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THE EFFICACY OF BAOBAB FRUIT PULP ON THERMOREGULATORY, GROWTH AND BLOOD INDICES OF HEAT STRESSED BROILER FINISHER CHICKENS

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ABSTRACT

The study was carried out to assess the efficacy of baobab fruit pulp meal in ameliorating heat stress in finisher broilers (day 29-49) using thermoregulatory parameters, growth performance, haematological and biochemical parameters. Two hundred and fifty six finisher broiler chickens were randomly divided into four treatments (64 birds each) in a completely randomized design Ascorbic acid was added to the diet at 0, 68, 136 and 204 mg/kg. Each treatment had four replicates. Data gathered during the investigation was processed using general linear model of statistical analysis system and means were separated using Tukey's methodology. Glutathione Peroxidase activity was improved (P<0.05) at 68 and 136 mg/kg ascorbic acid respectively, while increased (P<0.05) LDL level was recorded for broilers offered the control diet and diet supplemented with 136 mg/kg ascorbic acid. Natural ascorbic acid (204 mg/kg) was found to improve broiler chickens' blood indices, growth and thermoregulatory parameters. As a result, during time of heat stress, finisher broiler chickens should be offered 204 mg/kg natural ascorbic acid added to their feed.

Keywords: stress; baobab; ascorbic acid; physiology; blood

INTRODUCTION

Besides scarcity and costly feedstuffs for poultry feed in emerging nations, there is intense stress imposed by the environment, especially during dry season. Heat stress is so significant in light of the fact that all classes of poultry are at its mercy. The climate under which birds are raised under intensive system in tropical nations is nowhere near the ideal. Heat stress (HS) occurs when heat intensity delivered by a creature is beyond the creature's ability to disperse the intensity to its surrounding. This might be as a result of certain ecological elements and quality of the animal (Lara and Rostagno, 2013). The physiological results of HS are numerous and can impact livestock performance indices negatively. Livestock elicit an array of mechanisms to cope, such as, expanded body temperature, diminished willful feed consumption, reduced resistance to diseases, change of the electrolyte equilibrium and blood pH, obstruction in endocrine and conceptive capabilities, diminished energy accessibility to cells, modification in the absorbability and digestion of different feed, disturbance in the construction and capability of gastrointestinal epithelium, adjustment of the ordinary and defensive microbiota, and expanded circulatory cortisol and corticosterone levels (Yahav, 2009; Syafwan *et al.*, 2011; Renaudeau *et al.*, 2012; Lara and Rostagno, 2013). High temperature results to a surge in free radicals and reactive oxygen species (ROS) by beginning lipid damage in cell layers and conveying stress chemicals that impact carbohydrate and fat metabolism as well as protein breakdown, consequently resulting in oxidative harm (Zhang *et al.*, 2014; Imik *et al.*, 2012; Hosseini-Mansoub *et al.*, 2010). Under such antagonistic circumstances (>30 ^{0}C), the body cannot produce the catalysts expected to obliterate ROS or fix the harm. L-ascorbic acid is an ingredient added to feed or food at a fixation level below that of an oxidizable substrate, which will fundamentally hinder the oxidation of the substrate (Halliwell and Gutteridge, 1999). Ascorbic acid is a natural substance which is a fundamental dietary supplement known as a co-variable of numerous catalysts (Youssef, 2004) and is required in minute amount. During hot weather condition, adding ascorbic acid to the diet of poultry has been accounted to have beneficial outcomes, such as, weight gain, increased egg laying, egg shell strength, fertility, hatchability and improved resistance to diseases in poultry (Abdulrashid *et al.*, 2010). Ascorbic acid was shown to reduce gasping rates, rectal and body temperatures in broilers and layers by Kutlu and Forbes (1993). Baobab (*Adansonia digitata*), also called African tree is known to have a significant proportion of L-ascorbic acid. Adeosun (2012) and Ndubuisi *et al.* (2022) reported 299.75mg/100g and 340mg/100g of vitamin C in the Baobab dry fruit pulp respectively. Inclusion of natural vitamin C to the diet of chickens could be advantageous in easing stress issues related with avian farming. Therefore, it is pertinent to investigate the effect of adding ascorbic acid to the diet of heat-stressed broiler finisher chickens.

METHODOLOGY

Study Area

The research was conducted within Ahmadu Bello University, Samaru, Zaria. Zaria is situated in the Northern Guinea Savannah zone of Nigeria on Latitude 11^0 09' 01.78" N and Longitude 7^0 39' 14.79" E, at a height of 671 m above sea level (IARMS, 2019). The environment is characterized with three distinct seasons; specifically the hot dry season from March to May, the wet season from June to September, and a harmattan season from November to February with an average yearly precipitation of approximately 700-1400 mm. The environment possesses a mean relative humidity of 36.0% during the dry season and 78.5% for the rainy season and a surrounding temperature of 26-32^0 C (IARMS, 2019).

