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Haematological and Biochemical Parameters of Broiler Chicken Supplemented with Humic Acid in the Drinking Water

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ABSTRACT

This study was conducted to evaluate the effects of supplementing humic acid in the drinking water on some haematological and biochemical parameters of broiler chickens. One hundred and twenty (120), day-old Arbor Acre broiler chicks were randomly divided into three treatments in a completely randomized design. Each treatment of forty birds was replicated 4 times with 10 chicks per replicate and the experiment lasted for 56 days. Treatment I served as the control without humic acid in the drinking water while, treatment II was supplemented with 1 mL/L humic acid and treatment III was supplemented with 2 mL/L of humic acid. Results obtained showed that supplementation with humic acids had no significant effect ($P > 0.05$) on haematological indices of the broiler chickens. Significant ($P<0.05$) reduction in serum level of uric acid was achieved as the level of supplementation of humic acid increased while, serum phosphorus and calcium concentrations were significantly ($P<0.05$) increased with humic acid. Liver function enzymes were not significantly ($P>0.05$) affected because the activity of serum alanine transaminase and aspartate transaminase were similar among all treatments. The results indicated that humic acid supplementation had a beneficial effect on mineral absorption and detoxification as revealed through uric acid concentration of broiler chickens.

Keywords: blood biochemistry; blood minerals; drinking water; haematology; humic acid

INTRODUCTION

Antibiotics had been used widely world-wide in poultry industry in order to prevent poultry disease and improve production. However, with the unavoidable spread of bacterial resistance and cross resistance the use of antibiotics has been considered hazardous (Andremont, 2000). Several additives have been tested as growth promoters to avoid the excessive use of antibiotics or at least reduce or substitute their inclusion in feeds, while maintaining an efficient animal production to obtain safe edible products (Islam *et al*., 2005; Gomez *et al*., 2012). Among these additives are humic substances which have been tested in domestic animals in several studies around the world with promising results. The humic substances are very common in nature as they originate from the decomposition of organic matter, and are normally present in the drinking water and soil (Islam *et al*., 2005).

Humic acids are naturally occurring decomposed organic constituents of soil and lignite that are complex mixtures of polyaromatic and heterocyclic chemicals with multiple carboxylic acid side chains (MacCarthy, 2001).
Farmers utilize humic substances to accelerate seed germination and improve rhizome growth. These materials stimulate oxygen transport, accelerate respiration, and promote the efficient utilization of nutrients by the plant (Visser, 1987). These observations have prompted scientists to assess the specific properties of humates and their possible benefits in the improvement of health and wellbeing in animals (Wang *et al*., 2008). The use of humic acid to replace antibiotics in poultry has gained widespread interest (Mutus *et al*., 2006). Beneficial effects of humic acid are described concerning stress management (Enviromate, 2002) immune system (Hooge, 2004; Loddi *et al*., 2002), anti-inflammatory activity (Yang, 1996), antiviral properties (Huck *et al*., 1991). It has been observed that humic acid included in the feed and water of poultry promote growth (Kocabagli *et al*., 2002; Mirnawati and Marlida, 2013). It has also been proposed that humic acid plays an effective role on some blood parameters (Banaszkiewicz and Drobnik, 1994; Rath *et al*., 2006) and have been used to minimize health problems and potential losses (Yoruk *et al*., 2004; Kucukersan *et al*., 2005; Trckova *et al*., 2006).

Humic acids have been reported to improved immune responses and electrolyte balance in turkeys (Parks *et al*., 1998). It is considered as safe to be used as the feed additives in animal production (Celik *et al*., 2008).

This study was conducted to evaluate the effect of supplementing humic acid in the drinking water of broiler chickens on the haematological and serum biochemistry parameters.

MATERIALS AND METHODS

The commercial humic acid used is a product of Dynapharmlab Associate SDN. BHD. It contains chelated micronutrients, nitrogen 2.35%, phosphorus 4.44%, potassium 1.75%, magnesium 0.36%, iron 867ppm, manganese 223ppm, copper 144ppm, zinc 153ppm, boron 0.011%, molybdenum 0.002% and humic acid 0.68%.

Experimental birds:

A total of one hundred and twenty (120) day-old Abor Acre broiler chicks were randomly allocated into three (3) treatments. Treatment I (control) with no humic acid supplementation, treatment II supplementation with 1mL/L humic acid and treatment III with 2mL/L humic acid supplementation in drinking water. Each treatment was replicated four (4) times with ten (10) chicks per replicate. The duration of the experiment was eight (8) weeks; broiler starter mash was fed for the first four (4) weeks while broiler finisher mash was given at the last four weeks. The gross composition of the diets are presented in Table 1. Fresh water was given on daily basis.

