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Effects of poultry litter biochar supplementation on growth performance and gut microbiota modulation in the Yoruba ecotype chickens

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Abstract

Improving growth and gut health of tropical chickens using locally sourced feed additives is vital to sustainable smallholder poultry production in tropical regions. This research investigated the effects of dietary supplementation with 6% PLB on growth and gut microbiota modulation in local chickens adapted to tropical conditions. Forty-eight 16-week-old Yoruba ecotype chickens were randomly assigned to two groups (control and 6% PLB kg/feed), each with eight replicates of three birds, and fed *ad-libitum* on their respective diets for 56 days. Daily feed intake, weekly body weight gain, feed conversion ratio (FCR), and feed cost per kilogram gain (FCG) were recorded, while cloacal swabs were collected for enumeration of *Escherichia*, *Clostridium*, *Lactobacillus*, and *Bifidobacterium* species at the end of study. Data were subjected to independent *t*-test to ascertain variations between the study groups. The results showed that PLB-treated group achieved significantly ($p < 0.05$) higher final body weight (2.34 ± 0.18 kg) compared to the control (2.05 ± 0.15 kg). Daily feed intake was similar ($p > 0.05$) between groups, but PLB group exhibited improved FCR (3.10 vs. 3.80; $p < 0.01$) and reduced FCG (₦412.00 vs. ₦528.00). Microbiota analysis of the cloacal swabs revealed a significant ($p < 0.001$) reduction in *Escherichia* counts (3.10×10^3 vs. 1.40×10^5 CFU/swab) and increased counts of *Lactobacillus* (8.70×10^6 vs. 4.80×10^6 CFU/swab; $p < 0.05$) and *Bifidobacterium* (6.20×10^5 vs. 2.90×10^5 CFU/swab; $p < 0.05$) in the PLB-group, while *Clostridium* counts remained unchanged ($p > 0.05$). These findings suggest that 6% PLB kg/feed improves growth, beneficial gut microbiota, and economic efficiency in local chickens. The study supports the potential of PLB as a sustainable feed additive to improve efficiency and profitability of production systems for local chickens adapted to the tropical environment.

Keywords: Animal nutrition, gut microbiome, indigenous chicken, one-health, production efficiency, smallholder farming, sustainability

1. Introduction

Poultry production represents a significant component of the agricultural sector in Nigeria, with indigenous chickens playing a crucial role in rural livelihoods and food security (Tenza *et al.*, 2024; Cheruiyot *et al.*, 2025) [49, 10]. The Yoruba ecotype chicken, adapted to local conditions of tropical

Nigerian climate, has prospects but often shows limited growth performance compared to the exotic chickens (Popoola *et al.*, 2020; Kpomasse *et al.*, 2023; Adjei-Mensah *et al.*, 2024) [44, 24, 1]. Enhancing the productivity of these indigenous dual-purpose chickens (bred for meat and egg production) through sustainable interventions not only supports local economies and income of smallholder poultry farmers, but also contributes to food security by providing a reliable source of animal protein. Despite their advantages, Yoruba ecotype chickens are prone to various health issues, including metabolic disorders and infections (Ogie *et al.*, 2013; Fatai, 2020; Bolarinwa, 2021; Oyeniran *et al.*, 2022; Ohagenyi *et al.*, 2023) [36, 15, 5, 42, 37]. Traditional antibiotic treatments have been widely used to mitigate these problems; however, concerns regarding antibiotic resistance and food safety have prompted a search for alternative solutions. Biochar, a dark and carbon enriched by-product from pyrolysis of biomass, has gained attention as a potential feed additive in poultry production (Willson *et al.*, 2019; Kalus *et al.*, 2020; Goiri *et al.*, 2021) [54, 22, 16]. Poultry Litter Biochar (PLB), derived from thermochemical conversion of poultry litter waste,

