



INFLUENCE OF ASCORBIC ACID ON PHYSIOLOGICAL INDICES OF BROILER CHICKEN DURING DRY SEASON

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ABSTRACT

The study evaluated the physiological response of broiler birds to different levels of Ascorbic Acid (AA) supplementation during the dry season. 320 one-day-old Ross 308 broiler chicks were allotted randomly to four dietary treatments groups T1 (Control), T2 (50), T3 (100) and T4 (150 mg AA/kg diet) in a completely randomized design. The experiment lasted for 8 weeks during which data on growth parameters, body morphometry, haematology, serum biochemistry and other physiological parameters were collected. Findings from the study revealed that, body weight gain and feed intake were highest in T4, indicating that AA significantly ($p < 0.05$) elicited improvement in the growth performance parameters. Collagen synthesis of AA was also observed as there was an improved ($p < 0.05$) thigh and shank lengths in birds exposed to AA. Haematological analysis indicated that, AA induced erythropoiesis as evidenced by significant ($p < 0.05$) increase in red blood cell counts from T1 ($15.12 \times 10^6 \text{ cell}/\mu\text{L}$) to T4 ($27.57 \times 10^6 \text{ cell}/\mu\text{L}$), with a decrease in physiological leukocytosis often related to tissue damage by heat. The AA proved non-hepatotoxic as serum biochemical indices were within safe ranges. By week 8, there was a significant reduction ($p < 0.05$) in rectal temperatures from 42.37°C in the control group to 40.01°C in T4. Significant reduction in respiratory rates were observed with birds in T1 having 59.59 breath/min, while those T4 had 53.41 breath/min suggesting that AA induced lower thermal polypnea and inhibition of respiratory alkalosis. Conclusively, incorporation of AA up to 150 mg/kg efficiently reduced the adverse effects of thermal stress, while maintaining homeothermy and promoting the overall performance and welfare of broiler chickens during the hot season.

Keywords: Heat, Stress, Ascorbic-acid, Broiler, Chicken, Thermoregulation

INTRODUCTION

The world poultry sector remains a crucial industry of the agricultural economy that provides premium and affordable source of animal protein to a increasing world population. In tropical savannah zones of Africa, however, the sector is bedeviled by intense environmental restrictions, majorly as a result of the severe ambient temperatures and humidity figures usually experienced during the dry season. Genetically selected for quick growth and high efficiency of metabolism, broiler chickens are particularly vulnerable to thermal distress (Oni *et al.*, 2024). Generally, chickens lack sweat glands and must therefore sustain homeothermy through evaporative cooling by panting. Whenever environmental temperatures transcend the thermoneutral zone (usually 18 to 24°C for mature broilers), the chickens begin to experience heat stress, capable of inducing several hazardous physiological responses (Sumanuet *et al.*, 2024).

During the harmattan season in Nigeria, ambient temperatures, most times, range between 32 and 35°C or more, which significantly exceed the tolerance level broiler chickens (Jimoh *et al.*, 2022). This discomfort occasioned by a surge in environmental temperature often leads to considerable economic losses resulting from decreased feed intake, reduced growth rates, defective feed conversion ratios (FCR) and elevated mortality rates (Tavakolinasab & Hashemi, 2025). In addition, the physiological stress triggered by increase in ambient temperatures disturbs the homeostasis of the chickens, making interventions necessary both nutritionally and pharmacologically to maintain a good level of productivity and welfare of the birds.



The physiological reaction of broiler chickens to heat stress is one that is characterized by intricate changes in metabolic, hematological and biochemical pathways. In order to dispel excess metabolic heat, birds result to panting, a process that can induce unrestricted loss of carbon dioxide (CO₂) via the lungs. This exhaustion of CO₂ triggers an increase in blood pH level (respiratory alkalosis) (Oni *et al.*, 2024). In order to cope with this sudden change, there will be a rise in the excretion of bicarbonate ions by the kidneys. This process is capable of causing a further disruption in the acid-base balance of the birds (Sumanuet *et al.*, 2024).

