Rovedar

FAHN

Farm Animal Health and Nutrition. 2024; 3(3): 57-63.

DOI: 10.58803/fahn.v3i3.57

http://fahn.rovedar.com/

Research Article

Effects of α-Lipoic Acid Supplementation on Oxidative Indices in Broiler Chickens under Heat Stress

Lawrence Ademu *, and Alour Adum

Department of Animal Science, Federal University Wukari, Wukari, Taraba, Nigeria

* ***Corresponding author:*** Lawrence Ademu, Department of Animal Science, Federal University Wukari, Wukari, Taraba, Nigeria. Email: lawrenceademu@fuwukari.edu.ng

ARTICLE INFO

Article History:
Received: 09/07/2024
Revised: 10/08/2024
Accepted: 29/08/2024
Published: 03/09/2024

check for updates

Keywords:
Antioxidation
α-lipoic acid
Cytokine
Histopathology
Serum biochemistry

ABSTRACT

Introduction: Heat stress has become a significant concern for the global poultry industry as a result of climate change. This condition can adversely affect growth, gut health, immune function, production levels, and reproductive performance, and can lead to lipid peroxidation in poultry. Therefore, this study aimed to evaluate Interleukin 6 and 10 immune responses, anti-oxidant indices, serum biochemistry, and hepatic and splenic histopathology of heat-stressed broiler chickens supplemented with α-lipoic acid (α-LA) in their diet.

Materials and methods: A total of two hundred and four unsexed day-old Arbor acre broiler chicks were randomly divided into four treatment groups and replicated three times to receive α-LA at 0, 50, 100, and 200 mg/kg, respectively in a completely randomized design for 42 days after a one-week adjustment period. The average temperature in the morning and afternoon was 27.9℃ and 35.1℃, respectively, while the morning and afternoon humidity was 73.1% and 44.1% respectively. Feed and water were provided *ad libitum*. At the end of the feeding trial, samples were taken for cytokines (interleukin-6 and 10), anti-oxidant, serum biochemistry, and hepatic and splenic histopathology analyses.

Results: An inverse relationship was observed between pro-inflammatory (IL-6) and anti-inflammatory (IL-10) cytokines whereby, IL-6 levels decreased while IL-10 levels increased relative to increasing α-LA levels in treatment groups. The treatment groups also indicated an increasing trend with rising levels of α-LA for superoxide dismutase, catalase, glutathione, and total antioxidant capacity. The α-LA significantly influenced malondialdehyde production, showing its reduction with rising levels of α-LA. A decrease in serum glucose and low-density lipoproteins was observed with increasing levels of α-LA, while high-density lipoproteins increased with increasing levels of α-LA. Cholesterol, triglycerides, very low-density lipoproteins, aspartate transaminase, alanine transaminase, and alkaline phosphate remained unchanged across the treatment groups.

Conclusion: α-LA supplementation at 200mg/kg in diet had the highest effects on immune responses of interleukin 6 and 10, antioxidant, serum biochemical indices, and histopathology of heat-stressed broiler chickens.

1. Introduction

Environmental stressor factors, such as heat stress which causes lipid peroxidation, poor feed efficiency, immune malfunction, and death are faced by the poultry industry today. Stress has been defined as the sum of all biological reactions to physical, emotional, or mental stimuli that disturb an animal's homeostasis[1]. Heat stress affects biological defense mechanisms, including the immune response in addition to inducing metabolic disorders, leading to a low productivity of chickens[2]. Heat stress leads to the atrophy of immune system organs,

Cite this paper as: Ademu L, and Adum A. Effects of α-Lipoic Acid Supplementation on Oxidative Indices in Broiler Chickens under Heat Stress. Farm Animal Health and Nutrition. 2024; 3(3): 57-63. DOI: 10.58803/fahn.v3i3.57

including the spleen, in broiler chickens. Thus, the function of the immune mechanisms in the spleen might be modified under heat conditions[2]. Consequently, a range of cytokines, which are small essential proteins for immune responses, are produced in the spleen. For example, T helper 2 (Th2) type cytokines (interleukin (IL) -4, IL-10, and IL 13), Th1 type cytokine (interferon (IFN)-γ and IL-18), and pro-inflammatory cytokine (IL-6) are expressed in the chicken spleen[2]. Heat stress caused an increase in lipid peroxides and Reactive Oxygen Species (ROS), and a decrease in antioxidant enzymes in the thymus and spleen of broilers. Heat stress causes oxidative stress to the immune organs of broilers, further reducing immune function[3].

