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Research Article

Proximate, Anti-Nutritional and Ascorbic Acid Composition of Baobab Fruit Pulp Meal and its Efficiency in Ameliorating the Impact of Heat Stress on Growth and Blood Indices of Broiler Chicks

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Abstract

Background: As global warming is getting severe, the environmental temperature has continued to rise in the recent years. Heat stress disrupts the performance, physiological, hormones and behavioural attributes of chickens, hence antioxidants are required in micro quantities to counter the deleterious effect of reactive oxygen species [ROS] during heat stress.

Aim: A study was conducted to investigate the nutrient and anti-nutrient compositions of Baobab fruit pulp meal [BFPM] as source of organic ascorbic acid and its ameliorative effect on broiler chicks during heat stress. A total of two hundred and fifty-six, day old Cobb 500 broiler chicks were randomly allotted to four experimental treatments [0, 68, 136 and 204 mgAA/kg], with four replicates each in a completely randomized design. All the data that was generated during the study was subjected to general linear model of SAS and means were compared using Tukey's procedure.

Results: Results indicated that BFPM contains substantial amount of nutrients with limited anti-nutritional factors. Growth performance of broiler chicks were similar [$p > 0.05$] across the treatments except for FCR which was better [$p < 0.05$] for birds feed the control diet [1.39g] and 68 mgAA/kg [1.40g]. Feed cost/kg gain was least for birds feed the control diet. Haematological parameters were similar [$p > 0.05$] and within normal physiological ranges. Serum indices were similar except for low density lipoprotein [LDL] and Alanine Amino-Transferase [ALT]. Chicks fed 68 [111.00 mg/dL] and 136 [123.50 mg/dL] mgAA supplemented diet had higher [$p < 0.05$] values of LDL than other treatments while ALT was higher in chicks fed 136 [55.50 μ/L] mgAA supplemented diet. Cortisol was down-regulated [$p < 0.05$] in chicks fed both the control [6.0 ng/mL] and 68 [4.8 ng/mL] mgAA supplemented diet. Thyroxine was similar [$p > 0.05$] in all groups.

Conclusion: Ascorbic acid content of BFPM in this study did not negatively affect the growth and haematology of broiler chicks except for FCR. Supplementation of BFPM at 68 and 136 mgAA/kg in the diet of broiler chicks during heat stress conditions improved LDL. ALT and cortisol was also improved in chicks fed diet supplemented with 68 mg of ascorbic acid. BFPM can be used as excellent source of ascorbic acid in the diet of broiler chicks during hot temperatures to alleviate the deleterious effect free radicals.

Keywords: Ascorbic Acid; Baobab; Growth; Haematology; Serum; Temperature

Abbreviation

BFPM: Baobab Fruit Pulp Meal; AA: Ascorbic Acid; FCR: Feed Conversion Ratio

Introduction

There is increasing demand for poultry products globally [1]. This is due to the health benefits of poultry being high in beneficial unsaturated fatty acids, high profit over a short period, and acceptability in many culinary traditions and increase in human population. Global warming which can lead to heat stress [HS] is considered one of the most important challenges affecting the performance of birds [2,3]. Abdulrashid., *et al*. [4] described ascorbic acid as an antioxidant which improves performance and alleviates the deleterious effect of heat stress in poultry birds. Ascorbic acid (Vitamin C) is a water soluble antioxidant that protects animals under heat stress conditions and also protect against oxidation and it is easily synthesized by the kidney of healthy bird.

Baobab [*Adansonia digitata*] fruit pulp is known to contain a significant amount of ascorbic acid with limited anti-nutrients, addition of this natural source of vitamin C to poultry diets could be beneficial in alleviating stress problems associated with poultry production [5]. Therefore it is imperative to investigate the nutrient and anti-nutrient compositions of Baobab fruit pulp meal [BFPM] as source of organic ascorbic acid and its ameliorative effect on growth and blood indices of broiler chicks during heat stress.