Even at the cellular level, heat stress challenge causes excessive secretion of Reactive Oxygen Species (ROS) like superoxide anion and hydroxyl radical. Under normal conditions, endogenous antioxidant enzymes like superoxide dismutase and glutathione peroxidase neutralize these radicals. However, during the dry season when the heat stress becomes acute and chronic, the ROS production rate exceeds the chickens' natural antioxidant capacity, leading to oxidative stress (Tavakolinasab& Hashemi, 2025). This oxidative state destroys the DNA and macronutrients in cells, including lipids and protein thereby compromising the integrity of the gut and immune function (Hieu *et al.*, 2022).

Ascorbic acid, also known as vitamin C, has proven to be an efficient, water-soluble antioxidant capable of protecting biological systems from oxidative destructions. Although chickens can endogenously produce ascorbic acid in the liver and kidneys, this synthesis is often inadequate, such that it cannot meet the increased demands when environmental stress is being experienced (Hieu *et al.*, 2022). Thus, incorporating ascorbic acid in the diet or drinking water has now surfaced as a good strategy to promote the resilience of broilers to heat stress during the dry season. (Li *et al.*, 2024).

Ascorbic acid's mode of action revolves round its capacity as a strong reducing agent. Through the donating electrons to unstable ROS, ascorbic acid prevents the commencement of lipid peroxidation in cell membranes (Hieu *et al.*, 2022). Furthermore, ascorbic acid is popular for regulating the hypothalamic-pituitary-adrenal (HPA) axis, decreasing the production of corticosterone (the major stress hormone in poultry) which otherwise represses immune responses and enhances muscle catabolism (Sumanuet *et al.*, 2024). Recent study revealed that inclusion level up to 250 mg/kg can considerably lead to an improvement in body weight gain and reduction in the heterophil-to-lymphocyte (H/L) ratio, which is an indicator of physiological stress in poultry (Tavakolinasab& Hashemi, 2025).

With all the numerous studies conducted on heat stress, the actual "physiological response of broiler chickens to ascorbic acid during the dry season" still requires investigation partly because of volatility of the environment which has made indigenous heat stress management systems less effective, and partly due to the need for economic sustainability. This study therefore aims to achieve the following objectives:

- a) To generate precise data on how the birds respond to exogenous antioxidants during hot season.
- b) To confirm ascorbic acid as an easily administered supplement that can make production cycles stable during heat stress.
- c) Identify the optimum dosage of ascorbic acid that can improve the quality of life of the chickens.

MATERIALS AND METHODS

Experimental Site and Duration

The study was conducted at the Poultry Unit of Teaching and Research Farm, University of Ilesa, Nigeria. The site is characterized by a tropical climate with an extensive dry season, usually from October/November to March/April, when ambient temperatures are often greater than 33°C and relative humidity ranges between 30 and 60% (Weather Spark, 2025). The experiment lasted for a period of 8 weeks for both the brooding and finishing phases.

Experimental Birds and Management

A total of 320 one-day-old broiler chicks (Ross 308 strain) were purchased from a reputable hatchery. On arrival, the weights of the chicks were taken and recorded before they were randomly assigned to four treatment groups. The chicks were housed using a deep litter system with the floor covered by wood shavings. Clean water and a nutritionally balanced commercial starter (for the first 4 weeks) and finisher (for the final 4 weeks) mash were made available *ad libitum*.



Experimental Design and Treatments

The experiment employed a completely randomized design (CRD) where the birds were assigned randomly to four treatment groups T1, T2, T3 and T4 in. Each treatment group was replicated 4 times, with 20 birds per replicate. The ascorbic acid (AA) was administered as follows:

T1(Control): Basal diet + 0 mg/kg.

T2: Basal diet + 50 mgAA/kg

T3: Basal diet + 100 mgAA/kg

T4: Basal diet + 150 mgAA/kg

Data Collection

Ambient temperature and relative humidity within the pen were recorded thrice daily (08:00 a.m., 1:00 p.m. and 6:00 p.m. with the aid of a digital thermo-hygrometer. Records of body weight gain (BWG), body length, thigh length, wing length, breast girth, leg length and feed intake (FI) were taken weekly while the Feed Conversion Ratio (FCR) was obtained by finding the ratio of feed intake to weight gain. Monitoring of rectal temperature and respiration rate (panting) was done weekly at 2:00 p.m. when the hotness for each day was at the peak. At the 8th week, five birds per replicate were randomly selected for blood collection via the wing vein.