Severe oxidative stress produces excessive ROS, such as superoxide, hydroxyl, and hydrogen peroxide, which may result in damage to DNA, proteins, and other cell injuries[4]. Alpha (α) lipoic acid, especially in recent years, has been used as a food additive, to give beneficial effects in the management and treatment of various types of disorders, such as endothelial dysfunction, atherosclerosis, diabetes mellitus, and degenerative diseases[5]. Alpha lipoic acid (α-LA) also known as thioctic acid (1,2-dithiolane-3-valeric acid) is an organic, sulphate-based compound produced by plants, humans, and animals. As a potent antioxidant and a natural dithiol compound, it performs a crucial role in mitochondrial bio-energetic reactions. α-lipoic acid is both fat and water-soluble and therefore, it can be easily absorbed and transported across cell membranes resulting in optimal nutrient availability[6]. It exists in oxidized as well as reduced forms, characterized by growth-promoting, anti-inflammatory, antioxidative, immunostimulatory, and hypocholesterolaemia properties when fed as a dietary supplement to farm animals, particularly broiler chickens[7]. The present study aimed to investigate the ameliorative properties of α-lipoic acid in mitigating the effects of heat and oxidative stress in broiler chickens.

2. Materials and Methods

2.1. Ethics approval

The animal wellbeing and management practices were approved by the research project committee of the Department of Animal Production and Health, Federal University Wukari, Nigeria, following the regulations of the Nigerian Institute of Animal Science (NIAS).

2.2. Experimental design

The study was conducted at the Poultry Unit of the Teaching and Research Farm, Ibadan, Nigeria. A total of two hundred and four unsexed day-old Abor acre broiler chicks with an average weight, of 50g were purchased from CHI farms in Ibadan, Nigeria for the study. The chicks were randomly divided into four dietary treatments, with each treatment consisting of 51 chickens. Each treatment was replicated three times, resulting in seventeen chickens per replicate, all organized within a completely randomized design. The birds were raised in 4.5 m^2-sized pens on a deep litter system in two phases; starter (weeks 0-4) and finisher (weeks 5-7). A lighting schedule of 18 hours was implemented starting from the second week of the study.

2.3. Experimental diets and management

According to NRC[8], specifications for broiler starters and finishers, four experimental diet starters, including 23%CP, 2834Kcal/kg ME, and finisher, 19% CP, 2952Kcal/kg ME were formulated. The basal diet was supplemented with α-LA at varying levels as follows, 0mg (Basal diet + 0g α-LA, Group 1), 50mg (Basal diet + 50mg α-LA /kg of feed, Group 2), 100mg (Basal diet + 100mg α-LA /kg of feed, Group 3), and 200mg (Basal diet + 200mg α-LA /kg of feed, Group 4). The chickens were provided with diets for 49 days and all standard management practices were strictly adhered to[3].

2.4. Temperature-humidity index determination

Indoor temperature and relative humidity were recorded daily using a digital thermo-hygrometer. The recording was taken in the morning (8.00 am) and afternoon (3.00 pm) throughout the experimental period and used to calculate the morning and afternoon temperature-humidity index (THI) according to Tao and Xin[9].

$$THI = 0.85\ T_{db} + 0.15\ T_{wb}$$

Where, THI = Temperature-humidity index in °C; T_{db}, dry-bulb or ambient temperature in °C and T_{wb}, wet-bulb temperature in °C. According to Stull[10], wet bulb temperature was determined from ambient temperature and relative humidity using the empirical expression function. Heat stress was classified as the absence of heat stress (< 27.8), moderate heat stress (27.8-28.8), severe heat stress (28.9-29.9), and extremely severe heat stress (> 30.0)[11].

2.5. Serum analyses

Two ml of blood was drawn from the jugular vein of two chicks for each replicate, utilizing collecting tubes that did not contain ethylene diamine tetraacetate. Serum parameters, such as serum glucose, triglycerides, cholesterol, High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), and Very Low-Density Lipoprotein (VLDL) were determined through the procedure described by Li et al.[12]. Anti-oxidative indices were determined using commercial kits. The procedure described by Lala et al.[13] was used to determine the liver function, alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP). Cytokine Interleukins 6 and 10 (IL-6 and IL-10) were determined through ELISA kits and using an Immunoassay analyzer.