Blood parameters measurements:

At the end of the 56 days of the trial, blood samples (5mL/bird) were obtained from 2 chickens per replicate by puncturing the brachial vein. Blood samples were collected into vials containing ethylene diamine tetra-acetate for the determination of haematological indices and the remaining into universal bottles without anticoagulant for serum analysis. Haemoglobin concentration (Hb) was estimated using the cyanmethaemoglobin method (Cannan, 1958). Packed cell volume (PCV), red blood cell (RBC), and white blood cell count (WBC) were determined with Wintrobe haematocrit tube according to the method of Schalm *et al*. (1975). Differential leucocyte counts (heterophils, lymphocytes, eosinophils, basophils and monocytes) were carried out on blood smears stained with May-Grunwald–Giemsa stain. Sera were harvested from blood by centrifugation and kept inside the freezer until needed for biochemical analysis. Total serum protein and albumin were determined using bromocresol purple method (Varley *et al*., 1980). Serum uric acid concentration was estimated according to standard procedures of Wootton (1964). Serum creatinine level was determined using the method described by Tietz (1986). Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase were determined by standard methods using Randox test kits (Randox_ Laboratories, Antrim, UK). Calcium and phosphorus were determined using the methods of Gindler and King (1972) and Goodwin (1970) respectively.

Table 1: Percentage composition of broiler chickens basal diets.

Ingredient	Starter (0-4 weeks)	Finisher (4-8 weeks)
Maize	53.9	60
Fish meal	2.5	0
Soya bean meal	35.6	28
Wheat offal	4	7.7
Bone meal	1.3	1.8
Oyster shell	1.8	1.5
Premix	0.3	0.3
Salt (NaCl)	0.3	0.3
L-lysine	0.1	0.1
L-methionine	0.2	0.3
Total	**100**	**100**
Analyzed values		
Crude protein (%)	23.64	19.91
Ether extract (%)	3.61	3.51
Crude fibre (%)	3.22	3.27
Calculated values		
Calcium (%)	1.08	1.09
Phosphorus (%)	0.43	0.39
Metabolizable energy (MJ/kg)	11.98	12

Starter premix provided: Vitamin A-10,000,000iu, Vitamin D3-2,000iu, Vitamin E-40,000mg, Vitamin K-2,000mg, Vitamin B1-1,500mg, Vitamin B2-4,000mg, Vitamin B6-40,000mg, Vitamin B12-20mg, Niacin-40,00mg, Pantothenic-10,000mg, Folic-1,000mg, Biotin-100mg, Choline-300,000mg, Manganese-80,000mg, Zinc-60,000mg, Iron-40,000mg, Copper-80,000mg, Iodine-800mg, Selenium-200mg, Cobalt-300mg, Antioxidant-100,000mg.

Statistical analysis:

Data generated were subjected to analysis of variance using the general linear model procedure of the SAS (2000). A probability of (P<0.05) was considered to be statistically significant using Duncan Multiple Range Test of the same package. Polynomial contrast (linear and quadratic) was used to determine the effect of varying dosage of humic acid.

RESULTS AND DISCUSSION

The haematological data presented in Table 2 indicated that supplementation of humic acid did not show any significant (P>0.05) effect on packed cell volume, red blood cell, haemoglobin, white blood cell and white blood differential count. Table 3 showed (P<0.05) reduced uric acid concentration in birds supplemented with humic acid when compared to those in the control treatment. Higher (P<0.05) blood Calcium (Ca) and Phosphorus (P) concentrations were obtained in the treatments supplemented with humic acid than control. Serum Ca and P (P<0.05) increased as the level of humic acid supplementation increased. Total protein, albumin, globulin and the liver enzymes (aspartate aminotransferase, alkaline aminotransferase and alkaline phosphatise) showed no significant difference among the treatments. However, haemoglobin and serum globulin were numerically higher in birds supplemented with humic acid than birds in the control group. While creatinine and the liver enzymes were reduced in birds supplemented with humic acid.

Similar haematological values obtained from broiler chickens supplemented with humic acid with those in the control group is indicative of tolerance of the humic acid used and the quantity supplemented in the drinking water to the chickens. According to Rath *et al*. (2006) RBC, WBC and PCV values in broilers were not affected by humic acid supplementation.

Table 2: Haematological parameters of broiler chickens supplemented with humic acid.

