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Research Article
Growth Response, Serum Biochemistry and Organ Histopathology of Broilers Fed Diets supplemented with Graded levels of *Petiveria alliacea* Root Meal

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Abstract

Background and Objective: This study was conducted to assess the growth response, serum biochemistry and organ histopathology of broiler chickens. **Materials and Methods:** A total of 180 unsexed day old broiler chickens were used for this study. Broiler chickens fed diets supplemented with graded levels of *Petiveria alliacea* root meal (PRM) at 0, 500, 1000, 1500, 2000 and 2500 g/100 kg of feed in 8 weeks feeding trial. Data were collected on feed intake and weekly weight gain. Blood samples were collected from the animals through the wing web vein for serum biochemistry while samples of visceral organs and small intestine were collected from the animals after they were stunned and sacrificed. **Results:** Results revealed no significant difference ($p>0.05$) in all the growth response parameters examined. While Aspartate amino transferase were significantly ($p<0.05$) lower in T6, glucose and cholesterol of the experimental birds were significantly higher in T6 ($p<0.05$). Relative organ weight of various visceral organs examined apart lung, kidney and spleen were also significantly influenced by the dietary treatments. The results of histopathological examination revealed damages done by dietary treatments. Necrosis of the villi and tubular epithelium were observed in intestine and kidney in all the treatment except the control. The liver of bird fed PRM showed signs of hepatocellular necrosis and increased mononuclear/inflammatory cells in the hepatocyte, which increased as the inclusion of PRM increased. **Conclusion:** *Petiveria alliacea* root meal as a phytobiotics improved the growth performance of broiler chicken, does not have any negative effects on the serum biochemistry but may induce necrosis of the villi and hepatocytes if fed above 1500 g/100 kg of feed for a longer period of time.

Key word: Antibiotic, broiler feed, growth performance, histopathology, phytobiotics, root meal, serum biochemistry

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Antibiotics have been used as antimicrobial growth promoters (AGPs) in animal feeds worldwide for many years to improve food safety, animal health and reduce or remove certain exogenous pathogens[1]. However, in order to avoid the possible risk of developing resistant pathogens, as well as to meet the public pressure for antibiotic-free animal products, the use of antibiotic in poultry diets was totally banned in the European Community in January 2006. However, apart from preventing the potential hazard, the absence of AGPs in the diet has resulted in health problems in poultry, including significant increase in infection[2]. Hofacre[3] reported that the incidence of necrotic enteritis or Clostridial infection has engendered considerable complications related to animal welfare and resulted in severe economic losses in poultry industries. In order to reduce the disadvantages of sub- and clinical infections, the poultry and feed industries needs to find alternatives to AGPs. These alternatives should be safe for both animal and humans, environmentally friendly, applicable in the diets and address organic livestock issue[4]. Various alternatives of green additives have been studied in order to maximize the growth performance and product quality of poultry without antibiotics. Phytobiotics are plant derived compounds that can be incorporated into diets in order to enhance performance and well being of the animals. Their effects have been proven to improve feed palatability and quality, growth, gut function and nutrient digestibility, gut microflora[5]. It has been reported that herbs, spices and their extracts can stimulates appetite and endogenous secretion of digestive enzymes[6]. Supplementation of 200 ppm essential oil extract from oregano with combination of cinnamon and pepper improved the apparent faecal digestibility of dry matter and crude protein in broiler finisher diet[7]. They also concluded that 5000 ppm labiates extract from sage, thyme and rosemary gave same results in broilers finisher diets. In addition, Williams and Losa[8] found that feeding cinnamon to broilers significantly reduced the concentration of pathogenic microorganisms in the ileum, caecum and colon which was accompanied by an increased weight gain of birds.

Petiveria aliacea is a species of flowering plant in the pokeweed family, phytolaccaceae[9] that is native to Florida and the lower Rio Grande valley of Texas in the United States, Mexico and Central America. The roots and leaves have a strong acid, garlic- like odour-which taints the milk and meats of animals that graze on it.

Petiveria alliacea, has been found to contain a large number of biologically active chemicals including benzaldehyde, benzoic acid. The plant roots have been shown to contain cystein sulfoxide derivatives that are analogous to but different from those found in such plants as garlic and onion. These compounds serve as the precursors of several thiosulfinate such as S-(2 hydroxyethyl 1) 2-hydroxyethane) thiosulfinate, S-(2 hydroxyethyl) phenyl methane thiosulfinate, S-(benzyl 2-hydroxyethane) thiosulfinate and S-(benzyl phenyl methane thiosulfinate (petivenin). And they have been found to exhibit antimicrobial activity[10]. The role of *Petiveria alliacea* as a phytobiotic or growth promoter in poultry species has not been fully explored.