Materials and Methods

Study location

The experiment was conducted at the Poultry Unit of the Department of Animal Science Teaching and Research farm, Ahmadu Bello University, Samaru, Zaria, Kaduna State, Nigeria. Zaria is located 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 an altitude of 671 m above sea level [6]. The area is characterized by hot dry season from March to May, the warm rainy season from June to September, and a cool dry season from November to February with a mean annual rainfall of about 700-1400 mm [6]. The site is characterized by an average relative humidity of 36.0% during the dry season and 78.5% for the wet season and an ambient temperature ranging from 26-32^0 C [6].

Experimental design and management of birds

Two hundred and fifty six, day old [*Cobb 500*] broiler chicks were randomly allotted to four experimental treatments [0, 68, 136 and 204 mgAA/kg], with four replicates each in a completely randomized design. Baobab fruit pulp [*Adansonai digitata*] was purchased from Giwa market in Zaria, Nigeria and used as source of organic Ascorbic acid. The pulp was separated from the seeds by gentle crushing using a mortar to prepare a baobab fruit pulp meal [BFPM]. BFPM was also analysed for ascorbic acid content and was supplemented during the diet formulation at 0, 2, 4 and 6% to provide the ascorbic acid content of the feed. A maize/soybean meal based broiler starter diets (2900 Kcal/kg and 23% C.P) was formulated (Table 3) according to NRC [7] nutrient requirement. Feed and water was provided *ad libitum*. All standard routine management practices were strictly followed.

Nutrient assay

Chemical analysis of the dried baobab fruit pulp was carried out according to AOAC [8] methods to determine the proximate composition [dry matter, crude protein, crude fibre, ether extract and ash contents]. The ascorbic acid [vitamin C] content was also determined in the Biochemistry Laboratory of the Department of Animal Science, Ahmadu Bello University, Zaria. Anti-nutritional factors such as tannins, oxalate, phytate and saponin were determined according to the procedures described by [9-12] respectively.

Growth parameter

Initial weight was measured at the beginning of the experiment, while feed intake and weight gain were recorded weekly using a digital precision weighing balance [Satorius ENTRIS]. Measured volume of water was offered daily to the birds, left over was also measured at the end of each day, measured volume of water was poured into an open container and placed at intervals along the passage within the building and evaporation was accounted for daily by measuring the quantity of water left in the container and subtracted from the initial quantity using a calibrated measuring cylinder. The left over and evaporation was then subtracted from the quantity of water offered to the birds to determine the volume of water the chicks consumed daily. Mortality records were also kept.

Haematological and serum analyses

Blood samples [2 ml] was collected from the Brachial vein of two birds per replicate on day 28 for haematological and serum assay, 1 ml was collected into tubes containing EDTA [Ethylene diammine tetra-acetic acid] anticoagulant to analyze for haematological parameters and 1 ml was collected in tubes containing no EDTA for serum assay. Concentration of Calcium and Phosphorus in the blood was also analyzed at the Clinical Pathology Laboratory of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

Haematological parameters such as Packed cell volume [PCV] was determined by the microhaematocrit method, haemoglobin concentration [Hb] was determined photometrically at the wavelength of 540 nm, the erythrocyte [RBC] and leucocyte [WBC] counts were determined using the improved Neubauer haemocytometer and Differential counts were determined by the thin slide method [13]. Liver function and lipid profile test was determined according to the procedure of [13].

Hormonal assay and determination of biomarkers

Cortisol and thyroxine was determined according to the procedures of Foster and Dunn [18] and Chopra., *et al*. [19] respectively. Malondialdehyde, catalase, superoxide dismutase and glutathione peroxidase were determined according to the procedures of Atawodi, [14]; Abebi, [15]; Fridovich, [16] and Rajagopalan., *et al.* [17] respectively at the Physiology Laboratory of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

Data analyses

All data obtained from this experiment were statistically analyzed using General Linear Model Procedure of Statistical Analysis System software package while significant difference among means were compared using the Tukey Procedure [20].

