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Performance, blood profile and gut morphometry of broiler chickens fed diets supplemented with Yohimbe (*Pausynistalia yohimbe*) and Larvacide



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ABSTRACT

The need for making livestock products free from antibiotics residue and other synthetic chemicals has resulted to the use of herbal products in livestock production. A 42-day study was conducted to compare the influence of Yohimbe (Pausynistalia yohimbe) and Larvacide on the growth performance, blood profile and gut morphometry of broiler chickens. Two hundred and twenty five 14-day old broiler chickens were randomly allotted into five treatment groups comprising of forty five chicks each. The groups were triplicates of fifteen birds. The five treatment groups consist of Basal (control), Larvacide (5 mg/Kg), three levels of Yohimbe supplementation (60 mg, 120 mg, and 180 mg/Kg) diets. Each supplement was supplied with the specified diets for the two phases of feeding [starter diet (3-4weeks) and the finisher diet (5-8weeks)]. Data collected were subjected to One-way analysis of variance. Results show insignificant effect (p > 0.05) on the growth performance parameters at the starter phase. However, at the finisher phase, supplementation of Yohimbe resulted to a progressive reduction in the feed intake (p < 0.05) without consequential reduction in the body weights. The chickens fed Basal diet +180 mg Yohimbe had the best Feed Conversion Ratio (FCR). Furthermore, chickens fed basal starter diet + 180 mg Yohimbe had the highest (p < 0.05) total protein, globulin, Red Blood Cell (RBC), White Blood Cell (WBC), and Eosinophil counts while it had the least (p < 0.05) Alanine Transaminase (ALT), uric acid and creatinine. Also, chickens fed basal starter diet + 60 mg Yohimbe had the highest (p < 0.05) Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH) while chickens fed basal starter diet + 120 mg Yohimbe had highest (p < 0.05) High Density Lipoprotein (HDL), but lower Very Low Density Lipoprotein (VLDL) and triglyceride. At the finisher phase, chickens fed basal diet + 180 mg Yohimbe had the highest (p < 0.05) Pack Cell Volume (PCV), haemoglobin and basophils while chickens fed basal diet + 60 mg Yohimbe had the highest WBC. Chickens fed basal diet + 120 mg Yohimbe had the least ALT and uric acid. Triglyceride, and cholesterol, HDL and VLDL, were lowered (p < 0.05) in chickens fed basal diet + Larcacide. Also, chickens fed basal diet +180 mg Yohimbe had the highest (p < 0.05) villi height. The study concluded that feeding broiler chickens basal diet + 180 mg Yohimbe improved optimum growth performance, blood profile and gut morphology.

1. Introduction

The use of growth promoters in feed for animals under intensive management system have been in practice for long (Yang et al., 2018). However, due to animal, human and environmental health issues, antibiotic growth promoters have been restricted in the European Union and other parts of the world. Alternatives to antibiotic growth promoters used in poultry production includes: essential oils, organic acids, herbs, plant extracts and the mixtures of either of them (Ozogul et al., 2015; Gracia et al., 2016). These alternatives to antibiotic growth

promoters enhance animal growth by: reducing the incidence and severity of subclinical infections, reducing the microbial use of nutrients, improving absorption of nutrients, reducing the amount of growth depressing metabolites, inhibiting the excretion of cytokines by macrophages, and antimicrobial activities (Humphrey & Klasing, 2003). Also, aside from antibiotic activities, plant extracts do stimulate the secretion of digestive enzymes thereby improving digestion and making nutrients in feeds readily available for absorption (Chao, Young & Oberg, 2000). These synergic activities by plant active substances, may improve health and performance of livestock (Manzanilla et al., 2001).

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Larvacide are insect growth regulators which disrupt the development of insect (Graf, 1993). They are used in the control of housefly in the poultry house. They include chemicals like: juvenile hormones, inhibitors, chitin synthesis and triazine derivatives (Retnakaran, Granett & Ennis, 1985). Cyromazine which is a triazine derivate is popularly known for its use as larvacide. However, it has been mentioned amongst the three classes of antibiotics found in water samples from and around poultry farms in Jiangsu, China (Ruicheng, Feng, Siyu, Ming & Ran, 2011) and also as anthelmintic agent (Usui et al., 2019) while other derivates of triazine are known for their antibacterial and antifungal activity against various gram-positive, gram negative bacterial and fungal strains (Dhananjay and Madhav Mane, 2015).

Pausinystalia yohimbe is a medicinal plant which grows in tropical West Africa. It is found within the span of south-west Nigeria, extending to Gabon and Congo. It has been used in trado-medicine for the treatment of erectile dysfunction and as aphrodisiac (Jacks, Asala & Priasad, 2007). Also, other properties it exhibit includes: antibiotic, antidiuretic, anti-inflamatory, and lypolitic properties (Lebeouf, Cave, Mangeney & Bouquet, 1981 and Berlan et al., 1991). The active compound in the plant is Yohimbine which is an indole alkaloid (Zanolari, 2003). Isolated Yohimbine is being used by body builders to increase muscle mass. In addition, the antidiuretic properties have been exploited to reduce urinary water loss in animals (Farjam & Greven, 1989). Aqueous extract of same plant has been used at low levels to increase spermatogenesis while it also increases lipolysis by antagonizing the anti-lipolytic activity of α 2- adrenoreceptors on fat cells (Berlan et al., 1991; Galitzky, Riviere, Tran, Montastruc & Berlan, 1990). Also, quinolizidine alkaloid and naphthylisoquinoline alkaloids, which are close class of alkaloids found in the plant has been reported to possess fungistatic, fungicidal, molluscicidal, and insecticidal properties (Morel et al., 2005; Sas-Piotrowska, Aniszewski & Gulewicz, 1996; Soon-Il and Young-Joon, 2017).

