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RESEARCH ARTICLE

Serum Biochemistry and Haematological Indices of Broiler Chickens Fed Graded Levels of Frog (*Rana esculata*) Meal as Replacement to Fish Meal

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ABSTRACT

An experiment was conducted to assess the effect of graded levels of inclusion of frog (*Rana esculata*) meal as a replacement to fish meal on serum biochemical indices and haematological parameters of broiler chickens. One hundred and eighty 1-day-old Abor acre broiler chicks were used for the experiment that lasted 42 days. Birds were randomly allotted to 5 treatments of 6 replicates with 6 birds each. Frog meal was used to replace fish meal at a level of 0, 25, 50, 75 and 100%, respectively. Results showed there were no significant differences observed in the total serum protein and globulin of birds on the experimental diets. However, the albumin and albumin/ globulin ratio of birds on 50% and 100% frog meal level respectively compared with birds on the control diet. The urea level of birds on 100% fish meal and 100% frog meal was similar. There were no significant differences observed in the packed cell volume, mean corpuscular haemoglobin concentration, lymphocyte and heterophils of birds on the experimental diets. However, there were significant ($P<0.05$) differences observed in the haemoglobin, red blood cell, white blood cell, mean corpuscular volume, mean corpuscular haemoglobin, monocytes, eosinophils and basophils of birds fed the dietary treatments. Birds on 50% frog meal and 50% fish meal diets recorded the highest values of mean cell volume ($88.39\mu^3$) and mean cell haemoglobin (30.45µµg) respectively. It was therefore concluded that frog meal can adequately replace fish meal in broiler diets up to 100% without adverse effects on the blood profile.

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INTRODUCTION

In developing countries like Nigeria, the poultry industry has always been faced with problem of obtaining feed ingredients that are of adequate nutritional quality to support growth performance. Fish meal (FM) is among the best source of high quality protein for animals. It has been used as a supplemental protein for many years primarily for monogastrics. High quality FM normally contains between 65% and 72% crude protein by weight. However, FM used in poultry ration has been faced with adulterations in recent times, as a result, demand for high quality and feed grade fish meal significantly exceeds availability which has also resulted into sky-rocketed cost of fish meal. This has invariably necessitated the need to source for unconventional alternative protein source. According to Ravinder *et al*. (1996), by-products like meat meal, liver residue, silk worm pupae meal etc, though available in small quantities, have served to bridge the gap in supply of animal protein sources as well as to lower costs. Other unconventional protein sources that had been used in poultry nutrition include: cray fish meal (Ojewole *et al.*, 2005; Asafa *et al.*, 2012), sun-dried shrimp waste meal (Oduguwa *et al*., 2004), shrimp meal (Rosenfeld *et al.*, 1997; Gernat, 2001), grasshopper meal (Aduku, 1993; Ojewole *et al*., 2005), locally processed fish waste meal (Ojewole *et al*., 2005); maggot from poultry droppings (Atteh and Oyedeji, 1990), housefly pupa meal (El-Boushy, 1991), poultry and chicken offal meal (Salami, 1997; Udedibie *et al*., 1988) and shrimp waste (Nwokoro, 1993; Fanimo *et al.*, 1996; Rosenfeld *et al.*, 1997). Unconventional feed ingredients as marine waste and frog waste are also available for use.

Frog meal is a potential feed resource and it is a non conventional protein source that can be fed to poultry in place of fish meal because of its closeness in biological value. The use of frog meal may augment the problem of competitiveness and high cost of conventional protein ingredient like fish meal. However, measuring the blood metabolites and constituents of birds fed frog meal based-diets can be used as basis for comparison to fish meal. Blood is an important index in determining the nutritional and health status of the animals. It is based on this background information that the present study was conducted to evaluate the effect of substituting frog meal for fish meal on serum biochemical and haematological parameters of broiler chickens.