Results and Discussion

Proximate analyses and anti-nutritional factor contents of baobab fruit pulp

The result of the proximate composition (Table 1) indicates that Baobab Fruit Pulp Meal [BFPM] contains 95.95 dry matter, 6.38% crude protein, 4.31% crude fibre, 3.94% ether extract, 73.87% Nitrogen Free Extract and 11.50% Ash. The ascorbic acid content was 340 mg/100g of BFPM. Table 2 shows the results of the anti-nutritional factors present in the Baobab fruit pulp. The Baobab fruit pulp contains 3.5% Tannin, 0.28% Phytate, 1.14 mg/100g Oxalate and 22.6 mg/100g Saponin. The values of the chemical analysis fell within the range of values earlier reported [5,21]. Similarly the ascorbic acid content of 340 mg/100g obtained from the chemical analysis ranged between 150 to 499 mg/100g as reported by Manfredini., *et al.* [22]. Hence, high content of ascorbic acid makes it a good source of organic ascorbic acid for utilization in animal nutrition. The result of the chemical analysis of Baobab Fruit Pulp Meal showed that it has lower levels of anti-nutritional factors [5], hence cannot pose any serious threat to its utilization in poultry diets especially if used at low levels of inclusion.

Component	%
Dry Matter	95.95
Crude Protein	6.38
Crude Fibre	4.31
Ether extract	3.94
Nitrogen Free Extract	73.87
Ash	11.50
Ascorbic acid	340 mg/100g

Table 1: Proximate and Ascorbic Acid Composition of Baobab Fruit Pulp.

Anti-nutrient compound	Baobab fruit pulp
Tannin [%]	3.5
Phytate [%]	0.28
Saponin [mg/100g]	22.6
Oxalate [mg/100g]	1.14

Table 2: Anti-Nutritional Factor in Baobab Fruit Pulp.

Ingredients [%]	Ascorbic acid content of diets [mg/kg diet]			
	0	68	136	204
Maize	57.00	54.50	52.30	50.00
Soyabean cake	25.00	25.50	25.70	26.00
Groundnut cake	13.50	13.50	13.50	13.50
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]	2953	2943	2934	2924
Crude protein [%]	23.04	23.08	23.01	23.00
Ether extract [%]	4.77	4.74	4.71	4.68
Crude fibre [%]	3.76	3.92	4.06	4.22
Calcium [%]	1.34	1.34	1.34	1.34
Available phosphorus [%]	0.58	0.58	0.58	0.58
Lysine [%]	1.26	1.27	1.27	1.27
Methionine [%]	0.54	0.53	0.53	0.53
Ascorbic acid [%]	163.44	166.14	167.08	168.60
Cost [₦/kg]	119.47	128.57	136.63	145.17

Table 3: Ingredient Composition of Ascorbic Acid Supplemented Diets Fed to Broiler Starter Chickens

Vitamin-mineral premix provide per kg of diet: Vit. A, 10,000,000 IU; Vit. D_3, 2,000,000 IU; Vit. E, 20,000 UI; Vit. K, 2,250mg; Vit. B1, 1,750mg; Vit. B_2, 5,000mg; Vit. B_6, 2,750mg; Vit. B_{12}, 15mg; Niacin, 27,500mg; Panth. Acid, 7,500mg; Folic acid, 7,500mg; Biotin, 50mg; Choline Chloride, 400g; Antioxidant, 125g; Manganese, 80g, Iron, 20g; Zinc, 50g; Copper, 5g; Iodine, 1.2g; Cobalt, 200mg; Selenium, 200mg.

