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Full Length Research Paper

Effects of *Phyllanthus Amarus* (Stone-Breaker) Leaf Meal Supplementation on Haematology and Serum Biochemistry of Broiler Chickens

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ABSTRACT: This study was carried out to evaluate the effects of *Phyllanthus amarus* leaf meal (PALM) on haematology and serum biochemistry broiler chickens. One hundred and twenty (120) day old marshal broiler chickens were used for the experiment that lasted for 56 days. The birds were randomly allotted to four treatments of T_0 (control, no PALM), T_1 (2.5g/kg PALM), T_2 (5.0g/kg PALM), T_3 (7.5g/kg PALM) and T_4 (10g/kg PALM) in a complete randomized design (CRD). Each treatment was replicated thrice with 8 birds per replicate. The birds were fed with measured commercial Top^R feed whereas water was given *ad-libitum*. At the end of the experiment, blood samples were aseptically collected via venopuncture from a randomly selected bird per replicate to determine the haematology and serum biochemistry. The data were subjected to analysis of variance and statistical difference in means was separated using Duncan's Multiple Range Test. The haematology results showed no significant difference ($p>0.05$) across groups except for RBC count where T_4 was significantly different ($p<0.05$) from others and eosinophil count where T_0, T_1 and T_4 were similar but different ($p<0.05$) from T_2 and T_3. The serum biochemistry parameters equally showed no significant difference ($p>0.05$) except for the uric acid where T_4 was statistically different ($p<0.05$) from others whereas T_2 and T_3 also differed ($p<0.05$) from T_0 and T_1 and urea that had T_4 and T_3 ($p>0.05$) differed from T_2 and T_1 ($p>0.05$) and T_0. It can therefore, be concluded that PALM enhances positive haematological and serum biochemical parameters. It is recommended that PALM even up to 5g/kg can be used in broiler chicken without deleterious effects on haematological and serum biochemical parameters.

Keywords: Broiler chicken, haematology, *phyllanthus amarus,* serum biochemistry

INTRODUCTION

Attempt has recently been taken to reduce the cost of feed, including the incorporation of agro-industrial by-products in broiler diets as an energy source (Sugiharto and Ranjitkar, 2019). However, some limitations may exist when using the agro-industrial by-products as the ingredients in broiler rations. The high and low contents of fibre and protein in the by-products may limit the digestibility and thus inclusion level of such by-products (Sugiharto *et al.*, 2018). It is known that some foliage contain a number of bioactive compounds that are

beneficial for the health of chickens (Rama-Rao *et al*., 2019). These compounds include vitamins, phenolic acids, flavonoids, isothiocyanates, tannins as well as saponins (Vergara-Jimenez *et al*., 2017). The use of medicinal plants in animal production has increased research interests as a potential substitute for antibiotics (Lillehoj *et al*., 2018). In this regard, the use of leaf meal in rations may not only reduce the cost of feeds, but also elicit the health-promoting effect on broiler chickens (Sugiharto *et al*., 2019).

Phyllanthus amarus is widely distributed in all tropical and subtropical regions of the planet (Edeoga *et al*., 2006). In Nigeria, it is called "Oyomokeisoamank edem" in Efik, "Iyin Olobe" in Yoruba and "Ebebenizo" in Bini (Etta, 2008). *P. amarus* has various groups of compounds such as alkaloids, flavanoids, hydrolysable tannins, major lignans and polyphenols (Peters *et al*., 2015). Lignans like phyllanthin and hypophyllanthin, flavonoids like quercetin were isolated from the leaves of *P. amarus* (Meena *et al*., 2018). *Phyllanthus amarus* is a plant of the family Euphorbiaceae and has approximately 800 species which are found in tropical and subtropical countries of the world (Tahseen and Mishra, 2013). The name '*Phyllanthus*' means "leaf and flower" and named so because of its appearance where flower, fruit and leaf appears fused (Kumar *et al*., 2011). Its effect in excretory system is due to its antiurolithic property and is used in the treatment of kidney/gallstones, other kidney related problems, appendix inflammation and prostate problems (Sen and Batra, 2013; Ushie *et al*., 2013).