Yohimbine has been included in the drinking water of broiler chickens at the starter phase and an increase in protein accretion was observed in the carcass (Metin & Ahmet, 2016). The researchers also reported reduction in the lipid content of the meat without a decline in the average live weight of the chickens. However, due to the poor solubility of Yohimbine in water (Tadeusz, 2007), alternative route of administration should be considered so as to improve the effectiveness of the supplement. The supply of un-isolated Yohimbine in the bark of Pausynistalia yohimbe may ensure unrestricted access of chickens to the compound. Also, higher inclusion of Yohimbine and the extension of the supplementation to the finisher phase were recommended for the improvement in the growth performance of the chickens to be achieved (Metin & Ahmet, 2016). In addition, the use of Pausynistalia yohimbe bark in poultry feed can help evaluate its Larvacidal properties alongside with its growth promoting properties. Hence, this study was aimed at investigating the effect of Pausinystalia yohimbe bark meal and Larvacide supplement on the performance, blood profile and gut morphometry of broiler chickens at the starter and finisher phases.

2. Materials and methods

The test ingredient (*Pausinystalia yohimbe* bark) used for the experiment was purchased from a commercial market at Abeokuta after which it was cleaned and sundried until a constant weight was achieved. The dried bark was pounded using wooden mortar and pestle until it is reduced to small particles. The particles were sieved using metal sieve with sieve size of 3 mm. The sifting was added to the compounded diet at appropriate levels of inclusion while the shaft was discarded. Also, Larvacide was purchased from the veterinary store and was added to the feed at the prescribed level by the manufacturer.

2.1. Ethical permit

The practices adopted in the conduct of the study according to the guidelines as approved by the project review committee of the College of Animal Science, Federal University of Agriculture, Abeokuta, Ogun state, Nigeria.

2.2. Proximate composition of test ingredients

Proximate composition which includes: moisture, crude protein (CP), ether extract (EE), carbohydrate and ash content of *Pausynistalia yohimbe* and the test diets were determined by method described by (AOAC 2005).

2.3. Experimental animals and management

A total of 250 unsexed day old broiler chicks of Abore acre strains were collected from a reputable hatchery in Abeokuta. Prior to the arrival of the birds, the brooding house and rearing house with their equipment were washed with disinfectants. During the brooding period, chicks were floor brooded together for two weeks and brooding temperature was monitored using a digital room thermometer and maintained close to the standard broiler chickens brooding temperature regime as described by Gerry (2007). Test ingredients were not administered during the brooding period so that the birds may be physiologically stable and vaccines would be given prior to the introduction of the antibiotic growth promoter. After two weeks of brooding two hundred and twenty five chicks were selected and divided into five experimental groups. Each group has 45 chickens assigned to it with a relatively similar average weight. The treatment groups were made up of triplicates of fifteen birds each. Vaccination and medication were administered which include: vitamin, antibiotics; Infectious Bursal Disease and Newcastle diseases vaccines were given at prescribed dosage at intervals. Dry wood shavings were used as the litter bedding and the environment was routinely cleaned to keep it conducive and hygienic. Experimental diets were fed to chickens after two weeks of brooding. The chickens were fed formulated starter mash (3-4 weeks) and finisher mash (5-8 weeks).

2.4. Experimental diets and layout

Table 1 shows the gross composition of the experimental diet at the starter and finisher phase. Each of the diets contained varying levels of *Pausynistalia yohimbe* and Larvacide according to the treatment groups they were ascribed.

- T1 = Feed without additives
- T2 = Feed with 5 mg/Kg Larvacide
- T3 = Feed with 60 mg/Kg of *Yohimbe* bark meal
- T4 = Feed with 120 mg/Kg of Yohimbe bark meal
- T5 = Feed with 180 mg/kg of Yohimbe bark meal

2.5. Data collection

2.5.1. Performance evaluation

Records of feed intake, weight gain and mortality were taken on weekly basis. Also, the Feed conversion ratio (FCR) was obtained by the ratio of feed intake to weight gain. The following formulae were used for calculations.

Total Feed intake (g) = Total Feed supplied (g)

- Total Feed left over (g)

Table 1

Gross Composition of the experimental diet at the starter and finisher phases.

Ingredients	Starter phase (%)	Finisher phase (%)
Maize	52.50	55.00
Wheat bran	4.10	9.10
Soybean meal	18.50	14.00
Groundnut cake	16.50	15.00
Fish meal	2.50	1.00
Bone meal	3.00	3.00
Limestone	2.00	2.00
Salt (NaCl)	0.25	0.25
Vit/Min Premix	0.25	0.25
Methionine	0.20	0.20
Lysine	0.20	0.20
Total Calculated analysis (%)	100.00	100.00
Crude Protein	22.29	20.13
Calculated Metabolizable Energy (Kcal/ Kg)	3028.33	2864.77
Ether Extract	4.53	4.00
Crude Fibre	3.40	3.54
Calcium	2.00	1.94
Phosphorus	0.93	0.67

*Premix composition per kg diet: Vit A: 400,000.00 IU, Vit D3: 800,000.00 IU, Vit E: 9200.00 mg, Vit k: 800.00 mg, Vit B1: 1000.00 mg, Vit B6: 500.00 mg, Vit B12: 25.00 mg, Niacin: 6000.00 mg, Pantothenic acid: 2000.00 mg, Folic acid: 200.00 mg, Biotin: 8 mg, Mn: 300,000.00 g, Zn: 20,000.00 g, Cobalt: 80.00 mg, I: 40.00 mg, Choline: 80,000.00 g, Antioxidants: 800.00mg.

Total weight gain per bird (g)

 $= \frac{Final \ body \ weight \ (g) \ - Initial \ body \ weight \ (g)}{Number \ of \ birds \ in \ the \ replicate}$

Feed conversion ratio = $\frac{\text{Total feed consumed (g)}}{\text{Body weight gain (g)}}$

% Mortality = $\frac{Number of dead birds}{Total Number of stocked birds} X 100$

2.5.2. Blood collection

Blood was collected at weeks 2 and 6 of the experiment. Five mls of blood was collected from the neck vein of three birds per replicate. Two mls of the blood was released into tubes containing anti-coagulant Ethylene diamine tetra-acetate (EDTA) for haematological indices, while three mls of blood samples meant for serum analysis was released into plain bottles.

