Original Article

Journal of Food Safety and Hygiene

Journal homepage: http://jfsh.tums.ac.ir

Treatment of avian pathogenic *Escherichia coli* infected broilers with aqueous extracts of *Vernonia amygdalina* in a challenge experiment

Pere-ere Sarah Tobia[1], Elijah Ige Ohimain[2]*

[1]Department of Science Laboratory Technology, Federal Polytechnic Ekowe, Bayelsa State, Nigeria.
[2]Department of Microbiology, Niger Delta University, Wilberforce Island Bayelsa State, Nigeria.

ARTICLE INFO

Article history:
Received 10.01.2024
Received in revised form
24.03.2024
Accepted 27.03.2024

Keywords:
Antimicrobial resistance;
Alternative antimicrobials;
APEC;
Botanicals;
Colibacillosis;
ExPEC;
Phytobiotics;
Phytogenics;
Virulent genes

ABSTRACT

Colibacillosis, which is the leading cause of infectious diseases resulting in mortality and economic losses in poultry is triggered by Avian pathogenic *Escherichia coli* (APEC). Conventionally, antibiotic growth promoters (AGP) are incorporated into poultry diets to control infectious diseases and boost performance. However, because of the problem of antimicrobial resistance, synthetic antibiotics are now restricted or banned in animal production in some countries. In this study, we carried out a challenge experiment by inoculating healthy broilers with APEC (10^6 cfu/mL) by oral gavage on day 21 of the experiment and orally administering various treatments five days post challenge for five days; synthetic antibiotics (T2), *Vernonia amygdalina* (bitter leaf) extracts (T3), no treatment (T4), which were compared with the control that wasn't challenged (T1). The experiment lasted for 42 days. Performance, hematological and histological studies were carried out. The results show that the challenged birds became diseased with the development of visible lesions, which ameliorated over time to varying extents with the use of synthetic antibiotics and Vernonia treatments. Overall, the performance of the birds with the use of Vernonia (T3) was comparable with that of synthetic antibiotics (T2) without eliciting any adverse hematological effects on the broilers. We therefore conclude that Vernonia can be safely used as a supplement for disease prevention in broiler chicken production.

Citation: Tobia PS, Ohimain EI**. Treatment of Avian Pathogenic *Escherichia coli* infected broilers with aqueous extracts of *Vernonia amygdalina* in a challenge experiment.** J Food Safe & Hyg 2024; 10 (1): 73-90 DOI:10.18502/jfsh.v10i1.16446

1. Introduction

Colibacillosis refers to several infectious diseases of poultry caused by Avian pathogenic *Escherichia coli* (APEC). It is widely recognized as the leading cause of infection and mortality caused by bacteria in poultry (1-4) and has now been considered to be endemic in the poultry industry worldwide (5).

*Corresponding author. Tel.: 234-8037306520
E-mail address: eohimain@yahoo.com

Colibacillosis causes economic losses through a reduction in the live weight of the birds, egg production and feed conversion ratio; ultimately causing death and carcass rejection by customers (4).

Escherichia coli is among the commonest normal flora in the guts of birds and mammals including humans. But extraintestinal pathogenic *E. coli* (ExPEC) like APEC display enhanced ability to cause infections beyond the

intestine in different animals (6,7) perhaps due to their expression of virulent genes (8).

APEC have been found to be connected to human strains of ExPEC such as septicemic *E. coli* (SEPEC), neonatal meningitis-causing *E. coli* (NMEC) and uropathogenic *E. coli* (UPEC) (69) and shared at least 10 key virulence genes with them including *iutA*, *iucC*, *iucD*, *cvaA*, *cvaB*, *cvaC*, *cvi, hlyF, etsA*, and *ompT* (4,10). Hence, APEC is considered to have zoonotic potential (9,11,12).

The potential for APEC to cause zoonotic infections in humans is a public health risk. For instance, it has been reported that colibacilloses are among the leading cause of food-borne infections globally (13). They can cause the transmission of Shiga toxin-producing *E. coli* (STEC), which has been associated with abdominal cramps and diarrhoea, some of which are bloody (14). Hence, APEC-contaminated poultry is considered unsafe for human consumption (13). In addition, APEC strains are known to express multi-antibiotic resistance genes, which have also been shown to be transmissible to humans. The problem of antibiotic resistance has become global among farm animals and humans. It is thought that farm animals particularly poultry are among the major routes of antibiotic resistance gene transmission to humans (15). The extensive and intensive use of antibiotics in poultry has recently been observed to lead to the evolution of antibiotic resistance among APEC strains both in humans and animals (1, 8, 16).

Antibiotic growth promoters (AGP) are conventionally added to animal diet to boost performance and control infections particularly nuisance bacteria, fungi and parasites. But due to the potential dangers of antibiotic resistance, the use of synthetic antibiotics is either restricted or outrightly banned in poultry farming in some countries. Therefore, recent efforts are now focused on the development of alternative antimicrobial substances for the eradication of infections in poultry. Phytobiotics are now increasingly being considered as a credible alternative to synthetic antibiotics in poultry farming. Some of the plants that have been tried with encouraging results include Ocimum *gratissimum* (17-19), *Zingiber officinale* (20), *Alchornea cordifolia* (21), *Asplenium barteri* (22), Moringa Oleifera (23), *Talinum triangulare* (24-26), *Bidens pilosa* (27). Other substances that have been substituted as growth promoters in poultry include enzymes (28-31), probiotics (32) and mushrooms (33-35). In a challenge experiment, Willis et al (36) demonstrated the use of mushrooms in broilers for the control of coccidiosis through the prevention of oocysts excretion by the parasite, *Eimeria tenella*.

Bitter leaf (*Vernonia amygdalina*) is an herb commonly used in traditional African medicine and as an ingredient for the preparation of soup. Several authors have reported the use of *Vernonia amygdalina* as an alternative AGP to boost performance and control microbial infections in poultry, particularly bacteria, fungi and parasites. They mostly administer the plant orally either as water extracts (37,38) or as leaf meal (39,40). Ofongo et al (41) screened neem leaf and bitter leaf for potential use as AGP in Poultry Feed. Banjoko et al. (42) demonstrated the use of Vernonia as anticoccidial in broilers. Vernonia is now being used in small-scale traditional poultry farming (43). We previously isolated a strain of APEC from some chicken suffering from colibacillosis at the Teaching and

DOI:10.18502/jfsh.v10i1.16446

Research Farm (T&RF) of the Niger Delta University (NDU) and characterized it for virulent genes (44) and antibiotic resistance genes (45). We tested the antibiotic activity of Vernonia both in-vivo and in-vitro (46). Hence, this study aimed to demonstrate the ability of Vernonia to substitute for synthetic antibiotics in a challenge experiment using broiler chickens.