P. amarus like other tropical tree leaves contains some bioactive compounds which may affect nutrient utilization, haematological and serum biochemical parameters in animals (Ogbuewu *et al*., 2010). The anti-nutritional compounds contained in *P. amarus* include oxalate, phytate, hydrogen cyanide, nitrate and tannin (Gafar *et al*., 2012). Herbal medicines are believed to be safer than synthetic medicine because phytochemicals in the plant extract target its biochemical pathways (Sandigawad, 2015). Evaluation of haematological parameters are not only used to determine the extent of deleterious effect of herbal extracts, but also to explain functions of plant extracts or their products on the blood of animals (Bin-Jaliah *et al*., 2014). Due to the many applications of *P. amarus* plant in ethnomedicine, there is the need to investigate its effect on the haematology and serum biochemistry before using it as an alternative feed supplement and antibiotic in poultry production.

MATERIALS AND METHODS

Site of the study and ethical consideration

The experiment was carried out at the student's project site of the Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan, Oyo State, Nigeria. Ibadan is located approximately on longitude 3° 5' to 4° 36' E and latitude 7° 23' to 7° 55' N (Oladele and Oladimeji, 2011). Ibadan has a tropical wet and dry climate, with a lengthy wet season. It has mean total rainfall of 9,233.60 mm, mean maximum and minimum temperatures of 39.82 °C and 22.5 °C respectively (Egbinola and Amobichukwu, 2013) and relative humidity of 74.55%. Ethical conditions governing the conduct of experiments with life animals were strictly observed as stipulated by Ward and Elsea (1997). The experimental protocol was approved by the institutions ethical committee for the use of animals for experiment.

Animals, test ingredient, feed, design and duration

One hundred and twenty (120) day old unsexed broiler chicks were used for the experiment. The *Phyllanthus amarus* leaves used was obtained from the botanical garden of the above named institution. The leaves were air dried to crispiness, while still retaining the green colour. The dried leaves were ground with electric grinding machine (Sonik^{R}, Model SB-464) to produce *P. amarus* leaf meal (PALM). It was stored in air-tight container and kept in the fridge till used. Commercial Starter (3,200Me/Kcal/Kg, CP = 22%) and Finisher Top Feed^{R} (2800Me/Kcal/Kg, CP = 18%) were purchased and used for the experiment. Each grade of supplement represented treatment and was represented as T_0 (control, no PALM), T_1 (2.5g/kg PALM), T_2 (5.0g/kg PALM), T_3 (7.5g/kg PALM) and T_4 (10g/kg PALM). Birds were allotted to treatments using completely randomized design. The experiment lasted for eight weeks (56 days).

Management of the birds

The poultry pen was cleaned and washed with a solution of detergent, disinfected with solution of Dettol^{R} and later Azinol^{R} (diethyl-2-Isopropyl-6-methylprimidin-4-yl phosphorotionate, at manufacturer's recommended dilution). The pen was left without stocking for one week before the arrival of the chicks to enable disinfectant residues' elimination. The floor litter was laid with 5cm thick wood-shavings. On the arrival of the chicks, anti-stress solution (mixture of water, glucose and multivitamin) was served them. Routine vaccinations (NDV I/O, NDV lasota and IBD vaccine) as prescribed by the Veterinarian were strictly followed during two weeks of acclimatization whereas the commercial starter feed (Top Feed^{R}) and water was served *ad libitum*. After acclimatization, the birds were allocated to the five treatments. Measured quantity of fresh feed according to the weight was given to them by 7am and 5pm daily whereas clean borehole water was supplied *ad libitum* throughout the experiment. The PALM was measured and given as stated above throughout the experimental

period.

Blood collection and analyses

In the end of the eight weeks of study, 4 mL of blood was aseptically (methylated spirit swab) collected through the jugular veins from a bird per replicate using sterile syringe and needle, 2 mL emptied into a plain (without anticoagulant) test tube and allowed to coagulate to produce sera according to the methods described by Okeudo *et al*. (2003). Another 2 mL also emptied into EDTA sample bottle for haematological analysis.

Statistical analysis

All data obtained were subjected to analysis of variance (ANOVA) using a Statistical Package for Social Sciences (*SPSS*) version 20.0. Significantly different means were separated using Duncan's New Multiple Range Test (DNMRT) as described by Obi (2002).