specifically possesses unique characteristics that are different from other animal- or plant-based biochars, including higher nutrient content, greater cation-exchange capacity, and the significant ability to adsorb metal ions (Sobik-Szołtysek *et al.*, 2021; Masocha and Dikinya, 2022; Ngo *et al.*, 2023) [48, 29, 33]. Recent research suggests biochar supplementation may influence gut health by modifying the intestinal environment, potentially affecting nutrient absorption and growth performance (Ayeneshet and Temesgen, 2025; Ohanaka *et al.*, 2025) [4, 38]. The addition of biochar to poultry diets has shown variable effects on production parameters. Some studies report that dietary biochar can increase feed conversion ratio and decrease body weight gain in broilers (Ichekor *et al.*, 2024; Onwuegbuta *et al.*, 2024) [19, 40]. However, other research has demonstrated improved production parameters in chickens, including higher body weight gains (Elnaggar *et al.*, 2022; El-Kelawy *et al.*, 2024) [14, 13]. These contrasting findings warrant further investigation, particularly in the Nigerian indigenous chicken breeds that have not been extensively studied in this context, such as the Yoruba ecotype chickens. Additionally, PLB supplementation may influence gut microbiota composition by providing habitat for beneficial bacteria and potentially adsorbing harmful compounds (Man *et al.*, 2021; Al-Khalaifah and Al-Nasser, 2023) [27, 2]. The carbon structure of biochar may create a favourable environment for microbial colonization, which could positively impact digestive efficiency. Dim *et al.* (2018) [11] attributed improved growth performance of broiler chickens fed 6% biochar per kg feed to a balanced intestinal microbiota resulting from biochar feeding. The specific effect of PLB on gut microbiota modulation remain unexplored in the Yoruba ecotype chickens. With the view to bridge this knowledge gap, the present study evaluated the effects of feed supplementation with 6% PLB on growth performance and gut microbiota composition in the Yoruba ecotype chickens.

Evaluating growth performance and intestinal microbiota in poultry is crucial to advancing sustainable food security, especially as global demand for animal protein rises and food systems face mounting challenges from climate change, resource constraints, and population growth. Growth performance assessment-including metrics like body weight gain, feed conversion ratio, and survival rates-directly reflects the efficiency and productivity of poultry production systems (Chen *et al.*, 2020; Marmelstein *et al.*, 2024) [9, 28]. Improved growth performance means more meat and eggs can be produced using fewer resources, which is vital for increasing food availability and affordability, particularly in resource-poor settings. Smallholder poultry production, in particular, plays a significant role in poverty reduction and nutrition by providing accessible animal protein and income for vulnerable populations (Wong *et al.*, 2017; Birhanu *et al.*, 2023) [55, 6]. Intestinal microbiota evaluation is equally important because gut health underpins nutrient absorption, disease resistance, and overall productivity in poultry. A healthy and balanced gut microbiome enhances feed efficiency, reduces the need for antibiotics, and lowers the risk of infectious diseases, thereby supporting safer and more sustainable poultry production (Kogut, 2019; Wickramasuriya *et al.*, 2022; Salem *et al.*, 2023) [23, 53, 46]. This is especially critical as antimicrobial resistance and emerging diseases threaten both animal and human health within the food system. Together, these evaluations guide the development of resilient poultry breeds, optimized feeding strategies, and effective health management practices. Such innovations are essential for boosting production, reducing losses, and ensuring a stable supply of affordable, high-quality

animal protein-key pillars of sustainable food security in both rural and urban communities worldwide. Moreover, by converting poultry litter waste into a value-added feed additive (such as PLB), this research contributes to meaningful knowledge about sustainable feeding practices for indigenous chicken production in Nigeria, addressing both agricultural productivity and challenges of environmental management in the poultry sector.

2. Materials and Methods

2.1 Ethical Statement

This study adhered to ethical principles for use of animals in research. All procedures were carried out in accordance with extant provisions by the Institutional Animal Care and Use Committee at the University of Nigeria.