Source of Organic Ascorbic Acid

Baobab fruit (*Adansonai digitata*) was bought in Zaria and used as source of natural vitamin C. The pulp was separated from the seeds by gentle beating in a mortar to obtain the fruit pulp which was included in the feed of broiler finisher chickens at 2, 4 and 6 kg.

Chemical Analysis of Baobab Fruit Pulp

The proximate constituents (dry matter, crude protein, crude fibre, ether extract and ash) of the dry baobab fruit pulp was conducted in consonance with the method of AOAC (1990). The quantity of ascorbic acid (vitamin C) in the pulp was also analyzed.

Research Layout, Diets and Management of Birds

Two hundred and fifty six broiler finisher chickens were randomly allotted to four treatments (0, 68 136 and 204 mg of ascorbic acid/kg diet), each having 64 birds, replicated four times in a Completely Randomized Design. Broiler finisher diets (Tables 1) were formulated in accordance with NRC (1994) recommendation. Feed and water were provided *ad libitum*. Standard management practices were adhered to.

On arrival, the initial weights of the chicks were taken, while feed intake and weight gain were taken every week using a digital scale. Known quantity of water was given on daily basis to the birds, left-over was also estimated every day. Quantified water was poured into an open container and kept at strategic locations within the pen and was used to account for evaporation losses. Mortality was noted as it occurred.

Thermoregulatory Measurements

The pen temperature and relative dampness readings were taken two times every day (8.00 am and 1.00 pm) using a digital thermo-hygrometer all through the trial period and the readings were utilized to compute the morning and afternoon temperature-humidity index (THI). Chickens' (2 birds/replicate) rectal temperatures were measured by placing a digital thermometer 1 cm into the rectum, body heat was determined by placing the digital thermometer beneath the wing web while respiratory rate was obtained by counting of breath (breath/minute) while using a stopwatch. Heart beat was estimated by putting a stethoscope at the breast area and count taken with the aid of a digital watch (beat/minute). Temperature-humidity index (THI) was determined following the standard equation by Tao and Xin (2003) for poultry.

$THI = 0.85\ Tdb + 0.15\ Twb$

Where,

THI = temperature-humidity index in ^{0}C

Tdb = dry-bulb or ambient temperature in ^{0}C

Twb = wet-bulb temperature in ^{0}C

Wet bulb temperature was measured from environmental temperature and relative humidity utilizing the empirical expression function by Stull (2011). Heat stress was grouped as absence of heat stress (<27.8), mild heat stress (27.8-28.8), intense heat stress (28.9-29.9) and very intense heat stress (>30.0).

Haematological and Serum Analyses

Blood (2 ml) was drawn from the vein of two chickens in every replicate at the end (day 49) of the trial for haematological and serum assay according to the procedure of Lamb (1991). One ml was poured into EDTA (Ethylene di-ammine tetra-acetic acid) coated bottles for haematological assay and another 1 ml was poured into non EDTA coated bottles for serum assay. Levels of serum calcium and phosphorus were also determined. Also biomarkers were determined according to the procedure of Atawodi (2011), Abebi (1974), Fridovich (1989) and Rajagopalan *et al.* (2004) respectively.

Statistical Analysis

Data gathered from this trial were statistically analyzed using General Linear Model Procedure of SAS, while significant differences among means were compared using the Tukey Procedure (SAS, 2002).

RESULTS AND DISCUSSION

Thermoregulatory Parameters

The poultry house temperature humidity index (THI) during the trial is displayed in Fig. 1. The mean morning THI was 26.83 and 35.81 for afternoon. Figures 2-5 depict the thermo-regulatory responses of broiler chickens offered feed supplemented with different levels of ascorbic acid. Respiratory rate, heartbeat, rectal temperature and body temperature were similar (P>0.05) during the study. The Respiratory rate of broilers ranged from 79.69 – 87.94 cpm, heart rate ranged from 189.31 – 192.94 bpm, rectal temperature was 42.05 – 42.30 ^{0}C and body temperature was 42.17 – 42.27 ^{0}C.