2.5.3. Serum biochemistry profile

Blood samples collected in the heparinised tubes from the chickens were analysed for serum through the colorimetry method using Jenway 6405 UV/VIS Spectrophotometer (UK). Serum protein was determined by the process described by Tietz and Norbert (1995). Bromo Cresol Green (BCG) method was used for serum albumin determination as described by Donmas, Watson and Briggs (1971). Alanine Transaminase (ALT), Aspartate Transaminase (AST) were determined using the process outlined by (IFCC 1986a; IFCC 1986b) and Cholesterol values recorded using the method described by Gordon and Amer (1977). Beam spectrophotometer (492 nm) was used to determine the Creatinine levels while the concentration of Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL) and Triglycerides in the serum were determined using the procedures described by Burtis and Ashwood (1999).

2.5.4. Haematological indices

Haematological indices which include: Haemoglobin concentration (Hb) was determined using van slyke apparatus, Pack Cell Volume (PCV) – hacksley haematocrit centrifuge (UK), White Blood Cell (WBC), WBC differentials as determined using the Neuber count chamber according to procedure described by Fudge (2000) and Cray and Zaias (2004).

2.5.5. Gut morphometry

On the 42nd day of the experiment, a bird per replicate were randomly selected, slaughtered and the gastrointestinal tract were excised. The small intestine were divided into 3 segments: duodenum (from gizzard outlet to the end of the pancreatic loop), jejunum (from the pancreatic loop to Meckel's diverticulum), and ileum (from Meckel's diverticulum to the caecal junction) according to (AVMA 2007). Approximately 2 cm lengths of the duodenum, jejunum and ileum segments were removed for gut morphological measurements. The gut samples were flushed with ice-cold buffered PBS at pH 7.4 and immediately placed in 10% formalin solution. Thereafter, the samples were stained with the Feulgen reaction: hydrolysed in 1 N HCL at 60 °C for 6 min, it was rinsed three times with distilled water and stained with Schiff reagent for 30 min. The samples were rinsed in distilled water and stored in acetic acid/water (45/55, v/v) at 4 °C until further analysis. Intestinal villi with their crypts were separated individually under a dissecting microscope as described by Goodlad et al. (1991). The length and width of the villi were measured according to the procedure described by Hampson (1986) using an optical microscope and a camera.

2.6. Statistical analysis

Data obtained were subjected to one way Analysis of Variance and were analysed using the general linear model procedure of (Statistical Package for Social Sciences 2011) version 20. Treatment means were separated for significant difference at p < 0.05 using the Duncan Multiple Range Test (DMRT) of the same package.

2.6.1. Experimental model

- $Y_{ij} = \mu + T_i + \mathcal{E}_{ij}$
- Where:
- $Y_{ij\mathchar`-}$ Observed value of the dependant variable
- μ- Population mean

 T_i - Effects of graded levels of supplemented Yohimbe (0, Larvacide, 60, 120 and 180 mg/Kg)

E_{ii}- Random residual error

3. Results

3.1. Growth performance of broiler chickens fed supplemented diets at the starter (3–4weeks) and finisher phases(5–8 weeks)

Tables 2 and 3 show the effect of dietary inclusion of Larvacide and *Yohimbe* on the growth performance and feed cost ratio of broiler chickens fed starter (3–4 weeks) and finisher (5–8 weeks) diets respectively. At the starter phase, there were no significant differences (P > 0.05) in the final weight; total weight gain, total feed intake, feed conversion ratio (FCR) and mortality of chickens fed the supplemented diets. However, at the finisher phase, there was significant reduction (P < 0.05) in the total feed intake of chickens fed diet supplemented with 180 mg Yohimbe compared to chickens fed basal diet and Larvacide supplemented diets.

3.2. Effect of supplemented diet on overall growth performance of broiler chicken

Table 4 shows the effect of supplemented diet on the overall performance of broiler chicken. There were no significant differences (p > 0.05) in the Initial, final, total weight gain, Feed conversion ratio (FCR), mortality of chicken fed control diet and chicken fed supplemented Larvacide, 60 mg, 120 mg, and 180 mg Yohimbe. However, the

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Table	2
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Effect of supplemented diet on performance of broiler chickens at starter phase.

Parameters	Basal	Basal+5 mg Larvacide	Basal+ 60 mg Yohimbe	Basal+ 120 mg Yohimbe	Basal + 180 mg Yohimbe	SEM
Initial weight (g)	195.55	192.22	194.44	195.55	190.00	1.507
Final weight (g)	655.95	690.00	660.00	682.30	693.33	14.869
Total weight gain (g)	460.39	497.77	465.55	486.74	503.33	14.894
Total Feed intake (g)	895.96	946.66	948.00	978.98	963.33	17.418
FCR	1.94	1.90	2.06	2.01	1.93	0.037
Mortality (%)	2.22	0.00	0.00	2.22	0.00	0.605

FCR = Feed Conversion Ratio.

SEM = Standard Error of Mean.

total feed intake of chicken fed 180 mg Yohimbe at 4617.55 g was significantly reduced (p < 0.05) compared to chickens fed Larvacide at 5363.44 g. However, there were no significant differences (p > 0.05) between the control, 60 mg and 120 mg Yohimbe respectively.