Growth performance

The growth performance of starter broiler chicks fed varying levels of ascorbic acid is shown in table 4. All the parameters measured were similar [p > 0.05] except for feed conversion ratio [p < 0.05]. Chicks fed with the control diet and 68 mgAA/kg diet had a better feed conversion ratio [1.39 g] than the other groups, values ranged from 1.39 to 1.56 g. The similar performance of broilers in this study except for FCR is consistent with the report of Muhammad., *et al*. [23] but in contrast with the report of [24-27] who reported that ascorbic acid significantly improved the performance of broiler chickens during heat stress. FCR was higher in chicks fed with the control and 68 mgAA/kg diet. Adeosun [5] reported a better FCR in broiler chicks fed BFPM at 3.5 and 7% inclusion levels during the dry-hot season and 3.5% inclusion level during the wet-cool season. Similarly, Jahejo., *et al*. [26] reported a lower FCR in broilers fed 200 mgAA/kg than the control. Furthermore, Talebi and Khademi [25] reported a lower FCR in heat stressed broilers fed varying levels of glucose combined with either 200 or 300 ppm of AA in drinking water during a 42 days period. The high [p > 0.05] mortality recorded in 204 mgAA/kg supplemented groups could be due to the higher quantity of BFPM that made the feed more dusty in nature and might had led to more sever chronic respiratory disease [CRD] as revealed by the post mortem examination. The indoor temperature humidity index [THI] during the experimental period averaged 26.83% in the morning and 35.81% at noon.

Parameters	Dietary levels of Ascorbic acid [mg/kg diet]					
	0	68	136	204	SEM	P value
Initial weight [g/bird]	39.85	40.63	41.10	42.50	0.72	0.1178
Daily feed intake [g/b/d]	43.70	42.27	45.76	44.65	1.93	0.6350
Daily weight gain [g/b/d]	31.33	30.27	29.42	28.79	1.01	0.3525
Final weight [g/bird]	916.98	888.03	864.76	848.48	27.74	0.3684
Feed conversion ratio [g]	1.39^a	1.40^a	1.56^b	1.55^b	0.04	0.0143
Daily water intake [ml/b/d]	128.07	127.50	123.19	136.01	6.50	0.5840
Feed cost/kg gain [₦/kg]	166.06	179.99	213.14	225.01	-	-
Mortality [%]	4.69	6.25	4.69	10.94	2.89	0.4066

Table 4: Effect of Ascorbic acid Supplementation on Performance of Starter Broiler Chickens

^{a,b} Means with different superscript on the same row differ significantly [p < 0.05].

Haematological parameters

Table 5 shows the haematological parameters of broiler chicks fed varying levels of ascorbic acid supplemented diets during 28 days of the study. All the parameters measured were similar [p > 0.05]. PCV, haemoglobin and erythrocytes values range from 25.00-29.00%, 8.27-9.60 g/dl and 4.16- 4.78 x 10^{12}/l respectively. Leucocytes ranged from [10.68-13.08 x 10^9/l]. Heterophils, lymphocytes and H:L range from 14.50-17.25%, 80.25- 84.00% and 0.18-0.22. Monocyte and eosinophils ranged from [0.38-1.38% and 0.00-0.63%] respectively. The haematological parameters measured were within the normal physiological range, except for leucocytes which were slightly above the normal range and eosinophils which were below the normal range reported by Simra., *et al*. [28]. The arbitral values of leucocytes and eosinophils could be due to heat stress or underlying disease conditions that might have led to an alteration in the production of the components of white blood cells [29].

