



**BLOOD RESPONSE OF BROILER CHICKENS FED DIETS
CONTAINING BLACK FINGER MILLET (*ELEUCINE CORACANA*)
VARIETY WITH AND WITHOUT PHYTASE ENZYME
SUPPLEMENTATION**

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Abstract

*This study was conducted to evaluate the response of broiler chickens fed diets containing black finger millet (*Eleucine coracana*) with and without phytase enzyme supplementation in their blood parameters. Five hundred and seventy birds were allotted to 10 treatments with each having 3 replicates of twenty birds each. The phytase enzyme was supplemented at the rate of 100g/100kg of feed in a completely randomized designed (CRD). Treatment (T) 1 had enzyme without finger millet, T2, T3, T4 and T5 had enzyme and finger millet, T6 without enzyme and finger millet, T7, T8, T9 and T10 had finger millet without enzyme. Biochemical indices showed statistical differences in Aspartate aminotransferase (AST) and cholesterol. However, only WBC had significant ($P < 0.05$) differences in all the haematological parameters. Therefore, from the results obtained it can be concluded that feeding of black finger millet with and without enzyme supplementation to broiler chickens had no deleterious effects on their blood parameters.*

Keywords: *Blood parameters, Finger millet, Enzyme and Variety*

INTRODUCTION

Livestock agriculture has played a significant role in the livelihood for humans and in the supply of animal protein to man (Bettencourt *et al.*, 2015). Poultry is considered to be a means of livelihood and a way of achieving certain level of economic independence in Nigeria (Ogundipe and Sanni, 2002). 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 (Bot *et al.*, 2021). However, exogenous enzymes have been used extensively in the diets of poultry to improve productive performance and nutrient utilization especially from cereal grains (Akintunde, *et al.*, 2013, Torres *et al.*, 2013 and Oladipo *et al.*, 2015). Maize is a major ingredient used in livestock feed but competition between man and livestock for maize has resulted in high cost of the cereal, which has consequently resulted in high cost of feed and livestock production (Udokainyang *et al.*, 2019). An important measure that can be taken to alleviate this situation is the use of alternative energy sources such as sorghum and millet (Medugu *et al.*, 2010). D'Andrea *et al.* (1999) reported finger millet to be a native of East Africa-Ethiopia and Ugandan highlands. The crop is generally considered as a high drought tolerant and the very long storage time which may be up to 50 years. Serum biochemistry is a reliable biochemical system which can reflect the condition of the organism and the changes happening to it under the influence of internal and external factors (Toghyani *et al.*, 2010). Serum biochemistry provide useful information about viscera organ damage especially liver and kidney (Jurcik, 2007). Haematology is one of the cornerstones of medical diagnosis of diseases and is currently considered as an integral part of clinical laboratory diagnostics in avian

species (Harr, 2002). Changes in haematological parameters are often used to assess stress in animals due to environmental, nutritional and/or pathological factors (Afolabi *et al.*, 2010). Igwe *et al.* (2017) reported that any deviation from the normal state of health is detectable in the blood profile. Haematological and serum biochemistry parameters are good indicators of physiological status of animals and their changes are important in assessing the response of such animals to various physiological situations (Khan and Zafar, 2005). This study was designed to evaluate the effect of black finger millet on blood parameters of broiler chickens with and without phytase enzyme supplementation.

MATERIALS AND METHODS

The study was conducted at the Poultry Section of Federal College of Animal Health and Production Technology (FCAH&PT), located 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° 45' E and latitude 9° 44' N on an altitude

Table 3.6: Composition of Broiler chickens finisher diets containing different levels of Black Finger millet (*E. coracana*) varieties as replacement for maize (5-8 weeks) with and without Ronozyme® hyphose supplementation