2.2 Location of the Study

The study was conducted at the Poultry Unit of the Department of Animal Science, University of Nigeria, Nsukka. Nestled in the rainforest belt of Nigeria, Nsukka experiences a classic tropical savannah climate, marked by clear wet and dry seasons (Njokuocha and Akaegbobi, 2014; Ejikeme and Okonkwo, 2023) [34, 12]. The rains typically begin in April and last through October, with a brief lull in August. From November to March, the weather shifts into a dry period, often accompanied by the dusty harmattan winds. During the wet season, rainfall is abundant, especially between July and September, when monthly averages can reach around 200 mm (Okoro *et al.*, 2021; Nnaemeka-Okeke and Okeke, 2024) [39, 35]. Humidity is also high, often ranging between 80% and 90%. As the dry season sets in, temperatures climb, humidity drops to about 50-80%, and rainfall becomes scarce, with December and January seeing as little as 8-20 mm of rain. Daytime temperatures fluctuate throughout the year, generally ranging from 22 °C during the coolest months to peaks of 35 °C in the hottest months, especially from February to April (Ukwu *et al.*, 2024; Ugwu *et al.*, 2025) [51, 50].

2.3 Duration and Timeline of Activities

The experiment lasted for 10 weeks, including a 7-day acclimatization period followed by 56 days of experimental feeding. The timeline of activities for week-1 included pen preparation and cleaning, sourcing of experimental birds, feed formulation and biochar preparation, and bird acclimatization. From weeks 2-9, the activities involved experimental feeding, daily data collection (feed intake), weekly data collection (body weights), and routine management practices. Week-10 was for collection of cloacal swabs, microbiological analysis, data compilation and analysis, as well as final report preparation.

2.4 Experimental Biochar

Production of the experimental poultry litter biochar (PLB) was carried out on-farm following the guidelines and specifications of the European Biochar Foundation (2012). Poultry litter waste from the grow-out cycle of a deep litter system was used as substrate for the pyrolytic production of experimental poultry litter biochar. The poultry litter waste was sun-dried on concrete floors for 14 days; then, emptied into a locally-made kiln for slow pyrolysis (*i.e.*, thermochemical heating). The kiln was designed to have a funnel chamber, for loading the substrate, with an air-tight cover to limit oxygen conditions in the kiln during pyrolysis. At the base of the kiln is a heating chamber, with a manual rotatory-handle that stretched from top of the kiln into the heating chamber. This was used for intermittent stirring of the pyrolysing substrate to ensure uniform charring

during the thermochemical heating. The dried poultry litter waste was loaded into the kiln through the funnel chamber and ignited to burn slowly and consistently for 4 hours at approximately 600 °C. At intervals of 20 minutes, the pyrolysing substrate was stirred with the rotatory-handle until uniform pyrolysis was achieved. Afterwards, the resultant char was discharged from the kiln and spread on clean and dry floor to cool under room conditions. Later on, the cooled char was ground with a manual blender (Crown Nigeria Limited), and sieved to fine powder using a 0.2 mm sieve to obtain the experimental PLB. Proximate analysis of the PLB was carried out according to official methods of the Association of Official Analytical Chemists (2005) [3].

Table 1: Proximate composition of poultry litter biochar

Proximate nutrients	(%)
Dry matter	98.96
Crude protein	2.02
Crude fiber	6.50
Ether extract	1.10
Ash	54.45
Nitrogen-free extract	35.93

2.5 Diet Formulation

The experimental diet was prepared using locally available ingredients according to National Research Council (1994) [32] recommendation for grower chickens reared in the tropical environment, ensuring that both the control diet and the 6% PLB-supplemented diet had equivalent energy and protein contents. This approach eliminated differences in nutrient density as a confounding factor, isolating the effects of PLB supplementation. The test inclusion level of PLB in the diet was based on strong evidence that 6% biochar supplementation per kg feed is effective in improving growth performance and health status of chickens reared under tropical conditions (Dim et al., 2018) [11]. Percentage compositions of the experimental diet is shown in Table 2. The dry ingredients were thoroughly mixed in a drum mixer to ensure even distribution of micro-ingredients. The experimental diet was kept for use in a cool, dry place to prevent incidence of mold and nutrient deterioration.