Increased ambient temperature due to fluctuation in season is a significant stressor that affects broiler production in numerous areas of the world and high misfortunes on investment capital may accrue due to reduced growth, depressed immunity and mortality (Melesse *et al*., 2011). The outcome of this study shows that THI at noon was higher by 25.08% than morning THI which revealed reduced heat stress at morning and the presence of very intense heat stress in the afternoon (Ademu *et al*., 2018), hence the birds were severely stressed. Lin *et al*. (2004) had earlier revealed that sensitivity to elevated temperature increases as the body weight of the bird increases. The respiratory rate of broilers was not consistent but was within the normal range, a surge in respiratory rate was observed in broilers offered feed supplemented with 136 mg of ascorbic acid but was lower in broilers fed 204 mg/kg ascorbic acid supplemented diet. Broilers fed 136 and 204 mg ascorbic acid supplemented diet had an increased heart rate than the other groups. The inconsistency observed in these parameters could be as a result of birds attempt to resist restraint at the point of determining these parameters which could have increased the metabolic rate of the birds. Although broilers offered ascorbic acid supplemented feed at 68 and 204 mg had lower heart beat and respiratory rate respectively. This is in disagreement with the report of Abdelrafea *et al*. (2013), who detailed that increasing ascorbic acid content from 0.5-1 gram alone or in blend with ethylene diamine tetra acetic acid (EDTA) in the feed of broilers increased respiration. Similarly, Olukomaiya *et al*. (2015) reported that elevating the ascorbic acid content of broilers feed from 150-300 mg did not lower their respiratory rate. Chaiyabutr (2004) revealed that one of the physiological reactions to heat-stress in birds is elevation in respiratory rate and that gasping happens when the core internal heat of poultry reaches 41 - 43 ^{0}C because birds do not have sweat glands like ruminants. Rectal and body temperatures of broilers were similar and within the range reported (Abdelrafea *et al*., 2013; Olukomaiya *et al*., 2015; Jahejo *et al*., 2016). The increased rectal temperature observed in broilers offered feed supplemented with 204 mg of ascorbic acid could be due to the more severe chronic respiratory disease (CRD) observed in this group. The similar results observed for rectal and body temperatures revealed that ascorbic acid had no negative impact on these parameters since they were within the normal range (40.60 – 43.00 ^{0}C) reported by Robertshaw (2004). Broiler chickens are known as homeotherms and their body system might permit certain fluctuation in temperature range without considerable distortion within their body (St-Pierre *et al*., 2003). The increase in thermoregulatory parameters (except respiratory rate) as ascorbic acid supplementation increased concurs with the findings of Abdelrafea *et al*. (2013), who reported a huge increase in cloacal, skin, feather temperatures and respiratory rate of broilers fed 1 g of ascorbic acid. Thermobalance is the stability accomplished between the heat generated and heat expelled by living organism and this is at its maximum physiological level inside the thermoneutral scope of any given specie (Olukomaiya *et al*., 2015).

Growth Performance of Broiler Finisher Chickens fed Ascorbic acid Supplemented diet (day 29-49)

Table 2 shows the growth performance of finisher broiler chickens fed different amount of ascorbic acid. Daily weight gain, final weight, daily feed intake, feed conversion ratio, daily water intake and mortality were statistically the same (P>0.05) for all treatment groups. Chickens fed 68 mg/kg ascorbic acid diet had the least mortality record. Birds fed 68 mg/kg ascorbic acid diet had least feed cost per kg gain. The similarity in the growth development of broilers in this study is in agreement with the findings of Muhammad *et al*. (2016) but in disagreement with the reports of Lohakare, (2005); Talebi and Khademi, (2011); Adeosun, (2012); Jahejo *et al*. (2016); Youssef *et al*. (2017) who stated that ascorbic acid improved the growth of broiler chickens during stress, although the THI indicated that their birds were not as stressed as those of the present study.

The Role of Ascorbic Acid Supplementation on Haematological indices of Broiler Finisher Chicken

Table 3 shows the haematological indices of heat stressed finisher broiler chickens fed different levels of diets supplemented with ascorbic acid. All the parameters considered were not significant (P>0.05). Packed Cell Volume and haemoglobin decreased numerically with increase in ascorbic acid supplementation, ranging from 26.88 – 30.71% and 8.51 – 10.21 g/dL respectively. Broilers fed the control diet had higher numerical PCV (30.71%) value, haemoglobin (10.21 g/dL) and eosinophils (0.86%). Leucocytes ranged from 10.14 – 13.16 $x10^9/L$ and was numerically higher in chickens fed 204 mg/kg ascorbic acid diet. Broilers fed 68 mg ascorbic acid supplemented diet had more numbers of erythrocytes (4.93 $x10^{12}/L$) and lymphocytes (82.83%) with a decrease in heterophils (15.00%) and H:L (0.18). Monocyte and band cells ranged from 0.14 – 2.00% and 0.29 – 2.14% respectively.