Ingredient	With enzyme (%)					Without enzyme (%)				
	0	25	50	75	100	0	25	50	75	100
Maize	60.00	42.75	28.50	14.25	0.00	57.00	42.75	28.50	14.25	0.00
Finger millet	0.00	14.25	28.50	42.75	57.00	0.00	14.25	28.50	42.75	57.00
Groundnut cake	10.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Soya bean meal	20.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00	24.00
Maize offal	5.60	5.60	5.60	5.60	5.60	5.60	5.60	5.60	5.60	5.60
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	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.20	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
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	0.00	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	0.75	0.79
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)	107.70	121.20	134.70	148.20	161.70	111.40	124.90	138.40	151.90	165.40

*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, 1.86mg; 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 millet

of 4200 feet (1280m) above sea level. Relative humidity ranges from 22% in January to 78% July/August. The daily average environmental temperature ranges between 17°C - 28.6°C with mean monthly sunshine hours range of 177-288.30 (NVRI, 2018).

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 to increase the surface area for ease of digestion by the birds.

Statistical analysis

Data generated from the study were subjected to General Linear Model Procedures of SAS software package (SAS, 2008). Significant differences between the treatment means were separated using Duncan Multiple Range Test (Duncan, 1955).

3.8.8 Model

The model used for the study is as follows:

$$Y_{ij} = \mu + V_i + L_j + (V \times L)_{ij} + e_{ij}$$

Where μ = over all mean

Y_{ij} = any observation

V_i = Effect of variety (Red variety and Black variety)

L_j = Effect of level of replacement (0, 25, 50, 75 and 100 %)

e_{ij} = Random error

Serum biochemical analysis

Blood samples (2 ml) were collected in clean, dry plain bottles and the blood allowed to clot and form serum which was used for biochemical analysis. The serum biochemical parameters analyzed included; Total Protein Biuret method according to Reinhold (1953); Aspartate Aminotransferase and Alanine Aminotransferase were carried out according to the method described by Reitman (1957); Urea analysis was done as described by Diacetyl Monoxime method according to

Marsh *et al.* (1965); Creatinine was according to the method of Bod and Sirota (1948); for Albumin was determined according to BCG method as described by Spencer and Price (1977) and for cholesterol, enzymatic method was used as described by the procedures outlined in the Randox kit (2002). The analyses were carried out at the Biochemistry Laboratory Department, National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria.

Blood Collection and determination of haematological parameters of broiler chickens

At the end of the experiment ie the finisher phase, blood was collected from the wing vein of two birds from each replicate. The birds were randomly selected and 5 ml disposable syringe and needles were used in collecting 2 ml of the blood samples (bleeding). 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. The blood parameters determined include; Red Blood Cell (RBC), White Blood Cell (WBC), Packed Cell Volume (PCV), Haemoglobin (Hb), Neutrophils and Lymphocytes according to the method of Merck's Manual (1998).

RESULTS AND DISCUSSION

Serum Biochemical parameters of broiler finisher chickens (5-8 weeks)

The result of the effect of diets containing black finger millet replacement levels on the biochemical indices of broiler chickens with and without Ronozyme[®] hyphose

supplementation is presented in Table 4.23. There were no significant ($P>0.05$) differences in albumin, total protein, Alanine aminotransferase, urea and creatinine. Albumin and total protein had similar values across the dietary treatment with and without enzyme supplementation. Blood has been shown as an important index of physiological, pathological and nutritional status in animals (Olorede *et al.*, 1995; Ewuola *et al.*, 2004). Alanine aminotransferase values were similar in birds fed all the experimental diets with and without enzyme supplementation.

Urea and creatinine had similar values within the dietary treatments with and without enzyme supplementation. The range of 0.91–1.99 mmol/l for urea was recorded in this study which was lower than the range of 1.60- 2.45 mmol/l obtained by Opowoye *et al.* (2018).