The non-significant result observed in all the parameters measured is an indication that the ascorbic acid levels and anti-nutritional factors contained in the baobab fruit pulp meal posed no negative impact on the blood profile of the birds [30]. PCV, Hb and erythrocyte were higher in birds fed 204 mg of ascorbic acid [AA] supplemented diet, this is an indication that the feed was safe for consumption and improved the health status of broilers during heat stress. Isikwenu and Omeje [31], reported that PCV and Hb are nutritional indicators which are largely influenced by the diets fed to the animals. The result of this study agrees with Adenkola., *et al*. [32], who fed broilers 500 mg of AA supplemented diet and reported that PCV, Hb and erythrocyte were similar. Similarly, Alaeldein., *et al*. [33] demonstrated that addition of either 100 or 200 mg natural vitamin C to the drinking water of broilers during heat stress did not affect the Hb nor erythrocyte. Leucocyte and band cells were higher in broilers fed the control diet. Leucocyte, depicts

	Dietary levels of Ascorbic acid [mg/kg diet]						
Parameters	**0**	**68**	**136**	**204**	**SEM**	**P value**	**Ref-value**
PCV [%]	25.00	25.13	28.25	29.00	1.71	0.2399	24.00-40.00[w]
Haemoglobin [g/dl]	8.26	8.34	9.39	9.60	0.58	0.2493	7.00-15.00[w]
Erythrocytes [x10^{12}/l]	4.16	4.18	4.53	4.78	0.29	0.3894	1.59-4.10[w]
Leucocytes [x10^9/l]	13.08	12.16	12.75	10.68	1.18	0.4969	1.90-9.50[x]
Heterophils [%]	17.25	14.50	15.38	17.25	2.18	0.7653	15.00-40.00[x]
Lymphocytes [%]	80.88	84.00	83.88	80.25	2.18	0.4985	40.00-100.00[y]
H:L	0.22	0.18	0.19	0.22	0.03	0.7811	-
Monocytes [%]	0.38	1.38	0.38	1.38	0.68	0.5476	1.00-7.00[z]
Eosinophils [%]	0.25	0.00	0.38	0.63	0.31	0.5520	1.50-6.00[x]
Bands [%]	1.25	0.13	0.00	0.63	0.39	0.1212	-

Table 5: Effect of Ascorbic Acid Supplementation on Haematological Parameters of Broiler Starter Chickens.

PCV: Pack cell volume, [w]Mitruka and Rawnsely, 1997, [x]Simrak., *et al*., 2004, [y]Jain, 1986, [z]Jain, 1993.

the well-being of an animal and increases with stress or during a disease condition [34]. AA supplementation lowered the leucocyte count and was close to the normal range reported by Simrak., *et al.* [28]. Sorensen., *et al.* [35] reported that the presence of AA may limit free radical damage and free radicals may play a complex role in the healing response.

Hetrophil-lymphocyte ratio [H: L] is an indicator of stress and increases during stress [36]. The lower heterophil, H:L and increased lymphocytes recorded in broilers fed 68 mg of AA supplemented diet are indications that AA mitigated the deleterious impact of heat stress on the birds. Youssef., *et al*. [27], reported that vitamin C at 200 mg lowered the concentrations of heterophils, H:L and increased lymphocyte count during heat stress. Broilers with supplemented diets of 68 and 204 mg of AA had higher number of monocyte and within normal physiological range while broilers fed 204 mg supplemented diet had higher number of eosinophil. This is consistent with the work of Youssef., *et al*. [27] who reported higher monocyte and eosinophils at 200 mg vitamin C supplementation. The higher monocytes and eosinophils recorded could be an indication of AA involvement in repair of tissues damage caused by free radicals or ROS [35]. Monocytes and eosinophils are component of leucocyte. Monocytes remove dead, damaged cells as well as microorganism and are often found in higher numbers during a stress response or inflammation and eosinophils are increased during hypersensitivity responses especially parasitic infections [37].

