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The Effect of Graded Levels of Dietary Methionine on the Haematology and Serum Biochemistry of Broilers

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Abstract: Graded levels of dietary methionine on the haematology and serum biochemistry were investigated in broilers. One hundred and fifty broiler chicks (Arbor acre breed) were use, the chicks were divided into 5 treatments consisting of 6 replicates of five chicks each. The chicks were kept in floor pens. The study lasted for 56 days at the teaching and research farm of the university of Ibadan Nigeria. No significant differences ($p \geq 0.05$) were observed in the PCV, RBC and WBC values of broilers fed the different levels of methionine inclusion, at the finisher phase, but at starter phase significant differences ($p \leq 0.05$) were observed, with the WBC values increasing as the inclusion rate of methionine increased. Total protein value of 4.80g/100ml and 4.48 g/100 ml were obtained for treatments 4 and 5 respectively which were not significantly ($p \geq 0.05$) from each other. There were wide variations in the glucose concentration of the birds, the highest glucose concentration was observed with birds on diet 2 (220.90) while the least was observed for birds fed the control diet though significant differences ($p \leq 0.05$) were observed it did not follow a particular order.

Key words: Broilers, dietary methionine, haematology, serum biochemistry

INTRODUCTION

Poultry farming has become an important money spinning industry in Nigeria today. The poultry industry, which includes the rearing of domestic chicken, turkey, ducks, geese and certain other birds are kept throughout the world and Nigeria in particular. The prominence of poultry production in Nigeria today is primarily due to the short generation interval and relatively quick turn over on investment and high quality protein from poultry products.

The rapid growth of broilers demands that they be supplied with high quality diets to sufficiently cater for their nutrients requirements. The principal constituent of broilers is soft tissues which are mainly proteins. The protein required by broiler depends primarily on the amount needed for maintenance of health, tissue integrity and for productive purposes. For broilers to meet the protein requirement, the amino acids must be available in the proper amount. Olomu and Offiong (1980) reported that a protein level of 23% is recommended for starting broilers raised in Nigeria. An increase in the amino acid level in warm environment to suite the expected reduction in feed intake in the warm environment was also suggested by the National Research Council (1994).

The influence of amino acids in poultry nutrition and methionine in particular cannot be over emphasized, methionine is an essential nutrient for poultry. In addition to amino acid that may provide methyl group which are needed for several metabolic reactions such as the syntheses of carnithine and creatine, some authors reported methionine sparing effect on chlorine (Pestal *et al*., 1981; Baker *et al*., 1985).

More recently, the sensitive of breast meat in broilers to dietary methionine has repeatedly been demonstrated (Hugghabaert *et al*., 1994) Schuttle and De Jong (1996) indicated the betaine has potential to partially spare methionine in a situation of severe methionine deficiency and even then betaine appear to be less effective than methionine.

The influence of methionine in broiler diets also serve to improved energy utilization in the body (Brody, 1994) because, supplementing methionine resulted in lowering feed conversion ratio while at the same time elevating abdominal fat content.

The objective of this experiment was to determine the influence of methionine on Haematology and Serum Biochemistry of broilers fed graded levels of methionine.

MATERIALS AND METHODS

This experiment was carried out at the broiler unit of the teaching and research farm, faculty of Agriculture and Forestry, University of Ibadan. One hundred and fifty (150) day old Arbor acre broiler chicks were purchased from a reputable hatchery in Ibadan, Nigeria. The birds were randomly alloted into five (5) treatments with six replicates of five birds per replicate in a completely randomized design. Feeds were formulated on isocaloric and isonitrogenous basis with different levels of methionine, The treatment 1 was the basal diet, treatment 2 had 0.19% of methionine, treatment 3 contained 0.31%, treatment 4 had 0.53% and treatment

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Table 1: Gross composition of experimental starter diets fed to broiler chicks (Starter phase)

Ingredients (%)	1	2	3	4	5
Maize	52	52	52	52	52
Soya bean meal	35	35	35	35	35
Wheat offal	6.5	6.5	6.5	6.5	6.5
Fish meal	2.5	2.5	2.5	2.5	2.5
Bone meal	2.0	2.0	2.0	2.0	2.0
Oyster shell	1.5	1.5	1.5	1.5	1.5
Broiler premix	0.25	0.25	0.25	0.25	0.25
Methionine	0.00	0.19	0.31	0.53	0.64
Salt	0.25	0.25	0.25	0.25	0.25
Crude protein (CP) %	22.6	22.6	22.6	22.6	22.6
Metabolizable energy ME (Kcal/kg)	2925	2925	2925	2925	2925
Total	100.0	100.0	100.0	100.0	100.0