However, there were significant ($P<0.05$) differences in Aspartate aminotransferase (AST) and total cholesterol levels across the dietary treatments with and without enzyme supplementation. AST was significantly ($P<0.05$) higher in birds on diets containing 25 % level of replacement of finger millet and the control diet with enzyme supplementation compared to birds on the other dietary treatments. Birds on diets containing 75 % finger millet without enzyme supplementation had the least value (39.22 iu/l) but all other dietary treatments were similar. According to Yildirim *et al.* (2011) activities of aspartate aminotransferase (AST), alkaline phosphate (ALP) and alanine aminotransferase (ALT) in the blood are bioindicators of liver function and damage. Increase levels of these enzymes are associated with liver or muscle damage, resulting from the body's

4.23: Effect of Black Finger millet replacement levels on Biochemical indices of Broiler chickens with and without Ronozyme[®] hyphose supplementation

Parameter	With enzyme					Without enzyme					SEM	Ref. value*
	0	25	50	75	100	0	25	50	75	100		
Albumin (g/l)	18.82	18.97	22.55	17.93	17.62	23.85	18.14	20.68	19.14	19.53	1.87	23-35
Total Protein (g/l)	38.04	36.07	39.71	38.87	36.05	36.19	38.06	36.40	38.05	37.50	2.76	49-79
AST (iu/l)	60.19 ^a	68.07 ^a	57.09 ^{ab}	45.34 ^{ab}	42.52 ^{ab}	52.76 ^{ab}	48.40 ^{ab}	58.46 ^{ab}	39.22 ^b	50.60 ^{ab}	7.86	7-19
ALT (iu/l)	70.21	75.29	67.65	66.50	72.38	68.51	65.14	69.50	63.35	70.49	4.20	5-8
Cholesterol (mg/dl)	88.63 ^a	82.00 ^{ab}	71.64 ^{ab}	78.83 ^{ab}	91.42 ^a	83.39 ^{ab}	90.82 ^a	68.62 ^{ab}	63.13 ^b	83.29 ^{ab}	6.95	
Urea (mmol/l)	1.12	0.91	1.78	1.38	1.49	1.26	1.59	0.94	1.99	1.73	0.48	
Creatinine (μmol/l)	23.15	30.17	29.47	25.79	28.07	26.66	20.88	22.21	23.15	25.96	3.37	

ab: Means on the same row with different superscripts differ significantly (p<0.05)

SEM: Standard Error of Means

AST- Aspartate Aminotransferase

ALT- Alanine aminotransferase

Ref.value- Anonymous 4

response to stress (Lumeji, 2008). Aspartate aminotransferase has a range of 39.22-68.07iu/l which is above the normal range of 7-19 iu/l. Serum albumin is influenced by breed, age, physiological state, environments and antigen exposure and is highly variable (Simaraks, 2015).

Total cholesterol was significantly ($P<0.05$) higher in birds on diets containing 100 % finger millet with enzyme supplementation. However, birds on 75 % and 50 % diets without finger millet had the least values (63.13 and 68.62 mg/dl) while all other groups were similar. A range of 63.13–91.42 mg/dl was obtained for cholesterol which was in line with the values of 68.29–130.11mg/dl reported by Akingbade *et al.* (2018) for broiler chickens. Serum biochemistry provides useful information about visceral organ damage especially for the liver and kidney (Jurcik *et al.*, 2007).

Haematological parameters of broiler finisher chickens (5-8 weeks)

The effect of diets containing black finger millet replacement levels on haematological parameters of broiler chickens fed with and without Ronozyme[®] hyphose supplementation is presented in Table 4.24. There were no significant ($P>0.05$) differences in all the parameters measured across the dietary treatments with and without enzyme supplementation except for WBC. The WBC values were significantly ($P<0.05$) higher in birds fed diets containing 50 % finger millet level of replacement with enzyme supplementation compared to all other treatments which were similar. WBC has a range of 3.57-5.55 $\times 10^{12}/l$ which is within the normal range of 1.00-9.50 $\times 10^{12}/l$. Roberts *et al.* (2003) reported that the higher the values of WBC the better phagocytosis and hence the ability to fight diseases. Abnormally high WBC could suggest the invasion of foreign bodies in the body, which

will trigger off immune response by the production of more WBC (Ahamefule, 2005).