MATERIALS AND METHODS

Five dietary treatments were prepared containing graded levels (0, 25, 50, 75 and 100%) of frog meal in place of fish meal weight for weight as shown in Tables 1 and 2. The frog meal used for this study was in a processed form. It was purchased from Bodija market, Ibadan, Nigeria. It was then milled and added to the diets appropriately. One hundred and eighty (180) broiler chicks (Abor acre strains) were used for this study. The birds were brooded for 7 days, and then randomly distributed into 5 treatment groups on beddings of wood shavings in a well illuminated and ventilated pen. The dietary treatments consist of 6 replicates of 6 birds each in a completely randomized design. The five dietary treatments prepared were; treatment 1 (control diet) contained no frog meal while treatments 2, 3 4 and 5 contained 25, 50, 75 and 100% graded levels of frog meal respectively. Experimental diets were offered *ad libitum* and birds had free access to clean water throughout the study period that lasted for 42 days. The study was carried out at the Pullet Unit of the Teaching and Research Farm, University of Ibadan, Nigeria.

Blood collection

On day 42, two birds per replicate were selected and bled through the jugular vein into two vaccutiner tubes, one containing ethylene diamine tetra acetic acid (EDTA) for haematological study and the other sterile plasticine vaccutiner tubes without EDTA. The second set of tubes were covered and centrifuged, serum separated out, decanted, deep-frozen for serum biochemical analyses.

Haematological indices and corpuscular constants

The Packed Cell Volume (PCV) was determined using Micro haematocrit method as described by Kelly (1979). Red Blood Cell (RBC) and White Blood Cell (WBC) were determined using Neubauer haemocytometer after the appropriate dilution. Haemoglobin (Hb) concentration was determined by a Cyanmethaemoglobin method using Drabkin's solution as diluents. Blood indices and corpuscular constants: Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) were determined using appropriate formulae (Jain, 1986).

Serum biochemical analysis

Total protein was determined by Biuret method as described by Kohn and Allen (1995) while albumin was determined using Bromocresol green (BCG) method as described by Peters *et al*. (1982). The globulin concentration was obtained by subtracting albumin value from total protein, while albumin/globulin ratio was obtained by dividing the calculated albumin value by the calculated globulin value. Urea was determined by the dimethyl monoxide method.

Experimental Design

Completely randomized design

Proximate analysis

The proximate composition of the test ingredient and experimental diets were analyzed according to the methods of AOAC (2000).

Statistical analysis

Data obtained were analyzed using descriptive statistics and ANOVA (P=0.05) (SAS, 2012). Mean differences were separated using Duncan multiple range test (Duncan, 1955).

RESULTS

The results of proximate composition of the test ingredients and experimental diets are shown in Tables 3 and 4 respectively. The crude protein and gross energy of frog meal (71.19% and 4.62 Kcal/g) and fish meal (72.42% and 3.73 Kcal/g) respectively are as shown in Table 3. Also, the crude protein (CP) content of the experimental finisher diets ranged from 19.08 to 21.20%. It decreased as the level of frog meal (FRM) increases in the diets. Crude fibre increased from 2.22 to 3.80% as the level FRM increased across the dietary treatments. Appreciable ether extract values ranging from 10.22% to 16.79% were recorded across the diets.

The result of serum biochemical indices of broiler chickens fed the experimental diets is as shown Table 5. Total serum proteins and globulin of broiler chickens fed graded levels of FRM were not significantly influenced by the dietary treatments. However, the highest serum protein was recorded in birds on 100% frog meal diet. The albumin (ALB) and albumin/globulin (A/G) ratio of birds on experimental diets were influenced (P<0.05) by the dietary treatments. The ALB and A/G ratio of birds on 50% and 100% FRM inclusion compared favorably with birds on the control diet (100% fish meal level). Similarly, significant differences (P<0.05) were observed in serum urea of birds fed experimental diets with the highest value (1.86mg/dl) observed in birds on 100% frog meal diet which was not significantly different from birds on 100% fish meal diet (1.83mg/dl).

The results of haematological indices of birds fed graded levels of frog meal (FRM) in replacing fish meal (FM) are shown in Table 6. The haematological variables in this study showed that there were no significant differences observed in the Packed Cell Volume (PCV), Mean Corpuscular Haemoglobin Concentration (MCHC), lymphocytes and heterophils of birds among the dietary treatments. The PCV of birds decreased as the level of frog meal increases in the diet except in diet 3 (50% FRM and 50% FM levels). The MCHC, lymphocytes and heterophils of birds in experimental diets did not follow a particular trend but were comparable to those on the control diet.