Therefore, the present study was conducted to evaluate the effect of graded levels of *Petiveria alliacea* root meal on broiler's growth response, serum biochemistry and organs Histopathological changes.

MATERIALS AND METHODS

Experimental plan: *Petiveria alliacea* was obtained from different locations within and outside the premises of Federal College of Animal Health and Production Technology, Ibadan, Nigeria. The roots were cut from the stems, they were then cut into pieces, washed and air dried until a moisture content of about 10% was obtained. The air dry root were then milled into *Petiveria alliacea* root meal (PRM) and stored in an airtight container.

Six experimental diets comprising *Petiveria alliacea* root meal (PRM) as a supplement at 0, 500, 1000, 1500, 2000 and 2500 g/100 kg feed were formulated and were designated as T1 (control), T2, T3, T4, T5 and T6, respectively. The diets were prepared with the addition of other ingredients for both starter and finishers phase (Table 1). A total of 180 unsexed day old broiler chicks of Cobb strain were used in this study.

Table 1: Gross composition of the experimental diets (g/100 g DM) starter and finisher phase

Parameters (%)	Starter	Finisher
Maize	50.50	55.00
Groundnut cake	5.00	3.80
Soybean meal	30.00	30.00
Fish meal	2.00	1.00
Wheat offal	7.30	5.00
Bone meal	2.50	2.50
Salt	0.25	0.25
Premix	0.25	0.25
Lysine	0.10	0.10
Methionine	0.10	0.10
Limestone	2.00	2.00
Total	100.00	100.00
Crude protein	22.67	20.13
Crude fibre	5.89	6.38
Total ash	7.85	7.68
Ether extract	3.75	3.74
Nitrogen free extract	56.66	51.58

The birds were brooded together for seven days after which they were randomly distributed into six dietary treatments of 30 birds each. They were further divided into three replicates of 10 birds per replicate. Birds in T1 were medicated with 20% enrofloxacin solution at 1 mL per 4 litres of water for a duration of 5 days at starter and another five days at finisher phase. Birds in other treatments were not medicated with antibiotics throughout the period of the experiment. However, vaccination procedures were strictly adhered to. The experimental feed and water were provided *ad libitum* two times daily at 8.00 and 16.00 h and the experiment lasted for 7 weeks.

Growth response: Known quantity of feed was supplied to the birds and the left over removed and weighed to determine the actual feed consumed on daily basis. The daily feed consumption was added together over a period of 7 days to obtain the feed consumption per week. The body weights were taken on weekly basis. The difference between mean weights for two successive weeks was taken in order to obtain the average weight gain of birds per week.

Feed conversion ratio was calculated as a ratio of feed consumption and body weight gain:

$$\text{Feed conversion ratio} = \frac{\text{Feed intake}}{\text{Weight gain}}$$

Blood collection and evaluation: At the end of the feeding trial, two birds per replicate were randomly selected, bled through the wing web veins and blood samples collected into a well labeled sterile bottle without anticoagulant ethylene diamine tetra acetic acid (EDTA), immediately covered and centrifuged, Serum separated out, decanted, deep-frozen for serum biochemical analysis as outlined by Ochei and Kolhatkar[11].

Organ histopathology: Three birds were sacrificed per treatment at the end of the feeding trial; weight of liver, kidney, spleen, gizzard, heart and lungs were taken and recorded. Also samples of liver, spleen, kidney and portion of the large intestine were collected, fixed in 10% formalin solution and later processed for Histopathological examination at the department of Veterinary pathology of the University of Ibadan as described by Drury and Wallington[12] and Ewuola[13]. The pathological changes were observed under microscope using BA410E Histology Pathology Lab microscope while the Photomicrographs were taken with the help of computerized digital camera (Amscope MU900).

Data analysis: Data generated were subjected to one way analysis of variance using SAS statistical package[14]. Duncan's multiple range test was used to determine significant differences between the means. Probability values less than 0.05 considered as statistically significant.