2. Materials and Methods

2.1. Source of isolate

The APEC used for the challenge experiment was isolated from the droppings of sick birds suspected to be having colibacillosis at the T&RF of NDU. The strain used for the study was identified with the presence of virulent genes (44) and characterized to have antibiotic-resistant genes (45). The isolate, which expressed three virulent genes (iuta, iucD, ompT) and three resistant genes Gentamicin (*aac*(*3*)-*IV*), Sulfonamide (sul), and Cephalosporin (bla_{CMY}) and when inoculated into healthy chicken, developed the symptoms of colibacillosis with visible lesions, was used for the challenge experiment.

2.2. Vernonia amygdalina aqueous extract

The leaves of Vernonia plant used for the study was purchased from Swali Market, Bayelsa State, Nigeria. They were washed and drained of water. Crude extract of the leaves was prepared by weighing 100 g of fresh leaves in an analytical balance into which 150 mL of distill water was added and blended using a sterile Warring Blender. The milled leaves were sieved using a 2 mm Muslin cloth to obtain the crude extract. The Vernonia have been previously characterized for the presence of phytochemicals and demonstrated antibiotic activity in-vitro and in-vivo (46).

2.3. Animal challenge experiment

The study was conducted at the T&RF of NDU, Wilberforce Island, Bayelsa State, Nigeria. Ethical approval was secured for the study. A total of 240 in number, day-old Cobb broiler chicks were purchased from Amo Hatchery, Ibadan, Oyo State, Nigeria. The birds were orally given vitality and glucose on arrival (as a source of vitamins and glucose) and for the next 2 days. The chicks were not vaccinated against any disease. They were brooded for seven days and randomly distributed to the four treatment groups having five replicates each of 12 birds. The treatments were arranged in a completely randomized design manner (Table 1). Birds in Treatment 1 (T1) were administered just feed and water for the duration of the experiment. Birds in Treatment 2 (T2) in addition to feed and water, were given a dose of the challenge organism i.e., *E. coli* (10^6 cfu/mL) by oral gavage using a syringe on day 21 of the experiment and antibiotics five days post challenge for five days. Treatment 3 (T3) was given 1ml of *Vernonia amygdalina* orally for five days post-challenge. The *Vernonia* administration was done using a 2 mL syringe to ensure that each bird in Treatment 3 had a 1 mL dosage of *Vernonia amygdalina* leaf extract. Birds in treatment 4 (T4) were given feed, water and also a dose of the challenge organism (10^6 cfu/mL) on day 21 of the experiment, without any form of medication. The duration of the experiment was 42 days.

The housing and management of birds followed standard procedures. A dwarf wall deep litter system was used to carry out the study. The poultry house consists of individual pens demarcated with wire mesh, to prevent birds from crossing from one treatment to another.

Table 1. Challenge experiment treatment groups

Treatment code	Treatment Description	Poultry Feed & water	Challenge with *E. coli*	Antibiotic	Vernonia amygdalina	No treatment
T1	Positive control	X				
T2	Antibiotic	X	X	X		
T3	Vernonia	X	X		X	
T4	Negative control	X	X			X

The poultry house was thoroughly washed with the germicide "Izal" and clean water, a week before the introduction of birds. Treated wood shavings were evenly distributed on the floor of the deep litter pens a day before the coming of the birds. A 200 watts' electric bulbs were used during the brooding periods to supply heat to the birds. Drinkers and feeders were also washed and cleaned daily and kept orderly within each pen.

The birds were fed *ad libitum* from the start to the finish of the experiment. This was to ensure that they gained maximum body weight and to avoid unnecessary stress due to hunger. Also, since vaccination was not part of the experiment, it was ensured that the birds were fed thoroughly to stay healthy unto the completion of the experiment. Feeding was done once daily and it was ensured that enough feed was always in the feeder. Clean drinking water was readily available in the drinker from which the birds drank. The birds were fed with broiler starter from day one (1) to day twenty-eight (28) and broiler finisher feed from day twenty eight (28) to the terminating period of the experiment (day 42) Routine poultry farm management practices was followed, which included daily cleaning of feeding troughs, supply of fresh water and feed, and inspection for disease symptoms, stress and death (47).

At the end of the treatment, performance, heamatological characteristics and histological sections of the birds were measured. Performance indices, which included initial body and final weight, feed intake was measured using an analytical balance, which was used to compute weight gain and feed conversion ratio. Feed intake was measured by subtracting the feed left after feeding from the total feed given to the chicken. The feed conversion ratio was calculated by dividing the weight of feed consumed by the birds by the live weight of the birds.

2.3. Heamatological studies

Blood samples were obtained from the birds on the fifth day post-challenge. Four (4) birds were bled from 4 replicates in each treatment to collect blood samples. During sample collection, a sharp blade was used to cut through the jugular vein of the bird (which is found around the neck region), to cause bleeding. The blood was immediately collected and stored in sealed EDTA

DOI:10.18502/jfsh.v10i1.16446

bottles containing Lithium heparine (anti-coagulant). The samples were cooled and transported to the laboratory for haematological analysis using Leishman staining technique and viewing the slides under an oil-immersion objective lens of a compound microscope.

2.4. Tissue histopathological studies

One bird per replicate across each treatment was selected randomly. The birds were sacrificed by bleeding through the jugular vein. The birds were then placed dorsally and the ventral region was cut open and the visceral were brought out in the spleen and the proventriculus was then separated from other organs and placed in a 10% formaldehyde solution. Samples were collected on days 21, 29 and 42.

The appropriately labeled samples were brought to the laboratory and subjected to histological procedures. The samples were dissected and fixed in 10% neutral buffered formalin and processed in automatic tissue processor, embedded in paraffin wax and sectioned at 5 microns on a rotary microtome mounted on glass slides and examined using Olympus microscope camera under varying objectives for different tissues; low power (×4) objective lens for normal tissues, medium power (×10) objective for abnormal areas and high (×40) objective for doubtful cells or structures according to the procedure described by Winsor (48). Photomicrographs were taken with the aid of a computerized digital camera (Amscope MU900).

Statistical analysis

The data was compiled using Microsoft Excel while statistical analysis was carried out using SPSS version 25 (IBM-SPSS Inc). Analysis of variance was carried out followed by multiple comparisons with Duncan Multiple Range test at 0.05 probability level.

3. Results

Table 2 presents the performance of broilers under the APEC challenge experiment with Vernonia and other treatments. At the beginning of the experiment, the initial weights were not significantly different among the treatment groups, but there was however a significant difference ($P<0.05$) between the positive control T1 and the synthetic antibiotic treatment T2. At the end of the treatment, there was no significant difference in final live weight, weight gain and FCR among the various treatments. The feed conversion ratio of 1.60-1.79 recorded among the treatments, fell within the normal range of 1.5 – 1.9 for healthy chicken. The feed intake was highest ($P>0.05$) in the positive and negative controls (T1 and T4), which were not significantly different T2 ($P>0.05$), while T3 is in-between.