Table 1: Gross composition of experimental diets (g/100gDM) (starter phase)

	Frog Meal Inclusion Levels				
Ingredients	0%	25%	50%	75%	100%
Maize	56.50	56.50	56.50	56.50	56.50
Soyabean meal	16.00	16.00	16.00	16.00	16.00
Groundnut cake	20.00	20.00	20.00	20.00	20.00
Wheat offal	2.00	2.00	2.00	2.00	2.00
Fish meal (72.4% CP)	2.00	1.50	1.00	0.50	0.00
Frog meal (71.2% CP)	0.00	0.50	1.00	1.50	2.00
Dicalcium phosphate	1.50	1.50	1.50	1.50	1.50
Limestone	1.00	1.00	1.00	1.00	1.00
Broiler premix	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Common salt	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
Calculated nutrients					
Crude protein	23.15	23.14	23.13	23.12	23.11
Energy (kcal/g)	3.00	2.98	2.97	2.95	2.94
Crude fibre	3.36	3.36	3.35	3.35	3.34
Lysine	1.26	1.24	1.22	1.19	1.17
Methionine	0.59	0.58	0.57	0.56	0.55
Calcium	1.12	1.14	1.15	1.17	1.18
Phosphorus	0.48	0.49	0.49	0.50	0.51

*Composition of Premix per Kg of diet: vitamin A, 12,500 I.U; vitamin D_3, 2,500 I.U; vitamin E, 40mg; vitamin K_3, 2mg; vitamin B_1, 3mg; vitamin B_2, 5.5mg; niacin, 55mg; calcium pantothenate, 11.5mg; vitamin B_6, 5mg; vitamin B_{12}, 0.025mg; choline chloride, 500mg; folic acid, 1mg; biotin, 0.08mg; manganese, 120mg; iron, 100mg; zinc, 80mg; copper, 8.5mg; iodine, 1.5mg; cobalt, 0.3mg; selenium, 0.12mg; Anti-oxidant, 120mg.

Table 2: Gross composition of experimental diets (g/100gDM) (finisher's phase)

	Frog Meal Inclusion Levels				
Ingredients	0%	25%	50%	75%	100%
Maize	56.50	56.50	56.50	56.50	56.50
Soyabean meal	16.00	16.00	16.00	16.00	16.00
Groundnut cake	10.00	10.00	10.00	10.00	10.00
Wheat offal	12.00	12.00	12.00	12.00	12.00
Fish meal (72.4% CP)	2.00	1.50	1.00	0.50	0.00
Frog meal (71.2% CP)	0.00	0.50	1.00	1.50	2.00
Dicalcium phosphate	1.50	1.50	1.50	1.50	1.50
Limestone	1.00	1.00	1.00	1.00	1.00
Broiler premix	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Common salt	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
Calculated nutrients	(%)				
Crude protein	20.35	20.34	20.33	20.32	20.31
Energy (kcal/g)	2.91	2.89	2.88	2.86	2.85
Crude fibre	3.71	3.71	3.71	3.71	3.71
Lysine	1.20	1.22	1.25	1.26	1.29
Methionine	0.59	0.57	0.58	0.59	0.61
Calcium	1.10	1.12	1.13	1.15	1.16
Phosphorus	0.49	0.50	0.50	0.51	0.52

*Composition of Premix per Kg of diet: vitamin A, 12,500 I.U; vitamin D_3, 2,500 I.U; vitamin E, 40mg; vitamin K_3, 2mg; vitamin B_1, 3mg; vitamin B_2, 5.5mg; niacin, 55mg; calcium pantothenate, 11.5mg; vitamin B_6, 5mg; vitamin B_{12}, 0.025mg; choline chloride, 500mg; folic acid, 1mg; biotin, 0.08mg; manganese, 120mg; iron, 100mg; zinc, 80mg; copper, 8.5mg; iodine, 1.5mg; cobalt, 0.3mg; selenium, 0.12mg; Anti-oxidant, 120mg.