RESULTS

The results of the growth response of broilers fed diets supplemented with graded levels of *Petiveria alliacea* is presented in Table 2. All parameters measured were not significantly ($p>0.05$) affected by the dietary treatments. Apart from Aspartate amino transferase, glucose and cholesterol, all other serum biochemical indices (Table 3) examined were not significantly affected by the PRM. Aspartate amino transferase (AST) activity of birds fed on T1, T2, T3, T4 and T5 were similar but higher than those fed on T6. The results of the organ weights (Table 4) showed significantly ($p>0.05$) higher gizzard weight in T3 compared with other treatments. Birds fed diets T1, T3 and T6 had statistically similar heart weights which were significantly higher than those broilers fed diets T2, T4 and T5. While all other parameters were not significantly influenced by the dietary treatments. Birds fed diet T3 had the highest value for gizzard (3.57%) while those fed on T2 had the lowest value (2.40%). Heart weight ranged from 0.40-0.67%, while the liver weight ranged from 1.67-2.37%. Qualitative histopathological

Table 2: Growth response of broilers fed diets supplemented with *Petiveria alliacea* root meal

Parameters	T1	T2	T3	T4	T5	T6	±SEM
*PRM (g/100 kg feed)	0.00	500.00	1000.00	1500.00	2000.00	2500.00	
Initial weight (g bird^{-1})	130.00	130.00	125.33	126.67	128.33	125.33	0.00
Final weight (g bird^{-1})	1983.33	2050.00	1866.67	1883.33	2150.00	2050.00	62.16
Weight gain (g bird^{-1})	1853.33	1920.00	1741.33	1756.67	2021.67	1924.67	61.75
Feed consumed (g bird^{-1})	5781.73	5788.37	5901.50	5765.20	5835.63	5920.07	71.46
Feed conversion ratio	3.23	3.00	3.43	3.33	2.93	3.13	0.10
Mortality (%)	3.33	3.33	3.33	0.00	3.33	3.33	1.09

[a,ab,b]Means in the same row with different superscripts are significantly different ($p<0.05$) PRM: *Petiveria alliacea* root meal

Table 3: Serum Biochemical indices of broilers fed diets supplemented with *Petiveria alliacea* root meal

Parameters	T1	T2	T3	T4	T5	T6	±SEM
*PRM (g/100 kg feed)	0.00	500.00	1000.00	1500.00	2000.00	2500.00	
Total protein (g dL^{-1})	4.07	4.37	4.20	4.33	4.27	4.73	0.10
Albumin (g dL^{-1})	1.80	1.73	1.80	1.93	1.80	2.07	0.09
Globulin (g dL^{-1})	2.27	2.63	2.40	2.40	2.47	2.67	0.12
Albumin/Globulin	0.83	0.67	0.77	0.87	0.73	0.90	0.59
AST (Ui L^{-1})	186.00^{a}	172.33^{a}	178.33^{a}	171.00^{ab}	177.00^{a}	154.00^{b}	3.36
ALT (Ui L^{-1})	25.00	33.33	34.00	35.00	42.33	39.00	2.63
Glucose (mg dL^{-1})	190.00^{ab}	198.67^{ab}	247.67^{ab}	149.00^{b}	240.33^{ab}	275.00^{a}	14.38
Cholesterol (mg dL^{-1})	103.67^{ab}	90.33^{b}	106.67^{ab}	96.00^{b}	108.00^{ab}	140.00^{a}	5.85

^{a,ab,b}Means in the same row with different superscripts are significantly different ($p<0.05$) PRM: *Petiveria alliacea* root meal, AST: Aspartate amino transferase, ALT: Alanine amino transferase

Table 4: Organ weights of broilers fed diets supplemented with *Petiveria alliacea* root meal

Parameters	T1	T2	T3	T4	T5	T6	±SEM
*PRM (g/100 kg feed)	0.00	500.00	1000.00	1500.00	2000.00	2500.00	
Lungs	0.53	1.97	0.47	0.47	0.50	0.50	0.24
Kidney	0.53	0.53	0.53	0.60	0.50	0.60	0.02
Gizzard	2.90^{ab}	2.40^{b}	3.57^{a}	2.60^{b}	2.73^{ab}	3.17^{ab}	0.13
Spleen	0.10	0.08	0.10	0.10	0.10	0.10	0.00
Heart 0.67^{a}	0.53^{ab}	0.57^{a}	0.53^{ab}	0.40^{b}	0.63^{a}	0.03	
Liver 1.90^{ab}	1.67^{b}	1.97^{ab}	2.00^{ab}	1.83^{ab}	2.37^{a}	0.08	

