# BLOOD PROFILE AND INTESTINAL MICROBES OF PULLET CHICKENS FED *PHYLLANTHUS NIRURI* LEAF MEAL

**OLUFAYO, O. O.,[1] *AKINLADE, O. O.,[2] OKPARAVERO, O. O.[3] & IRIVBOJE, O. A[2]**
[1]Animal Production Technology Department, The Federal Polytechnic, Ilaro, Nigeria
[2]Agricultural Technology Department, The Federal Polytechnic, Ilaro, Nigeria
[3]Horticultural Technology Department, The Federal Polytechnic, Ilaro, Nigeria
*Corresponding author: olamilekan.akinlade@federalpolyilaro.edu.ng

**ABSTRACT**
The use of chemical growth promoters as additives despite their adverse effects on animal and public health has compelled researchers to source natural alternatives such as plant-based or phytogenic feed additives. This study assessed the impact of *Phyllanthus niruri* leaf meal (PNLM) on the caecum microbial population and layers chicken's blood profile during the growth stage. The collected *P. niruri* leaves were milled after being air-dried at room temperature (25ºC) for ten days and then added to the pullets' diet. 360 pullets were randomly allotted to 6 treatments (T) of 4 replicates each in a completely randomised manner with 60 and 15 birds per treatment and replicate respectively. Treatments (T) 1 to T6 had 0%, 0% +antibiotics, 0.2, 0.3, 0.4 and 0.5% inclusion levels of PNLM respectively. Some haematological and serum parameters including caecum microbial populations were investigated using standard procedures. Data were subjected to one-way ANOVA while significant differences were determined using Duncan's multiple range test in SAS ($P<0.05$). The inclusion of PNLM significantly ($P<0.05$) influence the white blood cell, Lymphocyte and creatinine. The highest WBC ($17.23 \pm 4.50 \times 10^9$/L) and creatinine values ($4.63 \pm 2.64$ mg/dL) were recorded in T2. T5 had the highest Lymphocyte value (70.50 %). Inclusion of PNLM also improved beneficial bacteria in the caecum especially *Pseudomonas* and *Bacillus spp*. The study concluded that PNLM enhanced the beneficial microbes of the caecum without adverse effects on the blood profile.

**Keywords:** Blood, Health, Microbes, *Phyllanthus niruri* leaf meal, Pullets

## 1.0. INTRODUCTION

Antibiotic growth promoters (AGPs) are generally added to the animal ration to enhance immunity, reduce stress, boost nutrient absorption in the intestinal wall and prevent illness, increasing feed efficiency and the quality of poultry products. According to Kulkarni, Gaghan, Gorrell, Sharif, and Taha-Abdelaziz (2022), AGPs contribute to the decrease of harmful bacteria in the intestine, which promotes the growth of good microbiota (like *Lactobacillus spp*.) and improves bird performance. Manyi-Loh, Mamphweli, Meyer, and Okoh (2018) reported the negative impacts of AGPs in poultry production which include; the development of germs resistant to antibiotics, the presence of leftover effects in meat and eggs, and the rise in production costs.

Researchers have looked for ways to reduce the cost of poultry production, addition or supplementation of a conventional feedstuff with agro-industrial byproducts and plants or forages in the poultry diet became a viable solution (Sugiharto et al., 2019; Unigwe et al, 2022). Incorporating leaf meal into rations can lower feed costs while improving hens' health (Sugiharto et al., 2019).

*Phyllanthus niruri* has been utilised in many nations to cure various human medical ailments, including fever, jaundice, liver and kidney diseases, and prostate issues (Nguyen, Brian and Nguyen, 2012). Research on the pharmacological properties of this plant's bark and leaves demonstrates strong antibacterial activity (Meena, Bibwe, Bhushan, Jalgaonkar, & Mahawar, 2018), anti-inflammatory function, antioxidant (Endang et al., 2020) and antiviral activities (Meena et al., 2018; Endang et al., 2020; Perera et al., 2021).

Haematological factors are associated with blood and organs that generate blood, and they serve as reliable markers of an animal's physiological state (Khan, and Zafar, 2005). According to Olafedehan et al. (2010), blood serves as a pathological reflector of an animal's condition after exposure to toxins and other factors. Animals with a healthy blood composition are likely to perform effectively (Okpe et al., 2016). An organism's physiology, nutrition, and pathological state can all be greatly impacted by the analysis of blood, which provides the chance to look into the existence of many metabolites and other substances in animal bodies (Doyle, 2006).