Broilers fed the control diet had higher PCV, Hb and eosinophils levels. PCV and Hb were within the normal ranges disclosed by Mitruka and Rawnsely (1997). This revealed that the addition of ascorbic acid to the diets had no negative impact on the birds, also it has been established that these parameters are indicators of the adequacy of feeds (Isikwenu and Omeje, 2007). Eosinophils were slightly below the normal range in all the groups and could be due to heat stress (Altan *et al*., 2000). This agrees with the work of Muhammad *et al.* (2016), who revealed that supplementation of the diet of broilers with 0.07, 0.15, 0.22 and 0.30 g of ascorbic acid did not result in any impact on the PCV and Hb . Leucocyte was above the normal range reported by Simrak *et al.* (2004) and higher leucocyte count was observed in broilers fed diet supplemented with 204 mg ascorbic acid/kg diet. This is consistent with the work of Muhammad *et al.* (2016), who observed higher leucocyte number in broilers fed 0.22 g of ascorbic acid.

Higher erythrocyte and lymphocyte count with a decreased heterophil and H:L was recorded in broilers fed 68 mg ascorbic acid/kg diet. Ascorbic acid showed its potency in ameliorating heat stress as indicated by elevated lymphocyte, lowered heterophils and H:L than the control group. This concurs with the report of Youssef *et al*. (2017), who revealed that vitamin C supplementation increased lymphocyte and lowered heterophil and H: L during chronic heat stress than vitamin E and probiotics.

Serum Indices of Broiler Finisher Chickens Fed Ascorbic Acid Supplemented Diet

Table 4 shows the serum indices of broiler finisher chickens fed diet supplemented with different levels of ascorbic acid. Except for LDL and GSHPx, other parameters measured were not significant. Chickens offered feed supplemented with 204 mg of ascorbic per kg had numerically higher glucose (175.95 mg/dL), ALT (57.50 µ/L), AST (52.00 µ/L), globulin (2.30 g/dL), cholesterol (132.75 nmol/L). LDL ranged from 56.00 – 198.75 mg/dL, chickens fed the control and 136 mg of ascorbic acid supplemented diet had higher (P<0.05) LDL. Triglyceride, ALT, AST and ALP ranged from 44.00-96.25 mg/dL, 23.75-57-50 µ/L, 23.00-52.00 µ/L and 44.50-68.50 µ/L. Broilers offered diet having 68 mg and 136 mg of ascorbic acid had higher (P<0.05) GSHPx. SOD, MDA and CAT ranged from 4.05-5.55 µmol/mL, 4686-20548 nmol/mL and 4.15-6.10 U/mL and calcium and phosphorus ranged from 9.03-11.85 mg/dL and 4.07-5.95 mg/dL respectively.

According to Borges *et al*. (2007) a rise in glucose level is directly proportional to an increase in glucocorticoids which can be as a result different stressors such as heat. The primary effect of glucocorticoids on metabolism is to initiate gluconeogenesis from muscle tissue proteins. Glucose values were within the normal physiological range documented by Goodwin *et al*. (1994). The glucose level observed in ascorbic acid supplemented groups could be due to the fact that cortisol secretion was higher in broilers fed 204 mg and might had led to gluconeogenesis from muscle tissue or it may have increased due to an increased quantity of Baobab fruit pulp meal which is a carbohydrate source. The increased ALT and AST could be due to higher liver synthesis of these enzymes as a result of inflammation of the liver caused by poor digestion of feed as seen during the post mortem examination. AST was below the normal range reported by LAVC (2009). Globulin was within the normal physiological range documented by LAVC (2009). The lowered cholesterol (136 mg/kg ascorbic acid), LDL (68 and 204 mg ascorbic acid/kg diet) and triglyceride (204 mg ascorbic acid/kg diet) might be due to the antioxidant property of ascorbic acid in preventing lipid peroxidation and metabolism. According to Seyrek *et al.* (2004), ascorbate is essential for the transformation of cholesterol to bile acids by controlling the microsomal 7α-hydroxylation and its deficiency causes a significant reduction of this reaction, resulting to cholesterol accumulation in liver and in blood. The increased cholesterol level observed in broilers fed 204 mg/kg ascorbic acid could be as a result of liver inflammation caused by poor digestion of feed as a result of heat stress observed during the post mortem.

Broilers fed diets supplemented with 68 and 136 mg of ascorbic acid had higher GSHPx activity. Contrary to the findings of Jena *et al*. (2013) and Adenkola *et al*. (2016), this study revealed that ascorbic acid did not raise the serum GSHPx level in broilers. According to Yoda *et al*. (1986), glutathione in its reduced form, metabolizes hydrogen peroxide and hydroxyl radicals, which prevent oxygen toxicity from occurring. A healthy body is marked by a balance between free radicals and antioxidants. When this balance is disrupted by over-abundance of free radicals, oxidative stress (OS) occurs.