RESULTS AND DISCUSSION

Haematological parameters of broiler chickens given *Phyllanthus amarus* leaf meal (PALM) supplement

Table 1 shows the haematological parameters of broiler chickens fed meals supplemented with *P. amarus* leaf meal. Statistical difference ($p<0.05$) occurred only in the red blood cell (RBC) and eosinophil counts. The RBC showed that T4 differed significantly ($p<0.05$) from other groups. There were however, numerical increases packed cell volume (PCV), haemoglobin (Hgb) and RBC as the quantity of PALM increased progressively. This is likely attributable to the PALM supplement that has been proven to have antibiotic properties (Uzor *et al*. 2016) inhibiting competition between host bird and pathogenic gut micro-flora, and by this, improved haematopoeitic activity. This is corroborated by the study of Nwankpa *et al*. (2014) who had significantly increased levels of RBC, Hb and PCV in rats given ethanol leaf extract of *P. amarus.* It has been noted that some foliage contain a number of bioactive compounds such as vitamins, phenolic acids, flavonoids, isothiocyanates, tannins as well as saponins (Vergara-Jimenez *et al*., 2017) that are beneficial for the health of chickens (Rama-Rao *et al*., 2019; Sugiharto *et al*., 2019). The findings of this study partly corroborate the claims of Omokore and Alagbe (2019) and Jimoh (2020) that *Phyllanthus amarus* does not influence the haematology of rabbits nor the general performance and health status of animals since most of the parameters were not statistically different and as well fell within physiologic normal for broiler chickens as stipulated by Mitruka and Rawnsley (1977).

More so, the current findings are in agreement with Sakthi *et al*. (2017) findings that revealed significant increase in RBC, WBC and PCV which are comparable with the findings of Ajit *et al*. (2014) and Vivian *et al*. (2015) who opined that the increase in the vital hematological constituents like PCV, Hb, RBC, and WBC in birds fed with the herbal ingredients (ginger, garlic and tulsi) is an indication of improved oxygen carrying capacity of the cells which translated to a better availability of nutrients for utilization to the birds consequently affecting their well being with an active immune system.

The WBC and its differentials revealed that they were not negatively affected since they all fell within normal ranges as found by Mitruka and Rawnsley (1977). The control (T_0) had greater leukocyte count which could be a pointer to existing microbial infection prior to the administration of PALM, suggesting a probable remediation no wonder the leukocyte counts decreased in treated groups. Tannin in PALM has toxic properties against bacterial cell membranes by inhibiting certain enzymes (Tomiyama *et al*., 2016). However, with respect to eosinophil count, T_0, T_1 and T_4 were statistically similar ($p>0.05$) but differed significantly ($p<0.05$) from T_2 and T_3. Eosinophilia is rarely found in chickens, however, if it occurs, it can be attributed to parasitism (Irizaary-Rovira, 2004). The low basophil count is suggestive of absence of infection since Maxwell *et al*. (1992) stated that low number of basophils indicates that chickens are in healthier conditions, just like the heterophil and lymphocyte counts are suggestive of state of healthiness in this present study. Certain infectious conditions, such as bacterial infections or extraordinary viral diseases in hematopoietic cells, can cause a decrease in the number of heterophils (Latimer and Bienzle, 2010) while a decrease in the number of lymphocytes can occur under stress conditions (Latimer and Bienzle, 2010; Huff *et al*., 2010).

Serum biochemical parameters of broiler chickens given *Phyllanthus amarus* leaf meal (PALM) supplement

Table 2 shows the serum biochemical parameters of broiler chickens given *Phyllanthus amarus* leaf meal (PALM) supplement. The results revealed that all the parameters except the uric acid, ALT and ALP were not statistically different ($p>0.05$). The total protein (TP) progressively increased as the concentration of the PALM increased from 2.5 (T_1) to 5 g/kg (T_2) whereas the TP values decreased progressively as the test ingredient increased beyond T_2. Meanwhile, the concentrations obtained for TP were in line with the reference values for poultry and birds (Harvey, 2012; Thrall *et al*., 2012). The gradual increase of TP from T_0 to T_2 could be due to the additional crude protein via the PALM as well as the

Table 1: Haematological parameters of broiler chickens on *Phyllanthus amarus* leaf meal supplements.

