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Haematological Response and Serum Biochemical Profile of Broiler Finishers Fed with Oxytetracycline and Stonebreaker (*Phyllanthus amarus)* Leaf Meal

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Authors' contributions

This work was carried out in collaboration between both authors. Author ADO designed the study and carried out the feeding trial. Author IOA managed the literature search, statistical analysis and wrote the first manuscript. Both authors approved the final manuscript.

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ABSTRACT

Aims: The aim of the study was to assess the effect of *Phyllanthus amarus* leaf meal on haematology and serum biochemical profile of broiler finishers vis a vis oxytetracycline, in order to serve a basis for further study focusing on antibacterial properties of *Phyllanthus amarus*.
Study Design: The design of the study was completely randomised design.
Place and Duration of Study: The study was carried out at the Teaching and Research Farm, University of Ibadan, Nigeria. The study lasted for 6 weeks.
Methodology: One hundred and eight mixed-sex (Hybro PG) four-week old chicks were used for the study.
Four dietary treatments were formulated. Each treatment had three replicates, while each replicate had nine birds. The experimental diets contained 0.25% of tetracycline (T1), 0.20%, 0.40%, and 0.60% of *Phyllanthus amarus* leaf meal for T2, T3, and T4 respectively.
Results: Except for packed cell volume (PCV) and haemoglobin (Hb), there were no significant

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differences across the treatments for all the haematological parameters measured. There were no significant differences across the treatments for all the serum biochemical profile measured except for albumin and alanine amino transferase (ALT). There was significant (*P=.05)* increase for albumin for all diets containing *Phyllanthus amarus* leaf meal. The highest mean value was recorded for birds in T3 (1.56 g/dl), followed by those in T2 (1.45 g/dl). Those in control diet (T1) had the least mean value (0.53 g/dl). ALT did not follow a specific pattern.
Conclusion: *Phyllanthus amarus* leaf meal in the diets of broiler finisher chickens did not pose any health hazards to the haematological parameters and serum biochemical profile of the broiler finishers at the levels of inclusion.

Keywords: Antibiotic; blood; broilers; Phyllanthus amarus.

1. INTRODUCTION

Poultry meat has been regarded as the second largest global food commodity [1]. High cost of feed ingredients and disease outbreak are major factors stressing poultry production in the tropics. Hence, the need for use of cheap feed ingredients and antibiotics. Antibiotics have been defined as natural metabolites of fungi, bacteria or yeast that inhibit bacteria proliferation by direct action, bactericidal effect or bacteriostatic effect on sensitive fungi and/or bacteria [2]. However, synthetic antibiotic may leave resistance and is also becoming unaffordable for livestock farmers. This then necessitates the need to look inward for alternative antibiotics in leaves and other plant parts that could be included as additives. Finding healing power in plants is an age long idea. The use of leaf meal of monogastric animals is however limited, due to inability of the animals to utilise fibrous materials like ruminants. In a study conducted by Ogbuewu et al. [3], neem leaf meal was reported to have mild depressive effect on haemoglobin (Hb) concentration and packed cell volume (PCV) of female rabbits. Esonu et al. [4] on the other hand reported slight increments in the values of Hb and PCV of laying hens fed neem leaf meal diets. *Microdesmis puberula* leaf meal had been reported [5] to significantly improve feed intake, body weight gain, feed conversion ratio, nutrient utilization and organ characteristics of broilers at 0%, 10%, and 15% inclusion levels. The result of the study carried out by James et al. [6] showed that aqueous extract of *Phyllanthus amarus* significantly affected PCV and Hb concentrations of albino rats. The variations were however within the normal range.

Phyllanthus sp. is used among indigenous peoples as a small annual herb to cure many diseases such as hepatitis and other liver diseases [7]. Previous studies had indicated that *Phyllanthus* sp has anticancer effect as well as the ability to reverse the negative impacts of aflatoxicosis of broiler chickens [8,9]. It has also been reported that *Phyllanthus amarus* could be used as a medicinal plant to treat hepatic disease, eye infection, snakebites and acnes [10]. *Phyllanthus amarus* extract has been suggested as having the potential to reduce the effects of fumonisins on liver [11]. *Phyllanthus amarus*, like other tropical tree leaves contains some bioactive compounds which may affect nutrient utilization, haematological and serum biochemical parameters in animals [3,12,13]. The anti-nutritional compounds contained in *Phyllanthus amarus* include oxalate, phytate, hydrogen cyanide, nitrate and tannin [14]. There is need to look for alternative antibiotic for inclusion in poultry production, but the effect of the proposed alternatives need to be tested on haematology and serum biochemical before testing their antibiotic effect. Hence, this study was conducted to assess the effect of *Phyllanthus amarus* on blood parameters of broiler finishers.