2.6. Histopathology

On day 49, two chickens were randomly chosen from

each replicate for the evaluation of their liver and spleen. The chicks were sacrificed by severing the jugular vein and eviscerated. Portions were removed from the spleen and liver and then transferred to a tube filled with sterile ice-cold phosphate-buffered saline. Histological analysis was carried out to identify cells involved in inflammation using the procedure described by Gurina and Simms[14].

2.7. Data analyses

The collected data were subjected to analysis of variance (ANOVA) using Fit Y by X function of JMP[15], version 6.12, 2012. Where the result of ANOVA was statistically significant, for multiple comparisons, the Tukey post-test was performed to compare the means of all groups. The level of significance was set at $p < 0.05$. Graphs were prepared using GraphPad Prism, version 6.

3. Results

3.1. Temperature humidity index

Figure 1 demonstrates that the mean THI was higher in the afternoon compared to the morning. The morning and afternoon average room temperatures were 27.9℃ and 35.1℃ respectively, while the morning and afternoon average room humidity were 73.1% and 44.1% respectively. The chickens experienced no heat stress in the morning (23.8) and extremely severe heat stress in the afternoon (33.4).

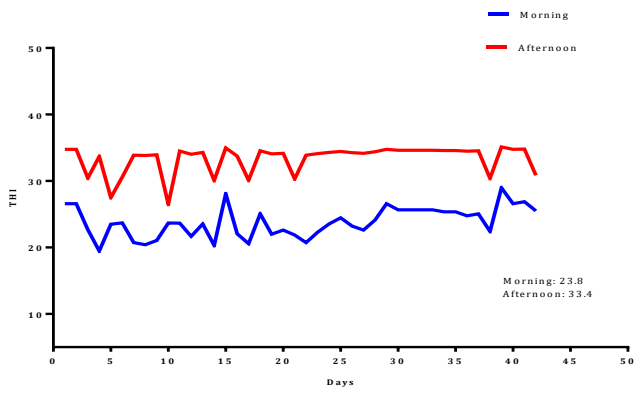

Figure 1. Daily THI inside the poultry house during the feeding trial period

3.2. Cytokines

The administration of α-lipoic acid (α-LA) exhibited significant effects on cytokine responses ($p < 0.05$). Specifically, interleukin 6 (IL-6), a pro-inflammatory cytokine, demonstrated a declining trend in expression as α-LA levels increased. Additionally, there was a significant impact on the anti-inflammatory cytokine interleukin 10 (IL-10, $p < 0.05$). The chickens receiving α-LA displayed elevated levels of IL-10 expression, with notable differences in groups 3 (100 mg) and 4 (200 mg) compared to the control group (Figure 2).

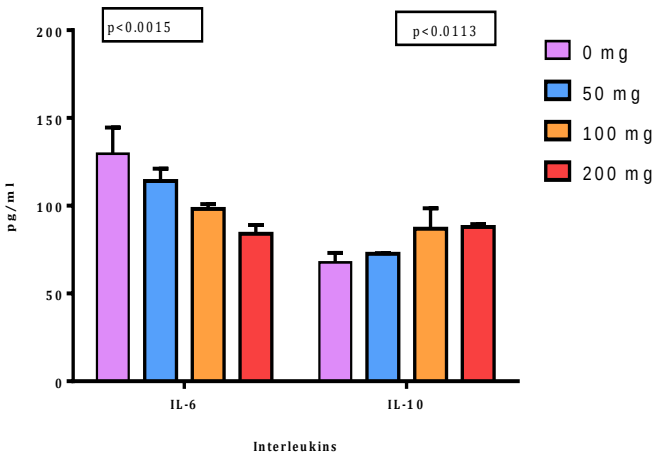

Figure 2. Interleukin levels of heat-stressed chickens fed α-lipoic acid

3.3. Anti-oxidant indices

Anti-oxidant indices showed that malondialdehyde (MDA) levels decreased significantly as α-LA levels increased ($p < 0.05$, Table 1). Chickens receiving 100 and 200 mg of α-LA exhibited similar MDA levels ($p > 0.05$), however, both differed significantly compared to the control group ($p < 0.05$). Superoxide dismutase (SOD) levels increased significantly with higher α-LA doses ($p < 0.05$). Catalase (CAT) and Glutathione (GSH) followed similar trends, with elevated levels observed at 100 and 200 mg of α-LA compared to the control group. Total antioxidant capacity (TAOC) also significantly increased with the rise of α-LA levels ($p < 0.05$).

