Archivos de Zootecnia
Journal website: https://www.uco.es/ucopress/az/index.php/az/

Growth response, organ development and blood indices of growing pullets administered aqueous extracts of *Petiveria alliacea*

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Additional keywords
Petiveria alliacea.
Growing pullets.
Poultry birds.
Herbal medication.

SUMMARY

A total of 288 eight-weeks-old Isa brown pullets arranged in a 2 × 4 factorial experimental layout were used to evaluate the growth response, organ development, haematology and serum biochemistry of growing pullets administered aqueous extract of Petiveria alliacea. After balancing for weight the birds were allotted into 8 treatments comprising pullets administered aqueous root extract at 4 concentrations (0, 15, 30 and 45 g/l) and aqueous leaf extract at 4 concentrations (0, 15, 30 and 45 g/l). Each treatment was replicated 3 times with 12 birds per replicate. Phytochemical analysis showed that Alkaloid, Phenolics, Antioxidant, Tannin, Phytate, Steroid, Anthraquinone and Oxalate concentrations were higher (P<0.05) in the root while Saponin, Flavonoid, Terpenoid and Carotenoid concentrations were higher (P<0.05) in the leaf. No difference (P>0.05) was recorded for growth performance of the growing pullets. Highest (P<0.05) weight for small intestine was recorded in control treatment (3.29%). Highest (P<0.05) caeca weight was recorded in birds maintained on 45 g/l concentration of leaf extract (0.89%). Birds administered 30 g/l leaf extract had the highest (P<0.05) proventriculus weight (0.56%). Birds maintained on 15 and 30 g/l root extract had higher (P<0.05) values for Red blood cell count compared to other treatments. Highest (P<0.05) white blood cell count was recorded at 45 g/l concentration of root extract. Highest (P<0.05) serum total protein was recorded in birds administered 45 g/l leaf extract (6.00 g/dl). Serum uric acid and cholesterol reduced to the lowest (P<0.05) in birds administered 45 g/l root extract. The study concluded that administration of aqueous extract of *Petiveria alliacea* root at 45 g/l concentration best improved health status of growing pullets without impairing growth.

Palabras clave
Petiveria alliacea.
Pollitas en crecimiento.
Aves de corral.
Medicamentos a base de hierbas.

Respuesta de crecimiento, desarrollo de órganos e índices sanguíneos de pollitas en crecimiento con extractos acuosos de *Petiveria alliacea*

RESUMEN

Se utilizaron un total de 288 pollitas marrones Isa de ocho semanas de edad dispuestas en un diseño experimental factorial 2 × 4 para evaluar la respuesta al crecimiento, el desarrollo de los órganos, la hematología y la bioquímica sérica de las pollitas en crecimiento administradas con extracto acuoso de Petiveria alliacea. Después de equilibrar el peso, las aves se asignaron a 8 tratamientos que comprendían aves administradas con extracto de raíz acuosa a 4 niveles de concentración (0, 15, 30 y 45 g / litro) y extracto de hoja acuosa a 4 niveles de concentración (0, 15, 30 y 45 g /litro). Cada tratamiento se repitió 3 veces con 12 aves por réplica. El examen fitoquímico mostró que las concentraciones de alcaloides, fenólicos, antioxidantes, taninos, fitatos, esteroides, antraquinonas y oxalatos fueron más altas (P <0.05) en la raíz, mientras que las concentraciones de saponina, flavonoides, terpenoides y carotenoides fueron más altas (P <0.05) en la hoja. No se registraron diferencias (P> 0.05) para el rendimiento de crecimiento de las pollitas en crecimiento. El peso del intestino delgado fue mayor (P <0.05) en el tratamiento de control (3.29%). El peso de Caeca fue más alto (P <0.05) en aves mantenidas con una concentración de extracto de hoja de 45 g / litro (0.89%). Las aves a las que se administró extracto de hoja de 30 had / litro tuvieron el mayor peso de proventrículo (P <0.05) (0.56%). Las aves mantenidas con 15 y 30 g / litro de extracto de raíz tuvieron valores de recuento de glóbulos rojos más altos (P <0.05) en comparación con otros tratamientos. El recuento de glóbulos blancos fue más alto a 45 g / litro de concentración de extracto de raíz. La proteína total en suero más alta (P <0.05) se registró en aves a las que se administró extracto de hoja de 45 g / litro (6.00 g / dl). El ácido úrico y el colesterol en suero se redujeron al nivel más bajo en las aves mantenidas con extracto de raíz de 45 g / litro. El estudio concluyó que la administración de extracto acuoso de raíz de *Petiveria alliacea* a un nivel de concentración de 45 g / litro mejoró el estado de salud de las pollitas en crecimiento sin afectar el crecimiento.