Heamatological characteristics of the birds under the challenge experiment are presented in Table 3. Neutrophils were highest in T1 being 54 ± 2.08%, which was not significantly different from T2 ($P>0.05$), but was significantly different from T3 and T4 ($P<0.05$), whereas T2, T3 and T4 were not significantly different ($P>0.05$). Lymphocytes were least in T1 being 33 ± 2.08%, which was not significantly different ($P>0.05$) from T2, but significantly lower than ($P<0.05$) values obtained from T3 and T4. Eosinophils count was the same in T1 and T2, being 4 ± 0.577%, but doubled in T3 and T4 ($P<0.05$). The monocyte counts were not significantly different ($P>0.05$) for all treatments

Table 2. Performance of broilers under APEC challenge

Parameters	T1	T2	T3	T4
Initial live weight, g	306.80±1.98[a]	281.20± 4.97[b]	291.60±6.43[ab]	288.40±4.06[ab]
Final live weight, g	1644.4±64.9	1585.8±37.7	1593.2±40.7	1577.0±37.0
Weight gain, g	1337.6±63.2	1299.6±35.3	1301.6±39.1	1288.6±33.4
Feed intake, g	2298.4±52.1[a]	2069.2±21.6[b]	2139.6±49.9[ab]	2297.0± 43.4[a]
FCR	1.73±0.08	1.60±0.05	1.65±0.07	1.79±0.08

*Means with different alphabets along the same row are significantly different (P<0.05).

Table 3. Heamatological characteristics during challenge of broilers with APEC

Parameters (%)	**T1**	**T2**	**T3**	**T4**
Neutrophils	54 ± 2.08[a]	49.67 ± 1.45[ab]	49.67 ± 1.45[b]	42.00 ± 2.08[b]
Lymphocytes	33 ± 2.08[b]	39 ± 2.08[b]	49.33 ± 1.86[a]	52.33 ± 1.45[a]
Eosinophils	4 ± 0.577[a]	4 ± 0.577[a]	2.33 ± 0.333[b]	2 ± 0.00[b]
Monocytes	9 ± 1.00[a]	7.33 ± 1.45[a]	6 ± 2.08[a]	3.667 ± 0.667[a]

* Means with different alphabets along the same row are significantly different (P<0.05

DOI:10.18502/jfsh.v10i1.16446

Table 4. Histopathology of the heart of broilers challenged with *E.coli*

Challenge phase	T1	T2	T3	T4
Before APEC Challenge, day 21	There is no observable lesion.	No observable lesion	Moderate myofibre atrophy	myofibre degeneration and inflammation
Post APEC Challenge & therapy, day 29	There is no observable lesion.	myofibre degeneration and cellular infiltrate in interstitium	moderate myofibre atrophy	There is moderate myofibre degeneration
End of study/post therapy, day 42	There is moderate myofibre atrophy	There is moderate myofibre atrophy	There is no observable lession	Myofibre degeneration