3.4. Serum biochemical indices

In the investigation of serum biochemical indices, glucose decreased significantly as α-LA levels increased ($p < 0.05$, Table 2). The chickens in the control group and the 50 mg group exhibited similar glucose levels ($p > 0.05$). The HDL levels increased significantly with higher α-LA doses ($p < 0.05$). chickens in the 100 mg and 200 mg groups had the highest HDL levels ($p < 0.05$), while those in the control group had the lowest. The LDL levels decreased significantly with rising α-LA levels ($p < 0.05$). No differences were observed for total cholesterol, triglycerides, and very low-density lipoprotein (VLDL, $p > 0.05$). The AST, ALT, and ALP showed similar levels across the treatment groups ($p > 0.05$).

3.5. Histopathology of liver and spleen

Liver histology of heat-stressed broiler chickens (Figure 3, H and E x250), showed normal histological features for the control group (A) with signs of aggregated inflammatory cells. At 50 mg/kg α-LA (B), there were signs of hepatic degeneration, with minimal inflammatory cell presence. For the 100 mg/kg α-LA (C) group, there were signs of mild infiltration of inflammatory cells. The 200 mg/kg α-LA (D) group showed features of hepatic degeneration, accompanied by a notable presence of

Table 1. Antioxidant indices of heat-stressed broiler chickens fed α-lipoic acid

Parameters	α-Lipoic acid (mg/kg)				SD	P-Value
	0	50	100	200		
MDA (µmol/mg)	18.27[a]	17.15[b]	15.65[bc]	15.16[c]	0.43	0.0002*
SOD(U/mol)	18.25[b]	19.34[b]	24.48[a]	23.79[a]	1.12	0.0013*
CAT (U/mg)	40.24[b]	45.22[ab]	50.16[a]	51.54[a]	1.62	0.0003*
GSH (µg/ml)	22.73[b]	27.31[ab]	29.00[a]	30.30[a]	1.38	0.0052*
TAOC	607.34[c]	673.38[b]	796.80[a]	781.01[ab]	31.15	0.0008*

[a,b,c] Means with difference on the same row differ significantly ($p < 0.05$). SD: Standard deviation, MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, GSH: Glutathione, TAOC: Total antioxidant capacity

inflammatory cells.

Splenic histology (Figure 4, Handa x 100) presented a poorly demarcated marginal zone and follicle in the white pulp in the control group (A), with slight cellular degeneration.

At 50mg/kg α-LA (B), there were few remains of hemorrhagic deposit around the vessel. At 100mg/kg of α-LA (C), severe cellular degeneration of the lymphoid cells. At 200mg/kg α-LA (D), normal splenic histological features of the white and red pulps were observed.

Table 2. Serum biochemistry of heat-stressed broiler chickens fed α-lipoic acid

Parameters	α-Lipoic acid (mg/kg)				SD	P value
	0	50	100	200		
Glucose (mmol/l)	10.75[a]	9.57[a]	7.85[b]	7.82[b]	0.40	0.0001*
Total cholesterol (mmol/l)	3.42	3.47	3.50	3.43	0.09	0.9166
Triglycerides (mmol/l)	0.32	0.35	0.33	0.34	0.04	0.9607
HDL (mmol/l)	0.62[c]	1.50[b]	2.38[a]	2.45[a]	0.07	0.0001*
LDL (mmol/l)	2.37[a]	1.48[b]	0.82[c]	0.48[c]	0.14	0.0001*
VLDL (mmol/l)	0.06	0.07	0.07	0.07	0.01	0.9607
ALP (U/L)	1388.17	1399.17	1344.17	1492.67	74.02	0.5564
AST (U/L)	45.28	44.57	39.60	40.37	2.91	0.4111
ALT (U/L)	145.52	160.98	125.68	155.07	12.56	0.2411

[a,b,c] Means with a difference on the same row differ significantly ($p < 0.05$). SD: Standard deviation, AST: Aspartate transaminase, ALT: Alanine transaminase, ALP: Alkaline phosphate, HDL: High-density lipoproteins, LDL: Low-density lipoproteins, VLDL: Very low-density lipoproteins, mmol/l: Millimoles per liter.