The makeup of the gut microbiota can influence growth parameters, including growth rate. Research has been done on the connection between production performance and microorganisms like *Enterobacteria*, *Lactobacilli*, *Escherichia coli*, and *Campylobacter* (Iqbal, Cottrell, Suleria, & Dunshea, 2020).

According to some research, phytobiotics influence the population of gut microbes by interfering with their metabolic processes, whereas gut microbes convert phytobiotics into simpler metabolites to make them into absorbable metabolites (Iqbal et al., 2020; Skoufos, Bonos, Anastasiou, Tsinas, & Tzora, 2020; Ahmed, Elwakeel, El-Zarkouny, & Al-Sagheer, 2024).

To reduce the cost of poultry production, and prevent the development of antibiotic-resistant pathogens and AGP residue in poultry products, the addition or supplementation of conventional feedstuff and drugs with agro-industrial byproducts and plants or forages in poultry diet became a viable solution. Thus, the current investigation looked into how *P. niruri* leaf meal will affect pullet hens' gut morphology, serum biochemistry, and haematology in comparison with conventional AGP.

## 2.0 Materials and Methods

### 2.1. Description of Experimental Location

The study was conducted at The Federal Polytechnic Ilaro Teaching and Research Farm's poultry unit in Ogun State, which is situated in the South-Western rainforest belt of Nigeria, with an average daily temperature of 28°C, an annual rainfall of 1500 mm, and longitudes of 3.7187ºE and 6.8940ºN relative to the equator (World Weather Online, 2024).

### 2.2. Collection and Preparation of Experimental Plant Material

A botanist verified *P. niruri* fresh leaves that were taken in and around the experiment location. The harvested *P. niruri* leaves were thoroughly rinsed to remove sand and debris after which they were dried at room temperature (25ºC) for ten days in the open air (Kamran, Hamlin, Scott, & Obied, 2015). The leaves were afterwards ground into powder using a blender (Pyramid® PM-B999) (Akinlade, Okusanya, & Okparavero, 2021). The resulting *P. niruri* leaf meal (PNLM) was then added to the birds' basal diets at various quantities during the experiment.

**Table 1: Proximate composition of *Phyllanthus niruri leaf* (PNL)**

| Nutrients | % Dry Matter |
|---|---|
| Dry matter | 91.06 |
| Crude fibre | 16.90 |
| Ether extract | 7.55 |
| Crude protein | 14.74 |
| NFE | 44.54 |
| Ash | 7.33 |

**Key: NFE: Nitrogen Free Extract,**

**Experimental Animal Management and Design**

In a fully randomized design, 360 growing pullets (Isa Brown) at nine weeks old were divided into six (6) treatments, with four duplicates of 60 and 15 birds, respectively. The following were the dietary interventions:

i) T1 = (without any additive)
ii) T2 = Antibiotics (Tylosin as tartrate 10 g, Doxycycline as hyclate 10 g)
iii) T3 = 0.2% PNLM
iv) T4 = 0.3% PNLM
v) T5 = 0.4% PNLM
vi) T6 = 0.5% PNLM

**Table 2: Composition of Experimental Diet (Basal Diet)**

| Ingredients | Growers |
|---|---|
| Maize | 50.00 |
| Wheat offal | 28.00 |
| Oyster shell | 2.00 |
| Soybean meal | 16.00 |
| Bone Meal | 1.25 |
| Oyster Shell | 1.00 |
| Limestone | 1.00 |
| Salt | 0.25 |
| Methionine | 0.25 |
| Premix | 0.25 |
| Total | 100.00 |
| Analyzed Result | % |
| Crude fibre | 4.24 |
| Crude protein | 20.55 |
| Ash | 4.26 |
| Ether Extract | 4.54 |
| Metabolizable Energy | 2967 (Kcal/kg) |

**2.3 Data Collection**

Data were collected on haematological parameters, serum biochemistry and gut microbes.

**Determination of Haemato-Biochemical Parameters**

In the twentieth week, 2 birds from each replicate were selected for blood evaluation, blood sample was collected through the wing web vein into bottles containing Ethylene Diamine Tetra-Acetic acid (EDTA) for haematological parameters. Parameters that were evaluated include: red blood corpuscles (RBC), packed cell volume (PCV), Haemoglobin (Hb), white blood cells (WBC), heterophils lymphocyte and differential counts (lymphocyte, heterophil, eosinophil, basophil, monocyte) ratio were calculated (Bordoloi, Jas, and Ghosh, 2012). Additionally, blood samples were drawn into sterile sample bottles devoid of anticoagulants to assess serum biochemical characteristics. Using commercial diagnostic kits, each serum sample was tested for cholesterol, total protein, albumin, globulin, low-density lipoprotein (LDL), high-density lipoprotein (HDL), aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) (Ogunbajo, Alemede, Adama, & Abdullahi, 2009; Alagbe et al., 2023).