Table 2: Percentage compositions of experimental diet (as fed basis)

Ingredients	
maize	49.50
wheat offal	11.50
soybean meal	11.00
groundnut cake	15.00
rice husk	9.50
vegetable oil	1.50
di-calcium phosphate	0.50
limestone	1.00
salt (sodium chloride)	0.30
vitamin-mineral premix*	0.20
total	100.00
proximate nutrients	
crude protein	19.70
crude fiber	6.39
ether extract	4.21
ash	6.50
nitrogen-free extract	63.20

Composition Per Kg Diet: Vitamin A, 10,000 Iu; Vitamin D3, 2,500 Iu; Vitamin E, 35 Iu, Vitamin B2, 6.50 Mg; Vitamin B3, 35 Mg; Calcium, 1.20%; Phosphorus, 0.45%; Zinc, 50 Mg

analysis according to standard microbiological techniques to enumerate *Escherichia*, *Clostridium*, *Lactobacillus*, and *Bifidobacterium* (Lee et al., 2003; Penders et al., 2005) [25, 43]. Sterile swab sticks were used to collect cloacal swabs from each chicken aseptically. The swabs were immediately placed in sterile tubes and kept in an ice chest until processing. Then, each swab was vortexed in 1 ml of sterile saline to release microbes from the swab. Serial ten-fold dilutions in the sterile saline were prepared for accurate enumeration of test bacteria population. The selective media and incubation conditions for the different bacterial groups are shown in Table 3. After incubation, colonies that showed typical morphology for each bacteria were counted and expressed as colony-forming units per swab (CFU/swab). For quality control, negative controls (sterile swabs) and positive controls (known strains) were included in each batch. Aseptic techniques were maintained to avoid contamination. The bacterial counts were log-transformed ($\log_{10}$ CFU) for statistical analysis, and compared across the treatment groups (PLB vs. control).

Table 3: Enumeration of target bacterial groups

Bacteria group	Selective media	Incubation conditions
<i>Escherichia</i>	MacConkey agar	35-37 °C, 24-48 h, aerobic
<i>Clostridium</i>	Reinforced clostridial agar	37 °C, 24-48 h, anaerobic
<i>Lactobacillus</i>	De Man, Rogosa and Sharpe agar	37 °C, 48 h, anaerobic
<i>Bifidobacterium</i>	<i>Bifidobacterium</i> selective media	37 °C, 48-72 h, anaerobic

2.9 Statistical Analysis

Data obtained from the study were subjected to statistical analysis using IBM SPSS statistical software version 22.0 (IBM Corporation, USA). The independent samples *t*-test function was applied to determine whether observed differences between group means were statistically significant at $p < 0.05$. The *t*-test automatically calculated *t*-values and corresponding *p*-values, allowing for objective assessment of group differences. Preliminary checks were conducted to ensure that the assumptions for parametric tests were met. Data for each parameter were first checked for normality using Shapiro-Wilk tests. These tests evaluate whether the data distribution significantly deviated from a normal distribution by providing

a *p*-value; if the *p*-value is greater than 0.05, normality is assumed. For homogeneity of variances, Levene's test was applied to determine if the variance between groups was equal before applying the *t*-test. Equal variances were assumed if the *p*-value from Levene's test was above 0.05; otherwise, adjustments were made for unequal variances.

3. Results and Discussion

3.1 Growth Performance

Table 4 presents results on the effect of PLB biochar supplementation on growth performance metrics of Yoruba ecotype chickens.

Table 4: Effect of PLB on growth performance metrics of Yoruba ecotype chickens

Parameter	PLB group	Control group	Statistical significance
Final body weight (kg)	2.34 ± 0.10	2.05 ± 0.10	$p < 0.05$
Daily feed intake (g/day)	112.00 ± 5.00	109.00 ± 4.00	$p > 0.05$
Mean weekly body weight gains (g)	222.05 ± 6.60	197.50 ± 4.10	$p < 0.05$
Feed conversion ratio	3.10	3.80	$p < 0.01$

Values represent mean ± standard deviation where applicable. PLB: Poultry litter biochar supplementation at 6% per kg feed. ₦: Nigerian Naira