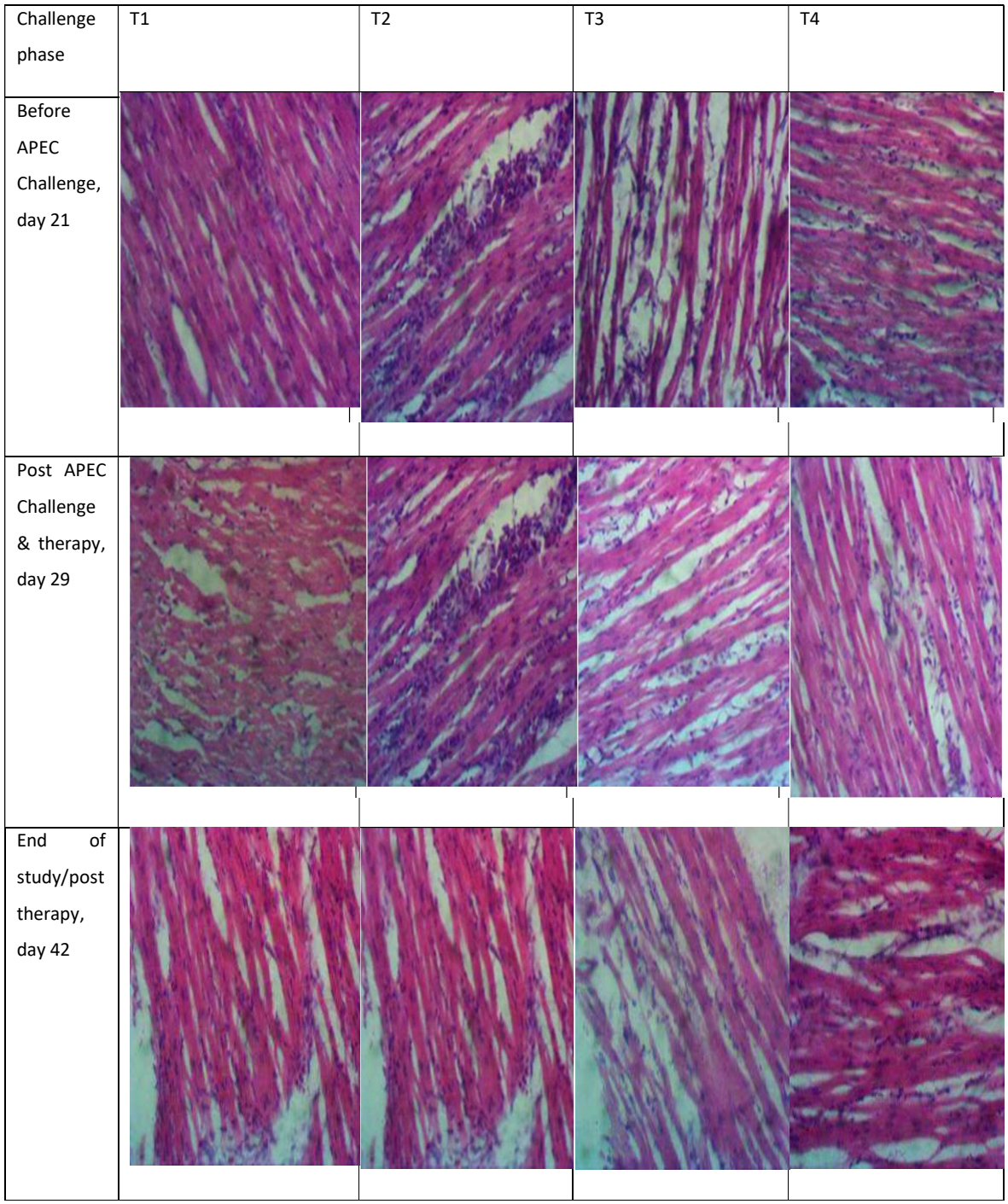


Figure 1. Photomicrograph of the heart

DOI:10.18502/jfsh.v10i1.16446

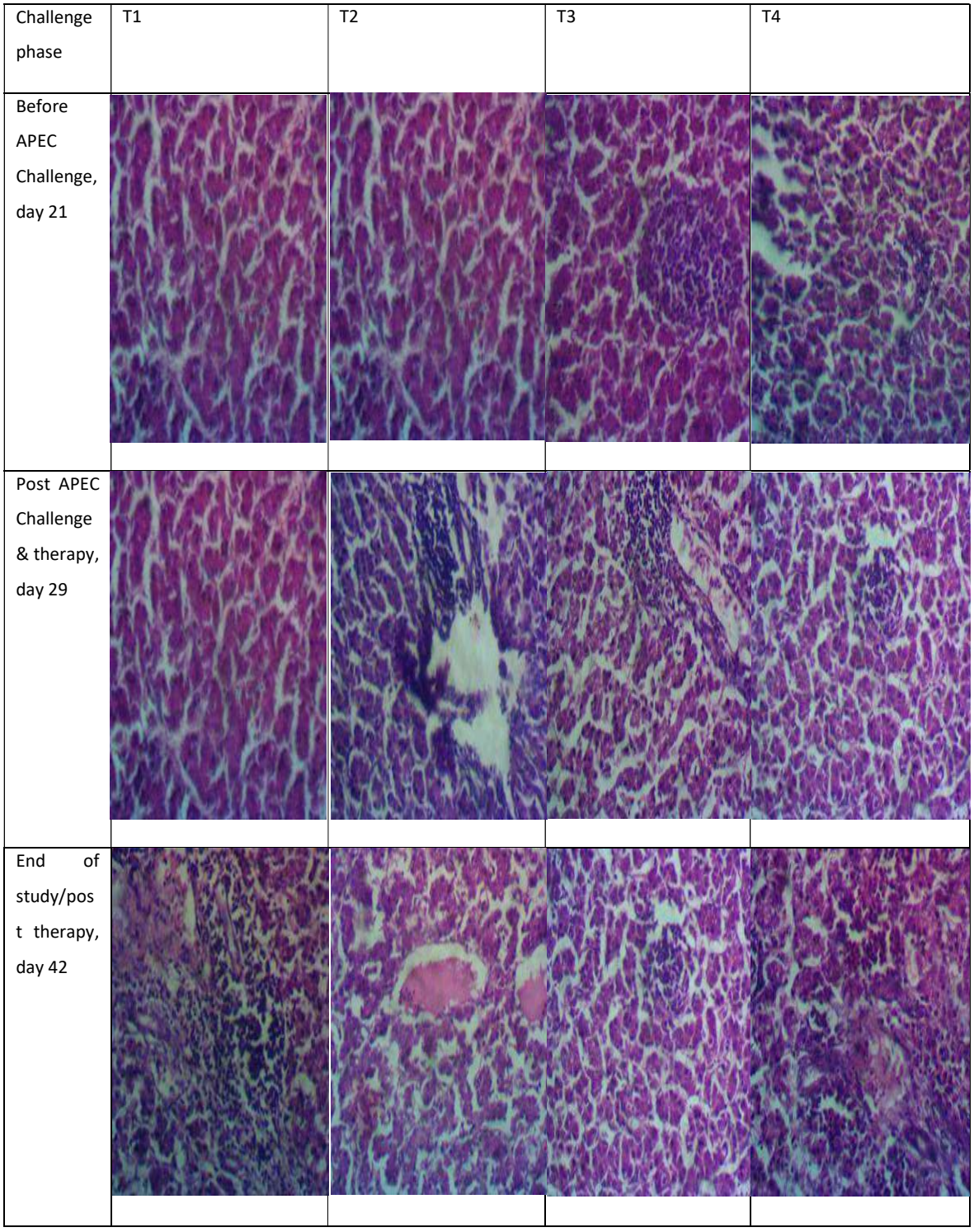


Figure 2. Photomicrograph of the liver

Table 5. Histopathology of the liver of broilers challenged with *E. coli*

	T1	T2	T3	T4
Before APEC challenge, day 21	There is no observable lesion	There is multifocal necrotising hepatitis	There is a focal nodular cellular aggregate in the parenchyma	There is moderate hepatocellular atrophy, and foci of necrotizing hepatitis
Post APEC challenge and therapy, day 29	There is no observable lesion	There is moderate centrrilobular hepatocellular atrophy and vasculitis	There is moderate centrilobular hepatocellular atrophy and perivascular cellular infiltrate	There is moderate hepatocellular atrophy, and foci of necrotizing hepatitis
End of study/post therapy, day 42	There is no observable lesion	There is moderate centrilobular hepatocellular atrophy and coagulation necrosis	There is moderate atrophy of hepatic plates	There is severe portal hepatocellular coagulation necrosis and inflammation

Table 6. Histopathology of the Lungs of broilers challenged with *E.coli*

	T1	T2	T3	T4
Before APEC challenge, day 21	There is no observable lesion	There is no observable lesion	There is moderate diffuse pulmonary congestion	There is moderate congestion and cellular infiltration in air spaces
Post APEC Challenge and therapy, day 29	There is moderate pulmonary congestion	There is moderate congestion of air capillaries and cellular infiltration in air spaces	There is moderate pulmonary capillary congestion	There is moderate pulmonary congestion
End of study/post therapy, day 42	There is no observable lesion	There is no observable lesion	There is no observable lesion	There is severe pulmonary congestion of vessels and capillaries

