels, decrease glycogen levels in the liver, increase respiration rate, dilate blood vessels, and heighten the sensitivity of the neurological system to stress (Siegel and Kempen, 1984; Naga *et al*., 2018). Corticosterone also elevates plasma glucose levels, leading to a notable increase in glucose in the control therapy. Prior studies on hens have also established a correlation between heat stress and cellular oxidative stress (Estévez, 2015; Surai *et al*., 2019). Oxidative stress induces the generation of an excessive amount of free radicals, causing detrimental effects on the cell's DNA, proteins, and lipids. The consequences of oxidative stress are contingent upon a multitude of conditions. Oxidative stress can range in severity, from mild and reversible alterations to severe cases that result in apoptosis and cell death (Lennon *et al*., 1991). The discrepancy in the body's antioxidant system, as described by Estévez (2015), accounts for the decrease in levels of glutathione peroxidase and monoaldehyde in the control therapy. The increase in AST and ALT enzyme activity observed in the T1 treatment may be attributed to the birds' exposure to stress. This increase is likely caused by the elevated corticosterone hormone resulting from acute heat stress experienced by the birds in all treatment groups. The elevated corticosterone hormone affects various liver enzymes, including ALT and AST, leading to an increase in their activity in the blood (Oriordan *et al*., 1982; Richard and Preston, 2006). The specific stimulation of this increase in ALT and AST activity can be attributed to the metabolic requirements for glucose production from non-carbohydrate sources, such as amino acids. These enzymes facilitate the transfer of a group, thereby promoting glucose synthesis. Amines encompass a variety of compounds, including alpha-type amino acids and ketoacids. The latter, being integral to the Krebs cycle, serve as a significant energy source for mitochondrial energy production (Stryer, 2000).

Neem leaves contain bioactive substances that enhance the body's immune system and has therapeutic characteristics that mitigate the impact of heat stress. Stress amplifies programmed cell death, while the active compounds included in neem, along with their antioxidant characteristics, decrease blood sugar levels by 26%. Badam *et al*. (1999), the inclusion of neem in dietary supplements at a concentration of 2.0% has been found to effectively inhibit the activity of free radicals. Neem leaves have been found to decrease oxidation and MDA levels in breast muscles, as demonstrated by Nakamura *et al*. (2022). In addition, they possess anti-inflammatory, antifungal, antibacterial, antiviral, and antimutagenic properties. The immune system is enhanced by these features, as well as the presence of safe bioactive substances. The effectiveness of neem leaf powder in increasing the physiological performance of birds has been demonstrated by Subapriya and Nagini (2005).

Conclusions and Recommendations

Prior research suggests that the use of neem leaf powder, due to its antioxidant capabilities and effects, increases the levels of cholesterol, triglycerides, glucose, glutathione, malondialdehyde, AST, and ALT in rats. To withstand thermal stress. Neem leaf powder may contribute positively to the growth performance and nutrient digestion of birds, hence improving their physiological performance and immunity, especially in conditions of heat stress, the results of the experiment were positive in terms of increasing the bird's ability to bear the burden of thermal stress, which reflects positively on the bird's productive performance and thus increases the profit return of broiler breeding projects, this may clarify the extent of conducting future studies on the use of neem leaves as an additive in feed or the use of aqueous extract, or alcoholic or nano to be added to the water may be more effective than adding neem leaf powder to the feed.

Declarations

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Ethical statement and IRB approval

The study followed the guidelines of the Research Ethics Committee of a College of Agriculture, Al-Qasim Green University, and complied with relevant Iraqi legislations with approved NO. (Agri.No.1.7.22).

Conflict of interest

The authors have declared no conflict of interest.

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