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Growth and haematological parameters of Isa-brown pullets fed *Phyllanthus niruri* leaf meal as additive at the chick phase

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Abstract

Phyllanthus niruri Linn. is an erect annual herb known for its medicinal values; thus, this study was conducted to determine the effect of graded levels of Phyllanthus niruri leaf meal (PNLM) as a substitute antibiotic feed additive on the performance and haematological parameters of pullets at the chicks phase. Four hundred and eighty, 4 weeks old pullets weighing between 204.38 - 215.63g were fed diets containing Phyllanthus niruri leaf meal for 35 days in a completely randomized design experimental layout. Treatments were: T1(0% PNLM), T2 (antibiotics), T3 (0.2%PNLM), T4 (0.3%PNLM), T5 (0.4%PNLM) and T6 (0.5%PNLM) replicated four times at 20 birds per replicate. The parameters investigated were feed intake, final weight, weight gain, feed conversion ratio, mortality, and blood profiles. The final weight of pullets fed 0.5%PNLM (552.50g) were significantly higher ($p<0.05$) than those on T1(0%PNLM) diet (530g). There was significant differences ($p<0.05$) in the haematology and serum parameters recorded among the treatments. Highest packed cell volume and haemoglobin concentrations of 32% and 10.9g/100 ml, respectively, was recorded in T4 (0.3%PNLM). The highest value (27 µ/l) of ALT was recorded in birds fed T6 (diet with 0.5%PNLM) and the least (16µ/l) in those on T5 (diet with 0.4%PNLM) and T1 (control diet). Inclusion of PNLM resulted in better weight gain in birds fed T6 (345g), average daily feed intake (46.03g/day/bird) in T4, feed conversion ratio (0.28) in T6 compared to T1(0% PNLM) and those treated with antibiotics (T2). Therefore, Phyllanthus niruri leaf meal may be considered as a potential alternative feed additives for layer chicken production at the chick's phase.

Keywords: Performance, haematology, pullets, *Phyllanthus niruri* leaf meal, additives

Croissance et paramètres hématologiques des poulettes ISA-Brown Feed Phyllanthus Niruri Feuille de feuilles comme additif à la phase de poussin

Résumé

Phyllanthus niruri Linn. est une herbe annuelle dressée connue pour ses valeurs médicinales; Ainsi, cette étude a été menée pour déterminer l'effet des niveaux de graduation du repas de feuille de niruri Phyllanthus (RFNP) en tant qu'additif d'alimentation antibiotique substitué sur les performances et les paramètres hématologiques des poulettes à la phase des poussins. Quatre cent quatre-vingt, 4 semaines de poulettes pesant entre 204,38 et 215,63 g étaient des régimes alimentaires contenant un repas de feuille de Phyllanthus niruri pendant 35 jours dans une structure expérimentale de conception entièrement randomisée. Les traitements étaient les suivants: T1 (0% RFNP), T2 (antibiotiques), T3 (0,2% RFNP), T4 (0,3% RFNP), T5 (0,4% RFNP) et T6 (0,5% PNLM) répliquée quatre fois à 20 oiseaux par réplication. Les paramètres étudiés étaient une consommation d'aliments, un poids final, un gain de poids, un ratio de conversion d'alimentation, une mortalité et des profilés de sang. Le poids final des poulettes nourris 0,5% RFNP (552.50g) était

significativement plus élevé (p <0,05) que celles du régime T1 (0% RFNP) (530 g). Il y avait des différences significatives (p <0,05) dans l'hématologie et les paramètres sériques enregistrés entre les traitements. Volume de la cellule emballé le plus élevé et les concentrations d'hémoglobine de 32% et 10,9 g / 100 ml, respectivement, ont été enregistrées dans T4 (RFNP 0,3%). La valeur la plus élevée (27 µ / L) d'ALT a été enregistrée dans les oiseaux nourris à T6 (régime alimentaire à 0,5% RFNP) et le moins (16µ / L) chez ceux sur T5 (régime avec 0,4% RFNP) et T1 (régime de contrôle). L'inclusion de RFNP a entraîné une meilleure prise de poids chez les oiseaux nourris à T6 (345 g), une consommation moyenne d'alimentation quotidienne moyenne (46,03 g / jour / oiseau) dans T4, rapport de conversion d'alimentation (0,28) en T6 par rapport à T1 (0% RFNP) et ceux traités avec des antibiotiques (T2). Par conséquent, le repas des feuilles de Niruri Phyllanthus peut être considéré comme un alternatif potentiel d'additifs alternatifs pour la production de poulet de couche à la phase du poussin.