**Determination of Bacterial Count in the Gastro-intestinal Tract**

Two randomly selected birds from each replicate were chosen and slaughtered, the caecum was extracted, preserved in peptone water, and stored at 40°C for additional examination. Before being used, glassware was allowed to cool after being sterilized in the oven for two hours. The autoclave was used to sanitize all of the media for 15 minutes at 121ºC. Each caeca content was homogenized for three minutes after being combined with nine millilitres of sterile dilution blank solution. One millilitre (ml) of each dilution was inoculated into different mediums, such as MacConkay agar for coliforms and MRS agar for LAB. After that, the inoculation plates were incubated for 24 to 48 hours at 37 to 40°C. Using an upgraded bacteria colony counter, the total number of bacterial colonies was counted after each incubation period (Gill, Penney and Nottingham, 1978; Siva, Subha, Bhakta, Ghosh, & Babu, 2012).

**2.4. Statistical Analysis**

A one-way analysis of variance (ANOVA) was performed on the collected data. Duncan's Multiple Range Test was used to identify significant differences between treatments as contained in SAS (SAS Institute, 1999)

## 3.0 Results and Discussion

### 3.1 Effect of PNLM inclusion on haematological parameters of pullets

Table 1 shows the impact of *PNLM* as an addition on the experimental birds' haematological parameters during the growing phase. Packed cell volume showed numerical differences (P>0.05), ranging from 28.50% (T1) to 37.75% (T2). The range of hemoglobin percentages was 9.70% to 12.03%. Moreover, T6 had the lowest red blood cell count ($2.75\times10^{12}$/L) and T4 had the greatest ($3.43\times10^{12}$/L). On the other hand, PNML significantly ($p<0.05$) influence the values of white blood cells and lymphocytes. T1 (control) had the lowest white blood cell value (13.18 $x10^9$/L) and T2 had the highest value (17.23 $x10^9$/L). T5 had the highest lymphocyte value of 72.5% while T4 had the lowest value of 68.00%.

**Table 3: Haematological parameters of pullets fed PNLM**

| Parameters | T1 | T2 | T3 | T4 | T5 | T6 | SEM± |
|---|---|---|---|---|---|---|---|
| Pack Cell Volume (%) | 28.50 | 37.75 | 32.25 | 31.25 | 32.25 | 28.75 | 3.58 |
| Haemoglobin (g/dl) | 9.70 | 12.03 | 11.00 | 10.10 | 10.18 | 9.70 | 2.51 |
| Red Blood Cell ($x10^{12}$/L ) | 2.78 | 2.93 | 3.25 | 3.43 | 2.78 | 2.75 | 0.22 |
| White Blood Cell ($x10^9$/L) | $13.18^b$ | $17.23^a$ | $15.45^{ab}$ | $15.50^{ab}$ | $14.28^{ab}$ | $14.43^{ab}$ | 4.50 |
| Lymphocyte (%) | $70.50^{ab}$ | $70.25^{ab}$ | $69.50^{ab}$ | $68.00^b$ | $72.50^a$ | $70.00^{ab}$ | 4.65 |
| Heterophils (%) | 28.75 | 29.25 | 29.50 | 30.00 | 26.75 | 28.75 | 4.78 |
| Monocytes (%) | 0.50 | 0.25 | 0.50 | 1.00 | 0.25 | 0.50 | 0.25 |
| Eosinophil (%) | 0.25 | 0.00 | 0.25 | 0.25 | 0.25 | 0.25 | 0.21 |

**Alphabetic superscript shows that Mean within the same row were significantly different (P<0.05)**

### 3.2 Serum biochemical parameters of pullets fed PNLM

The effect of PNLM as an additive on serum biochemical parameters of the experimental birds at the growing phase is presented in Table 2. The lowest cholesterol value (9.00mg/dl) was recorded for birds fed 0.2% PNLM (T3). Numerical differences (P>0.05) were observed for aspartate aminotransferase (AST) (118.15µ/l) in T5 and for alkaline phosphatase (ALP) (87.00µ/l) in T6 (500g PNLM). The lowest values in AST (97.18µ/l), ALP (64.50µ /l) and ALT (97.58µ/l) were observed in T2. In birds given T6, the highest ALT value (134.95µ/l) was observed (500g PNLM). There was a significant difference ($p<0.05$) in the value of creatinine. T2 had the highest value (4.63 mg/dl) while T1 had the lowest value (1.50 mg/dl) for creatinine, respectively.