DOI:10.18502/jfsh.v10i1.16446

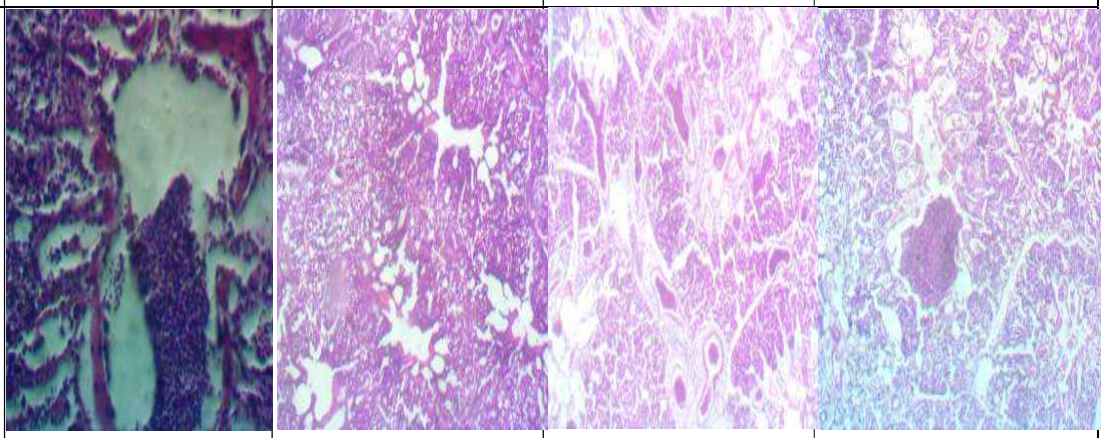
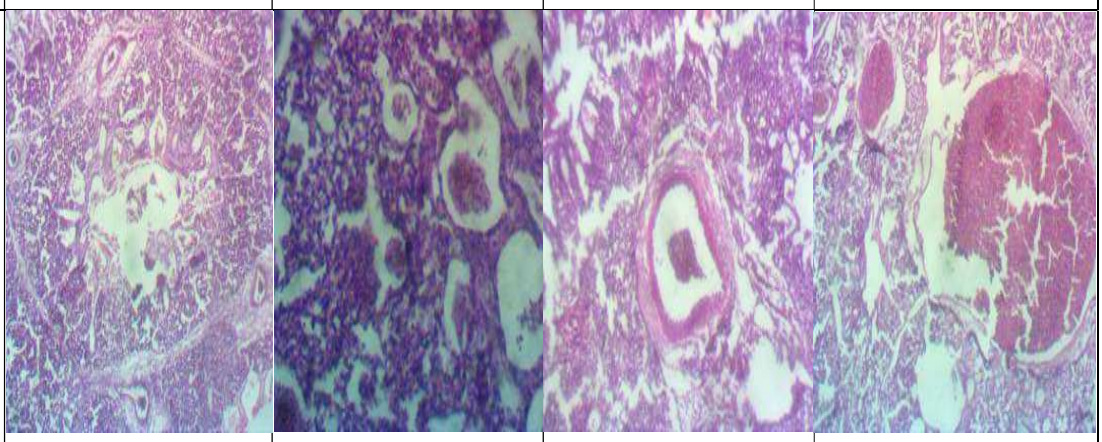
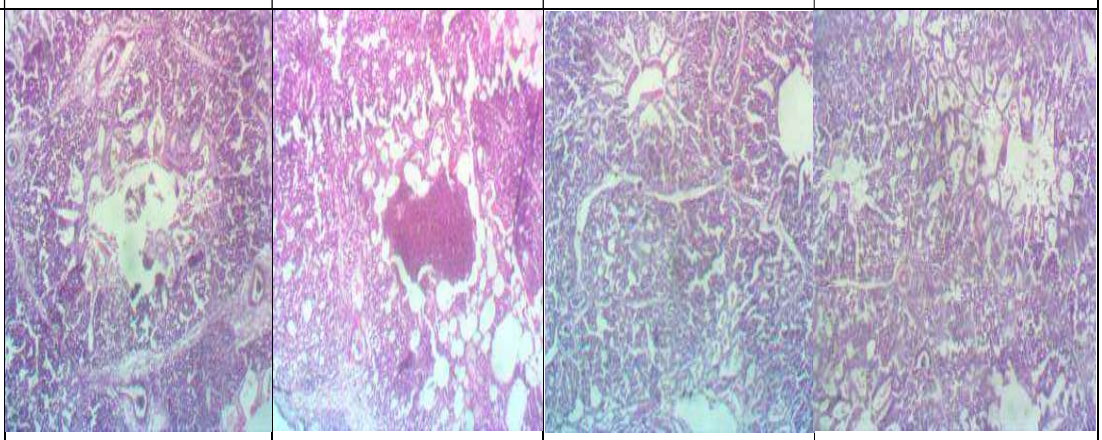
Challenge phase	T1	T2	T3	T4
Before APEC Challenge, day 21				
Post APEC Challenge & therapy, day 29				
End of study/post therapy, day 42				

Figure 3. Photomicrograph of the lungs

The histopathological observation of the tissues (heart, liver and lungs) of the birds before and after the APEC challenge followed by therapies is presented in Tables 4-6 and Fig. 1-3. The result of the microscopic observations (Table 4, Fig. 1) of the heart of the broiler birds before inoculation and after APEC challenge (inoculation) and after therapy show that the T3 group which is the Vernonia treatment displayed no observable lesion at the end of the experiment, while the antibiotics group, T2 showed moderate actions as the lesions were not worsened but sustained till the end of the study. There was degeneration in the negative control group (T4), which received no treatment.

The microscopic observation of the liver is shown in Table 5 and Fig. 2. After the challenge with APEC, there was moderate centrilobular hepatocellular atrophy in all treatment groups. However, after therapy and at the end of the experiment, it was observed that the Vernonia group, T3 did not progress further in the severity of the lesions, while the other treatments had either severe or multiple foci of hepatocellular coagulation necrosis and inflammation.

The microscopic observation of the lungs is shown in Table 6 and Fig. 3. After the challenge with APEC, there was moderate pulmonary capillaries congestion in all the groups. However, after therapy, and at the end of the experiment, it was observed that there were no observable lesions in both the antibiotic (T2) and Vernonia group (T3). However, there was severe pulmonary congestion in the negative control group (T4), which was not given any therapy.

4. Discussion

The results of the study show that the performance of the birds with Vernonia treatment is slightly superior to the synthetic antibiotics. The positive and negative controls (T1 and T4) also had the highest FCR, though not significantly higher than the other treatments ($P>0.05$). It should be noted that the lower the feed conversion ratio, the more proficient the bird can convert the feed to muscle. Overall, the results show that the performance of the birds with the use of Vernonia (T3) is comparable with that of synthetic antibiotics (T2). Our findings are in tandem with previous studies showing that Vernonia can be active against *E. coli* without reducing the performance of the chicken. The medicinal effects of Vernonia have been previously reported. Muhammad et al. (43) demonstrated the inhibitory effects of Vernonia against *E. coli* and other bacteria such as *Salmonella*, *Staphylococcus aureus* and *Pseudomonas*. The study recommended the utilization of plant extract as a substitute for synthetic antibiotics to control infections and enhance poultry production while minimizing the risk of antimicrobial resistance in a sustainable manner. Vernonia extract has been reported to increase the production of digestive enzymes, which enhances liver functions resulting in improved utilization of feeds (49). Japhet and Godgift (37) administered crude bitter leaf extract to broilers and pullets and found a significant increase in performance, particularly the carcass, live weight, heart, liver and gizzard, but no significant increase in kidney and spleen. Mandey et al (38) administered bitter leaf extract through drinking water to broilers and found that the plant exhibited antimicrobial activity, especially against *Salmonella*, which did not result in a significant increase in feed intake and carcass percentage, but resulted in a significant increase in final weight and FCR. Osho et al (50) orally administered graded levels of bitter leaf to

DOI:10.18502/jfsh.v10i1.16446

broilers and found that final weight gain, total feed intake and FCR were significantly different. Tokofai et al (40) studied the outcome of administering dietary *Vernonia amygdalina* leaf meal to broiler chickens and found that at 3 g/kg inclusion of Vernonia in the feed resulted in insignificant increases in organ weights but significant increases in digestive enzyme activities particularly amylase and trypsin, and improved mucosa immunity via an increase in Immunoglobulin A (IgA) and intestinal secretory IgG concentrations.

Blood measurements are used for the assessment of the health status of animals especially in feeding experiments. The results show that Vernonia can alter the distribution and occurrence of different types of white blood cells, which suggests the potential of Vernonia to act as an immunostimulant. However, relative to the positive control, neutrophil count was lower while lymphocyte was higher in the birds that were treated with the Vernonia extract. Note that neutrophils are the most abundant circulating granulocyte and migrate into tissues in response to infection or injury (51).