3.3. Serum biochemistry of broiler chickens fed dietary supplementation of Larvacide and Yohimbe

Table 5 and 6 show the effect of feeding dietary treatments on the serum indices of broiler chickens at the starter and finisher phases respectively. At the starter phase, significant differences (p < 0.05) were observed in the total serum protein, albumin, globulin, Alanine aminotransferase (ALT), creatinine, and High density Lipoprotein (HDL) values. Chickens fed basal diet without supplement and chickens fed 180 mg Yohimbe + basal diet had the highest (p < 0.05) total serum protein compared to other treatment groups. Also, chickens fed Control diet and chickens fed Basal diet + 60 mg Yohimbe had significantly higher (p < 0.05) blood albumin levels compared to chickens fed other supplemented diets. However, chickens fed basal diet + 180 mg Yohimbe recorded the highest globulin values followed by those fed the basal diet only while chickens fed basal + 180 mg Yohimbe has lower (p < 0.05) globulin levels compared with the chickens fed control diet. In addition, chickens fed basal diet + 0.12 and basal diet + 180 mg Yohimbe had the least (p < 0.05) ALT levels (p < 0.05) followed by those fed basal diet + 60 mg Yohimbe, while chickens fed basal diet + Larvacide had the highest (p < 0.05) ALT levels. There were no significant (p>0.05) differences in the Aspartate aminotransferase (AST), Total cholesterol, and Low Density Lipoprotein (LDL) of chickens fed various experimental diets. However, chickens fed basal diet + 60 mg Yohimbe and basal diet + 180 mg Yohimbe had reduced (p < 0.05) creatinine compared to chickens fed Basal diet only and chickens fed basal diet + Larvacide supplement. The HDL in chickens fed Basal diet + 120 mg Yohimbe was significantly higher (p < 0.05) compared to chickens fed Basal diet + Larvacide supplement while chickens fed other experimental diets had similar (p > 0.05) HDL.

At the finisher phase, total serum protein was higher (p < 0.05) in chickens fed basal diet + 180 mg Yohimbe than chickens fed basal diet + Larvacide supplement. The albumin level were higher (p < 0.05)

in chickens fed basal diet + 60 mg Yohimbe and basal diet + 180 mg Yohimbe than chickens fed basal diet + Larvacide supplement. Globulin levels were significantly (p < 0.05) higher in chickens fed basal diet + 120 mg Yohimbe and basal diet + 180 mg Yohimbe than chickens fed basal diet only and basal diet + 60 mg Yohimbe. However globulin level was lowest (p < 0.05) in group of chickens fed Basal diet + Larvacide supplement amongst all groups while ALT was lower in the chickens fed basal diet + 120 mg Yohimbe. There were no significant variation in the AST, Creatinine, and LDL of chickens fed the experimental diets. However, total cholesterol and HDL were lower (p < 0.05) in chickens group fed basal diet + 120 mg Yohimbe and basal diet + Larvacide supplement compared to chickens group fed other experimental diets.

3.4. Haematological indices of broiler chickens fed diets supplemented with Larvacide and Yohimbe

Table 7 shows the effect of supplemented starter diet on the haematological indices of broiler chickens. There were no significant differences (p > 0.05) in the Pack Cell Volume (PCV), haemoglobin, and basophils of chickens groups fed the experimental diets. However, there were significant differences (p < 0.05) in the Red Blood Cell (RBC) count, White Blood Cell (WBC) count, heterophils, lymphocyte, eosinophils, basophiles, monocytes, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC). RBC was increased in chickens groups fed basal diet only and basal diet + 180 mg Yohimbe while WBC was increased (p < 0.05) in the group of chickens fed basal diet + 180 mg Yohimbe. Heterophils was increased (p < 0.05) in chickens fed basal diet + 60 mg Yohimbe while Lymphocytes was reduced (p < 0.05) in chickens fed Basal diet + 60 mg Yohimbe than the chickens on Basal diet only and Basal diet + Larvacide supplement. Eosinophils was increased (p < 0.05) in the group of chickens fed Basal diet + 60 mg Yohimbe and Basal diet + 180 mg Yohimbe. Monocytes was reduced (p < 0.05) in chickens groups fed Basal diet + 60 mg Yohimbe and Basal diet + 180 mg Yohimbe. Mean corpuscular volume was increased (p < 0.05) in the group of chickens fed Basal diet + Larvacide and Basal diet + 60 mg Yohimbe while Mean corpuscular haemoglobin was

Effect of supplemented diet on performance of broiler chickens at finisher phase.

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Parameters	Basal	Basal + 5 mg Larvacide	Basal + 60 mg Yohimbe	Basal+ 120 mg Yohimbe	Basal+ 180 mg Yohimbe	SEM
Initial weight (g)	655.95	690.00	660.00	682.30	693.33	14.869
Final weight (g)	2141.39	2145.95	1996.03	2088.25	2027.77	46.715
Total weight gain (g)	1485.43	1455.95	1336.03	1405.95	1334.44	51.597
Total Feed intake (g)	4224.98 ^a	4416.77 ^a	4097.38 ^a	3934.04 ^a	3654.22 ^a	86.507
FCR	2.84	3.10	3.17	2.83	2.75	0.110
Mortality (%)	6.66	8.88	2.22	0.00	0.00	1.575

SEM = Standard Error of Mean.

g = gram/bird.

FCR = Feed Conversion Ratio.

g = gram/bird.

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Table -	4
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Effect of supplemented diet on overall performance of broiler chicken.

Parameters	Basal	Basal + 5 mg Larvacide	Basal + 60 mg Yohimbe	Basal+ 120 mg Yohimbe	Basal+ 180 mg Yohimbe	SEM
Initial weight (g)	195.55	192.22	194.44	195.55	190.00	1.507
Final weight (g)	2141.39	2145.95	1996.03	2088.25	2027.77	46.715
Total weight gain (g)	1945.83	1953.73	1801.58	1892.69	1837.77	46.584
Total Feed intake (g)	5120.94 ^a	5363.44 ^ª	5045.38 ^a	4913.03 ^a	4617.55 ^a	87.642
FCR	2.63	2.78	2.82	2.60	2.51	0.065
Mortality (%)	8.88	8.88	2.22	2.22	0.00	1.548

SEM = Standard Error of Mean.

g = gram/bird.