Blood Sample Collection

At the end of the feeding trial (Day 56), nine birds per treatment were randomly chosen for blood sampling. Approximately 5 ml of blood was drawn with the aid of sterile syringes and 23G needles from the brachial (wing) vein. Out of the 5 ml, 2 ml (Haematological sample) was transferred into blood tubes containing Ethylenediamine tetra-acetic acid (EDTA) so as to prevent coagulation; while the remaining 3 mL was released into plain tubes for biochemical, enzymatic and hormonal assays.

Statistical Analysis

Data collected were subjected to a one-way Analysis of Variance (ANOVA) using 2007 version of Minitab statistical software. Significant differences between treatment means were separated using Duncan's Multiple Range Test at $p < 0.05$ significance level.

Table 1: Experimental Diets (g/100kg)

Ingredients	Starter	Finisher
Corn	51.50	56.65
Soyabean Meal	21.50	13.96
Groundnut cake	10.75	17.00
Fish meal	5.50	4.50
Dicalcium Phosphate	4.50	0.20
Limestone	1.45	1.95
Corn bran	4.00	5.04
Vitamin-Mineral premix	0.20	0.20
Methionine	0.20	0.15
Lysine	0.20	0.10
Salt	0.20	0.25
Total	100	100
Calculated Nutrients		
Energy (MEKcal/kg)	3,000	3,100
Crude protein (%)	23.00	20.00
Crude fibre (%)	5.00	8.00

RESULTS AND DISCUSSION

Result of the effect of AA on growth performance of broiler chickens is presented in Table 2. The result revealed a linear and significant decrease in daily weight gain with the highest weight gain observed in T4. At high environmental temperatures which characterize the dry season, the release of corticosterone is often induced causing the breakdown of muscles. However, the significant increase in



the body weight gain at 150 mg/kg indicates that AA restricts the stress response successfully, thereby making it possible for the chickens to use the energy for accumulation of muscle tissues (Abidin, & Khatoon, 2013; Biswas *et al.*, 2024). A significant rise in feed intake was observed as the inclusion level of AA rose. The highest feed intake recorded in T4 suggests that AA, being an antioxidant, assisted the chickens to sustain their appetite by mitigating the distress and reducing the metabolic heat load (Albokhadaim *et al.*, 2019). Discomfort that arises from heat stress often triggers anorexia in birds, causing the birds to consume less feed in order to lower the heat generated during the process of metabolism (Teyssier *et al.*, 2022). Furthermore, an improved FCR was observed though not significant. This implies that AA improved the digestive system of the chickens, making them to efficiently convert feed into meat. This may be due to the fact that AA inhibited the occurrence of oxidative damage on the intestinal villi.

Table 2: Growth Performance of Broiler Chickens Exposed to Ascorbic Acid

Parameters	Ascorbic Acid Levels				SEM
	T1	T2	T3	T4	
Initial Live Wt. (g)	246.11	246.11	241.32	243.20	2.17
Final Live Wt. (g)	2257.30	2257.30	2123.2	2241.57	.46
Feed Intake (g/bird/day)	103.21 ^b	103.21 ^b	103.78 ^b	106.67 ^c	0.02
Body Weight Gain (g/bird/day)	31.45 ^a	31.45 ^a	33.81 ^b	36.58 ^c	0.01
Feed Conversion Ratio	3.42	3.42	3.15	3.11	0.21

The somatometric assessment of the birds is presented in Table 3, revealing the linear body measurements from the 5th week to the 8th week. At weeks 7 and 8, the birds exposed to AA showed superior body weight when compared to the control. This implies that AA supplementation enhanced protein sparing effect thereby conserving the muscles (Al-Khalaifah *et al.*, 2025). The bone strength (skeletal integrity) is significantly elevated by Vit C. as observed in the Thigh Length and Shank Length. AA is essential for the formation of collagen (Murad *et al.* 1981), an essential cofactor for proly hydroxylase which is an enzyme needed for the production of hydroxyproline. Furthermore, the significant elevation in the mean values of Thigh Length at week 8 is an indication that osteoblastic action was enhanced and that, collagen cross-linking, a concept that ensures a strong framework for the attachment of muscles, was equally increased, thereby mitigating bone deformities that usually accompany rapid growth of broilers (Al-Khalaifah *et al.*, 2025).

