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National Animal Production Research Institute
Ahmadu Bello University
P.M.B 1096, Shika-Zaria,
Kaduna State, Nigeria.
Email: japr@napri.gov.ng Url: journal.napri.gov.ng

BIOCHEMICAL AND HAEMATOLOGICAL PARAMETERS OF RED AND BLACK FINGER MILLET (*ELEUSINE CORACANA*) VARIETIES FED BROILER CHICKENS AS REPLACEMENT FOR MAIZE

[*1]Bot, M. H., [1]Udom, I. E., [2]Bot, D. Y., [3]Kpanja, E. J., [4]Tok, C. C., [1]Salihu, D. Y., [1]Hassan, I. M., [1]Aliyu, I. P., [1]Zungum, A. G. and [1]Idris, A. A.

[1]Federal College of Animal Health and Production Technology, NVRI, Vom, Plateau State, Nigeria.
[2]Department of Medical Laboratory Science, University of Jos, Plateau State, Nigeria.
[3]Department of Animal Science, Faculty of Agriculture, Ahmadu Bello University, Zaria, Nigeria.
[4]Federal College of Education, Pankshin, Plateau State, Nigeria.
[*]Corresponding Author: *maryambot@yahoo.co.uk*

ABSTRACT

An experiment was conducted using two varieties of red and black finger millet (RBFM) in feeding broiler chickens to determine the effects on their biochemical and heamatological parameters using a total of 540 birds in the experiment. The experiment was carried out at the College Poultry Unit of the Federal College of Animal Health and Production Technology (FCAH&PT), National Veterinary Research Institute (NVRI), Vom using 2 x 5 factorial arrangement in a completely randomized design with RBFM replacement rate at 0, 25, 50, 75 and 100% of maize in the diets. The result of biochemical parameters showed only albumin was significantly ($P<0.05$) different as main effect, however there was no significant effect in the replacement levels except in cholesterol. While the interaction results revealed that there was no significant ($P>0.05$) effect in all the parameters measured. The heamatological indices results showed that there was no significant ($P>0.05$) varietal effect in all the parameters measured except in WBC, however only heterophils showed significant effect in the replacement levels. Nevertheless, RBC showed significant ($P<0.05$) interaction across the dietary treatments. These results showed that red and black finger millet varieties could be fed to broiler chickens in place of maize since most of the parameters measured did not show significant effects in the broiler chickens that could affect their health and performance.

Key words: *Finger millet, Blood parameters, Broiler chickens, Maize, Biochemical parameters*

INTRODUCTION

FAO, (2010) reported that poultry has contributed to improved human nutrition and food security by being a source of high-quality protein, in addition to its economic, social and cultural significance in small societies. There is a need to expand the ingredient base to provide an array of alternative feedstuffs for poultry feed formulation (Dawfang and Shwarmen, 1996; Dawfang, 2006). Blood acts as a pathological reflector of the status of exposed animals to toxicants/disease and other conditions (Olafedehan *et al.,* 2010). Isaac *et al.* (2013) reported that animals with good blood composition were likely to show good performance. Test of blood in the laboratory could help to detect any deviation from normal in farm animals and humans (Ogunbanjo *et al*., 2009). The examination of blood gives the opportunity to investigate the presence of several metabolites and other constituents in the body of animals and it plays a vital role in the physiological, nutrition and pathological status of an organism (Aderemi, 2004; Doyle, 2006). The blood transports nutrients from one part of the body to another, therefore, whatever affects the blood will adversely or moderately affect the animal in terms of health, growth, maintenance and reproduction (NseAbasi *et al*., 2014). Blood analysis is a fast means of assessing clinical and nutritional health status of animals in feeding trials (Olabanji *et al*., 2007). The blood contains several metabolites which provide useful

information on nutritional status and clinical investigation of an individual organism hence, the use of blood parameters is recommended for medical and nutritional assessments (Egbunike *et al*., 2009).