**Table 4: Serum biochemical parameters of pullets fed PNLM**

| Parameters | T1 | T2 | T3 | T4 | T5 | T6 | SEM± |
|---|---|---|---|---|---|---|---|
| Cholesterol (mg/dl) | 20.00 | 15.00 | 9.00 | 12.25 | 14.25 | 10.00 | 5.19 |
| Aspartate aminotransferase (U/L) | 113.23 | 97.18 | 100.00 | 112.18 | 118.15 | 106.78 | 3.55 |
| Alanine transaminase (U/L) | 102.38 | 97.58 | 97.60 | 115.88 | 112.70 | 134.95 | 1.82 |
| Total Protein (g/dl) | 4.78 | 4.65 | 4.70 | 4.55 | 4.20 | 4.03 | 0.46 |
| Albumin (g/dl) | 2.95 | 2.90 | 3.10 | 2.95 | 2.48 | 2.58 | 0.27 |
| Globulin (g/dl) | 1.85 | 1.70 | 1.60 | 1.58 | 1.75 | 1.45 | 0.25 |
| Alkaline phosphatase (U/L) | 85.75 | 64.50 | 80.75 | 73.50 | 72.00 | 87.00 | 5.53 |
| Creatinine (mg/dl) | $1.50^b$ | $4.63^a$ | $2.10^{ab}$ | $2.63^{ab}$ | $2.53^{ab}$ | $1.60^b$ | 2.64 |

**Alphabetic superscript shows that Mean within the same row were significantly different (P<0.05)**

### 3.3 Effects of PNLM inclusion on the intestinal microbial population of pullets

The intestinal bacterial count of pullets fed diets with varying amounts of *Phyllanthus niruri* leaf meal (PNLM) as a supplement throughout the growing period is displayed in Table 3. The findings revealed that, except T1, none of the treatment groups had any Bacillus species in the caecum. There was no significant difference ($p<0.05$) in the caecum total bacteria count. The highest numerical value was observed in diet containing 0.5% PNLM (T6) ($2.0\ x10^6$ cfu/g) when compared to T1 control ($1.20x10^6$cfu/g) but almost closer to T2 treated with antibiotics ($1.70\ x10^6$cfu/g). However, the results showed a numerical increase ($p>0.05$) in the total bacteria count ($1.6\ x10^6$cfu/g, $1.8\ x10^6$cfu/g, $2.00\ x10^6$cfu/g) as the levels of PNLM increased (0.3%, 0.4%, 0.5g).

**Table 5: Intestinal microbial population of pullets fed PNLM**

| Treatment | TBC Caecum ($10^6$cfu/g) | *Staphyloccocus aureus* | *Bacillus Spp* | *Escherichia Coli* | *Streptococcus faecalis* | *Pseudomonas Spp* |
|---|---|---|---|---|---|---|
| 1 | 1.2 | + | + | - | + | - |
| 2 | 1.7 | + | - | + | - | + |
| 3 | 1.6 | + | - | + | - | + |
| 4 | 1.6 | - | - | + | + | - |
| 5 | 1.8 | + | - | + | _ | + |
| 6 | 2.0 | - | - | + | _ | + |

**TBC: Total Bacteria Count, Spp: Specie, P-value: 0.23**

### 3.4. Discussion

Major indicators for assessing circulatory erythrocytes and important in the diagnosis of anaemia include packed cell volume (PCV), and haemoglobin, (Peters, Gunn, Imumorin, Agaviezor, & Ikeobi). According to Chineke, Ologun, and Ikeobi (2006), they also function as helpful indicators of a mammal's ability to manufacture red blood cells in the bone marrow. The whole condition of chickens is influenced by their erythrocyte (RBC) count (Mitruka, Rawnsley, & Vadehia, 1977). PCV, haemoglobin, and red blood cell counts in the birds increased, suggesting that the blood's ability to carry oxygen was improved. According to Chineke et al. (2006), there has been a proposition that a high PCV reading, or polycythemia, signifies either a decrease in circulating plasma volume or an increase in red blood cell count. This could potentially be attributed to a physiological adjustment to a high pathological response to a chronic respiratory or circulatory illness. Most birds show signs of dehydration when their PCV value is higher than 56% (Pendl, 2001). The results obtained in this study, however, were within the reference ranges for layers of 25–45% (Al-Nedawi, 2018).