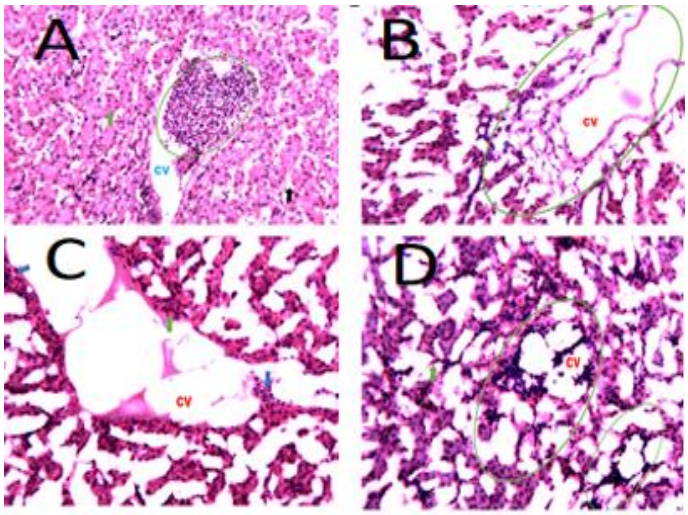

Figure 3. Histopathology of liver section of heat-stressed broiler chickens fed α-lipoic acid. **A:** Normal histological features of hepatocytes (green arrow), sinusoids (black arrow), and central vein (CV) with aggregated inflammatory cells in the portal triads (circle). **B:** Dilated CV and hepatic degeneration with few inflammatory cells (green circle). **C:** Mild infiltration of inflammatory cells (arrows) with severely dilated CV. **D:** Some features of hepatic degeneration (green arrow) with numerous inflammatory cells (circles).

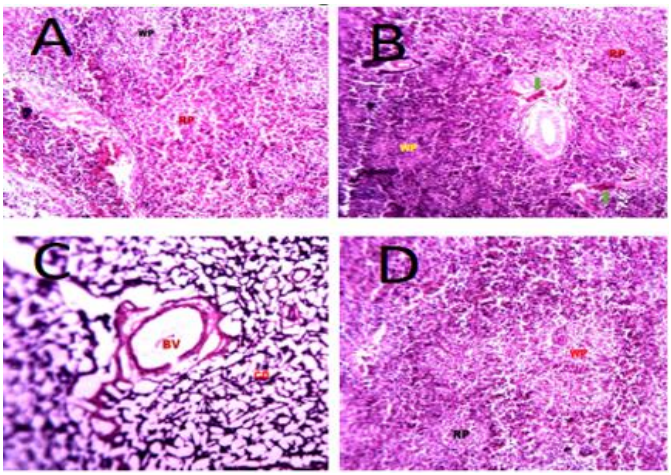

Figure 4. Histopathology of spleen section of heat-stressed broiler chickens fed α-lipoic acid. **A:** Poorly demarcated marginal zone and follicle in the white pulp (WP) with many lymphocytes surrounded by the red pulp (RP) with abundant erythrocytes. Note the appearance of slight cellular degeneration around the dilated blood vessels (circle). **B:** Few remains of hemorrhagic deposit (arrows) around the vessel (circle). There is poor demarcation of the marginal zone and the follicle of the WP and the RP. **C:** No clear demarcation of the marginal zone and the follicle in the WP. **D:** Normal splenic histological features of the WP and RPs with poor demarcation of the marginal zone and the follicle of the WP.

4. Discussion

The immune response to pathogens entails the swift activation of pro-inflammatory cytokines, which play a crucial role in initiating the host's defense mechanisms against microbial invasion. However, excess inflammation can raise the systemic metabolic and harmful hemodynamic disturbances to the host. As a result, the immune system has evolved parallel anti-inflammatory mechanisms that serve to curb the production of pro-inflammatory molecules to limit tissue damage and to maintain or restore tissue homeostasis. The IL-10 is a

cytokine characterized by its strong anti-inflammatory effects, which are crucial in regulating the host's immune response to pathogens. This regulation helps to avert potential harm to the host and ensures the preservation of normal tissue homeostasis[16]. The IL-6 promptly and transiently produces in response to infections and tissue injuries. It contributes to host defense through the stimulation of acute phase responses, hematopoiesis, and immune reactions[17]. There was an inverse relationship between IL-6 expression and levels of α-LA with a direct relationship between IL-10 expression and α-LA levels in the current study. The IL-6 expression in broiler chickens has been reported by Li et al.[12] to increase when exposed to an aflatoxin-contaminated diet. The α-LA alleviated aflatoxin-induced oxidative stress and immune changes and modulated the inflammatory response[12]. Heat stress is known to inhibit IL-10 production[3] and advocate the IL-6 production, due to injury at the sites causing inflammation. The α-LA supplementation promoted IL-10 expression, preventing inflammation in broiler chickens which agrees with previous studies[7,18].