Parameter	Humic acid supplemented level (mL/L)			SEM	P-value	
	0	1	2		Linear	Quadratic
Packed cell volume (%)	31.67	32.33	30.33	1.04	0.634	0.922
Red blood cell ($x10^6$/μL)	3.18	3.10	2.94	0.21	0.189	0.451
Haemoglobin (g/dL)	10.60	11.33	11.90	0.39	0.073	0.202
White blood cell ($x10^9$/L)	8.83	6.13	5.07	0.87	0.456	0.666
Lymphocytes (%)	66.33	66.67	64.00	1.18	0.92	0.982
Heterophil (%)	29.67	29.00	29.33	1.22	0.056	0.187
Monocyte (%)	2.00	3.00	4.00	0.44	0.381	0.171
Eosinophil (%)	1.67	1.00	2.33	0.28	0.244	0.422
Basophil (%)	0.33	0.00	0.00	0.11	0.067	0.12

SEM= standard error of mean

Table 3: Biochemical parameters of broiler chickens supplemented with humic acid.

Parameter	Humic acid supplemented level (mL/L)			SEM	P-value	
	0	1	2		Linear	Quadratic
Total protein (g/dL)	3.30	3.30	3.80	0.26	0.467	0.72
Albumin (g/dL)	1.83	1.47	1.63	0.14	0.593	0.627
Globulin (g/dL)	1.80	1.83	2.13	0.22	0.57	0.835
Uric acid (mg/dL)	4.53	3.50	3.63	0.18	0.031	0.009
Creatinine (mg/dL)	0.83	0.63	0.60	0.06	0.141	0.3
Aspartate aminotransferase (U/dL)	58.00	51.33	48.67	2.29	0.096	0.254
Alanine aminotransferase (U/dL)	33.67	31.67	25.00	1.90	0.053	0.143
Alkaline phosphatase (U/dL)	30.00	28.67	25.00	1.54	0.203	0.447
Calcium (mg/dL)	5.42	6.4	6.80	0.21	0	0
Phosphorus (mg/dL)	2.58	3.21	3.45	0.13	0	0

SEM= standard error of mean

Similarly, Cetin *et al*. (2006) reported that supplementation of humic acid to laying hens had no effects on WBC and PCV, but affected, RBC and Hb. However, Banaszkiewicz and Drobnik (1994) reported that Hb, PCV and RBC were observed to increase in rats treated with humic acid. The inconsistency of results obtained in the various studies might be attributed to the composition and quantity of the humic acid used.

The increase of Ca and P levels in blood serum produced by humic acid may be attributed to the lowering of gastrointestinal tract pH by using this acid, which increases the absorption of the minerals from the gut into the blood stream. Improvement in the utilization of calcium and phosphorus by organic acids supplementation was revealed by Boling *et al*. (2001). Also, Abdo and Zeinb (2004) observed an increase in blood calcium of broiler chicks fed on dietary acidifier. Furthermore, (Kishi *et al*., 1999) reported that dietary acetic acid prevented osteoporosis, through the reduction in bone turnover, as it enhanced intestinal Ca absorption by improving Ca solubility in ovariectomized rats.

After humate feeding, increased levels of some essential minerals (such as Ca, Al and Fe) in serum, liver and muscles were recorded by Stepchenko *et al*. (1991). Whereas, Klocking (1994) reported a reduction in the serum concentrations of Ca and P at 2.5 % level of humic acid, which may be due to its metal chelating effects caused by large number of carboxylic acid side chains.

Reduced uric acid concentrations with humic acid could be as a result of better utilization of protein and amino acid digestibility. As uric acid is the major end product of protein metabolism. The present results coincide with the findings of Abdo and Zeinb (2004) who obtained lower uric acid in broiler chicks due to citric acid and acetic acid inclusion. Sturkie (1986) also revealed that dietary addition of organic acid slightly reduced serum concentration of uric acid. Supplementation of humic acid showed no deleterious effects on liver functions enzymes as the values were similar (P>0.05) among the treatments. The result of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase means that broiler chickens could tolerate the addition of up to 2 mL/L of the humic acid without any deleterious effects on liver functions. Abdel-Azeem *et al*. (2000) showed that level of aspartate aminotransferase was reduced, although alanine aminotransferase was not significantly affected when citric acid was supplemented in the diets of rabbits.

CONCLUSION

The result showed that broiler chickens could tolerate the addition of humic acids in the drinking water up to 2 mL/L without any deleterious effect on the health status.

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