Serum biochemical parameters such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities provide a sensitive and specific measure of hepatic function or injury (Abbès *et al*., 2006). 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 (Toghyani *et al*., 2010). Serum biochemistry provides useful information about visceral organ damage especially for the liver and kidney (Jurcik *et al*., 2007).

Haematology refers to the study of the number and morphology of the cellular elements of blood – the red cells (erythrocytes), white cells (leucocytes), and the platelets (thrombocytes) and the use of these results in the diagnosis and monitoring of disease (Merck Manual, 2012). Haematology studies are useful in the diagnosis of many diseases and the extent of the damage to blood in animal and humans (Ovuru and Ekweozor, 2004). Haematological parameters are good indicators of the physical status of exposed animals (Khan and Zafar, 2005). This project was therefore designed to determine the effect of feeding red and black finger millet on the blood parameters of broiler chickens since blood status gives the health reflection of animals.

MATERIALS AND METHODS

Location of the 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 (NVRI, 2018).

Source of finger millet

The grain which is popularly called finger millet or *tamba* locally was purchased from Ganawuri local market which is located in Riyom Local Government Area of Plateau State and Manchok in Kaura Local Government Area of Kaduna State.

Serum biochemical Analysis

The blood samples were collected in clean, dry plain bottles, they were allowed to clot and to form serum which was collected and used for biochemical analysis. The serum biochemistry parameters analyzed include; Total Protein Biuret method (Reinhold, 1953); AST and ALT (Reitman and Frankel, 1957); Urea Diacetyl Monoxime method (Marsh *et al*., 1965); Method of Bod and Sirota, (1948) for Creatinine; Albumin (BCG method) (Spencer and Price, 1977) and cholesterol enzymatic method as described by Randox kit, (2002). 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 – the finisher phase, blood was collected from the wing vein of the birds, two birds from each replicate. The birds were randomly selected and a 5 ml disposable syringe and needles were used in collecting the blood samples (bleeding). 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 Merck's Manual, (1998). 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 procedure of SAS software package, (2008). Significant differences between the treatment means were separated using Duncan Multiple Range Test (Duncan, 1955). The model is as shown below;

Model

The model used for the study is as follows:

$Y_{ijk} = \mu + V_i + L_j + (VxL)_{ij} + eij_k$

Where μ = over all mean

Y_{ijk} = any observation

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

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

e_{ijk} = Random error

RESULTS AND DISCUSSION

Main effect of feeding diets containing different levels of red and black varieties of finger millet as replacement for maize on serum biochemistry of broiler chickens

The biochemical result of the main effect of variety and replacement levels of red and black finger millet on broiler chickens are presented in Table 1. There was a significant ($P<0.05$) main effect of variety on albumin, and there is also significant main effect of replacement level on cholesterol with a range 117-131.10 mg/dl, and there was no definite trend observed. The highest value of cholesterol was observed in the control (131.10 mg/dl) with birds fed maize based diets. This range was higher than 3.10-3.64 mg/dl reported by Duwa *et al*. (2012). High levels of blood cholesterol may result in fat deposition on the walls of the blood vessels and these deposits may eventually harden to form the atherosclerotic plague that might block important blood vessels and result in a myocardial infarction (Duwa *et al*., 2012). Viscosity – promoting potential of crude fiber had also been shown to reduce the overall digestive and absorptive efficiency by preventing nutrients from being available at the absorptive sites in the intestinal mucosa (Webel *et al*., 2003).

However, there was no significant ($P>0.05$) main effect of variety and replacement levels of finger millet on albumin, TP, AST, ALT, urea and creatinine. This may suggest that the fiber levels in these diets did not cause noticeable changes in the parameters measured to the detriment of the broilers. High fiber may cause reduced digestion and availability of minerals and vitamins to the birds, it also suggests that there was no distortion in the protein metabolism within the bird. 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 (Toghyani *et al*., 2010).