Table 3: Proximate composition (%) of frog meal and fish meal

Nutrient	frog meal	fish meal
Dry matter	93.90	91.05
Crude protein	71.19	72.42
Crude fibre	2.04	0.30
Ether extract	12.00	11.00
Ash	10.80	10.00
Nitrogen free extract	3.97	6.28
Gross energy (Kcal/g)	4.62	3.73

Table 4: Proximate composition of experimental diets (as fed basis) finisher phase (%)

	Frog Meal Inclusion Levels				
Ingredients	0%	25%	50%	75%	100%
Dry matter	89.71	89.33	89.43	89.00	89.00
Crude protein	21.20	20.77	20.62	19.47	19.08
Crude fibre	2.22	2.23	3.13	3.77	3.80
Ether extract	10.95	13.71	16.79	10.22	14.87
Ash	6.81	5.90	5.54	5.36	6.34
Nitrogen free extract	58.82	57.39	46.08	61.18	55.91

Table 5: Serum biochemical indices of birds fed graded levels of frog meal

	Frog meal inclusion levels					
Parameter	0%	25%	50%	75%	100%	SEM
Total protein (g/dl)	2.80	2.80	2.89	2.84	2.90	0.16
Albumin (g/dl)	1.75^a	1.58^b	1.77^a	1.50^b	1.70^a	0.17
Globulin (g/dl)	1.05	1.22	1.12	1.34	1.20	0.16
Albumin:Globulin	1.67^a	1.30^b	1.58^a	1.12^b	1.42^{ab}	0.16
Urea (mg/dl)	1.83^a	1.39^b	0.95^c	0.96^c	1.86^a	0.11

Means on the same row with different superscripts are significantly ($P<0.05$) different. SEM- Standard Error of Mean

There were significant ($P<0.05$) differences observed in the Haemoglobin (Hb), Red Blood Cell (RBC), white Blood Cell (WBC), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and some of the differential counts of birds on the experimental diets. Most of the values recorded did not differ significantly from the birds fed the control diet containing 100% FM. Birds on 50% FRM and 50% FM diets recorded the highest values of MCV (88.39μ^3) and MCH (30.45µµg).

DISCUSSION

The proximate composition of test ingredients (fish meal and frog meal) revealed that they had similar nutrient profile. Meanwhile, Crude Protein (CP) content of the diets decreased as the level of frog meal (FRM) increases in the diets. It could be as a result of lower CP content of frog meal (71.19%) as compared to that of fish meal (72.42%) that could have resulted from different processing methods. However, the CP value obtained for frog meal in this trial was higher than 47.31% reported by Ojewola *et al*. (2005) for frog probably because the oil in frog meal had been extracted which probably resulted in higher crude protein content. Appreciable ether extract values recorded across the diets in this study is probably an indication that frog meal based diets can supply sufficient energy in broiler diets. The proximate composition of the diets in the present study was comparable to other related previous findings (Fanimo *et al*., 1996; Rosenfeld *et al*., 1997; Fanimo *et al*., 1998).

Table 6: Haematological indices of birds fed graded levels of frog meal

Parameter	Frog meal inclusion levels					
	0%	25%	50%	75%	100%	SEM
Packed cell volume (%)	33.17	32.00	32.17	30.75	30.50	1.25
Haemoglobin (g/dl)	11.43[a]	10.64[ab]	10.70[ab]	10.18[b]	10.15[b]	0.37
Red blood cell ($x10^6/mm^3$)	4.14[a]	3.90[ab]	3.52[c]	3.78[bc]	3.90[ab]	0.10
White blood cell ($x10^3/mm^3$)	19.81[a]	19.05[ab]	18.24[b]	18.72[ab]	20.07[a]	0.45
MCV (μ^3)	83.52[a]	77.18[b]	88.39[a]	81.79[ab]	78.49[ab]	3.27
MCH (µµg)	27.76[ab]	25.56[b]	30.45[a]	27.07[ab]	26.12[b]	1.16
MCHC (%)	33.23	33.25	33.25	33.12	33.28	0.08
Lymphocytes (%)	62.92	66.25	64.25	64.50	63.67	2.74
Monocytes (%)	3.75[a]	2.83[b]	3.00[a]	2.75[b]	2.92[b]	0.35
Eosinophils (%)	2.57[a]	2.17[b]	2.42[b]	2.67[a]	2.80[a]	0.26
Basophils (%)	0.25[b]	0.00[c]	0.42[a]	0.25[b]	0.50[a]	0.24
Heterophils (%)	27.75	28.83	29.67	30.25	29.50	2.74

Means on the same row with different superscripts are significantly ($P<0.05$) different. MCV = mean corpuscular volume; MCH = mean cell haemoglobin; MCHC = mean corpuscular haemoglobin concentration; SEM- Standard Error of Mean.