Information

Cronología del artículo.
Recibido/Received: 23.01.2020
Aceptado/Accepted: 28.03.2020
On-line: 15.07.2020
Correspondencia a los autores/Contact e-mail:
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INTRODUCTION

A common segment of the Nigerian poultry industry is commercial egg-type chicken production. Commercial egg-type chickens are capable of producing averagely between 250-300 eggs/bird/year (FAO, 2003), this was achieved through improved management practices, high quality diets and genetic modification (Alabi & Samuel 2002, p.317). Due to rise in human population, there is increasing demand for eggs

as a substantial source of protein, vitamins and lipids of high nutritious value (Bamiro et al. 2006, p. 1164). To meet this ever increasing demand, there is need to identify techniques of improving growth and health status of poultry birds. Performance and health quality of pullets during growing period was noted to have symbolic effect on overall laying performance of the hen (Olawumi 2011, p. 1062), therefore improvement in growth and health condition at this period is of great importance.

Antibiotic growth promoters had been used widely in poultry production to enhance overall performance and productivity. However, use of antibiotic growth promoters has received great criticism due to emergence of antibiotic resistant microorganisms and its hazardous effect on consumer health which led to its restriction (Castanon 2007, p. 2469; Diarra and Malouin 2014, p. 2; Dhama et al. 2015, p. 152). Use of phytogenic materials is gaining recognition as a viable replacement for antibiotics due to their effectiveness, lower toxicity risk and environmental friendliness (Devegowda, 1996). Recent studies on phytogenic materials have revealed the potential of herbal plants to improve overall performance and health status of poultry birds (Jahan et al. 2008, p. 326; Rehman et al. 2011, p. 12; Oni et al. 2018, p. 77).

Petiveria alliacea belongs to the family Phytolaccaceae. It is native to the Caribbean, North, Central and South America with introduced population in Nigeria (Randle et al. 2018, p. 181). Various parts of the plant have been found useful in folk medicine for treating numerous ailments (Randle et al. 2018, p. 182). Likewise, scientific studies have confirmed its antibiotic (Kim et al. 2006, p. 191; Ekunseitan et al. 2016, p. 293), anti-inflammatory, anti-cancerous (Williams et al. 2007, p. 20; Lopes-Martins et al. 2002, p. 247) and Immunomodulatory (Santander et al. 2012, pp. 837-842) activities. Few studies conducted on *Petiveria alliacea* investigated the effect of dried and processed parts on poultry species (Sobayo et al. 2017, pp. 144-156; Sobayo et al. 2018a, pp. 299-311; 2018b, pp. 53-71; Muhammad et al. 2019, pp. 524-533; Odetola et al. 2019, pp. 45-50). The effect of fresh root and leaf of *Petiveria alliacea* on poultry species is yet to be documented. Therefore, this study seek to determine the growth response, organ development and blood indices of growing pullets administered aqueous extracts of fresh *Petiveria alliacea* root and leaf.

MATERIALS AND METHODS

Location of the Study

The study was conducted at the Poultry Unit of Livelihoods Support and Development Centre (SLIDEN AFRICA), Alabata road, Abeokuta, Ogun State, Nigeria. The site is located in the rainforest vegetation zone of South-Western Nigeria on Latitude 7° 13'29.01" N and longitude 3° 25' 26.40" E. Elevation 414ft above the sea level and eye altitude 1245ft. (Google Earth, 2020).

Sourcing and extraction of *Petiveria alliacea*

Fresh roots and leaves of *Petiveria alliacea* were sourced from Kotopo area of Abeokuta, Ogun State, Nigeria. Extraction was done according to the method described by Nodu *et al.* (2016, p. 1881). The freshly harvested roots and leaves were rinsed with water to remove sand and other dirt. Thereafter, 15 g, 30 g and 45 g of roots and leaves were weighed separately and blended individually in 1 litre of clean water. The blended mixture of each weighed sample of root or leaf was filtered using appropriate sieve. The filtrates were presented to birds as drinking water according to treatment. At all periods of administration, the volume of filtrates presented to birds was enough to meet and exceed daily water requirement of the pullets.

Management of experimental birds

A total of 288 eight-weeks-old Isa brown pullets were used for the study. The pullets were obtained from a reputable hatchery in Ibadan, Oyo State, Nigeria. The pullets were divided equally into two groups. Birds in the first group was administered aqueous extract of *Petiveria alliacea* root at 0 **g/l** (Control), 15**g/l**, 30 **g/l** and 45 **g/l concentrations** while the second group was administered aqueous extract of *Petiveria alliacea* leaf at 0 g/l (Control), 15 g/l, 30 **g/l** and 45 **g/l concentration**s. The experiment was laid out in 2 × 4 factorial arrangement, consisting of 8 experimental treatments replicated three times each with 12 birds per replicate. The experiment lasted between 8 and 18 weeks of age. Prepared aqueous extract of *Petiveria alliacea* root or leaf at the specified concentrations were presented to pullets in each treatment as drinking water on two consecutive days per week throughout the experiment. All treatments receiving aqueous extract of root or leaf of *Petiveria alliacea* were free of antibiotic drugs. Experimental birds were raised in a deep litter system and fed *ad libitum* on a formulated diet in mash form (**Table I**).