Serum indices

Serum indices of starter broiler chickens fed diet supplemented with varying levels of ascorbic acid is presented in table 6. All the parameters were similar except for LDL and ALT. Glucose, total protein, albumin and globulin ranged from 193.95-252.90 mg/dl, 3.65-4.70 g/dl, 2.25-2.95 g/dl and 1.30-2.20 g/dl. Cholesterol range from 46.00-94.25 mg/dl. LDL range from 55.25-123.50 mg/dl and was higher [$p < 0.05$] in broilers fed 68 mg and 136 mg of ascorbic acid supplemented diet. ALT varied from 8.25-55.50 μ/L and was higher [$p < 0.05$] for broilers fed diet supplemented with 136 mg of ascorbic acid per kg. AST and ALP range from 13.00-34.25 μ/L and 23.50-43.50 μ/L. GSHPx, SOD, MDA and CAT ranged from 1.64-2.56 μmol/mL, 1.50-4.35 μmol/mL, 17452-60526 μmol/mL and 3.78-9.45 U/ml. Calcium and phosphorus ranged from 6.45-9.25 mg/dL and 2.40-2.85 mg/dL. Glucose values were similar among the groups and within the normal range reported by Goodwin., *et al*. [38]. This implies that heat stress did not negatively affect the energy level of the birds during this period. Lin., *et al*. [39] stated that physiological stress induces a higher glucose level in the blood. Ismail., *et al.* [40], supplemented the diet of heat stressed broilers at two, four and six weeks with 1 g of vitamin C and reported non-significant difference in the glucose level among the groups.

The significant increase observed in ALT of broilers fed 136 mg of AA supplemented diet may be due to increased synthesis of ALT by the liver and could be as a result of inflammation of the liver

Parameters	Dietary levels of Ascorbic acid [mg/kg diet]						
	0	68	136	204	SEM	P value	Ref-value
Glucose [mg/dL]	193.95	211.50	203.40	252.90	19.65	0.2103	137-363^{w}
Total Protein [g/dL]	3.65	4.08	4.70	4.48	5.06	0.4646	3.60-5.50^{x}
Albumin [g/dL]	2.25	2.78	2.50	2.95	0.32	0.4526	1.10-2.20^{x}
Globulin [g/dL]	1.40	1.30	2.20	1.53	5.18	0.4722	1.20-3.20^{y}
Cholesterol [mg/dL]	53.25	89.50	94.25	46.00	16.14	0.1258	120-237
Low Density Lipoprotein [mg/dL]	73.25^{b}	111.00^{a}	123.50^{a}	55.25^{b}	13.40	0.0119	< 130.00
Triglyceride [mg/dL]	58.00	38.50	62.75	71.75	12.66	0.3415	< 135.00
Alanine-Amino Transferase [μ/L]	14.75^{c}	8.25^{c}	55.50^{a}	33.25^{b}	3.59	0.0001	-
Aspartate-Amino Transferase [μ/L]	13.00	34.25	29.00	17.50	5.59	0.0663	10-40^{y}
Alkaline Phosphatase [μ/L]	23.50	43.50	28.00	38.75	5.93	0.1145	10-106^{z}
Glutathione Peroxidase [μmol/mL]	1.64	2.56	1.82	1.79	0.37	0.3270	
Superoxide Dismutase [μmol/mL]	3.60	1.50	3.90	4.35	0.84	0.1327	
Malondialdehyde [nmol/mL]	60526	24138	28184	17452	14008.82	0.1886	
Catalase [U/ml]	3.78	4.39	9.45	7.49	1.69	0.1108	
Calcium [mg/dL]	8.55	6.45	9.25	7.55	1.67	0.6673	
Phosphorus [mg/dL]	2.45	2.75	2.85	2.40	0.36	0.7652	

Table 6: Effect of Ascorbic Acid Supplementation on Serum Indices of Broiler Starter Chickens.