Breast girth is regarded as an evidence of expansion of the muscle at the Pectoralis as well as an indication of the general meat yield. The observed increases in the breast girth of birds placed on diets supplemented with AA show that the AA mitigated the effect of Reactive Oxygen Species (ROS) produced as a result of exposure to high ambient temperatures by counteracting the activities of the radical, thereby conserving the structures of the muscle fibers and enhancing breast girth development (Barrio *et al.*, 2020; Al-Khalaifah *et al.*, 2025).

Table 3: Effect of Ascorbic Acid Supplementation on the Length of Primal Cuts of Broiler Chicken

Week	Treatment	Body Weight (g)	Body Length (cm)	Thigh Length (cm)	Wing Length (cm)	Breast Girth (cm)	Shank Length (cm)
5	T1	1240.31	30.28	10.35	8.33	10.14	7.20
	T2	1222.41	30.90	10.85	8.14	10.65	7.23
	T3	1212.27	30.28	10.55	8.41	11.00	7.33
	T4	1241.71	30.33	10.22	9.01	11.11	7.03
	SEM	12.49	2.72	1.58	1.67	1.16	1.62
6	T1	1600.18	34.37	11.34	9.44	10.66	8.38 ^a
	T2	1646.33	33.35	11.38	9.40	10.91	8.20 ^b
	T3	1644.38	33.24	11.40	9.36	11.98	8.55 ^b
	T4	1621.01	34.36	11.33	9.79	11.80	8.01 ^b
	SEM	13.41	2.80	1.40	1.15	1.24	1.34
7	T1	2081.72	36.20	12.24	10.28	11.90	9.71
	T2	2092.42	36.72	12.84	10.49	11.84	9.26
	T3	2189.58	36.60	12.23	10.31	12.48	9.52
	T4	2172.53	36.49	12.24	10.36	12.79	9.58
	SEM	12.39	2.82	1.18	1.17	1.16	0.27
8	T1	2201.23	37.82	13.41 ^c	12.51	12.83	10.23
	T2	2257.30	36.45	13.01 ^d	12.35	12.08	9.24
	T3	2123.21	37.44	12.93 ^b	12.33	13.25	9.14
	T4	2241.57	36.99	13.48 ^a	12.17	13.08	8.51
	SEM	13.46	0.83	1.02	1.16	1.21	0.06

The haematological profile of the birds is presented in Table 4. The mean values of the PCV showed significant modulations where the highest values (38.41% and 38.08% respectively) were recorded in T1 and T4. The elevated PCV in the control group shows hemoconcentration in the birds due to dehydration. In an attempt to dissipate heat through evaporative cooling, the birds pant (hyperventilate) at an increasing rate, leading to loss of considerable plasma volume (Ahmad *et al.* 2022) and an increase in the amount of cellular constituents (Oni *et al.*, 2024). On the other hand, increased PCV in T4 may be indicative of erythrocyte accumulation and not dehydration since AA facilitates the absorption of non-heme iron (Fe³⁺) from the GIT while preserving it in a reduced form (Fe²⁺) that is crucial for the production of heme (Cook & Reddy, 2001; Panet *al.*, 2024; Tavakolinashab & Hashemi, 2025).

There was a linear and significant increase in the RBC counts, from 15.12 in the control to 27.57 x10⁶cell/μL in T4. This significant rise indicates that ascorbic acid appears to trigger erythropoiesis in the bone marrow, thereby enhancing the capacity of the blood to carry oxygen so as to satisfy the metabolic requirements for growth during heat stress (Hassan & Asim, 2020).

