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Haematological and serum biochemical indices of broiler chickens fed diets containing red finger millet (*Eleucine coracana)* with and without phytase enzyme

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***Target Audience:** Nutritionists, Researchers, Feed millers and Livestock industries*

Abstract

The effect of feeding diets containing red finger millet with and without phytase (Ronozyme®) enzyme supplementation on haematological and biochemical indices of broiler chickens was evaluated. Five hundred and forty chickens were used and allotted to 10 treatments with each treatment having 3 replicates containing 18 chickens each with and without enzyme (Ronozyme®) supplementation at the rate of 100g/100 kg diet in a Completely Randomized Designed (CRD). Treatment 1 had enzyme without finger millet, T 2, 3, 4 and 5 with enzyme and finger millet, T 6 without enzyme and finger millet, T 7, 8, 9 and 10 with finger millet without enzyme. Only Alanine aminotransferase (ALT) and creatinine were significantly (P<0.05) affected by red finger millet in biochemistry indices. However, haematological parameters were not affected by red finger millet across the dietary treatments with and without enzyme supplementation. It can therefore, be concluded that red finger millet is safe to be incorporated in to broiler chickens' diet without causing any deleterious effect on the health of the broiler chickens.

***Keywords:** Eleucine coracana, Broiler chickens, Phytase, Haematology and Biochemistry*

Description of the Problem

Funds invested in poultry production are recovered faster than in any other livestock enterprise (1). In developing countries, global trading of all meat products as well as feed and related inputs has led to a significant growth in poultry (especially broiler chickens) production and consumption (2). Poultry birds are globally and religiously accepted animals whereby their meat has no taboo neither has it been discriminated against throughout the world's history (3). (1) stated that poultry is considered to be a means of livelihood and a way of achieving certain level of economic independence in Nigeria. Inadequate supply of feed, nutritionally poor rations, adulterated feeds and feed ingredients or stale feeds are some of the factors responsible for the low supply and decline in the quality of animal protein as a result of low productivity of livestock in tropics (4). Poor and inadequate protein has been incriminated for most of the malnutrition problems among all age groups especially in children and infants. As reported by World Health Organization (WHO), 35.3 % and 40.0 % of children in Nigeria between the ages of about 6 months and 6 years in urban and rural areas respectively, are

malnourished and underweight (5). (6) stated that the need to source, harness, process and utilize alternative feed stuff otherwise known as unconventional feed ingredients in the diets of poultry birds is more critical now than ever. Grains are food seeds that supply energy in the feed of livestock, and whenever these ingredients are in short supply it causes problems of different kind in the livestock industry. Blood has been shown as an important index of physiological, pathological and nutritional status in the animals (7; 8). (9, 10) reported that inadequate intake of protein and energy decreases packed cell volume (PCV) and haemoglobin (Hb) concentrations which indicates anaemia. Kidney malfunction could lead to an elevated level of serum urea level (11). Haematological indices provide essential information on the physiological status of animals and, they are related to blood and blood forming issues. Blood act as pathological reflector of the status of exposed animals to toxicants and other conditions found in the environment (12). Haematological parameters serve as indicators of the physiological state of birds (13). Serum biochemistry is a reliable biochemical system which could reflect the condition of the organism and the changes happening to it under the influence of internal and external factors (14). (15) reported that the serum biochemistry was routinely used for detection of organ diseases in domestic mammals. Therefore, this study was designed to evaluate the effect of red finger millet with and without phytase enzyme on blood parameters of broiler chickens.

Materials and Methods

Study area

The study was conducted at the Poultry Section of Federal College of Animal Health and Production Technology (FCAH&PT), in Livestock Investigation Division (LID), National Veterinary Research Institute (NVRI), Vom, Nigeria. Vom is located in the Guinea Savannah zone of Nigeria, with geographical location on longitude 8^{o} 45` E and latitude $9^{o}$ 44` N on an altitude of 4200 feet (1280 m) above sea level. Relative humidity ranges from 22 % in January to 78 % in July/August. The daily average environmental temperature ranges between 17 ^{o} C – 28.6 ^{o}C with mean monthly sunshine hours range of 177– 288.30 (16).

Source of finger millet (*E. coracana)*

This cereal crop popularly and locally known as finger millet or *tamba* was purchased from Ganawuri, a local market in Riyom Local Government Area, Plateau State, Nigeria. The cereal grain was purchased around November - December. The grain does not need any further processing but to be milled, because it was processed, that means dried and winnowed before being brought to the market for sale. Though, before feeding to the birds it was milled to increase the surface area for ease of digestion for the birds. The experiment lasted for a period of eight (8) weeks.