^{a,ab,b}Means in the same row with different superscripts are significantly different ($p<0.05$) PRM: *Petiveria alliacea* root meal

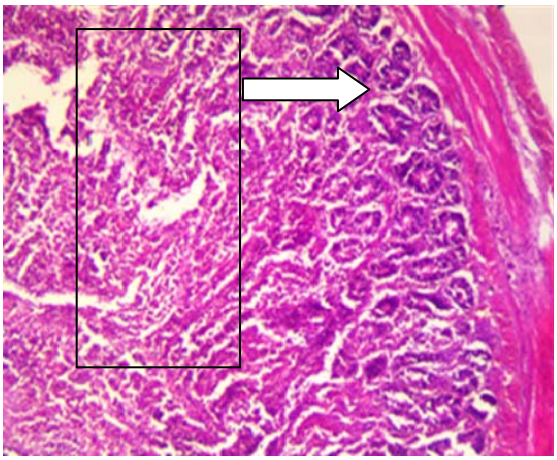

Fig. 1: Electron micrograph of the intestine of the birds fed the experimental diets showing marked necrosis (box) of the villi with numerous inflammatory cells and necrotic debris. The crypts (thick arrow) appears intact (T3, T4, T5 and T6).
Stain: Haematolin and Eosin. ×400

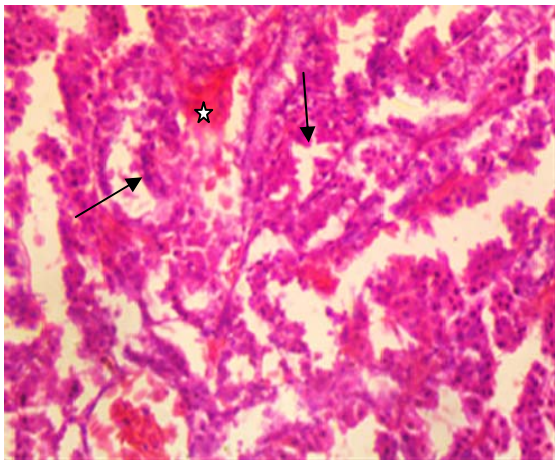

Fig. 2: Electron micrograph of the Kidney of the birds fed the experimental diets showing widespread moderate congestion of interstitial blood vessels (star) as well as necrosis of tubular epithelium (thin arrows) (T3, T4, T5 and T6).
Stain: Haematolin and Eosin. ×400

assessment showed variation in the selected organs of broiler fed diets supplemented with graded levels of PRM when compared with the control. Necrosis of the villi with numerous inflammatory cells and necrotic debris were observed in the intestine of the birds fed on T3, T4 and T5 respectively (Fig. 1). Congestion of interstitial blood vessels and necrosis of tubular epithelium were observed in the kidney of birds which fed on T3, T4 and T5 (Fig. 2). The liver of bird fed diets T4, T5 and T6 showed signs of hepatocellular necrosis and increased number of mononuclear/inflammatory cells in the hepatocyte (Fig. 3). The hepatocellular necrosis and increased number of mononuclear/inflammatory cells in the hepatocyte increased with increase in the quantity of PRM in the diet of the bird. There were numerous large discrete peri-arteriolar lymphoid sheaths and marked congestion of splenic sinuses and sinusoids in broilers fed on T4, T5 and T6 (Fig. 4).

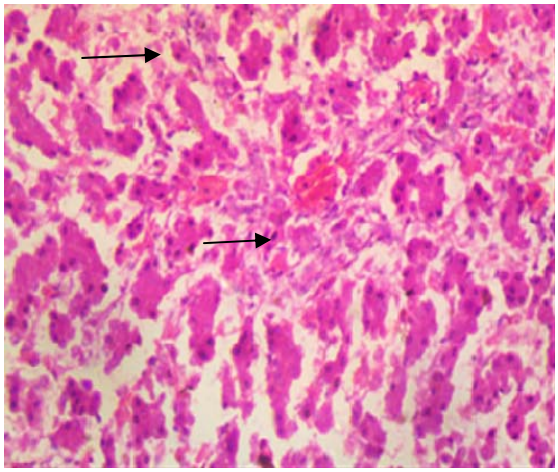

Fig. 3: Electron micrograph of the Liver of the birds fed the experimental diets showing multiple foci of hepatocellular necrosis (T4, T5 and T6).
Stain: Haematolin and Eosin. ×400