FCR = Feed Conversion Ratio.

^a Means on the same row with different superscripts differ significantly (p < 0.05).

increased (p < 0.05) in chickens group fed Basal diet + 60 mg Yohimbe. Mean corpuscular haemoglobin concentration was reduced (p < 0.05) in the group of chickens fed Basal diet + 60 mg Yohimbe.

Table 8 shows the effect of supplemented finisher diet on the haematological indices of broiler chickens. There were no significant difference (p > 0.05) in the RBC, mean corpuscular volume, and mean corpuscular haemoglobin concentration of chickens fed the experimental diets. However, PCV and haemoglobin count were increased (p < 0.05) in chickens group fed basal diet + 180 mg Yohimbe. WBC increased (p < 0.05) in chickens group fed basal diet + 60 mg Yohimbe. Heterophils was reduced (p < 0.05) in chickens groups fed basal diet + 60 mg Yohimbe and basal diet + 120 mg Yohimbe. Lymphocyte was increased (p < 0.05) in the group of chickens fed basal diet + 120 mg Yohimbe than chickens on basal diet only. Also, the eosinophils increased in chickens on basal diet + Larvacide. Also, the group of chickens fed basal diet + 60 mg Yohimbe and basal diet + 180 mg Yohimbe had increased (p < 0.05) basophils counts. Furthermore, chickens group fed basal diet + 120 mg Yohimbe had increased (p < 0.05) monocyte count, followed by chickens group fed basal diet + 60 mg Yohimbe and basal diet + 180 mg Yohimbe. Chickens on Basal diet + 120 mg Yohimbe had increased (p < 0.05) mean corpuscular haemoglobin count than chickens fed basal diet only.

3.5. Effect of supplemented diet on the gut morphometry of broiler chicken

Table 9 shows the effect of supplemented diet on the gut morphometry of broiler chicken. Duodenal villi height was significantly higher (p < 0.05) in Larvacide supplemented diet (975.87 µm) compared with the control (969.10 µm). However, it was significantly least (p < 0.05) in chicken fed 60 mg Yohimbe supplemented diet (584.08 µm) compared to the control (969.10 μm), followed by 180 mg Yohimbe (617.54 μm) and 120 mg Yohimbe (705.11 μm) respectively.

Duodenal crypt depth was significantly highest (p < 0.05) in chicken fed 120 mg Yohimbe supplemented diet (187.92 µm) compared with the control diet (67.07 µm), followed by 60 mg Yohimbe (167.48 µm), Larvacide (128.55µm), and 180 mg Yohimbe supplemented diet (115.10µm) respectively.

Duodenal apical width was significantly least (p < 0.05) in 120 mg Yohimbe supplemented diet (143.49 µm) compared with the control diet (319.51 µm), followed by 60 mg Yohimbe (183.10 µm), Larvacide (229.50 µm), and 180 mg Yohimbe supplemented diet (238.53 µm) respectively.

Duodenal basal width was significantly least (p<0.05) in 180 mg Yohimbe supplemented diet (0.18 µm) compared with the control diet (128.50 µm), followed by 60 mg Yohimbe (91.53 µm), 120 mg Yohimbe (94.04 µm), and Larvacide supplemented diets (109.52 µm) respectively.

Jejunum villi height was significantly higher (p < 0.05) in 180 mg Yohimbe supplemented diet (1333.93 µm) compared with the control diet (1001.46 µm). However, Larvacide (874.56 µm), 120 mg (50.90 µm), and 60 mg Yohimbe (564.45 µm) were progressively and significantly lesser (p < 0.05) villi height compared with the broiler chicken fed control diet.

Jejunal crypt depth was significantly lesser (p < 0.05) in chicken fed 120 mg Yohimbe (50.90 µm) compared to those fed control diet (113.04 µm). However, chicken fed 180 mg (124.96 µm), 60 mg Yohimbe (156.50 µm) and Larvacide supplemented diet (166.96 µm) has progressively higher Jejunal crypt depth compared with the chicken fed the control diet.

Jejunal apical width in broiler chicken fed 60 mg (203.46 µm) and

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Parameters	Basal	Basal+5 mg Larvacide	Basal+ 60 mg Yohimbe	Basal + 120 mg Yohimbe	Basal + 180 mg Yohimbe	SEM
Total Protein (g/dl)	3.975 ^a	3.350 ^a	3.600 ^a	2.950 ^a	3.950 ^a	0.112
Albumin (g/dl)	1.925 ^a	1.775 ^a	1.925 ^a	1.575 ^a	1.550 ^a	0.059
Globulin (g/dl)	2.075 ^a	1.575 ^a	1.650 ^a	1.350 ^a	2.425 ^a	0.110
ALT (U/L)	33.300 ^a	34.925 ^a	30.700 ^a	28.075 ^a	28.450 ^a	0.736
AST (U/L)	53.775	54.000	55.525	59.375	53.550	0.872
Creatinine (mg/dl)	0.750 ^a	0.732 ^a	0.617 ^a	0.662 ^a	0.585 ^a	0.019
Total Cholesterol (mg/dl)	126.000	118.000	119.000	127.250	123.750	2.402
HDL (mg/dl)	67.900 ^a	63.250 ^a	64.875 ^a	80.200 ^a	72.425 ^a	2.387
LDL (mg/dl)	34.700	27.300	30.475	30.250	30.875	1.644

SEM = Standard Error of Mean.

ALT = Alanine aminotransferase.

AST = Aspartate aminotransferase.

HDL = High Density Lipoprotein.

LDL = Low Density Lipoprotein.

g/dl = gram/decilitre.

U/L = Unit/Litre.

mg/dl = milligram/decilitre.

Table 6

Effect of supplemented finisher diets on Blood Serum and Lipid profile of broiler chickens.