2. MATERIALS AND METHODS

2.1 Location and Duration of the Study

The study was carried out at the Poultry Unit of Teaching and Research Farm, University of Ibadan, Nigeria. The study lasted for 28 days.

2.2 Experimental Diets and Animals

One hundred and eight mixed-sex (Hybro PG) four-week old chicks were used for the study and were fed for 28 days with test diets. Four dietary treatments were formulated. Each treatment had three replicates, with nine birds per replicate. The design of the study was a completely randomised design. The birds were raised in a deep litter system, under environmental temperatures (23ºC). *Phyllanthus amarus* used for the study was obtained from University of Ibadan, Nigeria. Ibadan ranges in elevation from 150 m in the valley area, to 275 m above sea level. It has a

tropical wet and dry climate. It has a lengthy wet season with relatively constant temperatures throughout the year. It has the mean total rainfall of about 1420.06 mm, falling in approximately 109 days. There are two peaks for rainfall, June and September. The mean maximum temperature is 26.46ºC, minimum 21.42ºC and the relative humidity is 74.55%. The harvested plants were air dried under shade until they were crispy to touch, while still retaining their greenish colour. The leaves were not sun dried to prevent destruction of bioactive molecules of the plant at high temperature. The dried leaves were milled using a kitchen blender to produce stonebreaker (*Phyllanthus amarus)* leaf meal (SBLM), suitable for incorporation into broiler diets. The diets contained 0.25% of tetracycline (T1), 0.20% of SBLM (T2), 0.40% of SBLM (T3), and 0.60% of SBLM (T4). Feed intake was determined by deducting the weight of left over from the weight of feed fed.

2.3 Data Collection and Analysis

At the end of the study, two birds were randomly selected from each replicate and blood samples were collected from them. Blood samples were collected from the birds using new, sterilized syringe and needles through their jugular veins. Blood samples were collected before the birds were served feed for the day. Blood samples for haematological analysis were collected into sterilized glass tubes containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant. Samples for serum biochemical analysis were collected into tubes without EDTA. Packed cell volume (PCV) was determined using micro haemotocrit and haemoglobin (Hb) concentrations were determined using cyanmethaemoglobin method [15]. Erythrocyte (RBC) and leukocytes (WBC) counts were determined using the improved neubrauer haemocytometer after appropriate dilution. Blood samples for serum biochemical analysis after being centrifuged were taken to the laboratory for the analysis. Aspartate amino transferase (AST) and alanine amino transferase (ALT) were determined using spectrophotometric methods. Proximate analysis was carried out using the standard procedures of Association of Official Analytical Chemists [16]. Gross composition of the experimental diets is shown in Table 1.

2.4 Statistical Analysis

Data obtained were analysed using one way analysis of variance, and significant means were separated using Duncan multiple range test [17].

Table 1. Gross composition of experimental diets (g/100% DM)

Ingredients	T1	T2	T3	T4
Maize (%)	65.50	65.80	65.60	65.40
Soya bean meal (%)	20.00	20.00	20.00	20.00
Groundnut cake (%)	8.25	8.00	8.00	8.00
Fish meal (%)	2.00	2.00	2.00	2.00
Dicalcium phosphate (%)	1.50	1.50	1.50	1.50
Limestone(%)	1.50	1.50	1.50	1.50
Salt (%)	0.25	0.25	0.25	0.25
EoD (%)	0.25	0.25	0.25	0.25
L-Lysine (%)	0.25	0.25	0.25	0.25
Dl-Methionine (%)	0.25	0.25	0.25	0.25
Oxytetracycline (%)	0.25	-	-	-
Phyllanthus amarus leaf meal *(%)*	-	0.20	0.40	0.60
Calculated nutrients				
Metabolisable energy (Kcal/kg	3064.00	3074.00	3072.00	3071.00
Proximate composition				
Dry matter	86.90	86.30	86.76	87.45
Crude protein (%)	19.69	20.13	20.13	21.88
Ether extract (%)	8.90	8.90	8.90	8.90
Ash (%)	12.00	13.00	13.00	13.00
Crude fiber (%)	4.20	3.70	3.70	3.70