Mots-clés: performance, hématologie, poulettes, Phyllanthus Niruri Feuilles de feuilles, Additifs

Introduction

Modern intensive poultry has made incredible progress in terms of producing high-quality, safe chicken meat, eggs, and poultry by-products in an efficient and cost-effective manner. The industry has had to optimize the health and well-being of the birds while minimizing the industry's environmental impact (Masud *et al.,* 2020). With the use of feed additives, the poultry sector has achieved worldwide success in supplying affordable animal protein (meat and egg production) (Augère-Granier, 2019). Antimicrobials have long been used in intensive poultry farms to reduce disease and boost growth and feed efficiency (Agyare, Boama, Zumbi and Osei, 2018). Alternatives to chemical therapies are now being evaluated by the industry, with the goal of enhancing animal performance and increasing the economic output of livestock producers (Lillehoj *et al.,* 2018). Most antibiotic growth promoters, on the other hand, have been linked to residues in meat, milk, and eggs, as well as the development of antibiotic resistance in bacteria. As a result, there is need to investigate the use of non-therapeutic alternatives as feed additives in animal production, such as prebiotics, probiotics, enzymes, immunological stimulants, organic acids, and phytobiotics (herbal plants, spices, and derivatives) (Agyare *et al*., 2018). Phytobiotics have been shown to be residue-free, natural, and safe feed additives in animal production, with beneficial impacts on animal development and health via antibacterial action and immunological boosting (Yang, Iji and Choct, 2019). Phytogenic feed additives (PFA) are natural bioactive substances obtained from plants that can be added to animal feed to improve livestock performance (Karaskova, Suchy and Strakova, 2015). Among the potential herbal plants is *Phyllanthus niruri* leaf which is found to be loaded with several bioactive chemicals of high pharmacological activities. *Phyllanthus niruri* is popularly known as stonebreaker, gale of the wind or seed-under-leaf which is a 2-feet-height weed and has small leaves that grow in an alternate arrangement in two rows. The leaves are membranous and unusually thin and glaucous under its surface, elliptical in shape and have a narrow base with two stipules (Oktavidiati, 2012).

Bioactive compounds in *Phyllanthus niruri* have antimicrobial (Adeolu and Sunday, 2013), anti-inflammatory (Dada *et al*., 2014), anti-diabetic (Evi and Degbeku,

2011), antioxidant (Lim and Murtijaya, 2017), anticonvulsant (Manikkoth *et al.,* 2011), anti-carcinogenic and anticancer (Rajeshkumar *et al*., 2002) as well as antiviral (Salazar *et al*., 2011 and Prasad *et al.,* 2013). Alkaloids, flavonoids, lignins, phenols, tannins, and terpenes are among the active ingredients found in *Phyllanthus niruri* (Calixto, 1998; Bahar *et al*., 2011). Any deviations from a normal state of health can be detected in the blood profile, according to Igwe, Eze, and Nwachukwu (2017). The effect of *Phyllanthus niruri* leaf meal as an alternative feed additive on pullet growth performance, haematology, and serum biochemical markers during the chick phase was investigated in this study.

Materials and methods

Experimental diet and animals

In a completely randomized design, 480, 28-day old Isa brown pullet chicks were assigned to six nutrition regimens with four replicates each. Twenty birds were grown in each replicate in a deep litter house. The birds were offered fed and given water *ad libitum* according to standard management methods. The experiment lasted 35 days. The dietary treatments were T1: (basal diet without additive); Treatment 2: (basal diet with Tylo-dox Extra WSP as antibiotics/100kg feed; Treatment 3: Diet with 0.2% (200g) of *Phyllanthus niruri* leaf meal (PNLM)/ 100kg of feed; Treatment 4: Diet with 0.3% (300g) of PNLM/100kg of feed; Treatment 5: Diet with 0.4% (400g) of PNLM/100kg of feed; Treatment 6: Diet with 0.5% (500g) of PNLM/100kg of feed.

Source and processing of test ingredient

Phyllanthus niruri plants were collected from Federal Polytechnic Ilaro in Yewa-South, Ogun State, South-western Nigeria. The leaves were air-dried for four days at room temperature (35˚C) to obtain a constant weight before being processed into *Phyllanthus niruri* leaf meal (PNLM). For storage, the air-dried leaves were crushed and stored in water-resistant zip-lock bags. The *Phyllanthus niruri* leaf meal was subjected to a proximate analysis (AOAC, 1990) and the result is presented in Table 1 below.