The MDA is a stable end product of lipid peroxidation and therefore can be used as an indirect measure of cumulative lipid peroxidation[19]. The α-LA demonstrated the capability to alleviate the impacts of heat stress that intensify lipid peroxidation. Heat stress has been shown to raise MDA levels[3,20]. The enzymatic antioxidant defense system is greatly associated with the immune system, reflecting the health status of animals. The first detoxification enzyme and the most powerful antioxidant in the cell is SOD[4]. The CAT is a common antioxidant enzyme with the highest turnover rate. It is present in living tissues and is a key clinical enzyme involved in the breakdown of hydrogen peroxide to water and molecular oxygen[21]. The elevation of α-LA correlates with an increase in CAT activity, indicating that α-LA effectively mitigates stress in broiler chickens while enhancing SOD levels. In a study by El-Rayes[22], remarkable improvements in plasma oxidative statuses, including TAOC, SOD, GSH-px, and MDA were observed in broiler chicks after a 42-day feeding period. Similarly, the SOD level was significantly increased in broiler chickens, when lipoic acid was supplemented at 900 ppm[23].

Serum biochemistry parameters are particular indicators of the physiological response as well as the general health condition of animals. When animals are under stress, glucose levels rise[24], inhibiting insulin production[25] hence conversion of excess glucose to glycogen is prevented. In the context of chronic stress, physiological stress leads to an increase in blood glucose levels, surpassed only by corticosterone[26]. The α-LA supplementation proved ameliorative in combating heat stress which reduced excess circulating glucose in the present study. By aiding the production of insulin which controls serum glucose levels in broiler chickens this makes α-LA a potent anti-diabetic[18]. Elevated levels of HDL cholesterol are associated with a reduced risk of heart disease and stroke[27]. Conversely, LDL cholesterol, that referred to as "bad" cholesterol, constitutes the majority of the body's cholesterol. When present at elevated levels, it has the potential to accumulate on the walls of blood vessels. It can cause health problems, such as heart disease and stroke[28]. Hypocholesterolaemia properties of α-LA were observed as its supplementation increased HDL and reduced LDL, showing its potency to prevent “plaque” and lipid-related cardiovascular diseases in broiler chickens.

Lu et al.[29] have reported that heat stress causes liver fat accumulation and inflammation, and impaired liver function in broilers. The incorporation of α-LA effectively mitigated heat stress, thereby inhibiting the oxidation and inflammation of hepatocytes and the portal triads, demonstrating the anti-inflammatory properties of α-LA[18]. Broilers exposed to heat stress for 1 or 2 weeks were reported to show necrotic points on the liver surface[30]. Liver histological changes indicated heat stress-induced inflammatory infiltration in the liver of heat-stressed broilers. The obtained results indicated that heat stress impaired liver growth and induced liver injury in the control while α-LA was able to combat the effect of stress in the 50 and 100 mg/kg α-LA.

The poorly demarcated marginal zone and follicle in the white pulp in 50mg/kg α-LA and the control indicate abnormality as a result of heat stress[31] which may lead to immune disorder because it may affect lymphoid tissues and lymphocyte production[32]. The appearance of slight cellular degeneration around the dilated blood vessels in the control and also poor demarcation of the marginal zone and the follicle of the red pulp in the 50mg/kg α-LA also show abnormal structure of the spleen. This is also associated with heat stress. The function of the immune mechanisms in the spleen might be modified under heat conditions[2]. Group control displayed the histological structure of a normal spleen’s white and red pulp that showed the proven effects of α-LA on adaptive immune cells.

5. Conclusion

The study demonstrated that dietary supplementation with α-lipoic acid (α-LA) up to 200mg/kg had a positive impact on various aspects of heat-stressed broiler chickens. Specifically, α-LA influenced immune responses (interleukin 6 and 10) which are crucial in maintaining immune homeostasis, improving antioxidant indices, modulating serum biochemical parameters, and affecting hepatic and splenic histopathology. The study findings underscore the potential of α-LA as a valuable nutritional strategy to mitigate the adverse effects of heat and oxidative stress in poultry. It is suggested further studies elucidate the signalling mechanisms underlying α-LA’s potential impact on other aspects of poultry health and productivity.

Declarations

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

The work was carried out in collaboration with the authors. Adum Aluor handled data collection and literature and wrote the original draft. Ademu Lawrence designed the methodology and managed the analyses of the study. Both authors read and approved the final draft of the manuscript.