Moreover, a significant reduction was found in the WBC with the highest value recorded in the control group, implying that the birds in the control group must have suffered physiological leukocytosis as a reaction to tissue destruction induced by thermal stress challenge (Sumanuet *al.*, 2024). This result however does not align with the findings of Unigweet *al.* (2022) who reported a significant increase.

Table 4: Effect of Ascorbic Acid Supplementation on Haematological Parameters

Treatment	PCV (%)	RBC ($\times 10^6$ cell/ μ L)	WBC ($\times 10^3$ cell/ μ L)	Hb (g/dL)	MCV (fL)	MCH (pg/cell)	MCHC (g/dL)
1	38.41 ^a	15.12 ^a	14.50 ^a	8.40	30.00	32.00	38.51
2	34.50 ^c	20.10 ^b	12.56 ^c	9.47	30.00	33.70	34.20
3	36.29 ^b	21.50 ^b	12.66 ^c	9.81	31.05	33.60	34.33
4	38.08 ^a	27.57 ^c	11.55 ^b	9.33	35.63	32.15	39.02
SEM	1.03	0.82	0.02	0.02	1.00	0.04	0.06

PCV = Packed Cell Volume; RBC = Red Blood Cell; WBC = White Blood Cell; Hb = Hemoglobin; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Hemoglobin; MCHC = Mean

Corpuscular Hemoglobin Concentration

Moreover, a significant reduction was found in the WBC with the highest value recorded in the control group, implying that the birds in the control group must have suffered physiological leukocytosis as a reaction to tissue destruction induced by thermal stress challenge (Sumanuet *et al.*, 2024). This result however does not align with the findings of Unigweet *et al.* (2022) who reported a significant increase.

The significant reduction observed in the albumin may be indicative of metabolic re-partitioning especially with an increase found in the TAG and Cholesterol. AA is an efficient antioxidant which poses an influence on the rate of hydroxylation reactions. This implies that the liver might have shifted its synthetic preference to lipid metabolism rather than synthesis of protein (Bhootet *et al.*, 2023). Furthermore, a change in the immune-globulin component could be the explanation for the decreases observed in the globulin mean values.