Parameters	T_0	T_1	T_2	T_3	T_4	±SEM
PCV (%)	18.50	20.50	21.00	22.00	24.00	0.83
Hgb (g/dL)	6.15	6.75	6.80	7.10	7.75	0.24
RBC ($x10^6$/L)	3.24^b	3.30^{ab}	3.50^b	3.29^b	3.78^a	0.15
WBC ($x10^3$/L)	16.4	11.5	15.4	12.1	13.6	0.10
Platelet ($x10^5$/L)	32.0	18.8	30.6	27.6	34.6	0.23
Lympho ($x10^9$/L)	67.50	67.00	70.00	67.00	62.50	1.14
Heterophil ($x10^9$/L)	24.00	25.00	24.00	27.00	28.50	1.08
Monocytes ($x10^9$/L)	3.50	2.50	4.00	4.00	3.50	0.27
Eosinophil ($x10^9$/L)	4.50^a	5.00^a	2.00^b	2.00^b	5.00^a	0.50
Basophil ($x10^9$/L)	0.50	0.50	0.00	0.00	0.50	0.15

a, b :- Means with different superscripts along the same row are significantly different ($p<0.05$). Key: PCV = Packed cell volume, Hgb = Haemoglobin, RBC = Red blood cell, WBC = White blood cell.

Table 2: Serum biochemical parameters of broiler chickens fed *Phyllanthus amarus* leaf meal supplemented diets.

Parameters	T_0	T_1	T_2	T_3	T_4	±SEM
T. P (gdl^{-1})	5.07	5.70	6.08	5.77	5.02	0.16
Alb (gdl^{-1})	2.21	2.30	2.63	2.25	1.96	1.09
Glo (gdl^{-1})	2.86	3.40	3.45	3.52	3.06	0.64
UA (mg/dL)	4.24^c	5.43^c	5.78^b	5.92^b	6.21^a	0.63
Urea (mg/dL)	3.50^c	4.97^b	5.07^b	6.15^a	5.47^a	0.58
Glu (mg/dL)	205.00	194.50	198.50	206.00	208.00	4.99

a,b,c :- Means with different superscripts along the same row are significantly different ($p<0.05$). Key: TP= total protein, GLU= Glucose, UA = Uric acid.

likelihood that PALM acted as antimicrobial hence a study by Babatunde *et al*. (2014) and Uzor *et al*. (2016) demonstrated the antimicrobial potential of *P. amarus* against normal intestinal flora thereby inhibiting the growth of pathogenic microorganisms that could have impacted negatively on the intestinal mucosal integrity where digestion and absorption of nutrients occur as well as altering the general wellbeing of the birds and concomitant increase in anti-nutritional factors that probably got excessive after 5g/kg supplementation. Probably, the maximum concentration of PALM required for enhancement of TP is 5g/kg. The anti-nutritional compounds contained in *P. amarus* include oxalate, phytate, hydrogen cyanide, nitrate and tannin (Gafar *et al*., 2012). Several physiological and pathological factors have been investigated to describe possible qualitative and quantitative alterations in the concentrations of blood proteins, reflecting the actual general health state and condition of the evaluated animals, including bird species (Tóthová *et al*., 2017) and plays important roles in determining the synthetic and excretory roles of the kidney and liver (Bashir *et al*., 2015). More so, the eventual gradual decrease of TP beyond T_2 could suggest that the extract might have interfered with the equilibrium in the rate of synthesis or destruction of total protein from the system of the animals (Yusuf *et al*., 2018). Such decrease could, however, lead to hydration which is detrimental to cellular homeostasis. This will negatively affect the metabolic activities of the liver and consequently the health of the animals (Lawal *et al*., 2016).