SOD was higher in all ascorbic acid supplemented groups compared to the control, this might be due to the fact that SOD scavenges for both intracellular and extracellular superoxide radicals and annihilate their deleterious activities by acting in combination with catalase and glutathione peroxide (Agarwal and Prabhakaran, 2005), and ascorbic acid is known to improve the activities of antioxidant enzymes. CAT level was higher in 136 mg ascorbic acid supplemented broilers with broiler fed the control diet being next and better than broilers fed 68 and 204 mg/kg ascorbic acid . Adenkola *et al*. (2016) reported that broilers fed control diet during heat stress had higher GSHPx, SOD and CAT than the ascorbic acid group. According to Draper and Hadley (1990), exposure to environmental oxidants increases MDA production *in vivo*. MDA was lowered by 68 mg of ascorbic acid supplementation but was higher in broilers fed 136 mg/kg ascorbic acid diet. The higher MDA at 136 mg/kg ascorbic acid supplementation could be due to the increased serum cortisol level. This is in contrast with the work of Jena *et al.* (2013) who revealed that supplementation of ascorbic acid at 200 and 400 mg in the diet of broiler breeder hen during summer lowered their MDA level (4.96 + 0.61 and 4.71 + 0.59 nmol/mg) Similarly, Adenkola *et al*. (2016) stated that supplementing the diet of broilers with 500 mg/kg ascorbic acid lowered serum MDA level (0.93 + 0.009 ng/mL).

In combating oxidative stress, both enzymatic (catalase and superoxide dismutase) and non-enzymatic (MDA) antioxidants play crucial roles. Catalase detoxifies hydrogen produced during different metabolic processes and also in stressful conditions by converting it to hydrogen peroxide and oxygen. Superoxide dismutase breaks down dismutation of superoxide radicals into water and oxygen (Kwiecient *et al*., 2004).

CONCLUSION

- Organic ascorbic acid when supplemented at 204 mg/kg diet, improved thermoregulatory parameters via lowered respiratory rate and increased heartbeat.
- Serum indices (low density lipoprotein and GSHPx) were improved at all levels of ascorbic acid supplementation.

Table 1: Ingredient Composition of Ascorbic Acid Supplemented Diets Fed to Broiler Finisher Chickens

	Ascorbic acid content of the diet (mg/kg diet)			
Ingredients (%)	**0**	**68**	**136**	**204**
Maize	59.00	56.50	54.00	51.50
Soyabean cake	20.00	20.50	21.00	21.50
Groundnut cake	9.50	9.50	9.50	9.50
Maize offal	7.00	7.00	7.00	7.00
BFPM	0.00	2.00	4.00	6.00
Bone meal	3.00	3.00	3.00	3.00
Limestone	0.50	0.50	0.50	0.50
Common salt	0.30	0.30	0.30	0.30
Vitamin premix	0.30	0.30	0.30	0.30
Lysine	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20
Total	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis				
ME (Kcal/kg)	2994.00	2982.00	2971.00	2962.00
Crude protein (%)	20.05	20.09	20.09	20.10
Ether extract (%)	4.52	4.50	4.47	4.45
Crude fibre (%)	3.86	4.01	4.17	4.32
Calcium (%)	1.32	1.33	1.33	1.33
Available phosphorus (%)	0.56	0.56	0.56	0.56
Lysine (%)	1.09	1.10	1.10	1.11
Methionine (%)	0.50	0.50	0.50	0.50
Ascorbic acid (%)	131.73	134.43	137.13	139.83
Cost (₦/kg)	112.07	120.77	129.47	138.17

Vitamin-mineral premix provide per kg of diet: Vit. A, 8,000,000 IU; Vit. D_3, 1,600,000 IU; Vit. E, 5,000 UI; Vit. K, 2000mg; Vit. B1, 1,500mg; Vit. B_2, 4,000mg; Vit. B_6, 1,500mg; Vit. B_{12}, 10mg; Niacin, 15,000mg; Panth. Acid, 5,000mg; Folic acid, 500mg; Biotin, 20mg; Choline Chloride, 200g; Antioxidant, 125g; Manganese, 80g, Iron, 20g; Zinc, 50g; Copper, 5g; Iodine, 1.2g; Cobalt, 200mg; Selenium, 200mg; BFPM= Baobab Fruit Pulp Meal.

