



(RESEARCH ARTICLE)



## Serum biochemistry and haematological indices of finisher broilers fed varied levels of frog (*Rana esculenta*) meal as a replacement for full fat soybean

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### Abstract

A 28-day feeding trial was conducted to evaluate the effect of varied levels of frog meal as a replacement for full fat soybean on the haematology and serum biochemistry of finisher broilers. One hundred and forty-four (144) day old broilers were allocated to four dietary treatments containing 0%, 25%, 50%, and 100% of frog meal (FML). Each treatment had thirty-six (36) birds, divided into three (3) replicates of twelve (12) birds. Observed results showed significant differences ( $P < 0.05$ ) in all the haematological parameters except MCH, MCHC and Lymphocyte with birds in treatment four (100%FML) showing improved levels of haematological indices. Significant differences ( $P < 0.05$ ) were also observed for total protein, globulin, all the blood serum parameters except the values for cholesterol. Values observed for both the haematology and serum biochemistry of the birds showed that frog meal significantly improved their values without having any negative effect on the health of the birds when compared to the control. Results from the feeding trial showed that full fat soybean could be replaced replaced with frog meal up to 100% without any negative implication on the health of the birds.

**Keywords:** Frog meal; Haematology; Serum biochemistry; Finisher broilers

### 1. Introduction

Poultry industry constitutes an important agricultural enterprise in terms of profitability and quick returns on capital invested. The industry also plays a major role in protein production from the livestock sub sector of the national economy. According to [1], birds constitute over 90% of the current national livestock population and are of appreciable economic and social value to the investors and consumers. Poultry products which are sold contribute about 15% to the annual income of a typical poultry producing household [2].

The poultry industry is not without its own challenges. Given the increasing number of people venturing into poultry business and the consequent high demand for commercial feeds, there is increasing tendency for feed manufacturers to produce substandard feeds especially as the quality control agencies in Nigeria are either less concerned or non-functional [3]. However, the prices of conventional energy and protein sources like maize, soybean, fishmeal etc. continue to soar and is becoming uneconomical to use them in poultry feed formulations [4]. This therefore necessitates the search for cheaper feedstuff to continue to be very central to the research efforts of animal nutritionist in the tropics rising from the critical need to find alternative feed ingredients that can substitute for more conventional feedstuffs which are not only very expensive but are more badly needed for human feeding [5].

Fish meal and soybean are two of the most popular proteins used in feed production, they are gotten from animal and protein sources respectively. Fish meal is very common as it is believed to be the main source of protein in poultry diet because of its high level of methionine and lysine. However, the high cost of fish meal among other several poultry feed

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ingredients made animal nutritionists venture into research on other unconventional available animal feed source that is closest to fish meal and relatively unacceptable to humans [6]. It has been observed that demand for fish meal significantly exceeds availability [7], this is also a major problem affecting the use and availability of soybean.

The increasing world price of soybean due to competition between food and biofuel industries has reduced its availability for poultry feeding. There has been increased research interest into alternative cheap protein sources for poultry feeding necessitating utilization of non-conventional feed resource [8]. Consequently, [9] advocated harnessing the potentials of good quality and relatively inexpensive feed ingredients as replacement for the more expensive conventional feed ingredients.

Some non-conventional protein sources that had been used in poultry nutrition are: Sun-dried shrimp waste meal [10] and locally processed fish waste meal. Other sources of animal protein that are sparingly used are blood meal, meat meal and recently frog meal [11].

Frog meal is a nutrient-rich by-product, with a composition similar to that of fish, protein (65- 71%), fat (7-17%) and ash (13-24%) [12]. It is high in digestible nutrient [13] and can be fed to poultry as replacement for fish meal because of its quality in biological value.

With the threat of a global food crisis looming large, it is very important to source for alternative feed ingredients which are not as expensive or in huge demand as the conventional ones and does not compromise the performance and health of the birds. It is based on this premise that this research was carried out, to assess the effect of using frog meal as a partial replacement of full fat soybean on the haematology and serum biochemistry of broilers.

