BLOOD PROFILE OF BROILER CHICKENS AS AFFECTED BY DIETS SUPPLEMENTED WITH GRADED LEVELS OF ASCORBIC ACID

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ABSTRACT

Effect of graded dietary ascorbic acid on blood profile of broiler chickens was investigated using a total of 270 1–day broiler chickens. The birds were randomly divided into six treatments. Each treatment was in triplicate of fifteen birds. Six isocaloric and isonitrogenous diets were formulated. Treatment 1 (T1) was the control diet with no ascorbic acid added while T2, T3, T4, T5 and T6 contained 0.1, 0.2, 0.3, 0.4 and 0.5g/Kg supplemental ascorbic acid respectively. The design of the experiment was completely randomized design. White blood cell counts ($x10^3/mm^3$) of birds on T3, T4, T5 and T6 at the starter phase were 2.65, 2.99, 2.83 and 3.13 respectively and were significantly higher ($p<0.05$) than those on T1 (1.69) and T2 (1.87). Red blood cells ($x10^6/mm^3$), monocytes (%), triglyceride (g/dL) and high density lipoproteins, (mg/dL) values differed significantly ($p<0.05$) among treatments while other parameters did not vary significantly ($p<0.05$) due to supplementation. A minimum of 0.2g/Kg ascorbic acid supplementation in diets of broiler starters improved the immune potentials and alleviated the effects of stress.

Keywords: Supplemental ascorbic acid, Chicken immune potentials, Heat stress alleviation, Broiler blood profile

Introduction

In Nigeria poultry production has evolved as one of the most efficient industries producing food for human consumption (Onu, 2009). Proper nutritional and management practices are the major factors that determine success in broiler chicken production resulting in the broilers reaching maximum weight within a shorter time (Rajput *et al.*, 2009). However, in Nigeria, environmental temperature is usually above the comfort zone of $18^0C – 22^0C$ for broiler chickens (Charles, 2002). Compared with other domestic animals, broiler chickens are more susceptible to changing environmental conditions (Nolan *et al.*, 1999). Due to the relatively higher cost and impracticability of cooling animal buildings, dietary manipulations have been one of the ways of alleviating the effects of heat stress on broiler chickens (Sahin *et al.*, 2003).

Supplemental ascorbic acid has been noted to play very important roles in poultry stress management (Roche, 1989). These roles include collagen formation, nutrient absorption, anti-oxidant functions etc. (Vasudevan *et al.*, 2011). The inclusion of ascorbic acid in the diet to maximize broiler chicken production in temperate region has also been documented (McCormack *et al.*, 2001). It has been reported (Majekodunmi *et al.*, 2012) that ascorbic acid and electrolytes supplementation in water for heat stressed broilers in Ibadan, Nigeria increased survivability though, did not positively affect the body weight and feed conversion ratio.

Conversely, other reports (National research council, 1994; McDowell, 2000; Konca *et al.*, 2008) have found no beneficial effect of vitamin C supplementation under any condition. National Research Council (NRC) (1994) and Agricultural Research Council (ARC), two major bodies that set nutrient requirement for livestock did not have any ascorbic acid recommendation or specification for broiler chicken on the basis that poultry have the innate ability to synthesize the vitamin (Roche, 1989). However, research (Kutlu and Forbes, 1993) has shown that for newly hatched, fast growing and matured stressed poultry, there was slow rate of endogenous biosynthesis and increased probability of ascorbic acid deficiency which may reflect in the blood picture.

Though, nutrient levels in the blood and body fluids are not valid indications of nutrient function at cellular level, they are however considered to be proximate measures of long term nutritional status (Doyle, 2006). Blood constituents have been very valuable both for clinical and nutritional evaluation (Aderemi, 2004), also a tool for diagnosing dietary deficiency and toxicity. This study was therefore undertaken to determine the effects of graded dietary ascorbic acid supplementation on the blood profile of broiler chickens.

Materials and Methods

The experiment was carried out at the Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria. A total of 270, 1-day old broiler chicks were randomly allotted to six dietary treatments of forty five chicks per treatment. Each treatment was a triplicate of fifteen birds per replicate. The birds were raised on partitioned deep litter house at stocking density of $0.1m^2$/bird. Six isocaloric and isonitrogenous starter and finisher diets were formulated. Details of the basal starter and finisher dietary composition is shown in Table 1. The experimental diets were obtained with appropriate graded inclusion of ascorbic acid as follows: Treatment 1 (T1) was the control diet with no ascorbic acid added while T2, T3, T4, T5 and T6 contained 0.1, 0.2, 0.3, 0.4 and 0.5g/kg supplemental ascorbic acid respectively. The design of the experiment was completely randomized design. Routine medication, vaccination and husbandry practices were administered on the birds (Oluyemi and Roberts, 2000).