Parameters	Basal	Basal + 5 mg Larvacide	Basal + 60 mg Yohimbe	Basal+ 120 mg Yohimbe	Basal+ 180 mg Yohimbe	SEM
Total Protein (g/dl)	3.800 ^a	3.175 ^a	4.450 ^a	4.100 ^a	4.575 ^a	0.157
Albumin (g/dl)	2.225 ^a	1.850 ^a	2.850 ^a	2.200 ^a	2.575 ^a	0.117
Globulin (g/dl)	1.575 ^a	1.325 ^a	1.600 ^a	1.900 ^a	2.000^{a}	0.068
ALT (U/L)	25.000 ^a	26.500 ^a	30.500 ^a	24.250 ^a	27.750 ^a	0.702
AST (U/L)	63.500	59.000	71.000	64.500	59.000	1.990
Creatinine (mg/dl)	1.100	0.900	1.175	1.125	1.175	0.085
Total Cholesterol (mg/dl)	132.850 ^a	89.675 ^a	119.575 ^a	95.375 ^a	119.400 ^a	5.088
HDL (mg/dl)	81.800 ^a	42.725 ^a	72.025 ^a	49.875 ^a	74.350 ^a	4.378
LDL (mg/dl)	29.300	28.975	27.150	24.300	26.475	1.228

SEM = Standard Error of Mean.

ALT = Alanine aminotransferase.

AST = Aspartate aminotransferase.

HDL = High Density Lipoprotein.

LDL = Low Density Lipoproteinre.

g/dl = gram/decilitre.

U/L = Unit/Litre.

mg/dl = milligram/decilitre.

^a Means on the same row with different superscripts differ significantly (p < 0.05).

180 mg Yohimbe (162.48 µm) was significantly increased (p<0.05) compared to chicken fed control diet (145.50 µm). However, chicken fed Larvacide (134.53 µm) and 120 mg Yohimbe supplement (9.46 µm) had significantly reduced (p<0.05) apical width compared with the control diet.

Jejunal basal width was significantly reduced (p < 0.05) in broiler chicken fed 120 mg Yohimbe supplemented diet (3.47 µm) compared with the control diet (61.50 µm). However, chicken fed 60 mg (158.48 µm), 180 mg Yohimbe (74.89 µm) and Larvacide supplemented diets (72.48 µm) had significantly reduced (p < 0.05) basal width compared to control diets.

Ileal villi height was significantly highest (p < 0.05) in broiler chicken fed 120 mg Yohimbe supplemented diet (1045.05 µm) compared with the control (561.07 µm), followed by Larvacide (828.55 µm), 60 mg Yohimbe (820.94 µm), and lastly 180 mg Yohimbe supplemented diet (735.08 µm).

Ileal crypt depth was significantly highest in broiler chicken fed 60 mg Yohimbe supplemented diet (213.94 μ m) compared with the control diet (83.57 μ m), followed by 120 mg Yohimbe (179.14 μ m), Larvacide (119.94 μ m), and 180 mg Yohimbe supplemented diet (112.05 μ m).

Ileal apical width and Illeal basal width was significantly highest in

Table 7

Effect of supplemented starte	r diets on Haematological	Indices of broiler chickens.
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broiler chicken fed 120 mg Yohimbe (213.12 μ m` and 113.46 μ m) compared to the control diet. However, broiler chicken fed 60 mg (179.95 μ m and 109.95 μ m), 180 mg Yohimbe (115.91 μ m and 93.54 μ m) and Larvacide supplemented diets (136.92 μ m 81.08 μ m) had significantly declining Illeal apical compared with 180 mg Yohimbe supplemented diet (155.91 μ m and 93.54 μ m).

4. Discussion

At the starter phase, there were no increase in the final weights, total weight gained per chickens, total feed intake, and FCR. The nonresponse of chicken at the starter phase may be because the digestive organs were not fully developed to process hard feed stuff. This is similar to the finding of Metin and Ahmet (2016) who supplied Yhombine in the drinking water of broiler chickens at the starter phase but recorded no significant changes in the performance parameters. However, at the finisher phase, *Yohimbe* reduces feed intake which consequentially reduced the feed conversion ratio (FCR) of the broiler chickens. The reduction in feed intake is a phenomenon once reported by Currie and Wilson (1992) who supplied dietary *Yohimbe* to rats and observed suppressed appetite and decreased energy level intake in same. The reduction in feed intake can be due to the presence of tannin

Parameters	Basal	Basal + 5 mg Larvacide	Basal + 60 mg Yohimbe	Basal + 120 mg Yohimbe	Basal + 180 mg Yohimbe	SEM
Pack Cell Volume (%)	33.000	32.500	33.000	32.000	33.750	0.363
Haemoglobin(g/dl)	11.000	10.725	10.850	10.675	11.250	0.133
$RBC(\times 10^{12}/L)$	3.025 ^a	2.700 ^a	2.675 ^a	2.675 ^a	3.025 ^a	0.053
WBC ($\times 10^{9}/L$)	11.125 ^a	13.025 ^a	11.225 ^a	13.500 ^a	14.325 ^a	0.444
Heterophils (%)	31.500 ^a	33.500 ^a	39.250 ^a	34.750 ^a	35.500 ^a	0.881
Lymphocyte (%)	67.250 ^a	65.250 ^a	59.750 ^a	64.750 ^a	63.000 ^a	0.900
Eosinophils (%)	0.250 ^a	0.500 ^a	0.750 ^a	0.000 ^a	0.750 ^a	0.101
Basophiles (%)	0.500	0.500	0.250	0.250	0.750	0.095
Monocytes (%)	0.500 ^a	0.250 ^a	0.000^{a}	0.250 ^a	0.000^{a}	0.060
Mean corpuscular volume (Fl)	112.125 ^a	120.700 ^a	123.650 ^a	119.600 ^a	112.625 ^a	1.531
Mean corpuscular haemoglobin (pg)	37.375 ^a	39.875 ^a	40.625 ^a	39.900 ^a	37.525 ^a	0.496
Mean corpuscular haemoglobin						
concentration (g/dl)	33.300 ^a	33.000 ^a	32.825 ^a	33.325 ^ª	33.300 ^a	0.069

SEM = Standard Error of Mean.

g/dl = gram/decilitre.