The result of our study agreed with many other studies on the innocuous nature of bitter leaves on the hematological properties of chickens. Olobatoke and Oloniruha (52) observed that powdered *Vernonia amygdalina* leaf was able to improve the FCR of cockerels without affecting their hematological profile. Omatsuli et al (39) studied the effect of Vernonia leaf meal diet at 0.1-2.5% inclusion in broiler diets and found no adverse effects on heamatological parameters (red blood cells, white blood cells, haemoglobin, packed cell volume) of the birds and concluded that that bitter leaf can be safely used as an alternative to synthetic antibiotics in broiler production. Osho et al (50) did not observe any significant difference in most hematological parameters when broilers were orally administered graded level of bitter leaf. They found that there was no significant increase in packed cell volume, red blood cells, basophils, neutrophils, eosinophils, monocytes, leucocytes, or haemoglobin. Several other authors have also tried Vernonia on poultry and found that it did not have a negative effect on the health and performance of the animals including their haematology and serum biochemistry (42, 53-55).

The results of the histopathology study of the birds before and after APEC challenge followed by treatments affected their organs including the heart, liver and lungs to varying extents. Lesions developed in the organs after the birds were challenged with APEX. The inflammation and necrotic lesions observed in the liver of the challenged birds could be a result of vascular injury and/or the expression of enterotoxin by APEC (46, 56). Other authors including Srinivasan *et al*. (57) have also reported necrosis in the liver and heart of birds suffering from colibacillosis. However, upon treatment, the antibiotic and Vernonia treatment groups recovered, while the disease progressed in the negative treatment group. The overall result, which shows improvements in the organs during the course of treatment, suggests that *Vernonia amygalina* could be used as a complement to antibiotics for the treatment of colibacillosis in broiler birds. The initial symptoms observed in some of the treatments even before APEC challenge might be because the birds were not vaccinated initially i.e., at the start of the experiment. Hence opportunistic infection could emerge. However, these subtle effects evened out at the end of the

experiment because all the treatments were equally exposed to the same experimental conditions.

5. Conclusion

Vernonia has been previously reported to have antimicrobial properties, which helped to control infections and boost poultry performance. In this study, we carried out a challenge experiment by artificially inducing colibacillosis in broilers followed by treatments with conventional synthetic antibiotics and bitter leaf extract. The results show a comparable performance in the broilers without any negative effects on hematological properties. Hence, we conclude that Vernonia can be used as a feed supplement for the treatment of colibacillosis in broilers without the risk of antibiotic resistance in sustainable poultry farming.

Ethical approval

Ethical approval was obtained from the Niger Delta University to work with animal subjects for the experiment

Funding

No financial support was received for the study and preparation manuscript.

Authorship contribution

The study was based on the Ph.D. research work carried out by the first author under the supervision of the second author. The second author conceived the work, carried out data analysis and wrote the initial draft of the manuscript, while both authors reviewed and approved the final version.

Declaration of competing interest

We declare no competing or conflict of interest in carrying out this research work.

Data availability

This publication contains the data generated from the study.

Acknowledgments

We thank the staff of the Teaching and Research Farm of the Niger Delta University for their support in the management of the birds during the study.

References

1. Usman S, Anjum A, Usman M, Imran MS, Ali M, Moustafa M, et al. Antibiotic resistance pattern and pathological features of avian pathogenic *Escherichia coli* O78: K80 in chickens. Brazil J Biol. 2022; 84: e257179. DOI: 10.1590/1519-6984.257179.
2. Aberkane C, Messaï A, Messaï CR, Boussaada T. Antimicrobial resistance pattern of avian pathogenic *Escherichia coli* with detection of extended-spectrum β-lactamase-producing isolates in broilers in east Algeria. Vet World. 2023; 16 (3): 449-54. https://doi.org/10.14202/vetworld.2023.449.
3. Delannoy S, Schouler C, Souillard R, Yousfi L, Le Devendec L, Lucas C, et al. Diversity of *Escherichia coli* strains isolated from day-old broiler chicks, their environment and colibacillosis lesions in 80 flocks in France. Vet Microbiol. 2021; 252: 108923. doi: 10.1016/j.vetmic.2020.108923
4. Bhattarai RK, Basnet HB, Dhakal IP, Alocilja EC. Virulence genes of avian pathogenic *Escherichia coli* isolated from commercial chicken in Nepal. Compar Immunol Microbiol Infect Dis. 2023; 95: 101961. doi: 10.1016/j.cimid.2023.101961
5. Wibisono FJ, Effendi MH, Wibisono FM. Occurrence, antimicrobial resistance, and potential zoonosis risk of avian pathogenic *Escherichia coli* in Indonesia: A review.

Int J One Health. 2022; 8(2): 76-85. doi: www.doi.org/10.14202/IJOH.2022.76-85

6. Sarowska J, Futoma-Koloch B, Jama-Kmiecik A, Frej-Madrzak M, Ksiazczyk M, Bugla-Ploskonska G, et al. Virulence factors, prevalence and potential transmission of extraintestinal pathogenic *Escherichia coli* isolated from different sources: recent reports. Gut Pathogen. 2019; 11: 1-16. doi: 10.1186/s13099-019-0290-0.
7. Mu X, Gao R, Xiao W, Gao Q, Cao C, Xu H, Liu X. EntE, EntS and TolC synergistically contributed to the pathogenesis of APEC strain E058. Microbial Pathogen. 2020; 141: 103990. doi: 10.1016/j.micpath.2020.103990.
8. Subedi M, Luitel H, Devkota B, Bhattarai RK, Phuyal S, Panthi P, et al. Antibiotic resistance pattern and virulence genes content in avian pathogenic *Escherichia coli* (APEC) from broiler chickens in Chitwan, Nepal. BMC Vet Res. 2018; 14(1): 1-6. doi: 10.1186/s12917-018-1442-z.
9. Kathayat D, Lokesh D, Ranjit S, Rajashekara G. Avian pathogenic *Escherichia coli* (APEC): an overview of virulence and pathogenesis factors, zoonotic potential, and control strategies. Pathogen. 2021; 10 (4): 467. doi: 10.3390/pathogens10040467.
10. Olsen RH, Christensen H, Bisgaard M. Comparative genomics of multiple plasmids from APEC associated with clonal outbreaks demonstrates major similarities and identifies several potential vaccine-targets. Veterinary Microbiol. 2012*158*(3-4): 384-393. doi: 10.1016/j.vetmic.2012.03.008.
11. LeStrange K, Markland SM, Hoover DG, Sharma M, Kniel KE. An evaluation of the virulence and adherence properties of avian pathogenic *Escherichia coli*. One Health. 2017; 4: 22-26. doi: 10.1016/j.onehlt.2017.08.001.
12. Jørgensen SL, Stegger M, Kudirkiene E, Lilje B, Poulsen LL, Ronco T, Christensen H. Diversity and population overlap between avian and human *Escherichia coli* belonging to sequence type 95. MSphere 2019; 4(1): e00333-18. doi: https://doi.org/10.1128/msphere. 00333-18.
13. Adeyanju GT, Ishola O. Salmonella and *Escherichia coli* contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria. Springerplus. 2014; 3:1-9. doi: 10.1186/2193-1801-3-139.
14. WHO. *Escherichia coli*. https://www.who.int/news-room/fact-sheets/detail/e-coli. 2018. Accessed 27 August 2024.
15. Ghorbani A, Khoshbakht R, Kaboosi H, Shirzad-Aski H, Ghadikolaii FP. Study on antibiotic resistance and phylogenetic comparison of avian-pathogenic *Escherichia coli* (APEC) and uropathogenic *Escherichia coli* (UPEC) isolates. Vet Res Forum. 2022; 13 (4): 569-576. doi: 10.30466/vrf.2021.527563.3160.
16. Ievy S, Islam MS, Sobur MA, Talukder M, Rahman MB, Khan MFR, et al. Molecular detection of avian pathogenic *Escherichia coli* (APEC) for the first time in layer farms in Bangladesh and their antibiotic resistance patterns. Microorganisms. 2020; *8*(7): 1021. doi: 10.3390/microorganisms8071021.
17. Alikwe PCN, Ohimain EI, Omotosho SM, Afolabi OO. Proximate, mineral, phytochemical and amino acid composition of *Ocimum gratissimum* and *Ocimum sanctum* leaf meals as potential feed additive for Monogastric animals. Continental J. Animal Vet Res. 2013; 5 (1): 22–30 https://www.academia.edu/80627803/Proximate_Mineral_Phytochemical_And_Amino_Acid_Composition_Of_Ocimum_Gratissimum_And_Ocimum_Sanctum_Leafmeals_As_Potential_Feed_Additives_In_Monogastric_Nutrition
18. Ohimain EI, Ofongo-Abule RTS, Zige DV. In-vitro antibacterial effect of *Ocimum gratissimum* on broiler gut