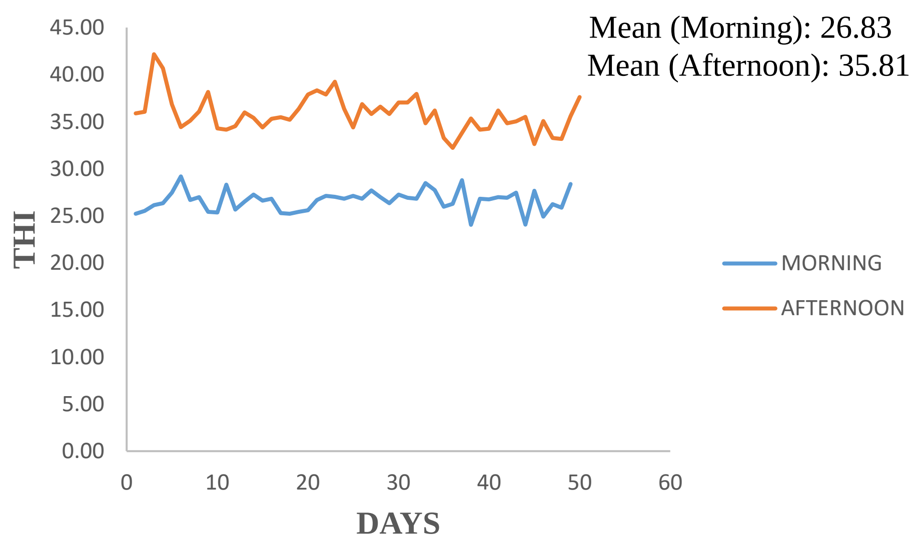

Fig. 1: Daily Temperature-Humidity index of the Poultry House during the study Period

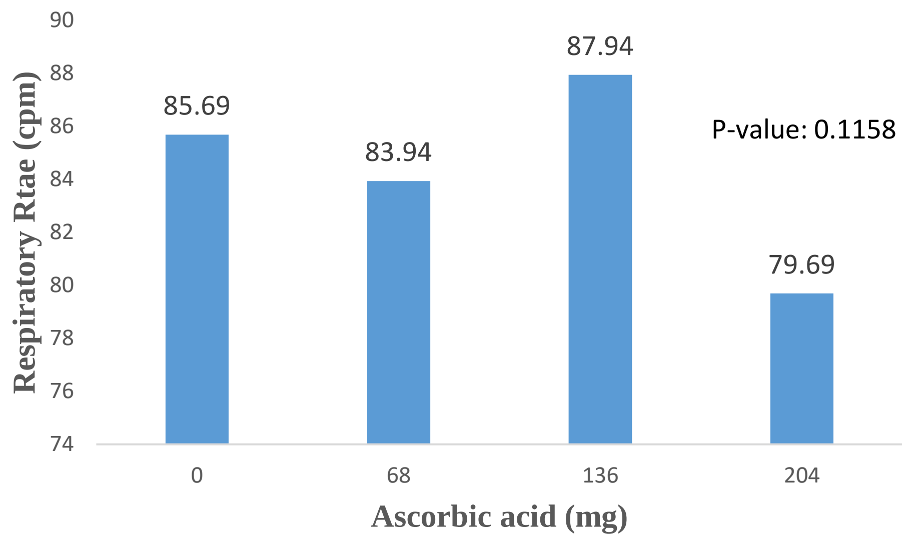

Fig. 2: Respiratory Response of Broiler Chickens to different Levels of Ascorbic Acid

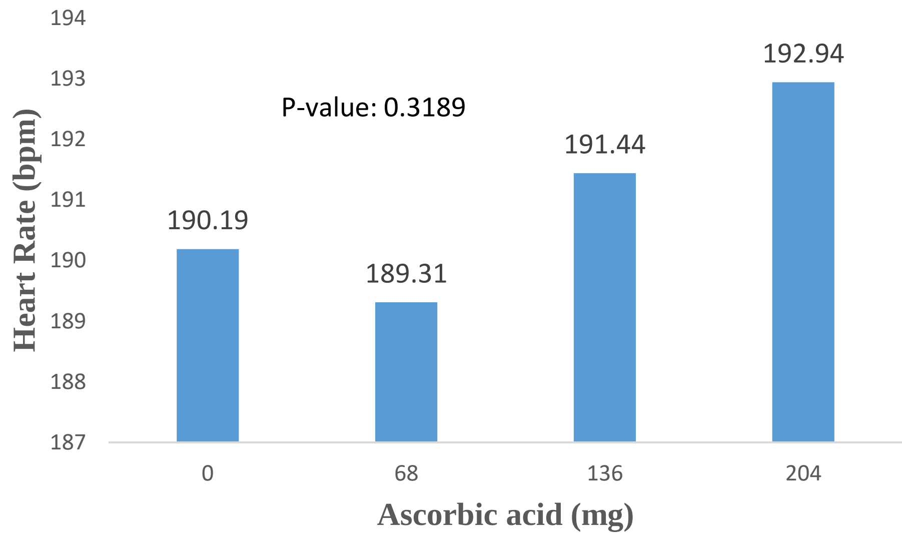

Fig. 3: Effect of different Levels of Ascorbic Acid on Heart beat of Broiler Chickens