Table 1: Proximate composition (g/100g DM) of *Phyllanthus niruri* leaf

Nutrients	% Dry Matter
Dry matter	91.06
Crude fibre	16.90
Crude protein	14.74
Ether extract	7.55
Ash	7.33
NFE	44.54
NDF	43.81
ADF	25.49
ME (MJ/Kg)	7.74
IVOMD	57.40

Key: NFE: Nitrogen Free Extract, NDF: Neutral Detergent Fibre, ADF: Acid Detergent Fibre, ME: Metabolizable Energy, IVOMD: In Vitro Organic Matter Digestibility

Management of experimental bird

The diet was formulated to suit the nutritional needs of pullet chicks as determined by the National Research Council (NRC) (as stated in Table 2) (NRC, 1994). The experiment was undertaken at the Poultry Unit, Teaching and Research farm, The Federal Polytechnic Ilaro, Ogun State located within longitude 3.71870E of the Greenwich meridian and latitude 6.8940°N of the equator in the South-Western rainforest belt of Nigeria with annual rainfall of 1500 mm and mean daily temperature 28°C. Before the chicks arrived, the pen houses were properly cleaned. Body weight of the chicks was measured, assigned to different treatments, and afterwards on a weekly basis for 35 days. The amount of feed consumed was monitored on a daily basis. The feed conversion ratio (FCR) was calculated by dividing the total amount of feed consumed by the amount of weight gained (Yi *et al.,* 2018). The treatment group's average values for these parameters were calculated and reported.

Table 2: Gross composition of pullet chick diet containing varying levels of *Phyllanthus niruri* leaf meal

Ingredient	0%(PN) T1	(antibiotic) T2	0.2% (PN) T3	0.3% (PN) T4	0.4% (PN) T5	0.5% (PN) T6
Maize	50.00	50.00	50.00	50.00	50.00	50.00
SBM	28.00	28.00	28.00	28.00	28.00	28.00
Fish meal (72%CP)	2.00	2.00	2.00	2.00	2.00	2.00
Wheat offal	15.00	15.00	15.00	15.00	15.00	15.00
Limestone	1.00	1.00	1.00	1.00	1.00	1.00
Bone Meal	1.00	1.00	1.00	1.00	1.00	1.00
Oyster Shell	1.50	1.50	1.50	1.50	1.50	1.50
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Methionine	0.40	0.40	0.40	0.40	0.40	0.40
Lysine	0.40	0.40	0.40	0.40	0.40	0.40
Premix	0.30	0.30	0.30	0.30	0.30	0.30
Total	**100**	**100**	**100**	**100**	**100**	**100**
Calculated analysis						
ME(Kcal/kg)						
Crude fibre (%)	2800.05	2800.05	2800.05	2800.05	2800.05	2800.05
	4.56	4.56	4.56	4.56	4.56	4.56
Crude protein (%)	20.80	20.80	20.80	20.80	20.80	20.80

ME: Metabolisable energy; Premix (Composition per kg diet): Vit. A (I.U.) 2,800,000; Vit E (mg) 16,000; Vit. K (mg) 800; Vit. B1 (mg) 1,200; Vit. B2 (mg) 1,600; Vit. B6 E.E4 (mg) 30; Folic Acid (mg) 0.4; Niacin (mg) 20,000; D Cal Pan (mg) 4,400; Co (mg) 120; Cu (mg) 3,200; I (mg) 600; Se (mg) 48; Zn (mg) 24,000; Fe (mg) 16,000; Mn (mg) 40,000; Choline Chloride (mg) 120,000; Antioxidant (mg) 48,000; T1: basal diet without any additive; T2: basal diet with Tylo -dox Extra WSP as antibiotics/100kg feed; T3: Diet with 0.2% (200g) of PNLM/ 100kg of feed; T4: Diet with 0.3% (300g) of PNLM/100kg of feed; T5: Diet with 0.4% (400g) of PNLM/100kg of feed; T6: Diet with 0.5% (500g) of PNLM/100kg of feed.

Collection of blood

On the 35th day of the trial, blood samples were taken following a 12-hour fast to determine haematological indices. Blood was taken through jugular vein from two randomly selected birds from each replicate and placed in sterilized vials containing the anticoagulant ethylene diamine tetra acetic acid (EDTA). The microhaematocrit method (Turkson and Ganyo, 2015) was used to assess packed cell volume (PCV), whereas the cyanomethaemoglobin method was used to determine haemoglobin concentration.

Statistical analysis

Analysis of Variance (ANOVA) was done using SAS (2010) package to analyse data on growth performance (feed intake, changes in body weight, feed conversion ratio) and haematological indicators. New Duncan's Multiple Range Test (DNMRT) of the same package (Duncan, 1955) was used to separate different means at $\alpha_{0.05}$.