The birds were offered starter and finisher' diets and water *ad libitum* in the first 3 and the latter 3 weeks of the experiment respectively. Blood samples were obtained from the jugular vein of three birds per replicate at days 21 and 42 into bottles with EDTA for haematological analyses and bottles without EDTA for serum biochemical analyses.

Haematological parameters; Packed cell volume (PCV), haemoglobin (Hb), red blood cells (RBC), white blood cells (WBC) and differential counts were determined using standard procedures (Schalm *et al*., 1975). Serum indices; total protein (Kohn and Allen (1995) albumin and globulin (Peter *et al*., 1982), cholesterol (Roschlan *et al*., 1974), triglycerides (Jacob *et al*., 1960), alkaline phosphatase (ALP) (Reitman *et al*., 1957), high density lipoprotein (Friedwald, 1972) and electrolytes (Calcium and Phosphorus) were also determined. Data obtained were analyzed using analysis of variance (SAS, 1999) while significant means were separated.

Results

The haematological parameters of broiler chickens fed diets with graded levels of ascorbic acid for blood sampled at days 21 and 42 respectively are shown in Tables 2 and 3. Values obtained for PCV, Hb, lymphocytes, eosinophils and basophils were not significantly different ($p<0.05$) among treatments. However, RBC ($x10^6/mm^3$), WBC ($x 10^3/mm^3$) and monocytes (%) which ranged from 3.98-5.20, 16.98-31.30 and 2.33-7.67 respectively were significantly different among treatments at day 21. At day 42, there were significant differences ($p<0.05$) in the values of RBC, WBC, heterophils and monocytes among treatments. Birds on 0.5g/kg ascorbic acid supplementation had significantly ($p<0.05$) higher value ($4.66 \times 10^6/mm^3$) of RBC which differed from the values recorded for birds on other treatment groups. Birds on 0.2, 0.3, 0.4 and 0.5g/kg ascorbic acid supplementation had significantly ($p<0.05$) higher values of WBC compared with those on the control and 0.1g/kg supplementation. Significant variation was observed in heterophil values at day 42 which ranged from 28.00-31.00% with birds on T3 (31.33%) and T6 (31.00%) having significantly ($p<0.05$) higher values compared to those on T5 (23.67%). Significantly ($p<0.05$) higher value (4.00%) of monocytes was also observed in birds on 0.3g/kg ascorbic acid supplementation which differed from those in the control group (1.00%). Results of serum biochemical parameters of broiler chickens fed diets with graded levels of ascorbic acid at days 21 and 42 respectively are shown in Tables 4 and 5. Significant variations ($p<0.05$) were observed in values of triglycerides among treatments at day 21 with birds on T5 having significantly higher value (102.72 g/dL) compared with those in the control group (58.99 g/dL). Likewise at day 42, significant ($p<0.05$) variations were observed in the values of total protein, triglycerides, cholesterol, high density lipoprotein (HDL), very low density lipoprotein (VLDL), phosphorous and alkaline phosphatase among treatments.

Table 1: Gross composition of basal starter and finisher diets fed to broilers

Ingredients		Starter	Finisher
Maize		52.00	51.00
Soyabean meal		35.00	25.00
Palm oil		2.50	3.00
Wheat offal		7.23	17.73
Oyster Shell		1.00	1.00
Dicalcium phosphate		1.50	1.50
Lasalocid		0.06	0.06
Broiler premix		0.25	0.25
Salt		0.25	0.25
DL Methionine		0.15	0.15
L Lysine		0.06	0.06
Ascorbic acid		xx	xx
Total		100.00	100.00
Total calculated nutrients			
Crude protein (%)		22.94	19.73
Crude fibre (%)		4.31	4.80
Metabolisable energy	Kcal/kg	3147	3121

Table 2: Haematology of broiler birds fed diets supplemented with graded levels of ascorbic acid (blood sampled at day 21)

Parameter	T1	T2	T3	T4	T5	T6	SEM
PCV (%)	30.00	31.33	30.00	29.00	28.67	29.67	1.72
Hb (g/dL)	10.00	10.44	10.00	10.33	9.56	10.22	0.68
RBC ($x10^6/mm^3$)	4.16^{ab}	4.45	3.98^{b}	4.37^{ab}	4.61^{ab}	5.20^{a}	0.33
WBC ($x10^3/mm^3$)	1.69^{b}	1.87	2.65^{a}	2.99^{a}	2.83^{a}	3.13^{a}	1.77
Lymphocytes (%)	48.33	46.00	48.67	58.33	55.00	61.67	8.92
Heterophils (%)	42.00	48.00	43.00	34.67	40.67	32.33	7.93
Monocytes (%)	7.67^{a}	3.33^{ab}	6.67^{ab}	5.00^{ab}	2.33^{b}	4.33^{ab}	1.54
Eosinophils (%)	2.00	2.33	1.67	2.00	1.67	2.00	0.85
Basophils (%)	0.00	0.33	0.00	0.00	0.33	0.33	0.24