Serum biochemical analysis

At the end of the experiment, blood was collected at the point of slaughter, blood samples were channeled into clean and dry plain bottles. The bottles were kept in a slanting position to allow the clotted blood form serum which was collected and used for biochemical analysis in the laboratory. The serum biochemistry parameters analyzed include; Total Protein by Biuret method (19); Aspartate Aminotransferase (AST) and ALT (20); Urea by Diacetyl Monoxime method (21); Method of (22) for Creatinine; Albumin-Bromocresol Green (BCG) method (23) and cholesterol by enzymatic method as described by (24). The analyses were carried

out at the Biochemistry Department, National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria.

Haematological Analysis

At the end of the experiment – that is finisher phase, birds were randomly selected, blood samples (bleeding) were collected from the wing vein of the birds. Two birds from each replicate, a 5 ml disposable syringe and needles were used in collecting the blood samples. The blood parameters for haematology that were considered included red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), haemoglobin (Hb), neutrophils and lymphocytes according to the method of (25). The blood samples were collected in Ethylene diaminetetraacetic acid (EDTA) embedded in bottles. The analyses were carried out at the Haematology Laboratory Section of the Central Diagnostic Division, National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria.

Statistical analysis

Data generated from the study were subjected to General Linear Model Procedures of SAS software package (17). Significant differences between the treatment means were separated using Duncan Multiple Range Test (18).

Model

$\mathbf{Y}_{ij} = \boldsymbol{\mu} + \mathbf{t}_i + \mathbf{e}_{ij}$ Where: $\mathbf{Y}_{ij}$= Dependent variable $\boldsymbol{\mu}$= Overall mean $\mathbf{t}_i$= i^{th} Effect of treatment $\mathbf{e}_{ij}$ = Random error.

Results and Discussion

The result of the effect of red finger millet on biochemical indices of broiler chickens fed diets supplemented with and without enzyme is presented in Table 1. Serum biochemistry is a reliable biochemical system which could reflect the condition of the organism and the changes happening to it under the influence of internal and external factors (14). There were no significant ($P>0.05$) differences in albumin, total protein, AST, cholesterol and urea concentrations. It was observed that there was no consistency in increase and reduction of the values in a particular pattern across the dietary treatments with and without enzyme supplementation in all the parameters measured. Albumin values were similar across the dietary treatments with birds on 25 % replacement level of finger millet having the highest value (14.26 g/l) and 0 % replacement level recording the lowest value (11.24 g/l) all in birds without enzyme supplementation. Albumin has a range of 11.24-14.26 g/l which is below the normal range of 23-35 g/l. This indicates that the birds have had some health challenges though it was not conspicuous because the birds were able to overcome their health challenges. Albumin is a strong predictor of health; a low albumin concentration is a sign of poor health and predictor of bad outcome (26). The highest total protein value was reported in birds on 75 % with enzyme supplementation and lowest in 100 % level of inclusion without enzyme supplementation.

Birds on diets without enzyme supplementation had higher values of cholesterol than birds on diets supplemented with enzyme while birds on 25 % with enzyme had the lowest (109.08 mg/dl) value and birds on diet of 50 % without enzyme supplementation had the highest (126.58 mg/dl) value. Urea had a range of 0.62-1.44 mmol/l. which was lower than the range of 1.50- 2.17 mmol/l obtained by (27).

Table 1: Composition of Broiler chickens finisher diets containing different levels of Red Finger millet (*E. coracana)* varieties as replacement for maize With and Without enzyme supplementation (5-8 weeks)

	With enzyme					Without enzyme				
Ingredients (%)	**0 (1)**	**25 (2)**	**50 (3)**	**75 (4)**	**100 (5)**	**0 (6)**	**25 (7)**	**50 (8)**	**75 (9)**	**100 (10)**
Maize	60.00	45.00	30.00	15.00	0.00	60.00	45.00	30.00	15.00	0.00
Finger millet	0.00	15.00	30.00	42.50	60.00	0.00	15.00	30. 00	45.00	60.00
Groundnut cake	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Soya bean meal	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Maize offal	5.60	5.60	5.60	5.60	5.60	5.60	5.60	5.60	5.60	5.60S
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Lime stone	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.20	0.50
Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.20	0.20	0.20	0.00	0.20	0.20	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Common salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Enzyme	0.10	0.10	0.10	0.10	0.10	0.00	0.0	0.00	0.00	0.00
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated analysis										
Crude Protein (%)	20.00	20.40	20.80	21.20	21.50	20.00	20.40	20.80	21.20	21.50
ME (kcal/kg)	2950	2915	2880	2845	2811	2950	2915	2880	2845	2811
Ether Extract (%)	3.62	3.58	3.53	3.49	3.44	3.62	3.58	3.53	3.49	3.44
Crude fibre (%)	3.77	4.38	4.99	5.60	6.21	3.77	4.38	4.99	5.60	6.21
Calcium (%)	1.34	1.37	1.40	1.44	1.47	1.34	1.37	1.40	1.44	1.47
Av. P (%)	0.79	0.78	0.77	0.76	0.75	0.79	0.78	0.77	0.76	0.75
Lysine (%)	1.22	1.22	1.21	1.20	1.20	1.22	1.22	1.21	1.20	1.20
Methionine (%)	0.50	0.54	0.62	0.63	0.67	0.50	0.54	0.62	0.63	0.67
Feed cost (? /kg)	109.75	122.50	135.25	148.00	160.75	120.75	133.50	146.25	159.00	171.75