## 2. Materials and methods

### 2.1. Experimental Site

The feeding trial was conducted in the Poultry Unit of the Livestock Teaching and Research Farm of Prince Abubakar Audu University, Anyigba. Anyigba is situated between Latitude 7° 15' and 7° 29'N of the equator and Longitude 7° 11' and 7° 32' E of the Greenwich meridian and with an average altitude of 420metres above the sea level. Anyigba town falls within the tropical wet and dry climate region of the Guinea savanna, with average annual rainfall of 1600mm and daily temperature range of about 25 °C - 35 °C [14].

### 2.2. Experimental Diets

**Table 1** Gross Composition of Experimental Diets Containing Frog Meal for Finisher Broilers (%)

Ingredients	T1(0%FML)	T2(25%FML)	T3(50%FML)	T4(100%FML)
Maize	35.10	35.10	35.10	35.10
Full fat Soybean	26.00	19.50	13.00	0.00
Frog meal	0.00	6.50	13.00	26.00
Maize offal	23.50	23.50	23.50	23.50
Bone meal	2.50	2.50	2.50	2.50
Lysine	0.15	0.15	0.15	0.15
Methionine	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
<b>Calculated analysis</b>				
Crude protein (%)	21.42	23.28	25.14	28.86
M.E.(Kcal/kg)	2962.15	2993.90	3025.64	3089.13

The dried frogs were ground and stored in airtight containers pending the time of its use as a partial replacement for soybean. Four experimental diets were compounded while the soybean were partially replaced at 0%, 25%, 50% and 100% with the frog meal. These were designated as T1(control), T2, T3 and T4 respectively as presented in Table 1.

### 2.3. Experimental birds and design

One hundred and forty-four (144) 7-day old unsexed Arbor Acre broiler chicks were used for the experiment. The birds were allotted to four (4) diets (0%, 25%, 50% and 100% frog meal. These were designated as T1 (control), T2, T3 and T4 respectively). Each treatment had thirty-six (36) birds, with each treatment having three (3) replicates of twelve (12) birds. The feeding trial was for a period of twenty-eight (28) days.

### 2.4. Data collection

At the end of the feeding trial (28 days), blood samples were collected from randomly picked birds in the various replicates representing each treatment, through the wing web using sterile disposable needles and syringes. The blood samples were collected into well labeled bottles containing ethylene diamine tetra acetic acid (EDTA) anti-coagulant for haematological profile evaluation. Another set of bottles without anticoagulant were used to collect another set of blood samples for evaluation of serum metabolites. Haematological parameters evaluated were packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC), haemoglobin (Hb), neutrophils, lymphocytes, mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), and mean corpuscular haemoglobin (MCH). Serum biochemical parameters evaluated were total protein, globulin, creatinine, albumin, urea, aspartate amino transferase (AST) and alanine amino transferase (ALT).

### 2.5. Statistical Analysis

All data obtained were subjected to Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 16. Fisher's Least Significant Difference (LSD) was used to separate significantly different ( $P < 0.05$ ) means.

## 3. Results

The haematology of finisher broilers fed diets containing frog meal as a replacement for full fat soybean is presented in Table 2.

The results showed significant ( $p < 0.05$ ) variation in most of the parameters analyzed except for MCH, MCHC and lymphocytes where no significant ( $p > 0.05$ ) differences were observed. The values of PCV, Haemoglobin, White blood cell, red blood cell, mean corpuscular volume, and neutrophils ranged from 26.67%(0%FML)-31.33%(100%FML), 8.13g/dl(0%FML)-10.38g/dl(100%FML),  $179.68 \times 10^9/l$ (25%FML)- $215.17 \times 10^9/l$  (50%FML),  $2.35 \times 10^{12}/l$  (0%FML)- $2.41 \times 10^{12}/l$ (25%FML), 114.17fl(0%FML)-122.50fl(25%FML),  $2.17 \times 10^9/l$  (25%,100%FML)- $3.50 \times 10^9/l$  (50%FML) respectively.