Table 1: Main effect of Finger millet varieties and levels of replacement on Biochemical indices of broiler chickens

	Varietal effect			Replacement levels						
Parameters	**Red**	**Black**	**SEM**	**0%**	**25%**	**50%**	**75%**	**100%**	**SEM**	**Ref. value***
Albumin (g/l)	13.76[b]	14.55[a]	0.31	14.67	13.30	14.10	14.02	14.69	0.49	23-35
TP (g/l)	49.40	49.10	1.42	49.52	49.10	49.86	50.04	49.71	2.25	49-79
AST (IU/l)	92.40	91.50	3.25	94.29	95.28	83.44	97.24	89.32	5.14	70-220
ALT (IU/l)	8.86	7.67	0.75	9.20	6.33	7.33	8.83	9.65	1.19	5-8
Cholesterol (mg/dl)	120.50	121.90	2.43	131.10[a]	117.40[b]	118.80[b]	117.00[b]	121.50[b]	3.84	
Urea (mmol/l)	0.99	1.00	0.08	0.98	0.92	0.93	1.08	1.06	0.12	
Creatinine (µmol/l)	28.80	29.10	1.52	33.29	31.31	27.05	26.53	26.53	2.40	

TP- Total protein
AST- Aspartate aminotransferase
ALT- Alanine aminotransferase
Reference value – chrome://global/skin/media/imagedoc-darknoise.png

Interaction effect of feeding diets containing different levels of red and black varieties of finger millet as replacement for maize on serum biochemistry of broiler chickens

Table 2 shows the interaction of red and black finger millet varieties and replacement levels on biochemical indices of broiler chickens. The result revealed that there was no significant (P>0.05) interaction in all the biochemical parameters measured. This suggests that the two varieties having different colours did not pose any health problem for the birds as indicated in the result obtained from parameters measured. It also suggested that all the diets were safe and adequate for the birds to be healthy. 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 (Toghyani *et al*., 2010). Although all parameters measured were not significantly different however, AST range was (81-97.50 IU/l) which was within the normal range of 70-220 IU/l as stated by Mitruka and Rawnsley, (1977) and ALT (5.14-9.99 IU/l) was slightly higher than normal range (5-8 IU/l), which suggested that the health of the birds were probably challenged at a certain time within the experimental period. The result of these enzymes (AST and ALT) gave idea about the liver condition since they were the liver marker enzymes of the birds. The elevated activities of these enzymes were indicative of cellular leakage and the loss of the functional integrity of the cell membranes in the liver which may be the cause (Naik and Panda, 2008). However, the birds were able to survive throughout the experimental period which suggested they had strong immunity to overcome health challenges that might have occurred in them.

Table 2: Interaction effect of finger millet varieties and replacement levels on Biochemical indices of broiler chickens

	Red variety (%)					Black variety (%)					
Parameter	**0**	**25**	**50**	**75**	**100**	**0**	**25**	**50**	**75**	**100**	**SEM**
Albumin (g/l)	14.67	13.10	13.16	13.61	14.29	14.67	13.51	15.04	14.43	15.09	0.70
TP (g/l)	49.50	47.70	50.30	51.70	47.60	49.50	50.50	45.40	48.30	51.90	3.18
AST (IU/l)	94.30	96.90	85.90	97.50	87.30	94.30	93.70	81.00	97.00	91.30	7.27
ALT (IU/l)	9.20	7.51	8.58	9.71	9.30	9.20	5.14	6.09	7.95	9.99	1.69
Cholesterol (mg/dl)	131.10	108.90	122.10	114.20	126.00	131.10	126.00	115.40	119.90	116.90	5.43
Urea (mmol/l)	0.98	0.86	0.98	1.03	1.09	0.98	0.99	0.88	1.12	1.03	0.17
Creatinine (µmol/l)	33.30	32.10	28.10	25.50	25.00	33.30	30.60	26.00	27.60	28.10	3.40