Table 5: Effect of Ascorbic Acid Supplementation on Serum Biochemical Parameters

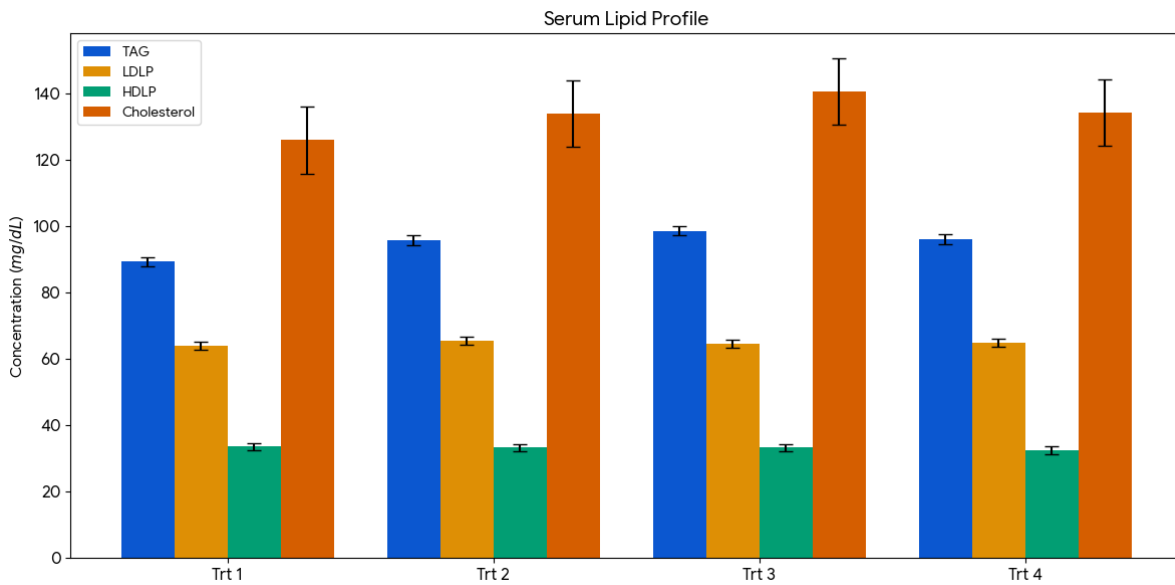
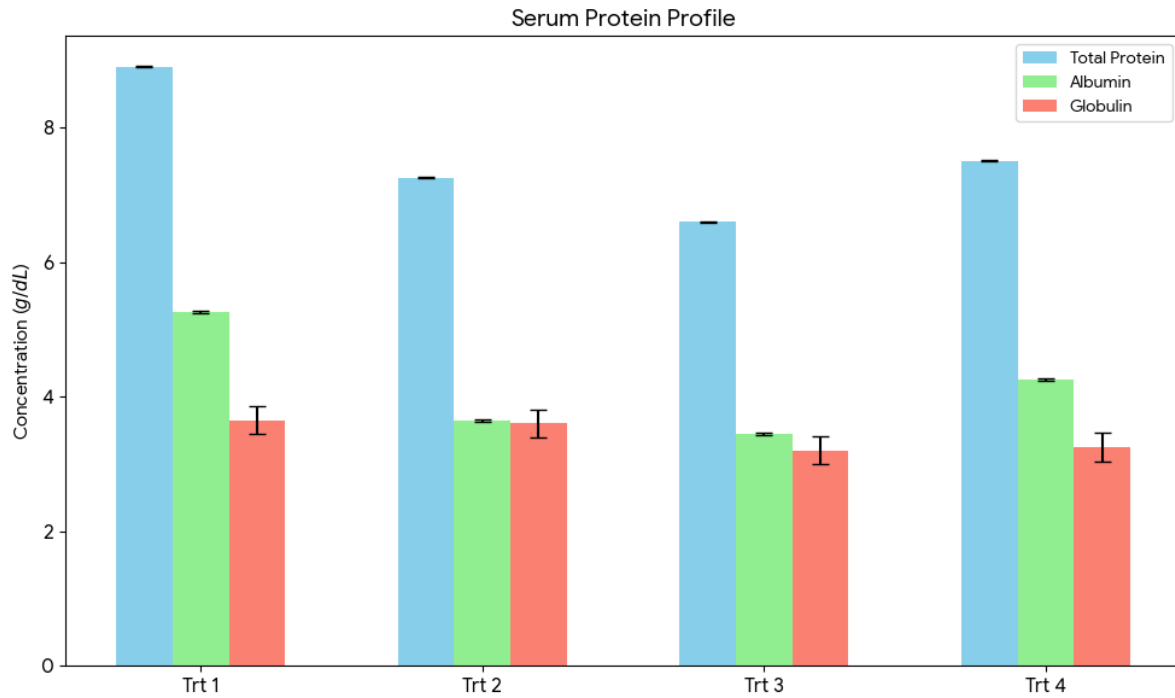
Trt.	T.P (g/dL)	ALB (g/dL)	GB (g/dL)	TAG (mg/dL)	LDLP (mg/dL)	HDLP (mg/dL)	CHOL. (mg/dL)	ALT(U/L)	AST(U/L)	ALP(U/L)
1	8.90	5.25 ^a	3.65	89.27 ^c	64.08	33.55	125.90 ^c	50.45	90.09	100.33
2	7.25	3.65 ^b	3.60	95.75 ^b	65.45	33.25	134.00 ^b	49.50	91.00	101.52
3	6.6	3.45 ^b	3.20	98.55 ^a	64.55	33.25	140.50 ^a	49.45	90.00	100.45
4	7.50	4.25 ^a	3.25	96.02 ^b	65.00	32.50	134.15 ^b	51.10	90.20	100.65
SEM	0.01	0.02	0.21	1.41	1.21	1.10	10.01	2.10	7.20	10.11