microflora. Bulletin Adv Sci Res. 2015; 1: 37-41. http://asdpub.com/index.php/basr/article/view/145

19. Ofongo-Abule RTS, Ohimain EI. Proximate composition and antimicrobial effect of *Ocimum gratissimum* on broiler gut microflora. J Adv Biol Basic Res. 2015a; 1 (1): 24–27. https://www.academia.edu/68286143/Proximate_composition_and_antimicrobial_effect_of_Ocimum_gratissimum_on_broiler_gut_microflora?hb-sb-sw=4313410
20. Ofongo-Abule RTS, Ohimain EI. Antimicrobial effect induced by fresh ginger root extracts in broilers. British Biotech J. 2015b; 9(1): 1-6 doi: 10.9734/BBJ/2015/18600.
21. Alikwe PCN, Tuagha B, Ohimain EI. Tropical christmas bush (*Alchornea cordifolia)* leaf meal as unconventional protein supplement in the diet of broiler chicks. Int J Appl Res Technol. 2014a; 3(2): 40–47. http://www.esxpublishers.com/images/ijrt-0214-0213.pdf.
22. Alikwe PCN, Iyoha ND, Ohimain EI. Performance, carcass and organ characteristics of finisher broilers fed graded levels of fern *(Asplenium barteri)* leaf meal. Int J Appl Res Technol. 2014b; 3(3): 105–10. http://www.esxpublishers.com/images/ijrt-0214-0229.pdf
23. Alikwe PC, Samuel IT, Ohimain EI, Akusu MO. Effect of Moringa Oleifera leaf meal as feed additive on layers performance. Biotechnol Res J. 2016; 2(1): 44-47. https://www.academia.edu/78558113/Effect_of_Moringa_Oleifera_Leaf_Meal_as_Feed_Additive_on_Layers_Performance.
24. Nworgu FC, Alikwe PCN, Egbunike GN, Ohimain EI. Performance and nutrient utilization of broiler chickens fed water leaf meal supplement. Int J Farm Allied Sci. 2014; 3 (8): 876-883. http://ijfas.com/wp-content/uploads/2014/08/876-883.pdf.
25. Nworgu FC, Alikwe PCN, Egbunike GN, Ohimain EI. Performance and haematological indices of broiler chickens fed Water Leaf (*Talinum triangulare*) meal supplement. J Agri Ecol Res Int. 2015a; 2 (1): 20-29. 10.9734/JAERI/2015/11754.
26. Nworgu FC, Alikwe PCN, Egbunike GN, Ohimain EI. Economic importance and growth rate of broiler Chickens fed with Water Leaf (*Talinum triangulare*) meal supplements. Asian J Agri Ext Econom Sociol. 2015b; 4(1): 49-57. DOI: 10.9734/AJAEES/2015/12542.
27. Alikwe PCN, Ohimain EI, Omotosho SM. Evaluation of the proximate, mineral, phytochemical and amino acid composition of *Bidens pilosa*as potential feed/feed additive for non-ruminant livestock. Animal Vet Sci. 2014; 2 (2): 18-21. https://www.sciencepublishinggroup.com/article/10.11648/j.avs.20140202.11.
28. Ohimain EI, Ofongo RTS. Effect of enzyme supplemented diet on gut microflora, digesta pH and performance of broiler chickens. J Microbiol Biotech Food Sci. 2013; 3(2): 127-31. https://www.academia.edu/64993659/Effect_of_enzyme_supplemented_diet_on_gut_microflora_digesta_pH_and_performance_of_broiler_chickens
29. Ofongo Abule RTS, Etebu E, Ohimain EI. Effect of diet type and enzyme supplementation on gut pH in broilers. Int J Agri Innovat Res. 2016; 4 (5): 964-67. https://www.ijair.org/index.php/issues?view=publication&task=show&id=723.
30. Ofongo Abule RTS, Ohimain EI. Enzyme supplemented wheat offal diet improves performance of broilers. Int J Agri Innovat Res. 2016; 4 (5): 968-71. https://ijair.org/index.php/issues?view=publication&task=show&id=736.
31. Ofongo RTS, Ohimain EI**.** Effect of enzyme supplementation and plant extracts on villus height and microbial counts in broilers. Niger J Animal Prod. 2019;

46 (2): 137 – 149. https://njap.org.ng/index.php/njap/article/view/28.