Results and discussion

Table 3 shows the growth performance of Isa-Brown pullets fed diets with varying inclusions of *Phyllanthus niruri* leaf meal (PNLM) during the chick's period. Pullet chicks in T4 (0.3% PNLM) and T6 (0.5% PNLM) groups showed an increased feed intake and body weight gain, respectively. T5 (92.19g) and T4 (102.4g) had the lowest and greatest average weekly feed intakes at the end of the chick period, respectively. The birds on T6 gained the most weight (345g) ($p>0.05$), whereas those on T2 (300g), the least. The T6 had the highest feed conversion ratio (0.28), while T4 (0.21) was lowest (as shown in Table 3). The results demonstrated that when larger quantities of PNLM were included in the diet, pullets in the chick phase performed better. Protein acts mostly in animal tissue growth, according to Olomu (1995). It's also possible that increasing the amount of PNLM in the Isa-Brown diet's meals would have a substantial impact on weight gain.

Table 3: Performance characteristics of Isa-brown pullet fed *Phyllanthus niruri* leaf meal at the end of chicks' phase

Parameters	T1	T2	T3	T4	T5	T6	SEM±
ILW (g)	215.60^{a}	207.50^{ab}	213.13^{ab}	214.38^{ab}	204.38^{b}	207.50^{ab}	4.83
FLW (g)	530.00^{ab}	507.50^{ab}	537.50^{ab}	533.75^{ab}	500.00^{b}	552.50^{a}	26.77
WG (g)	314.37	300.00	324.37	319.37	304.38	345.00	26.30
AWFI (g)	96.15^{ab}	93.08^{b}	97.09^{ab}	102.40^{a}	92.19^{b}	97.41^{ab}	1.20
ADFI (g/day/bird)	41.95^{b}	40.67^{b}	43.18^{ab}	46.03^{a}	40.68^{b}	43.86^{ab}	0.56
FCR	0.31	0.31	0.30	0.32	0.32	0.28	0.03
Mortality	0.50^{b}	3.00^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.28

^{a,b}: Mean within the same row with different superscript letters were significantly different ($P<0.05$);ILW: Initial live weight, FLW: Final live weight, WG: Weight gain, AWFI: Average weekly feed intake, ADFI: Average daily feed intake, FCR: Feed Conversion Ratio, T1: basal diet without any additive; T2: basal diet with Tylo-dox Extra WSP as antibiotics/100kg feed; T3: Diet with 0.2% (200g) of PNLM/ 100kg of feed; T4: Diet with 0.3% (300g) of PNLM/100kg of feed; T5: Diet with 0.4% (400g) of PNLM/100kg of feed; T6: Diet with 0.5% (500g) of PNLM/100kg of feed.

Table 4 shows the haematological and serum biochemical indicators of the chickens. The T4 (0.3% PNLM) had the highest packed cell volume (PCV) value of 32%, whereas T2 and T1 had values of 24% and 28%, respectively. The PCV value range of 24–32% is within 24.9 – 45.5% by Mitruka and Rawnsley (1997) and within the range of 30.75–34.25% obtained by Oyewole *et al.* (2018) for healthy birds. The inclusion of *Phyllanthus niruri* leaf meal in the diet of birds had no negative effects on their health. The T3 (0.2% PNLM) had the highest red blood cell (RBC) count of 5.5 $x10^6$ /mm^3, while T1 and T2 had values of 3.5 $x10^6$ /mm^3 and 4.2 $x10^6$ /mm^3, respectively. This is slightly higher than the typical range of RBC values for hens, which is 2.5-3.5 x $10^6 mm^3$ (Bounous and Stedman, 2000). Treatment T4 (0.3% PNLM) had the highest haemoglobin concentration of 10.9g/100 ml, while T1 and T2 had values of 9.3g/100 ml and 8.2g/100 ml, respectively. The results were within the typical range of chicken haemoglobin (HB) values of 7 to 13 g/dl (Bounous and Stedman, 2000). In white blood cell (WBC) count, The T3 had the highest white blood cell (WBC) count of 12.4 $x10^3$ /mm^3, whereas T5 had the lowest at 10.2 $x10^3$ /mm^3.With the exception of highest value recorded in T3, other results were lower than the normal range of WBC values for chickens of 12-30 µl (Bounous and Stedman, 2000). The recorded low WBC counts with increased levels of PNLM in diets may be due to the fact that PNLM helps to fight pathogens or reduce stress. The highest lymphocyte level of 72% was recorded in T2 and the lowest value of 68% was recorded in T4. The lowest AST (55u/l) and ALT (16u/l) were found in T1 (0%PNLM) and T5 (0.4% PNLM), as indicated in Table 4). Birds fed T6 (diet with 0.5% PNLM) had the highest ALT value (27u/l), while those on T5 (diet with 0.4% PNLM) and T1(0% PNLM) had the lowest value (16u/l). It is worth noting that the liver is at the centre of various digestive, metabolic, and productive processes, and as such, vulnerable to chemical and biological harm to varying degrees. The serum levels of particular enzymes originating from the liver reveals such damage. These enzymes may cause several biological functions to be disrupted, resulting in poor health and production performance, depending on their levels. The AST, ALP, and ALT are biomarkers of liver functions and injury in the blood (Yildirim *et al*., 2011). Increased levels of these enzymes have been linked to liver or muscle damage as a result of the body's stress response (Lumeij, 2008). Another enzyme generated primarily by the intestinal mucosa, liver, bone, kidney, and placenta is ALP. However, intestinal ALP does not contribute significantly to blood ALP levels (Hoffman and Solter, 2008). The ALP activity that is reduced could indicate a halt in bone formation (Szabo *et al.,* 2005). Enhanced osteoblastic activity, which involves the production and mineralization of bone in conjunction with increased skeletal development, is associated with higher serum levels of ALP (Lumeij, 2008). The T1 (0% PNLM) had the highest total cholesterol (135.4mg/dl), total protein (6.3g/dl), and globulin (3.1g/dl) values, all of which were statistically different ($p<0.05$) from the treated groups, whereas T3-T6 (diets with PNLM) had the lowest values for these parameters.