Data collection

Phytochemical analysis of *Petiveria alliacea* root and leaf

The root and leaf samples were washed, air dried at room temperature and then pounded to homogenous powder. 100 g each of powdered root and leaf were soaked in 300 ml of distilled water at room temperature for 2 days. The suspensions were filtered using a Whatman's No.1 filter paper, after which the filtrates were evaporated to dryness in a water bath at 40°C. Further test for phytochemical composition of major constituents was carried out as outlined by Harborne (1973, p. 279), Sofowora (1993, p. 97) and Evans (2000, p. 224).

Performance characteristics

Data were collected on initial body weight, feed intake, water intake, body weight gain, final weight, feed conversion ratio and mortality rate.

Determination of Haematological and blood biochemical indices

Blood collection and evaluation

At 18 weeks of age, two birds having similar weight with the replicates mean weight were selected from each replicate of all treatment. Two millilitre (2 ml)

of blood was drawn twice from the brachial vein of each selected bird into two separate labelled bottles for haematological and serum biochemistry examination. Blood samples for haematological examination were collected into bottles pre-treated with anticoagulant (Ethylene Diamine Tetra Acetate). Red blood cell (RBC) and White blood cell (WBC) counts were determined using Neubaur chamber method as described by Lamb (1981). PCV was determined using haematocrit reader as outlined by Benson *et al.* (1989). Haemoglobin was estimated using cyanomethaemoglobin method as described by (Dayyal, 2016). Blood samples for serum biochemical indices were collected into plain bottles and then centrifuged at 3,000 rpm, the sera were decanted into another labelled sample tubes. Serum total protein was determined by Biuret method (Reinhold, 1953) using a commercial kit (Randox Laboratories Ltd, U.K). Albumin and globulin values were determined using bromocresol green method as described by Doumas and Biggs (1971, p.175). Serum creatinine and urea nitrogen were estimated by de-proteinisation and Urease-Berhelot colorimetric methods, using a commercial kit (Randox Laboratories Ltd, U.K). Serum cholesterol was determined using a commercial kit (Quimica Clinica Applicada, S.A).

Intestinal organ weight

Table I. Percentage Nutrient and Chemical composition of experimental diet (Porcentaje de composición nutritiva y química de la dieta experimental).

Ingredients	(%)
Maize	40.00
Soya bean meal	5.00
Fish meal (72% CP)	1.50
Groundnut cake	5.00
Palm kernel cake	18.50
Wheat offal	26.80
Oyster shell	1.00
Bone meal	1.50
Lysine	0.10
Methionine	0.10
Common salt	0.25
Vit./Min. Premix	0.25
Total	100.00
Chemical Composition (%)	
Dry matter (%)	88.90
Crude Protein (%)	15.50
Crude Fibre (%)	7.50
Ether extracts (%)	3.45
Ash (%)	6.22
Metabolisable energy (Kcal/kg)	2,750.00

Vit./Min. Premix (Composition per kg diet): B1, B2, B6, 0.02g; K3; 3g; E, 30g; biotin, 0.05; folic acid, 1.5g, choline chloride, 250g; nicotinic acid, 30g; Ca- Pantothenate, 15g; Co, 0.4g; Cu, 8g; Fe, 32g I, 0.8g; Zn,40g; Mn, 64g; Se 0.16g, Butylated hydroxytoluene, 50.00g.

At 18 weeks of age, two birds having similar body weight with the replicate's mean weight were selected from each replicate of all treatment and slaughtered. The gastrointestinal organs were carefully separated from the carcass. Weights of the oesophagus, crop, proventriculus, gizzard, small intestine, large intestine, ceaca and liver were determined using an electronic weighing scale and expressed as a percentage of the live bodyweight.

Statistical analysis

Date obtained from phytochemical screening of *Petiveria alliacea* root and leaf were subjected to studentized t-test. Other data collected were arranged in a 2 × 4 factorial experimental layout in a completely randomized design. The significant ($p<0.05$) differences among each treatment means were separated using Duncan's Multiple Range Test as contained in SAS 2010 statistical package.

RESULTS

Concentrations of phytochemicals identified in *Petiveria alliacea* root and leaf are presented in **Table II**. Result showed that Alkaloid, Polyphenols, Tannin, Phytate and Oxalate were present in the root at higher ($P<0.05$) concentration compared to leaf while Saponin, Flavonoid and Terpenes concentrations were higher ($P<0.05$) in leaf compared to root.