**Table 2** Haematology of Finisher Broilers Fed Diets Containing Frog Meal

Parameters	T1(0%FML)	T2(25%FML)	T3(50%FML)	T4(100%FML)	SEM	LOS
PCV (%)	26.67 <sup>c</sup>	29.50 <sup>b</sup>	30.67 <sup>a</sup>	31.33 <sup>a</sup>	0.68	*
Hb (g/dl)	8.13 <sup>b</sup>	9.83 <sup>a</sup>	10.23 <sup>a</sup>	10.38 <sup>a</sup>	0.33	*
WBC ( $\times 10^9/l$ )	219.50 <sup>a</sup>	179.68 <sup>c</sup>	215.17 <sup>b</sup>	215.83 <sup>b</sup>	1.70	*
RBC ( $\times 10^{12}/l$ )	2.35 <sup>c</sup>	2.41 <sup>b</sup>	2.48 <sup>a</sup>	2.49 <sup>a</sup>	0.02	*
MCV(fl)	114.17 <sup>b</sup>	122.50 <sup>a</sup>	124.33 <sup>a</sup>	125.83 <sup>a</sup>	9.16	*
MCH (pg)	38.17	40.83	41.33	41.83	2.60	NS
MCHC(g/dl)	33.02	33.32	33.30	32.85	0.87	NS
Neutrophil ( $10^9/l$ )	3.00 <sup>a</sup>	2.17 <sup>c</sup>	3.50 <sup>b</sup>	2.17 <sup>c</sup>	0.12	*
Lymphocyte ( $10^9/l$ )	97.00	97.83	96.67	97.83	2.37	NS

abc: Means with different superscripts along the same row show significant difference at  $p < 0.05$ , SEM: Standard error of the mean, LOS: Level of significance, NS: Not significant at  $p > 0.05$ , \*: Significant at  $p < 0.05$ , PCV=Packed Cell Volume, Hb=Haemoglobin, WBC=White Blood Cell, RBC=Red Blood Cell, MCV=Mean Corpuscular Volume, MCH=Mean Corpuscular Haemoglobin, MCHC= Mean Corpuscular Haemoglobin Concentration

The serum biochemistry of finisher broilers fed diets containing frog meal as a replacement for full fat soybean is presented in Table 3.

The result obtained showed significant ( $p < 0.05$ ) influence of frog meal on all the serum parameters except for the cholesterol level. The values obtained ranged from 3.07-3.96g/dl for the serum total protein, 1.39-2.12g/dl and 1.61-1.84g/dl were obtained for the albumin and globulin respectively. Creatinine values steadily decreased significantly ( $P < 0.05$ ) across the treatments, with values ranging from 0.77(0%FML)-0.50mg/dl(100%FML). A similar observation was made for the alanine transaminase and aspartate transaminase levels with a steady decrease in value from 61.03 (0%FML)-52.30 $\mu$ /dl(100%FML) and 10.01(0%FML) to 9.44 $\mu$ /dl(100%FML) respectively.

**Table 3** Serum Biochemistry of Finisher Broilers Fed Diets Containing Frog Meal

Parameters	T1(0%FML)	T2(25%FML)	T3(50%FML)	T4(100%FML)	SEM	LOS
Total Protein (g/dl)	3.07 <sup>c</sup>	3.29 <sup>b</sup>	3.43 <sup>b</sup>	3.96 <sup>a</sup>	0.26	*
Globulin (g/dl)	1.39 <sup>c</sup>	1.61 <sup>b</sup>	1.82 <sup>ab</sup>	2.12 <sup>a</sup>	0.30	*
Albumin (g/dl)	1.68 <sup>b</sup>	1.68 <sup>b</sup>	1.61 <sup>b</sup>	1.84 <sup>a</sup>	0.13	*
Creatinine (mg/dl)	0.77 <sup>a</sup>	0.63 <sup>ab</sup>	0.54 <sup>b</sup>	0.50 <sup>b</sup>	0.06	*
ALT (iu/dl)	61.03 <sup>b</sup>	60.04 <sup>b</sup>	56.12 <sup>a</sup>	52.30 <sup>a</sup>	2.49	*
AST (iu/dl)	10.01 <sup>c</sup>	9.65 <sup>b</sup>	9.61 <sup>b</sup>	9.44 <sup>a</sup>	0.21	*
Cholesterol (mg/dl)	2.56	2.51	2.62	2.60	0.27	NS

abc: Means with different superscripts along the same row show significant difference at  $p < 0.05$ , SEM: Standard error of the mean, LOS: Level of significance, NS: Not significant at  $p > 0.05$ , \*: Significant at  $p < 0.05$ . ALT: Alanine amino transferase, AST: Aspartate amino transferase.