TP- Total protein
AST- Aspartate Aminotransferase
ALT- Alanine aminotransferase

Main effect of feeding diets containing different levels of red and black varieties of finger millet as replacement for maize on haematological parameters of broiler chickens

The result of the main effect of finger millet varieties and replacement levels of haematological parameters on broiler chickens is presented in Table 3. There was no significant (P>0.05) effect of variety on all the parameters measured except WBC and replacement levels on all the parameters measured except heterophils. The WBC revealed a range of 3.49-5.43 ($x10^{12}$/l) which was within the normal range (1.0-9.5 $x10^{12}$/l). This could suggest that immune system of the birds were challenged may be by disease such as coccidiosis in which their bodies immunity fought back the microorganisms. WBC is known to be among the body defense mechanisms that fight against non-self or pathogenic organisms (Ayo-Ajasa *et al*., 2015). 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 (Soetan *et al*., 2013). Heterophils range of 70-76.92% was above the normal range (50-65 %), probably the birds were stressed in one way or the other with resultant increase in the production of these cells. Heterophils are the primary phagocytic leucocyte, and proliferate in circulation in response to infection, inflammation and stress (Thrall, 2004).

The PCV range of 27-29.33% was within the normal range (27-42%), this revealed that the birds were normal and were neither anemic nor dehydrated. Reduction in the concentration of PCV in the blood usually suggests the presence of a toxic factor e.g. haemagglutinin which has adverse effect on blood formation (Oyawoye and Ogunkunle, 1998). Hb range of 8.93-9.78 g/dl was within the normal range (7-11 g/dl); this indicated that the birds did not find it difficult to transport oxygen round the body and to the vital areas where they were needed (Roberts *et al*., 2003). Lower levels of haemoglobin and PCV, according to Kumarnsit *et al*. (2006) caused acute inflammation from most pathogenic micro-organisms which resulted in haemolysis.

RBC range of 1.32-1.55 $x10^9$/l recorded in this study was below the normal range (2.2-4.0 $x10^9$/l), probably because the birds experienced shortage of blood, nevertheless, the available RBC was able to help them carry out their normal functions in the body effectively. Lower than normal red blood cells may be due to anaemia, bone marrow failure, haemolysis (red blood cells destruction due to blood vessel injury or other causes), haemorrhage and malnutrition (Bunn, 2011). RBC functions as a carrier of Hb which in turn carries oxygen to the tissues and carbon dioxide to the lungs (Mitruka and Rawnsley, 1977).

Lymphocytes range of 23.58-28.75% was also within the normal range of (20-50%). Lymphocytes are involved in the protection of the body from viral infection and harmful germs according to Akinsanya, (2010).

Table 3: Main effect of Finger millet varieties and levels of replacement on Haematology of broiler chickens

	Varietal effect			Replacement levels (%)						
Parameters	**Red**	**Black**	**SEM**	**0**	**25**	**50**	**75**	**100**	**SEM**	**Ref. value***
PCV (%)	28.30	27.37	0.97	27.17	27.58	27.00	28.08	29.33	1.53	27-42
Haemoglobin (g/dl)	9.41	9.13	0.33	9.14	9.07	8.93	9.43	9.78	0.52	7-11
RBC ($x10^9$/l)	1.36	1.42	0.06	1.35	1.55	1.35	1.32	1.40	0.10	2.2-4.0
WBC ($x10^{12}$/l)	3.68^b	5.22^a	0.62	3.63	5.43	4.94	4.76	3.49	0.98	1.0-9.5
Heterophils (%)	73.40	73.83	1.19	74.17^{ab}	74.92^{ab}	76.17^a	70.00^b	72.83^{ab}	1.88	50-65
Lymphocyte (%)	26.03	25.83	1.13	25.67	25.00	23.58	28.75	26.67	1.78	20-50

PCV- Park cell volume
RBC- Red blood cell
WBC- White blood cell
*****Reference value - Merck's Manual (1998)

Haematological parameters (Interaction)