The main effects of aqueous extract of different parts (root and leaf) and concentration of extraction of *Petiveria alliacea* on growth performance of growing pullets between 8-18 weeks of age are presented in **Table III**. Administration of aqueous extract of different parts of *Petiveria alliacea* had no significant ($P>0.05$) influence on the examined performance parameters. Similarly, concentration of extraction did not have any significant ($P>0.05$) effect on all growth performance indices assessed.

The interactive effect of aqueous extract of different parts (root and leaf) and concentration of extraction of *Petiveria alliacea* on growth performance of grow-

Table II. Phytochemical analysis of root and leaf of *Petiveria alliacea* (Análisis fitoquímico de la raíz y la hoja de Petiveria alliacea).

Parameters (mg/100g)	Root	Leaf
Saponin	96.66^{b}±0.88	388.66^{a}±0.88
Alkaloid	760.00^{a}±0.58	549.33^{b} ±0.88
Flavonoid	263.33^{b}±0.88	822.00^{a}±0.58
Terpenoid	189.33^{b}±0.88	229.00^{a}±0.58
Tannin	943.00^{a}±1.15	788.67^{b}±1.2
Phytate	190.67^{a}±1.2	152.00^{b}±1.15
Oxalate	1261.33^{a}±0.88	1138.00^{b}±0.58
Phenolics (GAE/g)	567.67^{a}±0.88	423.00^{b}±1.15

$^{a,b:}$ Means in the same row not sharing common superscript are significantly ($P<0.05$) different.; GAE: Gallic Acid Equivalent.

ing pullets between 8-18 weeks of age is presented in **Table IV**. Statistically similar values were recorded for all performance parameters evaluated and were not significantly (P>0.05) influenced by the interaction.

The main effects of aqueous extract of different parts (root and leaf) and concentration of extraction of *Petiveria alliacea* on haematological indices and some serum metabolites of growing pullet at 18 weeks of age is presented in **Table VI**. Haemoglobin value was

Table III. Main effects of aqueous extract of different parts (root and leaf) and concentration of extraction of *Petiveria alliacea* on growth performance of growing pullets between 8-18 weeks of age (Principales efectos del extracto acuoso de diferentes partes (raíz y hoja) y concentración de extracción de Petiveria alliacea en el rendimiento de crecimiento de pollitas en crecimiento entre 8-18 semanas de edad**).**

	Plant Part			Concentration of extraction				
	Root	Leaf	SEM	0 g/l	15 g/l	30 g/l	45 g/l	SEM
Parameter								
Initial weight (g/bird)	412.90	412.74	1.23	410.20	415.47	413.31	412.29	1.57
Final weight (g/bird)	1122.72	1117.31	13.57	1108.43	1108.69	1107.49	1155.45	16.49
Total weight gain (g/bird)	709.82	704.57	13.74	698.24	693.22	694.19	743.15	16.50
Weight gain (g/bird/day)	10.14	10.06	0.20	9.97	9.90	9.92	10.62	0.24
Total feed intake (g/bird)	4050.93	4044.08	5.83	4035.67	4049.03	4047.64	4057.70	7.44
Average feed intake (g/bird/day)	57.87	57.77	0.08	57.65	57.84	57.82	57.97	0.11
Feed conversion ratio	5.71	5.74	0.11	5.78	5.84	5.83	5.46	0.13
Total water intake (ml/bird)	14693.90	14272.90	353.95	14709.40	14856.40	13743.00	14624.80	479.67
Average water intake (ml/bird/day)	209.91	203.90	5.06	210.13	212.23	196.33	208.93	6.85
Mortality (%)	0.69	0.00	0.35	0.00	0.00	1.38	0.00	0.35

SEM: Standard error of mean

serum metabolites of growing pullets at 18 weeks of age are presented in **Table V**. Values recorded for PCV, RBC and WBC were significantly (P<0.05) higher in birds administered aqueous root extract compared to birds maintained on leaf extract. Blood uric acid and cholesterol levels were significantly (P<0.05) lower in pullets maintained on aqueous root extract (2.53 and 92.51 mg/dl respectively) compared with leaf extract (3.64 and 99.10 mg/dl respectively). White blood cell count increased significantly (P<0.05) from 10.03×10^9/l to 12.98×10^9/l as concentration of extraction increased. Similarly, lymphocyte differential increased as concentration of extraction increased. Heterophil differential decreased significantly (P<0.05) from 33.17 % to 27.17 % as concentration of extraction increased. Values for serum total protein and globulin were significantly (P<0.05) higher in pullets administered 45 g/l concentration (5.17 and 2.28 g/dl respectively) compared with other treatments.