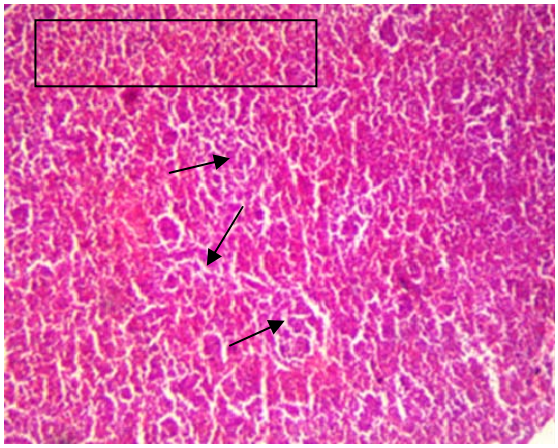

Fig. 4: Electron micrograph of the spleen of the birds fed the experimental diets showing numerous large discrete peri-arteriolar lymphoid sheaths (arrows). There is marked congestion of splenic sinuses and sinusoids (box) (T4, T5 and T6).
Stain: Haematolin and Eosin. ×400

DISCUSSION

The non significant difference recorded in the mortality of birds fed test ingredients *Petiveria alliacea* compared with the control may suggest that mortality recorded cannot be attributed to the test ingredient. David and Daum[15] and Dziezak[16] reported various feed additives to evaluate the growth performance of birds under experimental condition. Feed additives used as growth promoter improves palatability, nutrient utilization, stimulates appetite and increase flow of gastric juice[12]. Supplementation of different levels of feed additives in broiler diets significantly improved the live weight gain and fed conversion ratio[17]. Herawati[18] recorded an increase in weight gain and improved feed conversion ratio when broiler birds were fed diets containing ginger as an additive. However, *Petiveria alliacea* used as an additive in this study did not improve the weight gain and feed conversion ratio of broiler bird compared to the control. This may be due to variability in the type of additive and the level of inclusion which may not be high enough to cause improvement in their performance. This result corroborate the study of Onibi *et al.*[19] who reported no significant improvement in the weight gain and feed conversion ratio of broiler birds fed oregano oil and garlic as feed additive. Serum biochemical parameters may provide useful information for the evaluation of the health status of birds and reflect many metabolic alterations of organs and tissues[20]. Except for the aspartate amino transferase, glucose and cholesterol all the other indices measured were not significantly different across the treatments.

The results of the serum biochemical profile are similar to the findings of a previous study where no significant difference was noticed for most of the parameters studied for laboratory animals fed experimental diets containing *Moringa oleifera* leaf meal or crude extract from *Moringa oleifera* leaves[21]. The non-significant values for albumin and globulin obtained in this study suggests nutritional adequacy of the dietary proteins for broiler. It also suggests that the diets did not influence the serum albumin and globulin of the birds. A significant decrease was observed in AST activity in the birds fed on diet 6. Since liver is reported to contain enzymes like Alanine amino transferase (ALT) and AST, it releases these enzymes into the blood when damaged[22]. Elevation of AST and ALT activity can reflect reversible or irreversible changes in hepatocellular membrane permeability due to circulatory hypoxia, exposure to toxins and toxemia, inflammation, metabolic disorders or proliferation of hepatocytes[23]. Hence, the absence of significant differences among treatment diets in serum ALT and in the present study may reflect normal liver function of the birds fed diets containing PRM. This result is also supported by Olugbemi *et al.*[24] who reported *Moringa oleifera* leaves have a beneficial effect on the immune responses and improve intestinal health of broilers. Although the AST activity observed in birds fed on diets 1 (control), 3 (1000 g) and 5 (2000 g) was significantly higher than those of birds fed on

other diet, the decrease in AST activity observed in birds fed on diet 6 (2500 g) could suggest that PRM has hepato protective effect which can improve liver health.

CONCLUSION

Use of *Petiveria alliacea* root meal as phytobiotics improved the growth performance of broiler chicken and does not have any negative effects on the serum biochemistry. However feeding *Petiveria alliacea* root meal above 1500g/100kg of feed for a longer period of time may induce necrosis of the villi and hepatocytes.

SIGNIFICANCE STATEMENT

This study discovers the possible use of *Petiveria alliacea* root meal (PRM) as a natural growth promoter in broiler production. This study will enable researchers and farmers to uncover the beneficial role of PRM as a phytobiotics in reducing overdependence of farmers on the use of antibiotic growth promoter and eventual reduction of drug residues in broiler meat and possible development of resistance pathogens.

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