EOD = Enrichment of diet contained vitamin A (10000000 UI), vitamin D3 (2000 IU), vitamin E (40000 mg), vitamin K (2000 mg), Vitamin B1 (1500 mg), vitamin B2 (4000 mg), Niacin (40000 mg), panthothenic (10000 mg), folic (1000 mg) (400000 mg), vitamin B6 (100 mg), vitamin B12 (20 mg), biotin (100 mg), choline chloride (300000 mg), manganese (80000 mg), iron (40000 mg), zinc (60000 mg), copper (80000 mg), iodine (30 mg), cobalt (300 mg), selenium (200 mg), antioxidant (100000 mg)

3. RESULTS AND DISCUSSION

Tables 2 and 3 show the haematological parameters and serum biochemical profile of broiler finishers fed experimental diets. The mean values for all the haematological parameters fell within the reported normal range. Except for packed cell volume (PCV) and haemoglobin (Hb), there were no significant differences across the treatments for all the haematological parameters measured. Birds on T4 recorded the least mean value for PCV (26.6%), while those on T2 recorded the highest mean value (36.33%). Hb followed the same pattern. Birds fed diets containing *Phyllanthus amarus* leaf meal recorded higher mean values for red blood cell counts and white blood cell counts, while lower values were recorded for lymphocytes.

There were no significant differences across the treatments for all the serum biochemical profile measured except for albumin and alanine amino transferase (ALT). There was significant (*P=.05)* increase for albumin for all diets containing *Phyllanthus amarus* leaf meal. The highest mean value was recorded for birds in T3 (1.56 g/dl), followed by those in T2 (1.45 g/dl). Those in control diet (T1) had the least mean value (0.53 g/dl). ALT did not follow a specific pattern. Birds in T4 had the least mean value (4.83 IU/l) which was statistically similar to those on T1 (6.98 IU/l) and T3 (5.23 IU/l), but statistically (*P=.05)* lower than those in T2 (8.48 IU/l). Birds on diets containing *Phyllanthus amarus* leaf meal recorded lower mean values for low density lipoprotein (LDL) and triglycerides. Mean values for cholesterol did not follow a specific pattern, but birds on T2 recorded the least mean value (122.80 mg/l), while those on T3 (157.89 mg/l) had the highest mean value. Mean values for total protein, bilirubin, AST, urea and high density lipoprotein (HDL) were higher for birds fed diets containing *Phyllanthus amarus* leaf meal.

Blood parameters have been observed to be important factors used in assessing the response of animals to the diets they are fed [18,19]. The result of the study previously carried out by Ogbuewu [3] indicated that neem leaf meal had mild depressive effect on haemoglobin (Hb) concentration and packed cell volume (PCV) of female rabbits. James et al. [6] showed that aqueous extract of *Phyllanthus amarus* significantly affected PCV and Hb concentrations of albino rats. The result of this study indicated that all the haematological parameters measured fell within the normal (reference range) for healthy chickens [15], which suggested that the diets were well tolerated by the experimental animals. The increase in AST noticed in diets containing *Phyllanthus amarus* leaf meal is similar to the result of the study by Adedapo et al. [20] who reported significant increase in the levels of ALT and AST for rats fed diets containing 400 mg/kg and 1600 mg/kg doses of extracts of *Moringa oleifera* leaves. However, ALT in this study did not follow a similar trend with the result of Adedapo et al. [20]. ALT is known to increase in liver disease [20,21]. However, the increase in T2 in this study was not significantly different from the control diet. So, no liver damage is envisaged. Damaged liver would have been responsible for leakages of the serum enzymes into the blood. However, liver damage could not be suggested in this study as indicated in the result of the study. The result of this study therefore showed that inclusion of *Phyllantus amarus* at the levels of inclusion did not pose any adverse effects on the haematological parameters and serum biochemical profile of the experimental animals as there was no indication of organ toxicity from the serum enzyme assessed.