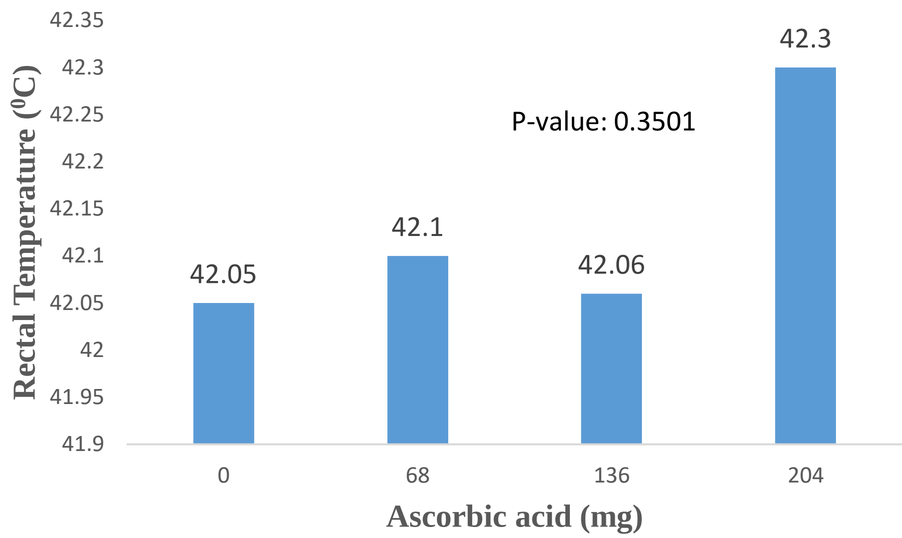

Fig. 4: The Response of Rectal Temperature of Broiler Chickens to Varied Levels of Ascorbic Acid

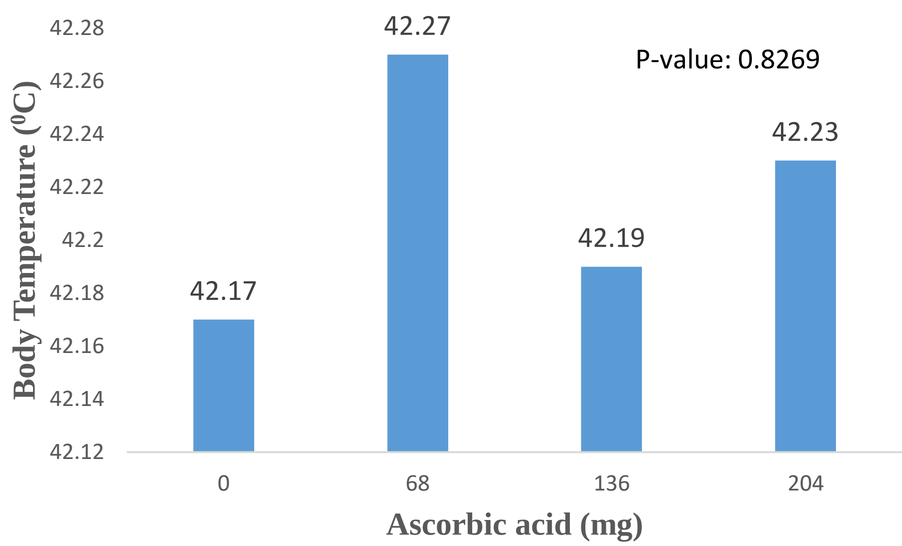

Fig. 5: Body Temperature Response of Broiler Chickens to Varying Levels of Ascorbic Acid

Table 2: Effect of Ascorbic acid Supplementation on Performance of Broiler Finisher Chickens

	Dietary levels of Ascorbic acid (mg/kg diet)					
Parameters	**0**	**68**	**136**	**204**	**SEM**	**P value**
Initial weight (g/bird)	916.98	888.03	864.76	848.48	27.74	0.3684
Daily feed intake (g/b/d)	133.08	127.51	122.12	133.09	5.61	0.4795
Daily weight gain (g/b/d)	61.44	64.61	64.55	66.66	2.71	0.6071
Final weight (g/bird)	2207.20	2245.00	2220.40	2248.40	73.87	0.9740
Feed conversion ratio	2.17	1.98	1.89	2.00	0.08	0.1292