^{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 Laboratory (1990), Collins (2018).

observed during the post-mortem examination. Changes in liver enzymes functions under stress conditions occurs due to malfunctioning of liver, as degenerating and necrotic cells leak enzymes from cytoplasm [41]. Oxygen free radicals can damage liver tissue and cause lipid peroxidation which is revealed by increase in serum ALT concentration, indicating the inability of liver to metabolize ALT [42] and attributed to the outflow of these enzymes from the liver cytosol to the blood stream [43]. In contrast, El-Habbak., *et al.* [44], argued that heat stress significantly decreased ALT level in the serum of broilers while Ismail., *et al*. [40] reported that ALT was non-significant when the diet of broilers was supplemented with 1 g of ascorbic acid or its combination with zinc bacitracin. Similarly, Adenkola., *et al*. [32] reported a non-significant effect in ALT of broilers fed 500 mg ascorbic acid supplemented diet. The increased ALT concentration observed as a result of higher AA supplementation agrees with the report of Youssf., *et al*. [27] who reported higher [$p < 0.05$] value of ALT in broiler fed 200 mg of AA supplemented diet.

The similar result obtained for ALP, total protein, albumin, globulin, cholesterol, triglyceride, calcium and phosphorus which were within the normal range implies that ascorbic acid improved the biochemical indices of broilers during heat stress. The result obtained for these parameters agrees with the report of Konca., *et al* [45]; Rashidi., *et al.* [46]; Ismail., *et al*. [40] and Alaeldein., *et al.* [33]. Rashidi., *et al*. [46] reported that heat stress increased levels of plasma glucose, triglyceride and cholesterol and reduced protein level. The increase in blood lipids under heat stress is due to the fact that high temperature reduces feed intake and broilers compensate their need for energy by lipolysis of body lipid which causes increase in blood cholesterol and triglycerides [46]. AA has been reported to increase serum calcium and phosphorus level [47].

Higher value of LDL was recorded in broilers fed 68 and 136 mgAA/kg diet but was lower in broilers fed the control diet and 204 mgAA/kg. This could be due to the fact that ascorbic acid reduced corticoid secretion and prevented the stimulation of lipoproteins and tissue lipases [48]. The synthesis and metabolism of LDL is similar to cholesterol and ascorbic acid is necessary for the conversion of cholesterol to bile acids by controlling the microsomal 7α-hydroxylation, this reaction limit cholesterol catabolism in the liver, but the deficiency of ascorbic acid slow down the rate of this reaction, leading to cholesterol accumulation in liver and blood [48]. Ascorbate reduces corticoid secretion and prevent lipoprotein and tissue lipases, hence lipids are not transported from tissue to liver and this also leads to reduced serum LDL level. Seyrek., *et al*. [48] reporteds that supplementing ascorbic acid at 150-500 mg/kg in the diet of quails during heat stress significantly lowered the level of serum lipoproteins.

GSHPx was higher in broilers fed 68 mg of AA supplemented diet, while 204 mg of AA supplementation increased SOD activity and lowered MDA concentration. Broilers fed diet supplemented with 136 mg of AA had higher CAT level than the other groups. In general AA in the diet of broiler improved the stress enzymes and lowered the MDA level of broilers. This is evidence of the antioxidant property of AA against free radical and ROS. The result obtained in this study agrees with the report of Jena., *et al.* [2013]; Adenkola., *et al*. [32].