Means along the same row with dissimilar superscripts are significantly different ($p<0.05$)
PCV-Packed cell volume; Hb-Haemoglobin; RBC-Red blood cells; WBC-White blood cells

Table 3: Haematology of broiler birds fed diets supplemented with graded levels of ascorbic acid (blood sampled at day 42)

Parameter	T1	T2	T3	T4	T5	T6	SEM
PCV (%)	29.67	31.00	31.67	38.67	37.00	29.00	1.53
Hb (g/dL)	9.89	10.33	10.56	9.56	9.00	9.78	0.50
RBC($x10^6/mm^3$)	3.31	3.04	3.69	3.00	3.69	4.66	0.16
WBC($x10^3/mm^3$)	23.33	24.10	28.75	25.08	17.83	17.75	1.21
Lymphocytes (%)	68.33	68.67	65.67	65.67	70.67	66.33	1.86
Heerophils (%)	28.00	28.33	31.33	27.33	23.67	31.00	2.15
Monocytes (%)	1.00	2.00	1.67	4.00	2.67	2.00	0.73
Eosinophils (%)	2.00	1.00	1.33	3.00	3.00	0.67	0.77
Basophils (%)	0.33	0.00	0.33	0.00	0.33	0.33	0.27

Means along the same row with dissimilar superscripts are significantly different ($p<0.05$)
PCV-Packed cell volume; Hb-Haemoglobin; RBC-Red blood cells; WBC-White blood cells

Table 4: Selected biochemical indices of broiler birds fed diets supplemented with graded levels of ascorbic acid (blood sampled at day 21)

Parameter	T1	T2	T3	T4	T5	T6	SEM
Total Protein (g/dL)	4.29	4.59	4.32	4.75	4.02	4.59	0.49
Albumin (g/dL)	1.23	1.35	1.39	1.38	1.18	1.44	0.11
Globulin (g/dL)	3.06	3.24	2.90	3.37	2.84	3.16	0.40
Triglyceride (g/dL)	58.98^{b}	80.55^{ab}	87.75^{ab}	82.13^{ab}	102.72^{a}	82.60^{ab}	10.01
Cholesterol (mg/dL)	97.15	93.74	86.40	95.38	95.77	92.02	9.91
VLDL (mg/dL)	18.46	16.11	17.55	16.43	20.55	42.50	11.09
HDL (mg/dL)	48.11^{b}	103.33^{a}	90.44^{a}	93.33^{a}	102.00^{a}	115.78^{a}	9.53
Calcium (mg/dL)	7.92	5.40	8.88	4.65	7.00	6.73	1.28
Phosphorus (mg/dL)	7.42	5.96	6.02	6.35	7.52	6.62	1.16
ALP (iu/L)	81.63	80.36	108.55	122.20	77.72	80.15	13.84

Means along the same row with dissimilar superscripts are significantly different ($p<0.05$)
VLDL-Very low density lipoprotein; HDL-High density lipoprotein; ALP-Alkaline phosphatase

Table 5: Selected biochemical indices of broiler birds fed diets supplemented with graded levels of ascorbic acid (blood sampled at day 42)

Parameter	T1	T2	T3	T4	T5	T6	SEM
Total Protein (g/dL)	2.44^{ab}	3.36^{a}	2.81^{ab}	2.62^{ab}	1.92^{b}	1.96^{b}	0.38
Albumin (g/dL)	1.05	1.50	1.52	1.01	1.13	1.37	0.02
Globulin (g/dL)	1.39	1.87	1.29	1.61	0.79	0.59	0.40
Triglyceride (g/dL)	23.36^{c}	32.28^{bc}	36.60^{ab}	43.91^{ab}	46.73^{a}	46.87^{a}	3.85
Cholesterol (mg/dL)	109.73^{ab}	163.10^{a}	143.70^{ab}	102.20^{b}	98.20^{b}	120.10^{ab}	16.12
VLDL (mg/dL)	4.67^{c}	$6.45b^{c}$	7.32^{ab}	8.78^{ab}	9.35^{a}	9.37^{a}	0.77
HDL (mg/dL)	16.68^{ab}	21.68^{a}	18.03^{ab}	17.92^{ab}	14.48^{b}	15.89^{ab}	1.70
Calcium (mg/dL)	3.54	5.49	8.55	5.39	8.04	8.48	1.56
Phosphorous (mg/dL)	13.09^{a}	8.52^{ab}	3.21^{b}	4.74^{b}	5.20^{b}	3.71^{b}	2.12
ALP (iu/L)	19.09^{b}	24.55^{b}	29.09^{ab}	30.03^{ab}	33.12^{ab}	46.12^{a}	5.22