*Vitamin-mineral premix provides per kg of diet: vit. A, 13,340iu; vit. D3, 2680iu; vit. E, 10iu; vit. K, 2.68mg; calcium pantothenate, 10.68mg; vit. B12, 0.022mg; folic acid, 0.668mg; choline chloride, 400mg; chlorotetracycline, 26.68mg; manganese, 13mg; iron, 66.68mg; zinc, 53.34mg; copper, 3.2mg; iodine, 1.1mg; cobalt,0.268mg; selenium, 0.108mg; ME-Metabolizable Energy, Av. P-Available Phosphorus
Diet 1-Maize based+enzyme; Diet 6–Maize based; Diets 2, 3, 4 and 5–finger millet+enzyme; Diets 7, 8 9 and 10–finger mill

There were significant (P<0.05) differences in ALT and Creatinine across the dietary treatments in birds fed diets with and without enzyme supplementation. However, there was no consistent pattern in terms of increase in values across the dietary treatments observed in ALT of birds fed with and without enzyme supplemented diets. ALT and AST are enzymes which when found in high concentration in the bloodstream signifies damage to the liver of the animal. Their concentration in the blood determines and suggests if the bird had liver health challenges or distortion in its normal functions. (29) stated that the values of AST and ALT were normally low in blood but are elevated when there was occurrence of liver damage by toxic substances. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities provide a sensitive and specific measure of hepatic function or injury (30).

Table 2: Effects of Red Finger millet with and without phytase enzyme on Biochemical indices of Broiler chickens

	With enzyme (%)					Without enzyme (%)						
Parameter	**0**	**25**	**50**	**75**	**100**	**0**	**25**	**50**	**75**	**100**	**SEM**	**Ref. value***
Albumin (g/l)	11.67	13.09	11.88	11.48	12.25	11.24	14.26	12.23	13.99	12.11	1.29	23-35
Total Protein (g/l)	19.73	22.17	18.76	25.14	19.10	16.80	15.97	21.97	18.84	15.70	3.46	49-79
Aspartate Aminotransferase (iu/l)	77.59	97.27	91.03	75.19	69.51	102.78	60.47	58.79	85.07	82.87	16.41	7-19
Alanine aminotransferase (iu/l)	1.72^{ab}	1.34^{ab}	2.31^{a}	0.78^{b}	0.62^{b}	1.00^{ab}	0.65^{b}	0.68^{b}	0.77^{b}	1.28^{ab}	0.44	5-8
Cholesterol (mg/dl)	119.74	109.08	117.42	115.54	114.96	111.29	122.39	126.58	123.80	124.06	9.66	
Urea (mmol/l)	0.46	1.09	0.73	0.67	1.10	0.47	0.50	1.44	0.62	1.20	0.42	
Creatinine (µmol/l)	41.48^{a}	27.16^{ab}	39.97^{a}	15.09^{b}	30.17^{ab}	26.40^{ab}	37.71^{a}	25.65^{ab}	30.17^{ab}	32.44^{ab}	5.50	

abc: Means on the same row with different superscripts differ significantly ($p<0.05$)
SEM: Standard Error of Means
(28) Ref. value* -

Table 3: Effect of Red Finger millet replacement levels on Haematology of Broiler chickens fed with and without phytase supplementation