The albumin (Alb) value also peaked at T_2 after which it gradually declined similar to the TP but did not stray out of normal range which corroborated the work of Ologhobo and Adejumo (2015) who reported significant ($p<0.05$) increase for albumin for all diets that contained *P. amarus* leaf meal. As adduced for TP, serum albumin and globulin depend on availability of dietary protein (Obikaonu *et al*., 2011) and their serum concentrations are associated with the recent nutritional status and reflect the balance between protein synthesis and degradation [Tóthová *et al*., 2017; Tóthová and Nagy, 2018]. There was however, no hypoalbuminaemia except for T_4 that fell slightly below normal physiologic range similar to report of Elsayed *et al*. (2014) that got 2.3 to 3.3 g dL^{-1} for broilers. This could be associated with high quantity of antinutritional factors induced by PALM (Gafar *et al*., 2012) or possible hepatotoxic concentration at 10mg/kg (T_4) since Lee (2012) had postulated that hypoalbuminaemia can be caused by a decreased hepatic production due to liver diseases such as chronic

hepatitis, cirrhosis or liver failure. Moreover, low albumin concentrations may indicate chronic malnutrition, inadequate protein intake, or might be associated with gastrointestinal diseases, internal parasitism and protein-losing enteropathy (Diogenes *et al*., 2010). It could also be an isolated normal for T_4 hence Simaraks (2015) stated that serum albumin is influenced by breed, age, physiological state, environment and antigen exposure and is highly variable.

The globulin followed similar trend as albumin except that it peaked at T_3 before declination but not lower than the control (T_0). As stated earlier, serum albumin and globulin depend on availability of dietary protein (Obikaonu *et al*., 2011). It could be that *P. amarus* has immunomodulatory properties since Venkatesh *et al*. (2002) observed that the increase of serum globulin indicates that birds are immunologically strong and suggested that herbs, spices and various plant extracts have appetite and digestion stimulating properties and antimicrobial effects.

The uric acid (UA) concentration in T_4 was the highest and significantly different ($p<0.05$) from others. However, T_0 and T_1 were statistically similar ($p>0.05$) but differed significantly ($p<0.05$) from T_2 and T_3. The urea followed similar trend with UA but had T_4 and T_3 statistically similar as also applied to T_2 and T_1 ($p>0.05$) whereas the least was the T_0 which was significantly different ($p<0.05$) from other treatments. The UA and urea also demonstrated gradual increases as the *P. amarus* increased from T_1 to T_4. The possibility that the birds reacted to higher protein as *P. amarus* supplement gradually increased was evidenced since the plant via proximate analysis has been reported to contain 18.77±0.15% protein (Okiki *et al*., 2015). Age and diet may influence the concentration of blood UA in birds (Kalita *et al*., 2018). This might be that *P. amarus* has predilection for kidney no wonder it has been reported to possess antiurolithic property (Ushie *et al*., 2013). The increase in the blood urea concentration observed in treated groups could be as a result of an impaired kidney function. Renal function in chickens is indicated by serum uric acid concentration (Sturkie, 1986). UA is the main end product of nitrogen metabolism in birds and is excreted via the faeces. It is relatively inert and substantially less toxic in comparison to ammonia and urea. UA is mainly synthesized in the liver by the metabolism of purine (Harr, 2006). Therefore, serum or plasma UA levels have been widely used in the detection of kidney damages and disease. In general, UA greater than 13 mg dL^{-1} suggests impaired renal function in birds (Thrall *et al*., 2012).

Although there was no statistical difference ($p>0.05$) in respect of the glucose (GLU), the concentration increased as the test ingredient gradually increased in quantity. They, however, fell within the normal physiologic range as reported by Mitruka and Rawnley (1977). In birds, glucose level below 150 mg/dl is life threatening (www.beautyofbirds.com- retrieved 06/07/2019).

In general, the blood GLU concentration in normal birds ranges from 200 to 500 mg dL^{-1} (Thrall *et al*., 2012). The main metabolite of animal metabolism is glucose, which is the primary metabolic fuel and is stored as glycogen in the liver (1–5% of wet matter) and muscles (~1% of wet matter). It is also a major energy substrate used by the brain (Fuller, 2004).

Conclusion

The use of *P. amarus* improved erythropoiesis and serum biochemical values in broiler chickens. Evidence of improved total protein is also a pointer that it can modulate immunity positively and aid in stability of peripheral blood pressure since there were enhanced albumin and globulin values particularly when administered at 5g/kg body weight. It also aided metabolic excretion of uric acid lending credence to its antiurolithic property.

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