Serum uric acid level decreased from 4.43 mg/dl to 2.13 mg/dl as concentration of extraction increased. Similarly, blood cholesterol level decreased significantly (P<0.05) with increase in concentration of extraction.

The interactive effect of aqueous extract of different parts (root and leaf) and concentration of extraction of *Petiveria alliacea* on haematological indices and some serum metabolites of growing pullet at 18 weeks of age is presented in **Table VI**. Haemoglobin value was significantly (P<0.05) higher in pullets administered 15 g/l concentration of root extract (10.53 g/dl) compared with other treatments. Red blood cell count was significantly (P<0.05) higher in pullets administered 15 and 30 g/l concentration of root extract (2.83×10^{12}/l and 2.77×10^{12}/l respectively) compared with other treatments. Highest (P<0.05) white blood cell count and lymphocyte differential was recorded in pullets maintained on 45 g/l root extract (13.17×10^9/l and 71 % respectively). Heterophil differential was higher (P<0.05) in control treatment compared with other treatments. The highest (P<0.05) values for serum total protein and globulin were recorded in pullets administered 45 g/l concentration of leaf extract (6.00 and 3.10 g/dl respectively). Blood uric acid decreased (P<0.05) as concentration of extraction increased for both groups administered root or leaf extract. The lowest (P<0.05) blood uric acid level (1.63 mg/dl) was recorded at 45 g/l concentration of root extract.

The lowest (P<0.05) blood cholesterol level (63.27 mg/dl) was recorded in pullets administered 45 g/l concentration of root extract.

Table IV. Interactive effect of aqueous extract of different parts (root and leaf) and concentration of extraction of *Petiveria alliacea* on growth performance of growing pullets between 8-18 weeks of age (Efecto interactivo del extracto acuoso de diferentes partes (raíz y hoja) y concentración de extracción de Petiveria alliacea en el rendimiento de crecimiento de pollitas en crecimiento entre 8-18 semanas de edad**).**

Plant part	Root				Leaf				
Concentration of extraction	0 g/l	15 g/l	30 g/l	45 g/l	0 g/l	15 g/l	30 g/l	45 g/l	SEM
Parameters									
Initial weight (g/bird)	409.00	415.55	414.39	412.64	411.39	415.39	412.22	411.94	2.37
Final weight (g/bird)	1095.06	1098.55	1138.33	1158.94	1121.80	1118.82	1076.65	1151.96	18.76
Total weight gain (g/bird)	686.06	683.00	723.94	746.30	710.41	703.43	664.43	740.01	19.11
Weight gain (g/bird/day)	9.80	9.76	10.34	10.66	10.15	10.05	9.49	10.57	0.27
Total feed intake (g/bird)	4045.55	4048.33	4045.56	4064.28	4025.78	4049.72	4049.72	4051.11	9.33
Average feed intake (g/bird/day)	57.79	57.83	57.79	58.06	57.51	57.85	57.85	57.87	0.13
Feed conversion ratio	5.90	5.93	5.59	5.45	5.57	5.76	6.10	5.47	0.15
Total water intake (ml/bird)	15193.00	15352.00	13369.00	14862.00	14226.00	14361.00	14117.00	14387.00	625.57
Average water intake (ml/bird/day)	217.04	219.31	190.99	212.32	203.23	205.16	201.68	205.53	8.94
Mortality (%)	0.00	0.00	2.77	0.00	0.00	0.00	0.00	0.00	0.35

SEM: Standard error of mean

The main effects of aqueous extract of different parts (root and leaf) and concentration of extraction of *Petiveria alliacea* on intestinal organ weights of growing pullets at 18 weeks of age are presented in **Table VII**. The lowest (P<0.05) proventriculus weight was recorded in the control treatment (0.49 %) while the highest (P<0.05) weight was recorded in birds administered 30 g/l and 45 g/l concentrations (0.54 %). Weight of small intestine decreased (P<0.05) from 3.29 % in control treatment to 2.58 % in pullets maintained on 45 g/l concentration of extraction. Moreover, caeca weight was significantly (P<0.05) lower in the control treatment compared with pullets maintained on all tested concentration of extraction of *Petiveria alliacea*.

The interactive effect of aqueous extract of different parts (root and leaf) and concentration of extraction of *Petiveria alliacea* on intestinal organ weights of growing pullets at 18 weeks of age is presented in **Table VIII**. The highest (P<0.05) proventriculus weight was recorded in pullets maintained on 30 g/l concentration of leaf extract (0.56%). Highest (P<0.05) caeca weight was recorded in pullets administered 45 g/l concentration of leaf extract (0.89%).