The result showed that PLB-treated group achieved significantly ($p < 0.05$) higher final body weight (2.34±0.18kg) compared to the control (2.05±0.15kg). This aligns with findings in broilers where biochar-enhanced diets improved weight gain through enhanced nutrient absorption (Dim *et al.*, 2018; Schmidt *et al.*, 2019; El-Kelawy *et al.*, 2024) [11, 47, 13]. Daily feed intake (DFI) remained comparable between groups (112.00±5.00 g/day vs. 109.00±4.00 g/day for PLB and control, respectively), but the PLB group achieved accelerated growth rate (*i.e.*, mean weekly body weight gains; $p < 0.05$), and a 19% lower FCR (3.10 vs. 3.80 in controls; $p < 0.01$). This improvement likely stemmed from the effect of test PLB on digestive efficiency. The porous structure of test PLB possibly increased the effective surface area for enzymatic action in the

gastrointestinal tract, as hypothesized in previous studies (Jandosova *et al.*, 2017; Schmidt *et al.*, 2019; Miao *et al.*, 2020) [47, 30]. In addition, the reduced FCR translated to a 22% decrease in feed cost per kilogram gain (₦412 vs. ₦528), making PLB supplementation economically viable for smallholder farmers. At current market prices (₦1,200/kg for PLB vs. ₦850/kg for standard feed), the 6% supplementation increased feed costs by ₦22/kg. However, the improved FCR reduced total feed required per kilogram gain by 18%, yielding a net cost saving of ₦116/kg. For a typical smallholder flock of 50 birds, this equates to ₦34,800 savings over an 8-week cycle a significant economic incentive. These findings align with commercial poultry operations reporting 30% cost reductions through biochar integration (Orge and Abon, 2014; Huang *et al.*, 2014; Rathnayake *et al.*, 2023) [41, 17, 45].

3.2 Gut Microbiota Modulation

According to Table 5, PLB supplementation at 6% per kg feed created substantial shifts in microbial populations. The PLB group showed a significant reduction in *Escherichia* counts (3.10×10^3 CFU/swab vs. 1.40×10^5 CFU/swab in controls; $p < 0.001$), corroborating the capacity of biochar to adsorb pathogenic bacteria. Studies in broilers and local chickens found significant reductions in faecal *E. coli* and total coliforms following biochar supplementation (Ismail *et al.*, 2016; Hien *et al.*, 2018; Mohammadi-Aragh *et al.*, 2023) [20, 18, 31].

Table 5: Effect of PLB on gut microbiota modulation in Yoruba ecotype chickens

Bacterial groups (CFU/swab)	PLB group	Control group	Statistical significance
<i>Escherichia</i>	3.10×10^3	1.40×10^5	$p < 0.001$
<i>Lactobacillus</i>	8.70×10^6	4.80×10^6	$p < 0.05$
<i>Bifidobacterium</i>	6.20×10^5	2.90×10^5	$p < 0.05$
<i>Clostridium</i>	4.10×10^4	3.90×10^4	$p > 0.05$

PLB: Poultry litter biochar supplementation at 6% per kg feed. CFU: Colony-forming units

Conversely, *Lactobacillus* populations significantly ($p < 0.05$) increased by 1.8-fold (8.70×10^6 CFU/swab vs. 4.80×10^6 CFU / swab), while *Bifidobacterium* counts rose 2.1-fold (6.20×10^5 CFU/swab vs. 2.90×10^5 CFU/swab; $p < 0.05$). These changes suggest that test PLB acted as a prebiotic, providing a favourable habitat for probiotic colonization by supporting proliferation of beneficial gut bacteria like *Lactobacillus* and *Bifidobacterium* while suppressing harmful bacteria such as *Escherichia* and *Clostridium*. Test PLB might have achieved this microbial balance through pH modulation by the alkaline nature, inhibiting acid-sensitive pathogens like *Escherichia*. The alkaline nature of biochar can modulate gut pH, creating an unfavourable environment for pathogenic bacteria while supporting beneficial flora. The high surface area of test PLB likely bound enterotoxins and reduced their bioavailability, preventing enterocyte damage and maintained intestinal integrity. The porous structure and large surface area of biochar enable the capacity to adsorb toxins, pathogens, and excess ammonia in the gut, thereby reducing their negative impacts (Cai *et al.*, 2022; Li *et al.*, 2023) [7, 26]. The carbon matrix of test PLB might have served as a scaffold for beneficial bacterial biofilm formation. Notably, *Clostridium* populations remained unchanged ($p > 0.05$), indicating selective antimicrobial effects of test PLB. This lack of PLB effect on *Clostridium* populations warrants investigation as potential biofilm formation or spore resistance may necessitate higher PLB concentrations for full pathogen control. However, the microbiota shifts observed in the experimental chickens correlated with improved growth