Table 2: Composition of experimental diets fed to broiler chicks (Finisher phase)

Ingredients (%)	1	2	3	4	5
Maize	56	56	56	56	56
Soya bean meal	27	27	27	27	27
Wheat offal	9.5	9.5	9.5	9.5	9.5
Fish meal (FM)	1.5	1.5	1.5	1.5	1.5
Bone meal (BM)	3.0	3.0	3.0	3.0	3.0
Oyster shell (OYS)	1.5	1.5	1.5	1.5	1.5
Broiler premix	0.25	0.25	0.25	0.25	0.25
Methionine	-	0.19	0.31	0.53	0.64
Salt	0.25	0.25	0.25	0.25	0.25
Crude protein (CP)%	20.1	20.1	20.1	20.1	20.1
Metabolizable energy ME (Kcal/Kg)	2,905	2,905	2,905	2,905	2,905
Total	100.0	100.0	100.0	100.0	100.0

5 had 0.64% of methionine. Management of the birds, vaccination and medication were followed as recommended by Oluyemi and Robert (1979).

Collection, processing and analysis of blood samples: Blood was sampled from three birds per replicate at the 4th and 8th week of the experiment 4 mls of blood was collected from the birds for hematological and serum biochemistry analyses, with the aid of sterile syringes and needles via the jugular vein at the neck of the birds, the blood samples were in 2 sets per bird into test tubes containing and devoid of anti coagulant, Ethylene Di-amine Tetra Acetic Acids (EDTA) serum was collected from the tubes without EDTA after centrifugation for serum analysis. the second set of test-tube contain 2 ml of blood was collected into test tube which already contain (EDTA) the blood sample was kept in fresh state (refrigerator) to make possible hematological studies.

The blood samples collected were analyzed by the methods stated by Kelly (1979).The analysis was carried out almost immediately after collection.

The packed cell volume carried out by the use of micro haematocrit method (Kelly, 1979) Principle: The whole blood was centrifuged for maximum red blood cell packing; the space occupied by the red blood cell is measured and expressed as a percent of whole blood volume.

Neubauer haemacytometer was used to carry out red blood cell count (Kelly, 1979) Principle: isotonic diluting fluid was used to dilute to facilitate counting and present lyses of the red blood cells.

Neubauer haemocytometer was used to carry out the white blood cell count (Kelly, 1979) Principle: the blood sample was mixed with weak acid solution to haemolyse the red blood cells and dilutes the blood. the cytoplasm of the white blood cell was digested by the acid PH of the solution. then the nuclear entity of the cells were stained with the gentian violet of the avian fluid for proper enumeration.

Serum metabolites: The serum protein and albumin were analyzed using sigma assay kits, glucose by the method of Cooper and McDaniel (1970) and cholesterol by the method of Roschlan *et al*. (1974). The biuret method was utilized in the determination of the total protein fraction while the serum was subjected to the direct colorimetric method for albumin with Bromocresol Green (BCG) as the dye as described by Peters *et al*. (1982). The globulin concentration was obtained by subtracting albumin from the total protein.

Albumin/globulin ratio was obtained by dividing the albumin value by the calculated globulin value as described by Peters *et al*. (1982).

The proximate composition of the experimental treatments using the method of the Association of official analytical chemist AOAC (1990).

RESULTS

No significant differences ($p \geq 0.05$) were observed in the PCV, RBC and WBC values of broilers fed the different levels of methionine inclusion, at the finisher phase (Table 4) but at starter phase significant differences ($p \leq 0.05$) were observed, with the WBC values increasing as the inclusion rate of methionine increases (Table 3).

However, birds on the basal diet (control diet) had highest total protein value (4.48) which was not significantly ($p \geq 0.05$) from birds fed diets in treatments 4 (4.80) and 5 (4.84) respectively. There were wide variations in the glucose concentration of the birds, the highest glucose concentration was observed with birds on diet 2 (220.90) while the least was observed for birds fed the control diet (Table 4).