L = Litre.

fl = femtolitre.

pg = picograms.

Table 8

Effect of supplemented finisher diets on Haematological Indices of broiler chickens.

Parameters	Basal	Basal + 5 mg Larvacide	Basal+ 60 mg Yohimbe	Basal+ 120 mg Yohimbe	Basal + 180 mg Yohimbe	SEM
PCV (%)	34.000 ^a	34.000 ^a	29.500 ^a	33.750 ^a	38.000 ^a	0.941
Haemoglobin(g/dl)	11.450 ^a	11.400 ^a	9.825 ^a	11.575 ^a	12.475 ^a	0.316
$RBC(\times 10^{12}/L)$	3.150	2.950	2.650	2.900	3.200	0.093
WBC ($\times 10^9$ /L)	10.275^{a}	11.650 ^a	11.850 ^a	9.975 ^a	10.700 ^a	0.236
Heterophils (%)	39.250 ^a	36.500 ^a	30.250 ^a	29.500 ^a	33.500 ^a	1.201
Lymphocyte (%)	60.250 ^a	62.500 ^a	68.250 ^a	69.500 ^a	65.000 ^a	1.135
Eosinophils (%)	0.000 ^a	0.750 ^a	0.250 ^a	0.250 ^a	0.250 ^a	0.081
Basophils (%)	0.250 ^a	0.250 ^a	0.750 ^a	0.000 ^a	0.750 ^a	0.093
Monocytes (%)	0.250 ^a	0.000 ^a	0.500 ^a	1.000 ^a	0.500 ^a	0.104
Mean corpuscular volume (fl)	109.650	115.775	113.175	117.425	118.775	1.605
Mean corpuscular haemoglobin (pg)	36.922 ^a	38.947 ^a	37.887 ^a	40.892 ^a	39.022 ^a	0.537
Mean corpuscular haemoglobin concentration (g/dl)	33.720	33.625	33.452	34.327	32.912	0.264

SEM = Standard Error of Mean.

g/dl = gram/decilitre.

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L = Litre.
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fl = femtolitre.
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pg = pictograms.

Means on the same row with different superscripts differ significantly (p < 0.05).

in the composition of Yohimbe as reported by Tam, Worcel and Wyllie (2001). The presence of tannin reduces feed intake in livestock generally. In addition, the reduction of Feed Conversion Ratio (FCR) despite the decline in feed intake can be due to the ability of Yohimbe to stimulate the production of digestive secretions as reported by Bagheri, Schmit, Barisn, & Montastruc (1997) who observed salivary stimulation when extracted Yohimbine was supplied orally to animals and humans. Furthermore, there were reductions in the mortality rates in broiler chickens. This can be due to the phytobiotic properties of alkaloids present in Yohimbe as reported by Morel et al. (2005). The presence of phytobiotics helps to maintain chickens health and protects against subclinical infections.

At the starter phase, the values of Serum biochemical indices recorded were within range with those reported by Adeleye et al. (2018) who evaluated the serum chemistry of broiler chickens at varying intervals of post hatch feeding although, uric acid and creatinine values were slightly higher. There was increase in total protein and globulin of broiler chickens fed dietary supplementation of Yohimbe in the starter ration. This agrees with the findings of Musa (2007) who supplemented Wista rat with Yohimbe. However, Yohimbe reduced blood Albumin, ALT, Uric acid, and creatinine; while Larvacide increased Uric acid levels when supplemented in the starter diet. The increase in the uric acid level due to Larvacide supplementation shows a level of protein

Table 9

wastage in the animal body (Fafiolu, 2007). Furthermore, the blood lipid profile was improved with the supplementation of Yohimbe as the VLDL and triglyceride were reduced with the inclusion of Yohimbe while HDL was raised at the same level of inclusion. However Larvacide reduced HDL in broiler chickens when supplemented in their diet. The increase in the HDL of broiler chickens fed Yohimbe supplements is traceable to its ability to increase plasma concentration of free fatty acids as reported by Galitzky et al. (1988).

The reduction in the HDL, VLDL, Triglyceride, and cholesterol in chickens fed finisher diet with Yohimbe supplement were contrary to the findings of Kucio, Jonderko and Piskorska (1991) who reported increase in blood free fatty acid concentration after the administration of yohimbine and also contradicts the finding of Sax (1991) who reported yohimbine had no effect on body fat and cholesterol levels. The contrast can be due to the physiological condition of the animal when the samples were collected; as they reported blood samples were collected during and after a physical exercise while samples were collected when chickens were not subjected to any physical stressor. The absence of stressors means lower adrenaline secretion. Low adrenaline secretion does not encourage the compensatory mobilization of body fat into the blood for energy metabolism which happens when there is a stressor (Galitzy et al., 1988).