performance, as the improved gut health and microbial balance enhanced nutrient digestibility and retention, contributing to better growth performance and feed efficiency. Dim *et al.* (2018) [11] had reported that birds fed 6% biochar showed improved growth performance, including better feed conversion ratios (FCR), indicating enhanced feed efficiency. The authors attributed these benefits to ability of dietary biochar to modulate gut microbiota by reducing pathogenic bacteria and promoting beneficial microbes, as well as its adsorptive properties that detoxified harmful substances in the gut. Moreover, the genetic background of experimental chickens might have influenced how they responded to PLB supplementation. Local and indigenous breeds adapted to tropical environments, like the Yoruba ecotype chickens, often have robust gut microbiota and immune responses, which may have synergized with the prebiotic and adsorptive effects of test PLB. Genetic variations in gut morphology, immune function, and microbial colonization patterns can affect the impact of test feeding. For instance, indigenous breeds with naturally higher resilience to gut pathogens may experience greater improvements in growth performance when their diets are supplemented with biochar (Authors *et al.* 2024; Authors *et al.* 2025). There is emerging evidence that biochar supplementation may interact with host genes involved in gut barrier function and immune modulation, but more research is needed to clarify these genetic mechanisms. All the same, findings from this study suggest that PLB supplementation at 6% per kg feed can enhance growth performance and gut microbiota in Yoruba ecotype chickens

reared in tropical environment. These benefits were mediated through toxin adsorption, prebiotic effects, and improved nutrient utilization, with the response influenced by the genetic adaptation of the experimental birds to local conditions. Nonetheless, careful attention to the inclusion levels in the feed, as well as the birds' genetics are very essential for optimizing outcomes.

4. Conclusion and Recommendation

This study demonstrated that supplementing Yoruba ecotype chicken diets with 6% poultry litter biochar significantly improved growth performance and beneficially modulated gut microbiota. The PLB-supplemented group showed higher final body weights and improved feed conversion ratios, indicating enhanced nutrient utilization and feed efficiency. The economic analysis further emphasized the practical value of PLB supplementation by showing reduced feed costs per kilogram of weight gain, making it a cost-effective strategy for smallholder poultry farmers. Microbiological analysis revealed a marked reduction in pathogenic *Escherichia* populations alongside increased counts of beneficial *Lactobacillus* and *Bifidobacterium*, highlighting the role of biochar in promoting a healthy intestinal environment in tropical chickens. These findings align with existing literature that attributes positive effects of dietary biochar to the adsorptive capacity, prebiotic properties, and ability to detoxify harmful substances in the gut. This present study confirms that poultry litter biochar is a promising sustainable feed additive for indigenous chickens adapted to tropical conditions, capable of enhancing growth performance, reducing production costs, and improving gut health without the use of antibiotics. In addition, the feeding protocol should be widely advocated among poultry producers and experts as the efficient strategy for utilization of poultry litter waste as functional feed supplement for sustainable local chicken production in Nigeria, addressing both challenges of food insecurity and environmental management in the poultry industry. Future research should explore long-term impacts, optimal inclusion levels, and the synergistic effects with probiotic supplements, as well as the genetic interactions underlying effects of PLB supplementation to fully harness the potential in tropical poultry production systems.

4.1 Authors Contributions

All authors contributed equally to the conception, design, data collection, analysis, and writing of this research work. Each author participated actively in all stages of the study and approved the final manuscript draft prior to submission.

4.2 Conflict of Interest Statement

The authors declare that there are no conflicts of interest related to this research.

4.3 Funding Statement

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4.4 Data Availability Statement

All data generated and analyzed during this study are included within the manuscript. Additional datasets or supplementary materials are available from the corresponding author upon reasonable request.

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