Total cholesterol was significantly ($P<0.05$) higher in birds on diets containing 100 % finger millet with enzyme supplementation. However, birds on 75 % and 50 % diets without finger millet had the least values (63.13 and 68.62 mg/dl) while all other groups were similar. A range of 63.13–91.42 mg/dl was obtained for cholesterol which was in line with the values of 68.29–130.11mg/dl reported by Akingbade *et al.* (2018) for broiler chickens. Serum biochemistry provides useful information about visceral organ damage especially for the liver and kidney (Jurcik *et al.*, 2007).

Table 4.24: Effect of Black Finger millet replacement levels on Haematology of Broiler chickens with and without Ronozyme[®] hyphose supplementation

Parameters	With enzyme					Without enzyme					SEM	Ref. value*
	0	25	50	75	100	0	25	50	75	100		
Pack Cell Volume (%)	28.17	26.83	28.83	28.83	27.67	27.50	27.50	28.00	27.67	28.50	1.19	27-42
Haemoglobin (g/dl)	9.38	8.88	9.62	9.73	9.10	9.27	9.32	9.35	9.07	9.50	0.43	7.-11
Red Blood Cell (x10 ⁹ /l)	3.97	4.32	4.45	4.30	4.18	3.57	3.95	4.33	4.12	5.55	0.63	2.2-4.0
White Blood Cell (x10 ¹² /l)	1.55 ^b	1.60 ^b	2.37 ^a	1.52 ^b	1.85 ^b	1.67 ^b	1.43 ^b	1.58 ^b	1.38 ^b	1.62 ^b	0.18	1.0-9.5
Heterophil (%)	41.67	55.33	46.29	45.67	44.83	52.67	38.17	43.50	42.33	38.83	6.65	50-65
Lymphocyte (%)	54.83	41.83	41.67	51.33	54.67	46.67	62.17	54.33	59.00	60.67	6.91	20-50

abcd: Means on the same row with different superscripts differ significantly (p<0.05)

SEM: Standard error of means

*- Reference value - Merck's Manual (1998).

PCV has range of 26.83-28.83 % which is within the normal range (27-42 %), this indicates that the birds had good quantity of blood within them hence they were not anaemic. A low level of packed cell volume was reported to be an indication of anaemia (Aster, 2004). Pendl, (2001) reported that as a guide any PVC greater than 56% is an indication of dehydration in most birds. However, the range in this study is not more than 35% percent which were within the reference ranges of 22-35% reported by (Jain, 1993) and 25-45% as reported by (Al-Nedawi, 2018) Haemoglobin has a range of 8.88-9.73 g/dl which is within the normal range (7-11 g/dl). This suggests that oxygen was transported to the vital parts of the birds' body and kept the birds healthy. This also explains why very minimal mortality rate was observed all through the experimental period. According to Minka and Ayo (2007) haematological values are used to assess stress and welfare in animals.

The highest value ($5.55 \times 10^9/l$) of RBC was found in birds with 100 % finger millet-based diet and the least value ($3.57 \times 10^9/l$) was in birds on 0 % maize-based diet all without enzyme supplementation. Red blood cells of birds in the experimental diets across the dietary treatments with and without enzyme supplementation were slightly above the normal range of $2.20-4.00 \times 10^9/l$. RBC functions as a carrier of Hb which carries oxygen to the tissues and carbon dioxide to the lungs (Mitruka and Rawnsley, 1977). The highest value (55.35 %) for heterophil was in birds fed 25 % diet with enzyme supplementation while the least value (38.17 %) was in birds on diet 25 % without enzyme supplementation. Heterophil range was 38.17-52.67 % and this is within the normal range (50-65%). Lymphocyte highest value (62.17 %) was in 25 % diet without enzyme supplementation and the least value (41.67 %) was in 50 % diet with enzyme supplementation. Lymphocyte range was

41.67-60.67 % in this study, however this was above the normal range of 20-50 %. This suggests that there was an invasion of foreign bodies within the birds which triggered the production of more lymphocytes in the body.

CONCLUSION

Based on this results, birds black fed finger millet diets supplemented with and without phytase enzyme did not show any problem emanating from the finger millet.

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