DISCUSSION

Phytochemicals identified in root and leaf of *Petiveria alliacea* were similar to those reported by Ekunseitan *et al.* (2016, p. 292) and Adesipo *et al.* (2017, p. 26). However, differences in quantitative amounts found may be attributed to variations in harvest environment and certain agronomical factors (Rehman & Munir 2015, p. 32). The identified phytochemicals have been reported as analgesic, stimulant, antioxidant, anti-inflammatory, anti-carcinogenic, hepatoprotective, antibiotic and immune-stimulating agents (Ayodele et al. 2015, p. 2). The presence of higher concentrations of most identified phytochemicals in root compared to leaf suggested that root of *Petiveria alliacea* may have superior beneficial properties compared to leaf.

Similar growth performance recorded across the treatments in this study indicated that aqueous extract of *Petiveria alliacea* root and leaf at all tested concentrations neither suppressed nor enhanced growth of pullets. This suggested that the extracts administered consists majorly phytochemicals which were reported to be non-nutritive bioactive compounds (Liu 2013, p. 3855) and may not have a direct effect on growth. It could also be said that anti-nutritional agents such as tannins, phytates, saponins and oxalates found in root and leaves of the plant are present in minimal amount at all tested concentrations. Similar result was reported by Odetola et al. (2019, p. 47) where all growth performance parameters examined were similar in broiler chickens fed diets supplemented with graded level of *Petiveria alliacea* root meal and un-supplemented diet. However, Sobayo et al. (2018b, p. 59) reported that diet supplementation with 1000mg *Petiveria alliacea* leaf meal per kilogram feed enhanced growth performance of growing pullets. The difference in the results suggested that apart from the phytochemicals present, the whole leaf meal incorporated in their study might have added to the nutritive value of the diet against aqueous extract used in this study.

Elevation in PCV and RBC of pullets administered aqueous root extract suggested that the root extract enhanced erythropoiesis. Rise in haemoglobin level indicated efficient oxygen supply to body tissues in pullets maintained on 15g/l root extract. This coincides with the report of Sobayo et al. (2018b, p. 62) who found higher haemoglobin in blood of growing pullets fed diet supplemented with *Petiveria alliacea* root meal at lower levels (1000, 1500 and 2000 mg/kg) compared to a higher level (2500 mg/kg). The concentration dependent increase in white blood cell count and lymphocyte differential in pullets administered aqueous extract of *Petiveria alliacea* root or leaf suggested enhancement in immune system. The observed immune stimulation can be attributed to Dibenzyl trisulphide earlier reported present in the plant and identified to be responsible for its immunomodulatory ability (Quadros et al. 1999, p. 118; Rosner et al. 2001, p. 170). Moreover, Randle et al. (2018, p. 181) reported that water extract of *Petiveria alliacea* enhanced lymphocyte, interferon and interleukin production. This result is identical to that of Sobayo et al. (2018a, p. 303), they also reported an increase in lymphocyte count in broiler chicks fed 500 and 1000 ppm dietary inclusion of *Petiveria alliacea* meal. However, result of Sobayo et al. (2018b, p. 62) showed lower WBC count in growing pullets fed diets containing *Petiveria alliacea* leaf meal at 1000, 1500 and 2000 mg/kg feed and those fed root meal at 1000, 1500 and 2500 mg/kg feed when compared with control birds. Differences in these results could be attributed to processing methods used which may affect the stability of bioactive compounds in the plant (Poojary et al. 2017, p. 30).

Ability of root extract to better enhance white blood cell production compared to leaf extract could be because many of the identified phytochemicals were found at higher concentrations in the root.

Similar observation was made by Sobayo et al. (2018a, p. 303) when they investigated the effect of *Petiveria alliacea* root and leaf meal on broiler chickens. Values for WBC count recorded in this study fell within the range (5.0-15.00×10^9/l) reported by McDonald (1996, p. 2).

Table V. Main effects of aqueous extract of different parts (root and leaf) and concentration of extraction of *Petiveria alliacea* on haematological and some serum metabolites of growing pullets at 18 weeks of age (Principales efectos del extracto acuoso de diferentes partes (raíz y hoja) y concentración de extracción de Petiveria alliacea en metabolitos hematológicos y algunos metabolitos séricos de pollitas en crecimiento a las 18 semanas de edad)**.**