#### 4. Discussion

The PCV values ranged from 26.50%-32.50% which agrees with the range of 26.0%-45.20% obtained by [15] and close to the range of 30%-35% reported by [16]. A low PCV is a good indicator of anaemia while a high PCV is indicative of dehydration or polycythemia [17]. The observed values suggest that the bird's health was not compromised by the inclusion of frog meal in their diets.

Haemoglobin is directly involved in the transport of oxygen and carbon dioxide. Any drop in this ability of Hb affects the wellbeing of the birds [18]. The values observed ranged from 8.13 g/dl -10.38g/dl which falls within the values of 9.43g/dl - 10.50g/dl reported by [19].

The RBC values obtained in the study were lower than the value ranges of  $3.65 \times 10^{12}/l$  -  $3.94 \times 10^{12}/l$  reported by [20] but within those observed by [18] and [21] who reported a value range of  $2.6 \times 10^{12}/l$  -  $3.3 \times 10^{12}/l$  as the normal red blood cell count of domestic chickens. No sign of anaemia and its attendant symptoms were observed in the birds which suggested RBC and haemoglobin count were within healthy ranges which may suggest that frog meal contains sufficient minerals and vitamins required for red blood synthesis as the test diets contained significantly higher values than the control.

Packed cell volume (PCV), haemoglobin and mean corpuscular haemoglobin (MCH) are key parameters for assessing circulatory red blood cells and are important in the diagnosis of anaemia. They also help as suitable indices in evaluating the capacity of the bone marrow to produce red blood cells in mammals [22]. The number of red blood cells in chickens impacts the overall conditions of the birds [23]. Consequently, the numerical increases in PCV, haemoglobin and RBC counts of the birds fed the test diets are an indication that the oxygen-carrying capacity of the blood was improved.

White blood cell values range from  $179.68 \times 10^9/l$  -  $219.50 \times 10^9/l$ , which was close to the value range of  $197.03 \times 10^9/l$  -  $229.17 \times 10^9/l$  reported by [8] while the neutrophils range of  $2.17 \times 10^9/l$  -  $3.00 \times 10^9/l$  was within the range of  $2.83 \times 10^9/l$  -  $3.17 \times 10^9/l$  observed by [19].

The white blood cells are the cells of the immune system that are involved in protecting the body against infectious diseases and foreign invaders. If the result of the neutrophil and white blood cells are below the normal range, it implies

that the birds may not be able to perform its normal functions. The major function of the white blood cells and its differentials are to fight infections.

The MCV, which is an expression of the average volume of individual red blood cell had a value range of 114.17fl-125.83fl which was within the range of 97.07fl - 166.45fl reported by [24] for healthy birds. This is an indication that the iron intake from the diets by the birds was sufficient.

The observed serum total protein values increased steadily across the treatments and were within the range of 3.80g/dl-4.36g/dl reported by [25] while the albumin values ranged from 1.61g/dl-1.84g/dl which were close to the values of 1.55g/dl-1.70g/dl observed by [26] for healthy birds. This could be as a result of increased protein digestion and utilization in the chicken. [27] stated that total protein and albumin are directly responsive to protein intake and quality. Globulin had a value range of 1.39g/dl-1.82g/dl which was within the range of 1.15g/dl-2.85g/dl reported by [28].

Creatinine values of 0.50mg/dl-0.77mg/dl was within the ranges of 0.51mg/dl-0.66mg/dl and 0.52mg/dl-0.80mg/dl recorded by [29] and [30], suggesting that the nutrients were sufficient for the birds without having to draw from body reserve which could lead to muscle wastage.

The concentrations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the blood are bio-indicators of liver function and damage [31]. Increased levels of these enzymes are associated with liver or muscle damage, resulting from the body's response to stress [32]. In this study, it was observed that the treatment groups had significantly lower values of both enzymes compared to the control, with T4 (100% FRM) having the least values. The ranges observed for ALT and AST were below those reported by [33] and [18]. This therefore suggests that T4 (100% FRM) improved the liver functions.

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## 5. Conclusion

Haematological and serum biochemistry parameters showed that the birds fed the treatment diets generally showed significantly improved values when compared to the control diet, without showing any deleterious effect on the health of the birds.

Based on the results observed in this experiment, frog meal can be used to completely replace (100%) full fat soybean in the diets of finisher broilers without compromising the health of the birds.

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

All authors declare no conflict of interest exist.

### *Statement of ethical approval*

The research was conducted in accordance with the ethical standard of the institution.

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