The result show no significant ($p \geq 0.05$) differences in the Packed Cell Volume (PCV) and Total Protein (TP) values of the bird fed different levels methionine supplement, the RBC however, had the highest significant value of 3.84 and the least value of 1.91.

DISCUSSION

Haematological parameters: The haematological parameters particularly WBC was significantly affected by increasing levels of Methionine in the first 4 weeks of

Table 3: Haematological indices and serum analysis of broiler starters fed varying dietary levels of methionine

Variables	1	2	3	4	5	SEM
PCV (%)	28.67	29.33	28.53	30.00	27.83	1.04
RBC ($_{10}^{6}$/mm^3)	2.84	2.74	2.56	2.77	2.91	0.53
WBC (X^{10}/mm^3)	7.35^b	10.90^a	10.87^a	10.37^a	12.23^a	0.64
Total protein (g/100 ml)	4.68	4.48	5.16	5.56	5.12	0.59
Albumin (g/100 ml)	1.07	0.93	1.27	1.21	1.14	0.35
Glucose (g/100 ml)	28.75^b	34.64^a	25.23^b	30.72^{ab}	25.76^b	4.92
Cholesterol (g/100 ml)	79.36^b	68.78^c	85.71^b	95.44^a	101.59^a	2.55

Note: Means in the same row with different superscripts are significantly different ($p<0.05$)

Table 4: Haematological indices and serum metabolites of broiler finishers fed varying dietary levels of methionine

Variables	1	2	3	4	5	SEM
PCV (%)	26.17	27.50	27.53	26.17	27.50	1.40
RBC ($_{10}^{6}$/mm^3)	3.84	3.95	4.48	3.44	4.23	0.43
WBC (X^{10}/mm^3)	11.23	11.00	10.13	11.60	11.60	0.81
Total protein (g/100 ml)	4.48^a	3.24^b	4.12^b	4.80^a	4.84^a	0.35
Albumin (g/100 ml)	1.39	1.26	1.20	1.50	1.27	0.37
Glucose (g/100 ml)	161.32^c	220.90^a	176.47^b	207.58^b	199.75^b	4.19
Cholesterol (g/100 ml)	91.0^a	74.92^b	85.08^{ab}	97.15^a	74.07^b	4.74

Note: Means in the same row with different superscript are significantly different ($p<0.05$)

age. WBC and different types of leukocytes are influenced by age, hormones and stress (Maxwell, 1993; Latimer and Bienzle, 2000). Although young birds demonstrate a great variability in total leukocyte count until 4-6 months of age (Fudge, 2000) birds often become excited when handled. Thus, the blood collection process usually results in a physiologic leukocytosis, that represents a transient phenomenon and this physiologic response increases the concentration of lymphocytes in the peripheral blood (Thrall, 2004). This transient phenomenon was demonstrated by the broilers at 8 weeks of age their WBC values were similar across treatments. The significant higher value of WBC than the control is an indication that the birds were resistant to diseases as the methionine level increases and have higher immunity status which is reflective of the adequacy of the dietary treatments as reported by Lindsay (1977).

The values obtained in PCV, RBC, were not influence by dietary treatments. Reference ranges for avian RBC and hemoglobin vary significantly among reports and among species sampled (Fudge, 2000). In general, the total erythrocyte count increases with age (Thrall, 2004) and the red cell mass of birds is also influenced by sex and environmental factors (Herbert *et al*., 1989). Slight increases in the peripheral RBC mass may occur in the excited or stressed avian species (Fudge, 2000).

Serum metabolites: Hyperproteinemia in most birds is indicated by plasma total protein concentrations of greater than 4.5 g/dL. (Thrall, 2004). We found that most mean values for serum total obtained in this study were higher, indicating that dietary methionine did affect the concentration of total serum protein and albumin. albumin is a blood transport protein binds many biomolecules and drugs including hormones, lipoproteins and amino acids (Ritchie and Navolotskaia, 1996; Beutler and Williams, 2001) This hyperproteinemia is associated with an increase in vitellogenin and lipoproteins which is induced by estrogens because they are necessary for yolk production (Thrall, 2004).

The values of the biochemical composition in Table 3 and 4 revealed significant differences ($p\leq0.05$) in glucose and cholesterol, in the first four weeks and after 8 weeks of feeding, but it seemed birds adjusted to the diets as the time of exposure to the diets increases.

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