Authors' relationships and activities

All authors are responsible for disclosing all relationships and activities that might bias or be seen to bias their work.

Funding

This study received no external funding.

Availability of data and materials

All used data and materials are available by reasonable request from authors.

Ethical considerations

The authors declare that this manuscript is original and is not being considered elsewhere for publication. Other ethical issues, including consent to publish, misconduct, fabrication of data, and redundancy, have been checked by the authors.

Acknowledgments

The authors thank everyone who helped in this study.

References

1. Asres A, and Amha N. Effect of stress on animal health: A review. J Biol Agric Healthc. 2014; 4(27): 116-122. Available at: https://core.ac.uk/download/pdf/234660471.pdf
2. Haruhiko O, Yamazaki M, Abe H, Murakami H, and Toyomizu M. Heat stress modulates cytokine gene expression in the spleen of broiler chickens. J Poult Sci. 2015; 52: 282-287. DOI: 10.2141/jpsa.0150062
3. Wang Y, Yang X, Li S, Wu Q, Gou H, Wang H, et al. Heat stress affects immune and oxidative stress indices of the immune organs of broilers by changing the expression of adenosine triphosphate-binding cassette subfamily G member 2, sodium-dependent vitamin C transporter-2 and mitochondrial calcium uniporter. J Poult Sci. 2023; 102(8): 102814. DOI: 10.1016/j.psj.2023.102814
4. Ighodaroab OM, and Akinloye OA. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria J Med. 2018; 54: 287-293. DOI: 10.1016/j.ajme.2017.09.001
5. El Barky AR, Hussein SA, and Mohamed TM. The potent antioxidant alpha lipoic acid. J Plant Chem Ecophysiol. 2017; 2(1): 1016. Available at: https://austinpublishinggroup.com/jpceonline/fulltext/jpce-v2-id1016.php
6. Bustamante J, Lodge JK, Marcocci L, Tritschler HJ, Packer L, and Rihn BH. α-Lipoic acid in liver metabolism and disease. Free Radic Biol Med. 1998; 24: 1023-1039. DOI: 10.1016/S0891-5849(97)00371-7
7. Sohaib M, Anjum FM, Nasir M, Arshad MS, and Hussain S. Alpha-lipoic acid: An inimitable feed supplement for poultry nutrition. J Anim Physiol Anim Nutr. 2018; 102: 33-40. DOI: 10.1111/jpn.12693
8. National research council (NRC). Nutrient requirements of poultry. 9th Revised ed. Washington, DC, USA: National Academic Press; 1994. Available at: https://www.agropustaka.id/wp-content/uploads/2020/04/agropustaka.id_buku_Nutrient-Requirements-of-Poultry_Ninth-Revised-Edition-1994-NRC.pdf
9. Tao X, and Xin H. Acute synergistic effects of air temperature, humidity and velocity on homeostasis of market-size broilers. Trans ASAE. 2003; 46(2): 491-497. DOI: 10.13031/2013.12971
10. Stull R. Wet-bulb temperature from relative humidity and air temperature. J Appl Meteorol Climatol. 2011; 50: 2267-2269. DOI: 10.1175/JAMC-D-11-0143.1
11. Marai IFM, Ayyat MS, and El-Monem UM. Growth performance and reproductive traits at first parity of New Zealand White female rabbits as affected by heat stress and its alleviation under Egyptian conditions. Trop Anim Health Prod. 2001; 33: 1-12. DOI: 10.1023/A:1012772311177
12. Li Y, Ma Q, Zhao L, Wei H, Duan G, Zhang J, and Ji C. Effects of lipoic acid on immune function, the antioxidant defense system, and inflammation-related genes expression of broiler chickens fed aflatoxin contaminated diets. J Mol Sci. 2014; 15: 5649-5662. DOI: 10.3390/ijms15045649
13. Lala V, Zubair M, and Minter DA. Liver function tests. Treasure Island (FL): StatPearls Publishing; 2023. PMID: 29494096
14. Gurina TS, and Simms L. Histology, staining. Treasure Island (FL): StatPearls Publishing; 2023. PMID: 32491595
15. JMP® software, version 6.12 of the SAS System for Windows. SAS Institute, Inc., Cary, NC, USA. 2012.
16. Chan IH, Wu V, Bilardello M, Mar E, Oft M, Van Vlasselaer P, et al. The Potentiation of IFN-γ and induction of cytotoxic proteins by pegylated IL-10 in human CD8 T cells. J Interferon Cytokine Res. 2015; 35(12): 948-955. DOI: 10.1089/jir.2014.0221
17. Tanaka T, Narazaki M, and Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol. 2014; 6(10): a016295. DOI: 10.1101/cshperspect.a016295
18. Tripathi AT, Anup KR, Mishra SK, Bishen SM, Mishra H, and Khurana A. Molecular and therapeutic insights of alpha-lipoic acid as a potential molecule for disease prevention. Rev Bras Farmacogn. 2023; 33: 272-287. DOI: 10.1007/s43450-023-00370-1
19. Masia M, Padilla S, Fernandez M, Rodriguez C, Moreno A, Oteo JA, et al. Oxidative stress predicts all-cause mortality in HIV-infected patients. PLoS One. 2016; 11: e0153456. DOI: 10.1371/journal.pone.0153456
20. Murali P, and Sherin GK. Supplementation of alpha lipoic acid on serum biochemical, minerals and antioxidant status in broiler chicken fed diet with animal fat. J Entomol Zool Stud. 2020; 8(4): 1622-1626. Available at: https://www.entomoljournal.com/archives/2020/vol8issue4/PartZ/8-3-327-933.pdf
21. Mahomoodally MF, Désiré D, and Rosette EM. Catalase. Antioxidants effects in health. 2022, p. 81-97. DOI: 10.1016/B978-0-12-819096-8.00022-7
22. El-Rayes TK. Assessment of the effect of α-lipoic acid supplementation to the diet with different dietary energy levels on broiler performance and antioxidants status. Egyptian J Nut Feed. 2020; 23(3): 515-526. DOI: 10.21608/ejnf.2020.148160
23. Zhang Y, Hongtrakul K, Ji C, Liu L, Hu X, and Ma Q. Effects of dietary alpha-lipoic acid on anti-oxidative ability and meat quality in Abor acre broilers. Asian-Australian J Sci. 2009; 22(8): 1195-1201. DOI: 10.5713/ajas.2009.90101
24. Qu Q, Li H, Bai L, Zhang S, Sun J, Weijie L, et al. Effects of heat stress on gut microbiome in rats. Indian J Microbiol. 2021; 8(3): 221-229. DOI: 10.1007/s12088-021-00948-0
25. Starzec JJ, and Berger DF. Stress and age-related serum glucose changes in Spontaneously Hypertensive and Sprague-Dawley rats. Bullet Psychonomic Soc. 1986; 24(3): 222-224. DOI: 10.3758/BF03330555
26. Lin HS, Sui HC, Jiao KJ, Zhao JP, and Dong H. Effects of diet and stress mimicked by corticosterone administration on early postmortem muscle metabolism of broiler chickens. Poult Sci. 2007; 86: 545-554. DOI: 10.1093/ps/86.3.545
27. Pappan N, Awosika AO, Rehman A. Dyslipidemia. StatPearls (Internet). Treasure Island (FL): StatPearls Publishing, 2024. Available at: www.ncbi.nlm.nih.gov/books/NBK560891/
28. Gelatt C. Understanding cholesterol: HDL, LDL, and triglyceride. Healthlines. Riverview health blog. 2017. Available at:

https://riverview.org/blog/nutrition-2/understanding-cholesterol-hdl-ldl-and-triglycerides/
29. Lu Z, He XF, Ma BB, Zhang L, Li JL, and Jiang Y. Increased fat synthesis and limited apolipoprotein B causes lipid accumulation in the liver of broiler chickens exposed to chronic heat stress. Poult Sci. 2019; 98: 3695-3704. DOI: 10.3382/ps/pez056
30. Tang L, Lui Y, Zhang J, Ding K, Lu M, and He Y. Heat stress in broilers of liver injury effects of heat stress on oxidative stress and autophagy in liver of broilers. Poult Sci. 2022; 101(10): 102085. DIO: 10.1016/j.psj.2022.102085
31. Elsaadawi HA, Kama TK, and Khalaf AA. Dietary alpha-lipoic acid effects on the mitigation of the negative impact of heat stress in broilers. Zagazig Vet J. 2022; 12 (4): 312-322. DOI: 10.21608/zvjz.2022.156750.1188
32. Roushdy EM, Zaglool AW, and El-Tarabany MS. Effects of chronic thermal stress on growth performance, carcass traits, antioxidant indices and the expression of HSP70, growth hormone and superoxide dismutase genes in two broiler strains. J Therm Biol. 2018; 74: 337-343. DOI: 10.1016/j.jtherbio.2018.04.009