Table 2. Haematological parameters of broiler finisher chickens fed graded levels of *Phyllanthus amarus leaf meal*

Parameters	T1	T2	T3	T4	Reference values*
PCV (%)	31.00(±0.12)^{b}	36.33(±1.49)^{a}	31.67(0.08)^{b}	26.60(±1.45)^{c}	29.00- 44.00
Hb (g/l)	10.33(±0.04)^{b}	12.11(±0.49)^{a}	10.53(±0.02)^{b}	8.89(±0.48)^{c}	9.10- 13.90
RBC (X 10^{6}/l)	3.16(±0.17)	3.93(±0.07)	4.01(±0.09)	3.74(±0.01)	1.58 – 4.10
WBC (X 10^{3}/l)	16.42(±0.28)	17.00(±0.10)	18.67(±0.40)	17.25(±0.03)	9.20- 31.00
Monocytes (X10^{6}/µl)	3.00(±0.10)	2.00(±0.20)	2.67(±0.001)	3.00(±0.10)	0.06- 5.00
Lymphocytes (X10^{6}/µl)	70.67(±0.83)	69.00(±0.33)	65.00 (±0.88)	67.00(±0.28)	47.20- 85.00

*Means with different superscripts on the same rows indicate significant difference (P=0.05); T1= contains 0.25% oxytetracycline; T2= contains 0.20% of Phyllanthus amarus leaf meal; T3= contains 0.40% of Phyllanthus amarus leaf meal; T4= 0.60% of Phyllanthus amarus leaf meal; * Mitrika and Rawnsley (1977)*

Table 3. Serum biochemical profile of broiler finisher chickens fed with graded levels of *Phyllanthus amarus leaf meal*

Parameters	T1	T2	T3	T4
Total protein (g/dl)	1.89(±0.32)	2.83(±0.06)	3.92(±0.24)	3.57(±0.14)
Albumin (g/dl)	0.53(±0.20)[b]	1.45(±0.08)[a]	1.56(±0.11)[a]	1.27(±0.02)[a]
Urea (mg/dl)	3.77(±0.14)	4.18(±0.01)	4.60(±0.12)	4.39(±0.06)
Creatinine (mg/dl)	0.66(±0.01)	0.62(±0.01)	0.58(±0.02)	0.68(±0.01)
AST (IU/l)	52.26(±2.35)	54.42(±1.70)	68.34(±2.50)	65.18(±1.55)
ALT (IU/l)	6.98(±0.18)[ab]	8.48(±0.63)[a]	5.23(±0.35)[ab]	4.83(±0.41)[b]
Cholesterol (mg/l)	148.10(±0.82)	122.80(±6.81)	157.89(±3.77)	152.72(±2.21)
Triglyceride (mg/l)	171.70(±5.04)	152.10(±0.87)	151.89(±0.93)	144.25(±3.24)
Bilirubin (mg/dl)	0.12(±0.06)	0.18(±0.01)	0.17(±0.003)	0.18(±0.01)
HDL (mg/l)	12.89(±3.30)	21.53(±0.69)	31.39(±2.28)	29.48(±1.71)
LDL (mg/l)	34.34(±1.05)	30.42(±0.14)	30.31(±0.17)	28.40(±0.75)

Means with different superscripts on the same rows indicate significant difference (P=0.05); T1= contains 0.25% oxytetracycline; T2= contains 0.20% of Phyllanthus amarus leaf meal; T3= contains 0.40% of Phyllanthus amarus leaf meal; T4= 0.60% of Phyllanthus amarus leaf meal; HDL=high density lipoprotein; LDL= Low density lipoprotein; AST= aspartate amino transferase; ALT= alanine amino transferase

4. CONCLUSION

It can be concluded from the result of this study that inclusion of *Phyllanthus amarus* leaf meal in the diets of broiler finishers do not pose any health hazards to the haematological parameters and serum biochemical profile of the broiler finishers at the levels of inclusion. However, antibacterial properties of *Phyllanthus amarus* should further be verified vis a vis oxytetracycline, so as to be able to recommend it to replace oxytetracycline for antibacterial properties in chickens.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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