White blood cells levels in birds have phagocytic activity and are utilized as sensitive biomarkers essential to immune function as well as a sign of stress response. Low white blood cell counts put animals at high risk of contracting diseases. In contrast, moderate counts allow for the production of antibodies during phagocytosis, increase disease resistance, and improve adaptation to both local environmental conditions and disease-prevalent conditions (Soetan, Akinrinde,& Ajibade, 2013). The result of WBC in this study points to the treatment groups had a relatively lower WBC than those reported by Pewan et al. (2019), though, within the range reported by (Pewan et al., 2019), this may mean that the birds were stressed hence the significant ($p<0.05$) reduction in WBC. This tends to confirm the report of Talebi, Asri-Rezaei, Rozeh-Chai, and Sahraei, (2005) that nutrition affects the blood profiles of birds and this implies that up to 2.5% inclusion of leaf meal had a positive effect on the relative quantity of blood cell as well as the total volume of blood. Lymphocytes are mediator cells of the adaptive immune response and play a crucial part in the body's defence against infection. The maturation process takes place in the bursa of fabricius and thymus in birds and mammals. The observed values are consistent with normal ranges reported for healthy birds (Lee et al., 2011).

It should be mentioned that the liver is prone to different degrees of chemical and biological damage because it is the hub of several metabolic, digestive, and productive processes. Serum levels of particular liver-derived enzymes indicate such damage. Liver function and damage can be bio-indicated by the blood levels of the enzymes AST, ALP, and ALT. (Yildirim, Yalchinkaya, Kanbur, & Oruc, 2011). The body's reaction to stress is linked to elevated levels of these enzymes, which might cause damage to the liver or muscles (Lumeij, 2008). There were notable variations ($P<0.05$) in the value of metabolites creatinine among the treatment groups. This shows that feed supplements with P. *ninuri* had a substantial ($P<0.05$) impact on the levels of creatinine. The cationic amino acid-derived nephrotoxic effect of accumulated antibiotics can cause damage to the kidney's proximal tubular epithelial cells by binding phosphoinositides. Consequently, the filter process is inhibited and levels of creatinine rise (Pazhayattil & Shirali, 2014). The high levels of creatinine seen in the control group indicate that antibiotics tend to impair kidney function. The reason for this is that antibiotics not only eradicate all bacteria but also combat infectious ones, which negatively impact poultry birds' health, particularly the liver and kidneys (Śliżewska, Cukrowska, Smulikowska, and Cielecka-Kuszyk, 2019). Probiotics, prebiotics, enzymes, organic acids, immunostimulants, bacteriocin, bacteriophage, carotenoids, phytoncides, nanoparticles, and essential oils are examples of frequent feed additives that are advised to be used instead. According to Mehdi et al. (2018) and Habibu, Dzenda, Ayo, Yaqub, and Kawu, (2018), the alternative feed additives are safer because they enhance chicken performance without interfering with kidney function or leaving behind residue in the meat, and liver, kidneys, skin, and fat. This suggests that using PNLM as a supplement for antibiotics in layers chicken production is both safe and healthy. The findings showed that the beneficial bacteria (*Pseudomonas spp*. and *Escherichia coli*) in T6 were enhanced by the addition of 0.5%PNLM. The presence of *Bacillus spp* in T2-T6 at the caecum and in T1 and T5 respectively, was not detected by PNLM supplementation. The microbiome contains useful, innocuous bacteria called *Bacillus* species. By growing the good bacteria and pushing out the bad bacteria, phytobiotics have demonstrated beneficial effects. According to Yildirim et al. (2011), the colonization of the gastrointestinal tract by helpful bacteria such as *Bacillus*, *Escherichia coli*, and *Pseudomonas spp*. inhibits the growth and presence of potentially harmful species. The findings imply that the phytobiotics found in *Phyllanthus niruri* leaf meal work in a bacteriostatic manner, which lessens the pathogenic bacteria's pathogenicity and aids in the colonization of the GIT by good bacteria. The findings of Murugesan, Syed, Haldar, and Pender (2015), who found that a phytogenic feed additive boosted the gut microbiota with beneficial bacteria and reduced the coliform population in the cecum are consistent with this work. Because of their quick colonization, rapid growth, and ability to cause acidity in the GIT, *Bacillus* species have the potential to selectively prevent pathogens from sticking once they become established (Alagbe et al., 2023).

### 4.0. Conclusion

This study's findings indicate that PNLM in layer chicken diet does not negatively impact the birds, suggesting that the product from such birds is safe for human consumption without risk of health problems. Furthermore, PNLM helps in the proliferation of beneficial microbes in the caecum. Therefore to enhance the health

status of layer chickens, the addition of *Phyllanthus niruri* leaf meal is recommended because it has no negative effect on the blood profile and enhances the proliferation of beneficial microbes in the caecum.

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