Table 4: Haematological and serum biochemical parameters of pullets fed *Phyllanthus niruri* leaf meal at the end of chicks' phase

Parameters	T1	T2	T3	T4	T5	T6	SEM±
PCV (%)	28	24	31	32	27	30	0.56
Hb (g/dl)	9.3	8.2	10.4	10.9	9.1	10.2	0.19
RBC ($x10^6$ /mm^3)	3.5	4.2	5.5	3.4	4.7	3.2	0.17
WBC ($x10^6$ /mm^3)	11.2	10.7	12.4	11.9	10.2	11.5	0.15
Lymph($x10^3$/L)	69	72	70	68	70	69	0.26
Heterophils	30	26	30	31	28	29	0.34
Monocytes($x10^3$/L)	0	1	0	1	1	1	0.09
Eosinophils	1	0	0	0	1	1	0.10
Basophils	0	1	0	0	0	0	0.07
SERUM							
Chol (mg/dl)	135.4^a	113.3^b	112.8^c	80.1^f	81.9^e	111.1^d	4.03
AST (U/L)	55^f	90^b	58^e	84^c	67^d	96^a	3.29
ALT (U/L)	16^e	19^d	20^c	21^b	16^e	27^a	0.77
TP (g/dl)	6.3^a	6.1^c	6.2^b	6.0^d	5.9^e	5.8^f	0.04
ALB (g/dl)	3.2^b	3.1^c	3.3a	3.0^d	3.0^d	2.9^e	0.03
Globulin (g/dl)	3.1^a	3.0^b	2.9^c	3.0^b	2.9^c	2.9^c	0.02
ALP (U/L)	32^a	30^c	31^b	29^d	30^c	29^d	0.22
HI titre	80^c	80^c	160^b	80^c	320^a	160^b	17.80

$^{a,b,c,d,e,f:}$ Mean within the same row with different superscript letters were significantly different ($P<0.05$);Key: PCV- Packed Cell Volume, Hb - Haemoglobin, RBC- Red Blood Cell, WBC - White Blood Cell, Lymph – Lymphocyte, Chol- Cholesterol, AST- Aspartate Aminotransaminase, ALT – Alanine Transaminase, TP- Total Protein, ALB- Albumin, ALP- Alkaline Phosphatase, T1: basal diet without any additive; T2: basal diet with Tylo-dox Extra WSP as antibiotics/100kg feed; T3: Diet with 0.2% (200g) of PNLM/ 100kg of feed; T4: Diet with 0.3% (300g) of PNLM/100kg of feed; T5: Diet with 0.4% (400g) of PNLM/100kg of feed; T6: Diet with 0.5% (500g) of PNLM/100kg of feed.

Conclusion

Pullet chicks fed *Phyllanthus niruri* leaf meal (PNLM) gained more weight than those treated with Tetra-Dox extra or no additive at all. As seen by the blood parameters of pullet chicks in this study, *Phyllanthus niruri* leaf meal (PNLM) did not cause toxicological side effects. The addition of *Phyllanthus niruri* leaf meal (PNLM) to pullet feed improved weight gain, reduced mortality and the maintained the haematological parameters of pullets in chicks phase.

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