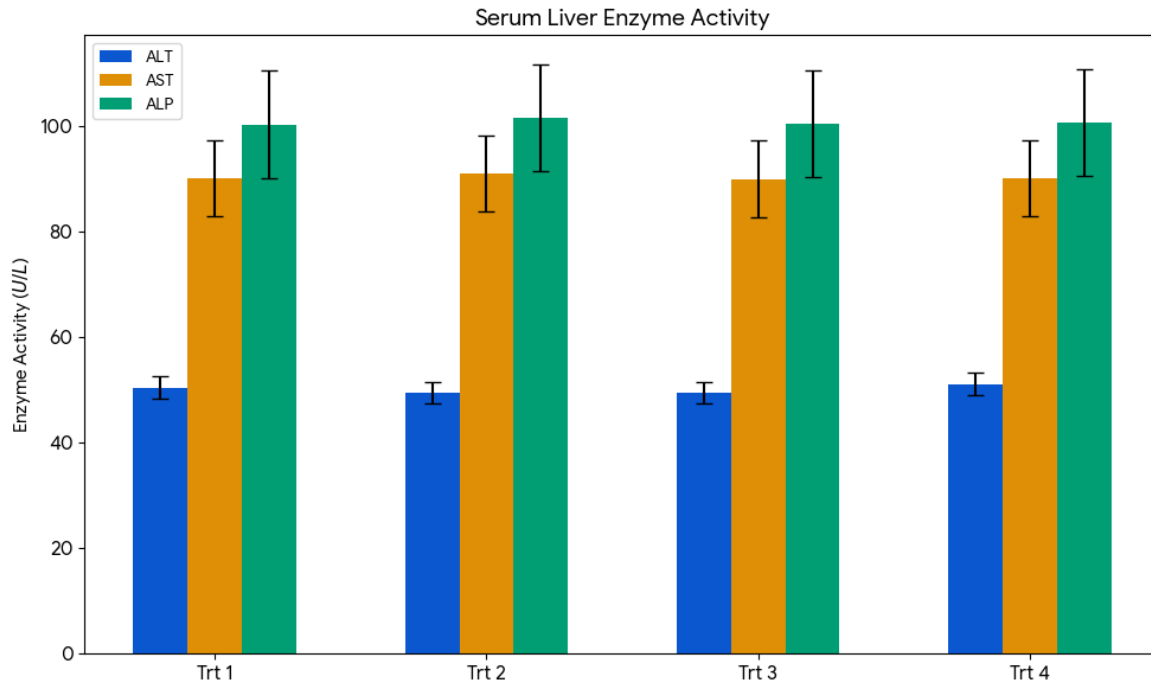
T.P = Total Protein; ALB = Albumin; GB = Globulin; LDLP = Low Density Lipoprotein; HDLP = High Density Lipoprotein; CHOL = Cholesterol; ALT = Alanine Aminotransferase; AST = Aspartate

Aminotransferase; ALP = Alkaline Phosphatase

The profile of the lipid components indicates a significant response to AA supplementation with peak values of 98.55 mg/dL and 140.50 mg/dL recorded in Treatment 3 for Triacylglycerol (TAG) and Total Cholesterol (Chol.) respectively. Cholesterol showed a significant lipid elevation up to 11.60%, while Triacylglycerol indicated an elevated lipid mobilization up to 10.40% between T1 and T3. The relative stability discovered in the LDLP and HDLP in all the treatments shows that although the cholesterol level was elevated, the AA supplementation did not significantly affect the fractions of the good cholesterol (HDLP) to the bad cholesterol (LDLP).

It appears that the AA dosage used in this study did not trigger hepatotoxicity but rather sustained hepatic homeostasis as the values recorded for ALT, AST and ALP were consistently non-significant across the treatments. This implies that AA supplementation is safe within the range of the dosage tested and that, the stability of transaminase values after administration of an antioxidant shows the capacity of AA in prohibiting an heat stress effect on the liver against lipid peroxidation (Sahin *et al.*, 2002; Du *et al.*, 2022).