The Haematological indices of chickens collected at the starter

Parameters	Basal	Basal + 5 mg Larvacide	Basal + 60 mg Yohimbe	Basal + 120 mg Yohimbe	Basal + 180 mg Yohimbe	SEM
		Ŭ				
Duodenum						
Villi Height (µm)	969.10 ^a	975.87 ^a	584.08 ^a	705.11 ^a	617.54 ^a	45.362
Crypt depth(µm)	67.07 ^a	128.55 ^a	167.48 ^a	187.92 ^a	115.10 ^a	11.271
Apical width (µm)	319.51 ^a	229.50 ^a	183.10 ^a	143.49 ^a	238.53 ^a	15.815
Basal width (µm)	128.50^{a}	109.52 ^a	91.53 ^a	94.04 ^a	68.10 ^a	5.364
Jejunum						
Villi Height (µm)	1001.46 ^a	874.56 ^a	564.45 ^a	50.90 ^a	1333.93 ^a	115.989
Crypt depth (µm)	113.04 ^a	166.96 ^a	156.50 ^a	8.49 ^a	124.96 ^a	15.054
Apical width (µm)	145.50 ^a	134.53 ^a	203.46 ^a	9.46 ^a	162.48 ^a	17.418
Basal width (µm)	61.50 ^a	72.48 ^a	158.48 ^a	3.47 ^a	74.89 ^a	13.239
Ileum						
Villi Height (µm)	561.07 ^a	828.55 ^a	820.94 ^a	1045.05 ^a	735.08 ^a	41.848
Crypt depth (µm)	83.57 ^a	119.92 ^a	213.94 ^a	179.14 ^a	112.05 ^a	12.735
Apical width (µm)	97.89 ^a	136.92 ^a	179.95 ^a	213.12 ^a	155.91 ^a	10.402
Basal width (µm)	75.11 ^a	81.08 ^a	109.95 ^a	113.46 ^a	93.54 ^ª	4.063

SEM = Standard Error of Mean.

 $\mu m = micrometre.$

phase were within range compared with the values reported by Talebi, Asri-Rezaei, Rozeh-Chai and Sahraei (2005). There was improvement in the haematological indices of chicken fed diets containing Yohimbe supplements. RBC, MCV, MCH, and MCHC were increased with Yohimbe supplementation. The PCV, Haemoglobin, MCH, MCHC are indicators for assessing the circulatory electrolytes (Peters, Gunn, Imumorin, Agaviezor & Ikeobi, 2011). It is suggested by Chineke, Ologun and Ikeobi (2006) that high PCV implies increase in RBC production and the values of MCH and MCHC are preferred to be high as low level implies anaemia (Aster, 2004). The improvement in the haematological indices of chickens fed Yohimbe supplemented diets is traceable to its' ability to stimulate the production of Red Blood Cells as it is reportedly used as blood tonic in western African medicine (Clark & Sunderland, 2004). In addition, there were increase in the White Blood Cell (WBC) counts and their differentials with the supplementation of Yohimbe in chickens diets. The White Blood Cells (WBC) and its differentials are responsible for the combat of disease infections. They transport antibodies during immune response. Animals with low WBC are susceptible to disease infections which makes them less adaptable to some environments (Soetan, Akinrinde & Ajibade, 2013). The high values of WBC, Heterophyls, Eosinophils of chickens fed Yohimbe supplement contradicts the findings of Musa (2007), who reported reduction in the WBC of rat with the inclusion of Yohimbe. The contradiction could be due to the specie of animal used in the two studies. The increase in the WBC with Yohimbe supplementation shows its immune stimulatory properties (Vidanarachchi, Mikkelsen, Sims, Iji & Choct, 2006). However, the Lymphocytes and monocytes were reduced in chickens fed Yohimbe supplemented diet. The decline in Lymphocytes and monocytes were also reported by Musa (2007) who supplemented Yohimbe in rat. However, dietary Larvacide supplementation reduced the heteropils of broiler chickens when administered in the starter diet.

The increase in PCV, Haemoglobin and MCH, with the supplementation of Yohimbe in the finisher diet shows that the supplement enhances the production of circulatory electrolyte. This agrees with the findings of Kuhlmann (1999) who reported Yohimbe bark extract was originally used in Africa tropics as stimulant and a tonic for men. Also the WBC differentials Lymphocyte, Basophils, Monocytes, increased with the supplementation of Yohimbe. This is traceable to the immunostimulatory properties of the herb (Vidanarachchi et al., 2006) while the Heterophils, Eosinophils reduced with the supplementation of Yohimbe. However, WBC increased at low inclusion rate of Yohimbe but declined as the inclusion rate increased.

The supplementation of chickens feed with Yohimbe affected the intestinal morphometric measurement at the duodenum, ileum, and jejunum. The villi height and crypt dept indicates intestinal prolificative and absorptive capacity(Lenhardt and Mozeš, 2003) and it shows how developed are the intestinal components (Franco, Murakami, Natali, Garcia & Furlan, 2006). The improvement of the crypt dept of chickens fed supplemented *Yohimbe* at the duodenum and jejunum shows the supplement encourages fast intestinal cell renewal and maintains the natural proximodistal decline of the morphometric indices from the duodenum to the ileum (Biasato et al., 2017). This also shows improved secretion of digestive enzymes as most digestive enzymes are secreted in the jejunum (Iji, Saki & Tivey, 2001). However, the improvement of the villi height, apical and basal width at the jejunum and ileum but not at the duodenum in chicken fed *Yohimbe* shows nutrient absorption occurs most at the jejunum and ileum.

5. Conclusion

This study concluded that chickens fed Basal diet + 180 mgYohimbe performs better than chickens fed diets with Larvacide and lower levels of Yohimbe inclusion. Also, chickens fed Basal diet + 180 mg Yohimbe had better blood profile and highest villi height compared to chickens fed with other diets. Consequently, this study recommends that the increased inclusion of Yohimbe in chickens diet positively influence its' growth performance and health status, and gut morphometry of chickens over larvacide.

Ethics statement

The following conditions were met by the investigators:

- 1 All phytogenics must be processed in Animal nutrition laboratory. Be sure you ensure proper bio-safety and biosecurity in Animal Production and Health laboratory
- 2 All un-used/improperly processed phytogenics must not be used for the study. Final samples of the processed phytogenics must be made available to the committee
- 3 Principal investigator must confirm that the samples of the phytogenics will not be used in any form or way or associated with biological weapon and transferred to another institution
- 4 All animal experimentation must be conducted with adequate space and use of appropriate methodology that will avoid pain to the animal will strictly be adhered to.
- 5 Your experimental station shall be open to committee's inspection whenever it is required
- 6 This permission is valid for 24 months starting from the date of start of the experiment and it is subject to cancellation should conditions necessitating doing so arise.

Declaration of Competing Interest

Author declares there is no conflict of interest of any form on the journal article

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