	Plant Part			Concentration of extraction				
	Root	Leaf	SEM	0 g/l	15 g/l	30 g/l	45 g/l	SEM
Haematological indices								
PCV (%)	35.50^{a}	32.42^{b}	0.81	33.00	34.33	34.17	34.33	1.31
Hemoglobin (g/dl)	9.95	9.45	0.18	9.27	10.00	9.68	9.85	0.25
RBC (×10^{12}/l)	2.63^{a}	2.39^{b}	0.08	2.53	2.67	2.48	2.37	0.12
WBC (×10^{9}/l)	11.97^{a}	11.14^{b}	0.35	10.03^{d}	11.10^{c}	12.10^{b}	12.98^{a}	0.22
Heterophil (%)	30.50	30.33	0.81	33.17^{a}	31.67^{ab}	29.67^{b}	27.17^{c}	0.67
Lymphocyte (%)	67.58	66.67	0.87	63.50^{c}	66.33^{b}	68.17^{b}	70.50^{a}	0.63
Eosinophil (%)	0.67	0.83	0.15	1.00	0.50	0.67	0.83	0.18
Basophil (%)	0.83	0.75	0.17	1.17	0.83	0.67	0.50	0.23
Monocyte (%)	0.67	1.17	0.27	1.17	0.67	0.83	1.00	0.41
Serum metabolites								
Total protein (g/dl)	4.53	4.65	0.17	4.23^{b}	4.35^{b}	4.62^{b}	5.17^{a}	0.21
Albumin (g/dl)	2.87	2.86	0.06	2.78	3.00	2.80	2.88	0.08
Globulin (g/dl)	1.66	1.79	0.18	1.45^{b}	1.35^{b}	1.82^{ab}	2.28^{a}	0.23
Albumin : Globulin	1.77	2.23	0.27	2.01^{ab}	2.91^{a}	1.60^{b}	1.48^{b}	0.34
Uric acid (mg/dl)	2.53^{b}	3.64^{a}	0.30	4.43^{a}	2.98^{b}	2.80^{b}	2.13^{c}	0.33
Creatinine (mg/dl)	1.63	1.57	0.05	1.72	1.60	1.57	1.52	0.07
Glucose (mg/dl)	143.59	139.62	4.15	147.87	147.13	139.52	131.90	4.72
Cholesterol (mg/dl)	92.51^{b}	99.10^{a}	6.59	130.93^{a}	90.18^{b}	90.32^{b}	71.78^{c}	2.24

SEM: Standard error of mean; PCV: Packed Cell Volume; RBC: Red Blood Cells; WBC: White Blood Cells; $^{a,b,c,d;}$ Means in the same row not sharing common superscript by factor are significantly (P<0.05) different

Table VI. Interactive effect of aqueous extract of different parts (root and leaf) and concentration of extraction of *Petiveria alliacea* on haematological and some serum metabolites of growing pullets at 18 weeks of age (Efecto interactivo del extracto acuoso de diferentes partes (raíz y hoja) y concentración de extracción de Petiveria alliacea en metabolitos hematológicos y algunos metabolitos séricos de pollitas en crecimiento a las 18 semanas de edad).

Plant part	Root				Leaf				
Concentration of extraction	0 g/l	15 g/l	30 g/l	45 g/l	0 g/l	15 g/l	30 g/l	45 g/l	SEM
Haematological indices									
PCV (%)	34.00	36.67	37.00	34.33	32.00	32.00	31.33	34.33	1.64
Hemoglobin (g/dl)	9.27 ^{b}	10.53 ^{a}	10.17 ^{ab}	9.83 ^{ab}	9.27 ^{b}	9.47 ^{ab}	9.20 ^{b}	9.87 ^{ab}	0.28
RBC ($\times 10^{12}$/l)	2.47 ^{ab}	2.83 ^{a}	2.77 ^{a}	2.47 ^{ab}	2.60 ^{ab}	2.50 ^{ab}	2.20 ^{b}	2.27 ^{b}	0.14
WBC ($\times 10^{9}$/l)	10.10 ^{d}	11.97 ^{c}	12.63 ^{b}	13.17 ^{a}	9.97 ^{d}	10.23 ^{d}	11.57 ^{c}	12.80 ^{ab}	0.15
Heterophil (%)	33.33 ^{a}	31.00 ^{abc}	29.67 ^{bc}	28.00 ^{cd}	33.00 ^{a}	32.33 ^{ab}	29.67 ^{bc}	26.33 ^{d}	0.89
Lymphocyte (%)	63.33 ^{e}	67.00 ^{cd}	69.00 ^{abc}	71.00 ^{a}	63.67 ^{e}	65.67 ^{de}	67.33 ^{bcd}	70.00 ^{ab}	0.87
Eosinophil (%)	1.00	0.67	0.33	0.67	1.00	1.00	0.33	1.00	0.24
Basophil (%)	1.33	1.00	0.33	0.67	1.00	0.67	1.00	0.33	0.79
Monocyte (%)	1.00	0.33	0.67	0.67	1.33	1.00	1.00	1.33	0.59
Serum metabolites									
Total protein (g/dl)	4.43 ^{bcd}	4.73 ^{b}	4.63 ^{b}	4.33 ^{bcd}	4.03 ^{cd}	3.97 ^{d}	4.60 ^{bc}	6.00 ^{a}	0.17
Albumin (g/dl)	2.83	2.90	2.90	2.87	2.73	3.10	2.70	2.90	0.11
Globulin (g/dl)	1.60 ^{bc}	1.83 ^{b}	1.73 ^{b}	1.47 ^{bc}	1.30 ^{bc}	0.87 ^{c}	1.90 ^{b}	3.10 ^{a}	0.20
Albumin : Globulin	1.81 ^{b}	1.58 ^{b}	1.72 ^{b}	1.95 ^{b}	2.21 ^{b}	4.27 ^{a}	1.47 ^{b}	1.00 ^{b}	0.31
Uric acid (mg/dl)	4.53 ^{a}	1.90 ^{cd}	2.06 ^{cd}	1.63 ^{d}	4.33 ^{ab}	4.07 ^{ab}	3.53 ^{b}	2.63 ^{c}	0.22
Creatinine (mg/dl)	1.83	1.63	1.57	1.50	1.60	1.57	1.57	1.53	0.10
Glucose (mg/dl)	148.63	150.23	148.87	126.63	147.10	144.03	130.17	137.17	5.69
Cholesterol (mg/dl)	131.70 ^{a}	86.97 ^{c}	88.10 ^{bc}	63.27 ^{e}	130.17 ^{a}	93.40 ^{b}	92.53 ^{bc}	80.30 ^{d}	1.87