a,b,c- means along the same row with dissimilar superscripts are significantly different ($p<0.05$); SEM- Standard error of mean; VLDL-Very low density lipoprotein; HDL-High density lipoprotein; ALP-Alkaline phosphatase

Discussion

Ascorbic acid is a multi functional biological agent. It plays important physiological roles as coenzyme in enzymatic biosynthesis of collagen, carnitine, catecholamine and peptide neuro-hormones (Wilson, 2002). In addition, ascorbic acid prevents injurious effects of oxidants because it reduces reactive oxygen and nitrogen species to stable molecules (Wilson, 2002). Ascorbic acid consistently increased RBC in birds on 0.4 and 0.5g/kg supplementation in this study. This may perhaps be due to the roles of vitamin C in improving the availability of iron found in the storage tissue site (NPA, 2003), and in facilitating iron absorption in the gastro intestinal tract by its ability to reduce ferric iron to the absorbable ferrous form in the intestinal system (Vasudevan *et al.*, 2011). Nguyen *et al.* (2001) reported that ascorbic acid acts as antioxidant to protect RBC membrane against the harmful oxidation processes and thus reduce the RBC haemolysis via increasing the flexibility of the cell membrane.

The WBC values obtained at both days 21 and 42 were within the reference range of 9-31x10^3mm for broiler chicken (Mitruka and Rawnsley, 1977; Mmereole, 1996 and Ikhimioya *et al.*, 2000). The WBC count greater than normal range is suggestive leukocytosis (Ewuola and Egbunike, 2008). General causes of leukocytosis include infection, trauma, toxicities, haemorrhage into a body cavity, rapidly growing neoplasm and leukemia. Vitamin C assists the immune system in two of its primary functions to rid the body of foreign invaders and to monitor the system for any sign of tumour cell. It accomplishes these vital tasks by stimulating the production of white blood cells primarily neutrophils, which attack foreign antigens such as bacteria and viruses. Apart from the values of monocytes that were lowered by increased administration of ascorbic acid at the starter phase only, other differential leukocyte counts were not affected significantly both at the starter and finisher phases.

Kutlu and Forbes, (1993) reported that vitamin C supplementation reduced serum glucose, cholesterol, and triglycerides whereas total protein, albumin concentration increased in stressed broiler birds. In this study, ascorbic acid increased the serum total protein up to 0.3% supplementation and reduced cholesterol concentration at 0.3 and 0.4g/kg supplementation. One of the metabolic pathways involved in the reduction of lipid mobilization and catabolism when birds are supplemented with ascorbic acid is the transformation of cholesterol to bile acids by controlling the microsomal 7α-hydoxylation thereby decreasing cholesterol concentrations in liver and in serum. Because cholesterol is transported in blood by lipoprotein complexes (VLDL, LDL and HDL), cholesterol and lipoprotein concentrations were positively correlated (Linne and Ringsrud, 1999). However, this report is at variance with the observation in this study as VLDL value was higher in ascorbic acid supplemented group.

Sahin *et al.* (2003) reported remarkable elevations of ALP activity in Japanese quails exposed to high temperature, given diet supplemented with vitamin C. Similarly, results of this study indicated progressive increased serum ALP concentration as the ascorbic acid supplementation increased at day 42. The ALP is known to be responsible, under normal conditions, for liberating inorganic phosphate from organic combination and thus increasing at the site of ossification, the ionic products particularly, Ca^{2+} X $PO4^{3-}$. This may be interpreted to mean a progressive increased osteoblast and osteoclast the duo dynamic processes in bone remodeling (Cheeke and Dierenfeld, 2010). This is in agreement with earlier reports (Cheeke and Dierenfeld, 2010; Bhattacharya, 2010; Vasudevan *et al.*, 2011) on the much orchestrated roles of ascorbic acid in bone metabolism.

Conclusion

Inferential indications from this study revealed positive effects of ascorbic acid supplementation on blood profile of broiler. There was marked improvement on blood profile of birds at supplementation levels from 0.1 to 0.3g/kg which was optimized at 0.3% supplementation.

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