32. Willis WL, Isikhuemhen OS Ibrahim SA. Performance assessment of broiler chickens given mushroom extract alone or in combination with probiotics. Poultry Sci. 2007; 86: 1856-60. https://www.sciencedirect.com/science/article/pii/S0032579119392090?via%3Dihub.
33. Ogbe AO, Atwod SE, Abdu PA, Sannusi A, Itodo AE. Changes in weight gain, fecal oocyst count and packed cell volume of *Eimeria tenella*-infected broilers treated with a wild mushroom (*Ganoderma lucidum*) aqueous extract. JIS Afric Vet Ass. 2009; 80: 97-102. doi: 10.4102/jsava.v80i2.179.
34. Willis WL, Isikhuemhen OS, Ibrahim S, King K, Minor R, Ohimain EI. Effect of dietary fungus myceliated grain on broiler performance and enteric colonization with *Bifidobacteria* and *Salmonella*. Int J Poultry Sci. 2010a; 9 (1): 48-52. doi: 10.3923/ijps.2010.48.52.
35. Willis WL, Isikhuemhen OS, Hurley S, Ohimain EI. Effect of phase feeding supplemental fungus myceliated grain on oocyst excretion and performance of broiler chickens. Int J Poult Sci. 2011; 10 (1): 1-3. doi: 10.3923/ijps.2011.1.3.
36. Willis WL, Isikhuemhen OS, Minor RC, Hurley S, Ohimain EI. Comparing the feeding of fungus myceliated grain with other anticoccidial control measures on oocyst excretion of *Eimeria* challenged broilers. Int J Poult Sci. 2010b; 9 (7): 648-651. doi: 10.3923/ijps.2010.648.651.
37. Japhet YB, Godgift T. Effect of Vernonia amygdalina leaf extract on growth performance, carcass characteristics of pullet and broiler birds. Int J Sci Res Arch. 2021; *3*(2): 209-13. https://ijsra.net/content/effect-vernonia-amygdalina-leaf-extract-growth-performance-carcass-characteristics-pullet.
38. Mandey JS, Sompie M, Wolayan FR. Growth assessment of broiler chickens given bitter leaves (Vernonia amygdalina) as phyto-additive, potentially antimicrobial agents of lipids and amino acids. In 9th International Seminar on Tropical Animal Production (ISTAP 2021) 2022; (pp. 21-25). Atlantis Press. doi 10.2991/absr.k.220207.005.
39. Omatsuli M, Imouokhome J, Enabulele B. The effect of varying levels of Vernonia amagdalina Del. leaf on the hematological parameters of broiler birds. BIU J Basic Appl Sci. 2021; 6(1): 127 – 135. https://www.biu.edu.ng/wp-content/uploads/2021/06/BIUJBAS-61_11.pdf.
40. Tokofai BM, Orounladji BM, Idoh K, Oke OE, Agbonon A. Effect of Vernonia amygdalina leaf meal on growth performance, intestinal mucosa activity, digestive enzymes, absorption capacity, and immunity in broiler chickens. J Appl Animal Nutr. 2023; 1-8. https://doi.org/10.3920/JAAN2022.0006.
41. Ofongo RTS, Ohimain EI, Iyayi EA. Qualitative and quantitative phytochemical screening of bitter and neem leaves and their potential as antimicrobial growth promoter in poultry feed. Europ J Medicin Plant. 2021; 32:38-49. doi: 10.9734/ejmp/2021/v32i430383.
42. Banjoko OJ, Ia A, Osho, IB, Olumide MD. Evaluation of varying levels of Vernonia amygdalina leaf meal on growth, hematological parameters and as anticoccidial. Int J Livestock Product. 2019; 10(8): 192-97. https://academicjournals.org/journal/IJLP/article-abstract/5D47ED461619.
43. Muhammad AB, Doko HIM, Ibrahim A, Abdullahi B, Yahaya H, Sharfadi RS. Studies on inhibitory effects of extract of Vernonia Amygdalina used in traditional poultry farming against some bacteria isolated from poultry droppings. Scholars J Appl Med Sci. 2014; 2(6E):3185-3192 doi: 10.36347/sjams.2014.v02i06.067
44. Tobia PS, Ohimain EI. Molecular determination of virulent genes from avian pathogenic *Escherichia coli* isolated from poultry farm droppings in Bayelsa State,

Nigeria. Microbes Infect Dis. 2023a. doi 10.21608/mid.2023.218901.1548.

45. Tobia PS, Ohimain EI. Molecular detection of antibiotic resistance genes in avian pathogenic *Escherichia coli* isolated from poultry droppings in Bayelsa State, Nigeria. Microbes Infect Dis. 2023b. doi 10.21608/mid.2023.225157.1572.
46. Tobia PES. Studies on *Vernonia amygdalina* as an alternative to antibiotic growth promoters in broilers. Ph.D. Dissertation. Niger Delta University. 2023.
47. Oluyemi JA, Roberts SA Poultry production in warm climate. 2nd Edition, Ibadan Spectrum books Limited. 2000. 244. https://www.cabidigitallibrary.org/doi/full/10.5555/19811410413.
48. Winsor L. Tissue Processing: In Woods AE, Ellis RC (eds) Laboratory Histopathology: A complete reference. 1st Edition. 1994Publisher, Churchhill-Livingstone, Edinburgh. Pp – 1- 42. https://www.researchgate.net/profile/Leigh-Winsor/publication/260272795_Tissue_Processing/links/00b7d530711a98e13a000000/Tissue-Processing.pdf.
49. Hernandez F, Madrid J, Garcia V, Orengo J, Megias MD. Influence of two plant extracts on broilers performance, digestibility and digestive organ size. Poultry Sci. 2004; 83: 169-74. doi: 10.1093/ps/83.2.169.
50. Osho IB, Akindahunsi A, Igbasan FA, Adekunle DJ. Effect of orally administered bitter leaf (Vernonia amygdalina) extract on the growth performance and haematological parameters of broiler chicken. J Vet Med Animal Health. 2014; 6(10): 251-25. https://academicjournals.org/article/article1411748886_Osho%20et%20al.pdf.
51. Weir DM, Stewart J. Immunology, 8th Edition, 1999. Churchhill Livingstone, New York. P 362.
52. Olobatoke RY, Oloniruha JA. Haematological assessment of bitter leaf (*Vernonia amygdalina)* efficiency in reducing infections in cockerels. Proceedings of the World Congress on Medicinal and Aromatic Plants, November 9-14, 2008, Cape Town, South Africa. 2009; 472-73. https://journals.athmsi.org/index.php/ajtcam/article/view/921.
53. Abosede OO, Shuaib AH. Haematology and serum biochemistry of cockerels given Vernonia amygdalina leaf extract in a sub-tropical environment. Wayamba J Animal Sci. 2018; *10*: 1622-29. https://wayambajournal.com/archives/papers/haematology-and-serum-biochemistry-of-cockerels-given-vernonia-amygdalina-leaf-extract-in-a-sub-tropical-environment.
54. Daramola OT, Oloruntola OD, Ayodele SO. Nutritional evaluation of bitter leaf meal (Vernonia amygdalina): effects on performance, carcass and serum metabolites of broiler chickens. Niger J Animal Sci. 2018; 20(2): 211-21.
55. Ukoha OA, Onunkwo DN, Ewetola IO, Goodluck CN. Effect of Bitter leaf (Vernonia amygdalina) meal on Haematology and Serum Chemistry of Broiler chickens. Nigerian J Animal Sci. 2022; *49*(4): 88-96. doi: https://doi.org/10.51791/njap.v49i4.3706.
56. Miller MA, Zachary JF. Mechanisms and morphology of cellular injury, adaptation, and death. Pathol Basis Vet Dis. 2017; 2:43.e19. https://doi.org/10.1016/B978-0-323-35775-3.00001-1.
57. Srinivasan P, Balasubramaniam GA, Murthy TR, Balachandran P. Pathomorphological studies of polyserositis in commercial caged layer chicken. Asian Pacific J Tropic Med. 2014; 7(Suppl 1): S313-S320. doi: 10.1016/S1995-7645(14)60252-2.