	With enzyme (%)					Without enzyme (%)						
Parameters	**0**	**25**	**50**	**75**	**100**	**0**	**25**	**50**	**75**	**100**	**SEM**	**Ref. value***
Pack Cell Volume (%)	26.50	27.67	27.17	28.00	27.33	29.17	28.67	27.00	26.17	24.17	2.20	27-42
Haemoglobin (g/dl)	8.82	9.22	8.50	9.17	8.67	9.72	8.93	8.68	8..73	8.03	0.81	7-11
Red Blood Cell ($x10^{9}$/l)	1.75	1.60	1.82	1.88	1.77	1.92	1.80	1.82	1.92	2.02	0.22	2.2-4.0
White Blood Cell ($x10^{12}$/l)	4.18	4.18	4.57	3.67	3.67	3.72	3.30	3.32	3.92	4.07	0.31	1.0-9.5
Heterophil (%)	50.67	51.33	54.33	57.83	54.33	55.83	44.67	48.33	52.50	53.17	7.50	50-65
Lymphocyte (%)	51.83	49.67	49.00	45.00	45.67	45.17	54.83	45.83	50.83	52.50	7.51	20-50

*Reference value – (25)

Creatinine had a range of 15.09-41.48 µmol/l which is higher than the range of 4.73-7.60 µmol/l reported by (31). Abnormally high blood creatinine level indicates muscle wastage and implies that the animal was surviving at the expense of the body reserves which also results in weight loss (32).

Haematological parameters of broiler chickens fed diets containing red finger millet supplemented with and without enzyme is presented in Table 2. All parameters measured were not significantly ($P>0.05$) different in birds fed diets with and without enzyme supplementation across the dietary treatments. Haematological indices provide essential information on the physiological status of animals and, they are related to blood and blood forming issues. (12) stated that blood act as pathological reflector of the status of exposed animals to toxicants and other conditions. The PCV values were similar in birds fed diets with and without enzyme supplementation across the dietary treatments. The PCV range of 24.17-29.17 % was within the normal range, this indicates that the birds had enough quantity of blood within the body system to carry out their normal body functions. PCV is taken to indicate the good status of the bird's health, as low PCV indicate anaemia which is a condition in which there are too few RBC or RBC are deficient in haemoglobin resulting in poor health (33). Birds fed diets with and without enzyme supplementation across the dietary treatments had similar values in haemoglobin. Haemoglobin had a range of 8.03-9.72 g/dl and is within the normal range of 7-11 g/dl. This suggests that the birds were able to overcome their health challenges and therefore normal transportation of oxygen within the body system was not disrupted. Hb influences transport of oxygen from respiratory organs to peripheral tissues (34).

Birds fed diet on 100 % inclusion of finger millet without enzyme supplementation had the highest (2.02 $x10^9$/l) value of red blood cells and birds on enzyme supplemented diet with 25 % finger millet had the least 1.60 $x10^9$/l values. Red blood cells range of 1.60-2.02 $x10^9$/l was slightly below the normal range of 2.2-4.0 $x10^9$/l. (35) reported that RBC functions as a carrier of oxygen to the tissue and carbon dioxide to the lungs. WBC of birds fed diets containing finger millet with and without enzyme supplementation across the dietary treatments had similar values. WBC has a range of 3.30-4.18 $x10^{12}$/l which is within the normal range (1-9.5 $x10^{12}$/l). The major functions of WBC are to fight infections, defend the body by phagocytosis against invasion of foreign organism (36). Thus, animals with low white blood cells are exposed to high risk of disease infection, while those with high counts are capable of generating antibodies in the process of phagocytosis and have high degree of resistance to diseases (37)

Birds had similar values for heterophil across the dietary treatments with and without enzyme supplementation. Heterophil had a range of 44.67-57.83 % without any particular pattern in terms of increase or decrease across the dietary treatments in birds fed diets with and without enzyme supplementation, this is slightly below the normal range of 50-65 %.

The highest value of 54.8 % lymphocyte was recorded in birds fed 25 % diet without enzyme supplementation and the least value of 45 % was in birds fed 75 % diet with enzyme supplementation. Lymphocytes ranged from 22.50-29.67 % in this study which is within the normal range (20-50 %); lymphocytes act to defend the body immune system from infections that the birds may come in contact with. The inclusion of phytase in the diets of broiler

chickens did not have any significant effect on their blood parameters.

Conclusion and Application

1. It can be concluded from the results obtained that the feeding of red finger millet to broiler chickens did not affect albumin, total protein, AST, cholesterol and urea. However, parameters such as ALT and creatinine were significantly affected.
2. The feeding of red finger millet did not pose any significant health challenges in the biochemical indices of the broiler chickens.
3. From the blood parameters measured, the result indicated that the birds did not encounter health challenges that could have cause damages to them and the inclusion of phytase enzyme did not affect the blood parameters negatively.
4. All the blood parameters measured did not show any negative effect caused by phytase enzyme supplementation in the feed of the broiler chickens.

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