The results of interaction effect of red and black finger millet variety and replacement levels on haematological parameters in the diets of broiler chickens is presented in Table 4. The results showed that there was significant ($P<0.05$) interaction of variety and replacement levels only on RBC. It revealed RBC range of 1.05-1.60 $x10^9$/l which was lower than the normal range of 2.2-4.0 ($x10^9$/l); this suggested that there might have been coccidia infection that the chickens might have come down with which probably affected the free transportation of oxygen and carbon dioxide within the body system. NseAbasi *et al*. (2014) reported that a decrease in the red blood cell count indicated a reduction in the level of oxygen that would be carried to the body tissues.

However, there was no significant ($P>0.05$) interaction effect of finger millet and replacement levels on PCV, Hb, WBC, heterophils and lymphocytes. The birds fed diets containing finger millet competed favourably with birds fed the control diets. PCV range of 25-31.17% was within the normal range of 27-42%. This suggests that the birds were neither anaemic nor dehydrated. PCV is taken to indicate the health status of birds as low PCV indicate anaemia which was a condition in which there were either too few RBC or RBC was deficient in Hb resulting in poor health (Yip, 2000). Packed cell volume was involved in the transportation of oxygen and absorbed nutrients (Isaac *et al*., 2013). Hb range of 8.27-10.40 g/dl was within the normal range (7-11 g/dl), this also may indicate that oxygen was transported to the vital areas within the body of the birds. According to Minka and Ayo (2007), haematological values were used to assess stress and welfare in animals.

WBC range of 3.05-7.80 $x10^{12}$/l was within the normal range (1.0-9.5 $x10^{12}$/l), this suggested that the birds were able to overcome health challenges that came their way within the growing period. WBC played a prominent role in disease resistance especially with respect to the production of antibodies and the process of phagocytosis (Soetan *et al*., 2013).

Heterophils range of 68.17-77.50% was above the normal range of (50-65%). Lymphocyte range of 22.50-29.67% was within the normal range of 20-50 %, this was an indication that the body of the chicken was able to fight foreign bodies within them implying increased immunity.

Table 4: Interaction effect of finger millet varieties and replacement levels on haematology of broiler chickens

	Red variety (%)					Black variety (%)						
Parameter	**0**	**25**	**50**	**75**	**100**	**0**	**25**	**50**	**75**	**100**	**SEM**	**Ref. value***
PCV (%)	27.50	30.17	25.33	31.00	27.50	26.83	25.00	28.67	25.17	31.17	2.16	27-42
Haemoglobin (g/dl)	9.17	9.87	8.45	10.42	9.17	9.12	8.27	9.40	8.45	10.40	0.73	7-11
RBC ($x10^9$/l)	1.20^{ab}	1.53^{a}	1.30^{ab}	1.58^{a}	1.20^{ab}	1.50^{ab}	1.57^{a}	1.40^{ab}	1.05^{b}	1.60^{a}	0.14	2.2-4.0
WBC ($x10^{12}$/l)	3.87	3.05	3.70	3.90	3.87	3.40	7.80	6.18	5.62	3.12	1.39	1.0-9.5
H (%)	74.00	73.33	77.50	68.17	74·00	74.33	76.50	74.83	71.83	71.67	2.66	50-65
L (%)	25.67	26.67	22.50	29.67	25.67	25.67	23.33	24.67	27.83	27.67	2.52	20-50

PCV- Park cell volume
RBC- Red blood cell
WBC-White Blood Cell
H- Heterophil
L- Lymphocyte
*****Reference value - Merck's Manual (1998)

CONCLUSION

Based on these results, it could be concluded that finger millet had no deleterious effects on blood parameters of broiler chickens. The biochemistry results showed that the birds did not encounter disease that could affect the normalcy of the values. All the haematological parameters measured indicate that the values were within the normal range except for heterophils that was above the reference values. This suggested that the body of the chickens were able to fight foreign bodies within them implying increased immunity provided by high values of heterophils.

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