The chemistry of serum is routinely used for detection of organ diseases in domestic mammals and the amount of available protein in the diets (Iyayi and Tewe, 1998). It has been reported that serum biochemical constituents are positively correlated with the quality of the diet (Brown and Clime, 1972; Adeyemi *et al*., 2000). It is accepted that serum protein profile and the absolute values of individual fractions are an excellent basis for a tentative diagnosis (Kaneko, 1997). Total serum proteins and globulin of broiler chickens fed graded levels of FRM were not significantly influenced by the dietary treatments. However, the highest serum protein was recorded in birds on 100% frog meal diet. This implies that the frog meal-based diets possessed identical dietary quality with the control (100% fish meal) diet. Although Agbede and Aletor (2003) have reported that total serum protein, albumin and globulin syntheses were not affected by sources of dietary protein (quality of protein). However, similar evidence of positive linear correlation between dietary protein quality and quantity has been reported (Tewe, 1985; Eggum, 1989). Globulin carries essential metals through the bloodstream to the various parts of the body and helps the body to fight infections. Elevated globulin levels are often pronounced in birds with serious infections because of abnormally increased production of antibodies. In this study, the globulin of birds on FRM diets compared favourably with those on 100% fish meal inclusion, which is an indication that the test diet did not precipitate any severe effects on the health status of the birds.

According to Deldar (1994), albumin is the most abundant protein in blood plasma. The similarity recorded between the values of albumin (ALB) and Albumin/Globulin (A/G) ratio of birds on 50% FM and 50% FRM levels when compared with those on 100% fish meal inclusion showed a synergistic effect of the protein quality of the test ingredients. The ratio of albumin to globulin can also help to determine whether certain disorders are occurring. An elevated A/G ratio could mean an underactive thyroid, called hypothyroidism, or elevated levels of glucocorticosteroids. Low globulin levels can also cause a rise in the A/G ratio. Serum urea can be used as a test of renal function, protein breakdown, hydration status, and liver failure. However, the concentration of urea also depends on diet especially those with high protein content. Serum urea of birds on 100% frog meal diet was similar to those on 100% fish meal diet (Table 5). This probably suggests that there was a better digestion, utilization and absorption of protein in the test ingredient used which invariably improved protein utilization. The values of the blood parameters across the diets were comparable to those reported in literature for broiler chickens (Swenson, 1970; Campbell *et al*., 2003) indicating nutritional adequacy of the diets.

Blood represents a means of assessing clinical and nutritional health status of animals in feeding trial and the haematological parameters most commonly used for assessment in nutritional studies include Packed Cell Volume (PCV), Red Blood Cell (RBC), Haemoglobin concentration, Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV) and clotting time (Aletor and Egberongbe, 1992; Olorede and Longe, 2000; Adeyemi *et al.*, 2000). The PCV of birds decreased as the level of frog meal increases in the diet except in birds fed 50% FRM and 50% FM levels. However, the values obtained for PCV and RBC were higher than those reported for chicks fed fish meal replaced with leaf protein concentrate from gliricidia (Agbede and Aletor, 2003). The MCHC, lymphocytes and heterophils of birds in experimental diets did not have a definite trend but were similar to those on the control diet. This suggests that both the control and test diets have similar dietary quality that resulted into identical haematological variables. PCV and MCHC are very important in anaemia diagnosis and also serve as a useful index of the capacity of the bone marrow to produce red blood cell. The values of PCV, MCHC, lymphocytes and heterophils are within the reported haematological values for normal chickens according to Hodgers (1977). The values of PCV and RBC were within the ranges of 30-35% and 2.88-4.12 x 10^6 mm^3 reported by Swenson (1970) and Campbell *et al*. (2003).

White Blood Cell (WBC) and some of the differential counts were significantly affected by increased levels of frog meal. The higher value of WBC observed in birds fed 100% FRM is an indication that the birds were resistant to diseases which probably signifies higher immunity status. This is reflective of the adequacy of the dietary treatments as reported by Lindsay (1977). All the haematological indices measures were within the normal range reported for broiler chickens (Mitruka and Rawnsley, 1981).

Conclusion

This study showed that frog meal is a valuable replacement for fish meal up to 100% in broiler diets without adverse effects on the blood metabolites. The results of haematological variables in this study suggest that the test diets did not precipitate any severe effects on the health status of the experimental birds.

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