Serum cortisol and thyroxine level

Figure 1 and 2 shows the cortisol and thyroxine levels of broiler chicks fed varying levels of ascorbic acid supplemented diets during 28 days of the study. Cortisol was lower [$p < 0.05$] in broilers fed control diet [6.00 ng/mL] and 68 mgAA/kg [4.80 ng/mL] and higher in broilers fed 136 [12.40 ng/mL] and 204 [11.90 ng/mL] mg ascorbic acid supplemented group. Numerical increase level of thyroxine was recorded in broilers fed 68 [50.51 nmol/L] and 204 [46.92 nmol/L] mgAA/kg, although there was a decrease in broilers fed 136 mg [35.88 nmol/L] ascorbic acid supplemented diet compared to the control group [38.36 nmol/L]. High environmental temperatures causes an increase in plasma corticosterone and reduction in the activity and performance of lymphoid organs and total leukocytic count, but ascorbic acid has been found to cause a reduction in high plasma corticosterone levels with subsequent maintenance of normal leukocytic count [49]. This was observed when the diet of broilers was supplemented with 68 mgAA/kg, the lower cortisol level of the control birds could be due to the basal ascorbic acid [163.44%] content of the control diet. The higher cortisol recorded in broilers fed 136 and 204 mgAA/kg could be due to the involvement of ascorbic acid in the bio-synthesis of corticosterone [49] or it pro-oxidant activity at high doses, matrix environment or chemical interaction. Sotler., *et al*. [50] reported that vitamin C is a potent antioxidant but can intervene as a pro-oxidant depending on the dose [antioxidant; 30-100 mg/kg body weight and pro-oxidant; 1000 mg/kg body weight] or its interaction with iron or copper. The report of the present study agrees with Mahmoud., *et al.* [51] who reported an elevated corticosterone level in broilers fed 250 mgAA/kg during heat stress but is in contrast to Poungpong., *et al.* [52] who reported a decreased cortisol level in broilers fed diet supplemented with 286 ppm ascorbic acid during heat stress. Mosleh., *et al*. [53] reported a significantly similar corticosterone level in broilers administered control or 12 gAA/100L of drinking water during heat stress. Thyroid hormones are known to be negatively influenced by stress, lowering plasma concentration of T3 and T4. The higher thyroxine level reported in broilers fed 68 mgAA/kg diet could be due to the attenuating role of ascorbic acid against the deleterious effect of heat stress while the decrease observed in broilers fed 136 mgAA/kg could be as a result of elevated cortisol level in the birds which might have interrupted thyroid activity. Mahmoud., *et al*. [51] reported that supplementing the diet of broilers with 250 mgAA/kg during heat stress elevated thyroxine level.

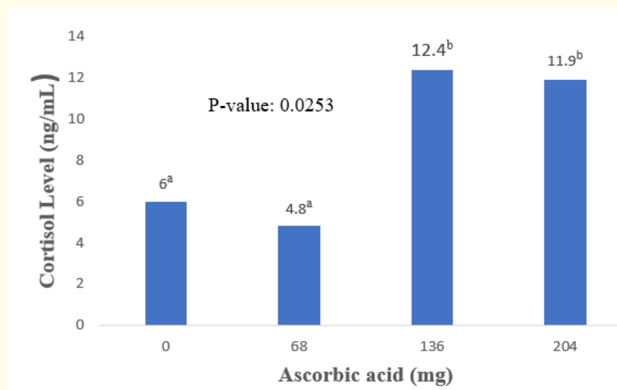

Figure 1: Effect of varying levels of Ascorbic acid on Cortisol level of Broiler Starter Chicks.

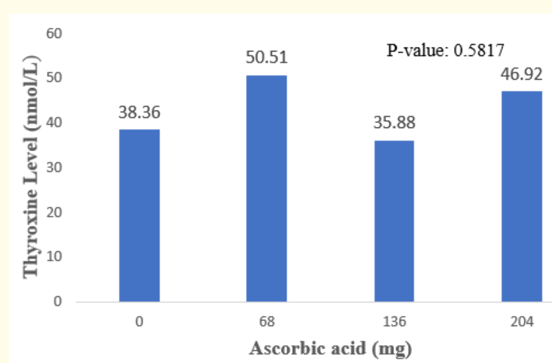

Figure 2: Effect of varying levels of Ascorbic acid on Thyroxine level of Broiler Starter Chicks.

Conclusion

BFPM is a good source of natural ascorbic acid and contain other nutrients that are required for poultry production and should be supplemented in the diets of poultry at 68 and 136 mgAA/kg during adverse [heat] temperature conditions. Further research should be conducted towards supplementing BFPM at levels other than those in the present study as natural source of ascorbic acid during hot temperatures.

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Conflicts of Interest

The authors declare no conflict of interest.

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