The respiratory dynamics of the birds are presented in Table 6. A continuous rise in respiratory rates was observed from the 5th week to the 8th week across all the treatments. This indicates a physiological reaction of the birds to the rise in metabolic heat production as they gained more weight, alongside the elevated environmental temperatures. It can also be observed that birds placed on AA supplementation groups (T3 and T4) showed significantly reduced respiratory rates, suggesting that AA preserved the central nervous system from damage and prevented respiratory alkalosis thereby mitigating the intensity of the discomfort caused by thermal stress (Ayoola *et al.*, 2025). Sumanuet *al.* (2024) reported that AA plays a role in lowering the corticosterone circulation, which is known for inducing serious respiratory response in heat stress chickens. In addition, at the cellular level, thermal stress produces Reactive Oxygen Species (ROS) that are capable of affecting the hypothalamus at the thermoregulatory zone. AA as an antioxidant neutralizes the ROS thus inhibiting damage to the neurons regulating panting during heat stress (Hieu *et al.*, 2022). This explains why the chicken in T4 could tolerate the elevated ambient temperature without exhibiting polypnea at the same rate as the birds in T1. Finally, respiratory rates appear to be more stable in T4 (53.1 bpm) relative to T1 (59.59 bpm) at week 8. The implication is that AA supplemented chickens produced endogenous heat lower than the control group. This finding aligns with the result of Tavakolinasab & Hashemi, (2025) who concluded that AA supplementation can enhance metabolic efficiency, which can result in lowering the heat generated from feeding.

Table 6: Effect of Ascorbic Acid Supplementation Respiratory Rates

Week	Ascorbic Acid Levels				SEM
	T ₁	T ₂	T ₃	T ₄	
	Breath/min				
5	39.89	39.68	39.68	38.20	0.21
6	48.62	46.22	45.61	45.02	0.71
7	58.11 ^a	57.45 ^a	56.41 ^b	56.67 ^b	0.29
8	59.59 ^a	59.21 ^a	56.66 ^b	53.41 ^c	0.31

Table 7 shows the physiological evaluation of the thermoregulatory ability of the birds during exposure to high thermal stress. The rectal temperatures in T1 were persistently high (42.35 °C to 42.98 °C) and since the values recorded in T1 and T2 are greater than the normal physiological temperature of a healthy broiler chicken (40.60 °C to 41.70 °C) (Oni *et al.* 2024), it implies that the broilers had been in a chronic heat stress. It can also be observed that the hypothermic influence of AA was dose-dependent as a statistically significant decrease was recorded for the rectal temperature as the AA level increased. At the 8th week, there was a decline in the temperature from 42.37 °C in T1 to 40.01 °C in T4. This decline affirms the anti-stressor potency of AA by preventing the production of corticosterone at the adrenal glands. This hormone is capable of initiating an increase in metabolic rate and consequently, heat generation. However, since AA inhibited the secretion of this hormone, which ultimately resulted in decreasing the rectal temperature, the birds were maintained within their thermoneutral zone even when exposed to dry season heat (Sumanuet *al.*, 2024; Ayoola *et al.*, 2025).

Table 7: Effect of Ascorbic Acid Supplementation on Rectal Temperature

Week	Ascorbic Acid Levels				SEM
	T ₁	T ₂	T ₃	T ₄	
	Rectal Temperature (°C)				
5	42.98	41.51	41.37	40.12	0.01
6	42.80	41.31	41.17	40.22	0.02
7	42.35	41.55	41.27	40.40	0.01
8	42.37 ^a	42.22 ^a	40.61 ^b	40.01 ^b	0.03

CONCLUSION AND RECOMMENDATION

The study has clearly shown that ascorbic acid is an effective physiological and metabolic antioxidant for broiler chickens raised under the high environmental temperature of the dry season. Ascorbic acid supplementation at an inclusion level of 150 mg/kg is most effective in mitigating the negative effects of the hypothalamic-pituitary-adrenal (HPA) axis activation, thus inhibiting muscle damage and enhancing body weight gain. The remarkable decrease in rectal temperatures and respiratory rates establishes the ability of AA in ensuring that birds are maintained within their thermoneutral zone, halting the activities of reactive oxygen species and prohibiting respiratory alkalosis. Moreover, the improvement of erythropoiesis and bone development emphasizes the numerous benefits of AA in enhancing both the oxygen-carrying capacity and physical framework of the birds. As a result, poultry farmers in tropical regions are hereby encouraged to supplement broiler chicken diets with 150 mg/kg of AA for economic sustainability as well as optimal welfare of the birds particularly during hot seasons.

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