Daily water intake (ml/b/d)	426.55	435.85	409.77	473.49	22.60	0.2840
Feed cost/kg gain (₦/kg)	242.92	238.97	245.22	276.65	-	-
Mortality (%)	6.25	1.56	9.38	6.25	2.16	0.1388

Table 3: Effect of Ascorbic Acid Supplementation on Haematological Parameters of Broiler Finisher Chickens

	Dietary levels of Ascorbic acid (mg/kg diet)						
Parameters	**0**	**68**	**136**	**204**	**SEM**	**P value**	**Ref-value**
PCV (%)	30.71	29.83	28.86	26.88	1.31	0.2456	24.00-40.00[w]
Haemoglobin (g/dl)	10.21	9.92	9.59	8.51	0.44	0.0705	7.00-15.00[w]
Erythrocytes ($x10^{12}/l$)	4.83	4.93	4.73	4.56	0.24	0.7658	1.59-4.10[w]
Leucocytes ($x10^9/l$)	11.56	10.73	10.14	13.16	1.32	0.4479	1.90-9.50[x]
Heterophils (%)	20.00	15.00	18.43	15.88	2.16	0.4276	15.00-40.00[x]
Lymphocytes (%)	78.71	82.83	76.43	82.50	2.06	0.1496	40.00-100.00[y]
H:L	0.26	0.18	0.25	0.20	0.03	0.4026	-
Monocytes (%)	0.14	0.50	2.00	0.75	0.57	0.1826	1.00-7.00[z]
Eosinophils (%)	0.86	0.33	0.57	0.25	0.32	0.5861	1.50-6.00[x]
Bands (%)	0.29	1.33	2.14	0.63	0.57	0.1619	-

PCV: Pack cell volume, [w]Mitruka and Rawnsely, 1997, [x]Simrak *et al*., 2004, [y]Jain, 1986, [z]Jain, 1993, H:L= Heterophils-lymphocytes ratio.

Table 4: Effect of Ascorbic Acid Supplementation on Serum Indices of Broiler Finisher Chickens

	Dietary levels of Ascorbic acid (mg/kg diet)						
Parameters	**0**	**68**	**136**	**204**	**SEM**	**P value**	**Ref-value**
Glucose (mg/dL)	134.55	168.30	135.90	175.95	15.69	0.1861	137-363[w]
Total Protein (g/dL)	4.75	4.58	3.88	4.45	0.38	0.4265	3.60-5.50[x]
Albumin (g/dL)	2.75	2.70	2.68	2.15	0.37	0.6413	1.10-2.20[x]
Globulin (g/dL)	2.00	1.88	1.20	2.30	0.45	0.3974	1.20-3.20[y]
Cholesterol (nmol/L)	130.25	123.25	92.75	132.75	13.74	0.2003	120-237
Low Density Lipoprotein (mg/dL)	198.75[b]	81.00[a]	192.25[b]	56.00[a]	35.47	0.0270	<130.00
Triglyceride (mg/dL)	96.25	70.50	86.25	44.00	18.85	0.2743	<135.00
Alanine-Amino Transferase (μ/L)	34.75	41.75	23.75	57.50	12.88	0.3500	-
Aspartate-Amino Transferase (μ/L)	23.00	33.00	31.25	52.00	9.09	0.1974	10-40[y]
Alakaline Phosphatase (μ/L)	68.50	58.00	44.50	48.50	8.29	0.2269	10-106[z]
Glutathione Peroxidase (μmol/mL)	1.24[c]	2.14[ab]	2.53[a]	1.84[b]	0.28	0.0405	
Superoxide Dismutase (μmol/mL)	4.05	4.05	4.80	5.55	0.83	0.5413	
Malondialdehyde (nmol/mL)	14132.00	4686.00	20548.00	10280.00	5877.21	0.3234	
Catalase (U/ml)	5.49	4.15	6.10	4.39	1.19	0.6299	
Calcium (mg/dL)	11.85	9.03	10.73	11.05	2.23	0.8347	
Phosphorus (mg/dL)	5.50	4.70	5.23	5.95	0.78	0.7210	

[abc] Means with different superscript on the same row differ significantly (P<0.05), Reference values: [w]Goodwin *et al*. (1994), [x]Ross *et al*. (1976), [y]LAVC (2009), [z]Bounous and Stedman (2000), Clinical Diagnostic Division (1990), Collins (2018).

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