SEM: Standard error of mean; PCV: Packed Cell Volume; RBC: Red Blood Cells; WBC: White Blood Cells; $^{a,b,c,d,e;}$ Means in the same row not sharing common superscript are significantly ($P<0.05$) different

Elevation of serum total protein and globulin levels observed at high concentration of leaf extract (45 g/l) suggested an enhancement in protein production within the liver (Oni et al. 2018, p. 76). Similarly, result from the study of Sobayo et al. (2018b, p. 65) showed higher serum total protein and globulin in growing pullets fed *Petiveria alliace*a leaf meal at 2500 mg/kg feed compared with other treatments. However, Sobayo et al. (2018a, p. 305) reported that broiler chicks fed *Petiveria alliacea* root meal obtained more appreciative value for serum protein, albumin and globulin compared to leaf meal. All values recorded for serum protein in this study fell within the range (3.55 g/dl to 6.03 g/dl) reported by Sobayo et al. (2018b, p. 65). The concentration dependent reduction in serum uric acid suggested improved renal function (Oni et al. 2018, p. 76) and better protein utilization in *Petiveria alliacea* treated pullets, considering that high serum uric acid signals poor dietary protein utilization (Awosanya et al. 1999, p. 92). More pronounced reduction in serum uric acid observed in pullets administered root extract compared with leaf extract can be attributed to high concentration of most of the identified phytochemicals present in the root compared with the leaf. This corresponds with result from the study of Sobayo et al. (2018a, p. 305) where feeding diet containing *Petiveria alliacea* root meal better reduced serum urea in broiler chickens compared to leaf meal. They also reported reduced serum urea level at higher inclusion level. Similarly, result of study conducted by Oni et al. (2018, p. 75) showed that high inclusion level (10 g/kg feed) of phytomix (ginger, garlic and chaya leaf) reduced serum urea in pullet chicks. Organosulfur compounds present in *Petiveria alliacea* similar to allicin (found in garlic) and responsible for its characteristic garlic odour (Randle et al. 2018, p. 181) has been reported to inhibit squalene epoxidase essential in the synthesis of cholesterol (Khan et al. 2007, p. 25).

This might be the reason for the observed concentration dependent reduction in serum cholesterol in pullets administered aqueous extract of root or leaf of *Petiveria alliacea*. Elson and Qureshi (1995, p. 205) also surmised that extracts from plants may lower blood cholesterol in chickens through inhibition of 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, an important enzyme involved in cholesterol synthesis. Stronger hypocholesterolemic ability of root extracts in this study can be associated with the presence of higher concentrations of most identified

Table VII. Main effects of aqueous extract of different parts (root and leaf) and concentration of extraction of *Petiveria alliacea* on intestinal organ weights of growing pullets at 18 weeks of age (Principales efectos del extracto acuoso de diferentes partes (raíz y hoja) y concentración de extracción de Petiveria alliacea en los pesos de órganos intestinales de pollitas en crecimiento a las 18 semanas de edad).

	Plant Part			Concentration of extraction				
	Root	Leaf	SEM	0 g/l	15 g/l	30 g/l	45 g/l	SEM
Organs (% of body weight)								
Oesophagus	0.34	0.34	0.01	0.33	0.35	0.33	0.34	0.01
Crop	0.60	0.61	0.02	0.60	0.64	0.59	0.58	0.02
Proventriculus	0.52	0.52	0.01	0.49^{b}	0.52^{ab}	0.54^{a}	0.54^{a}	0.01
Empty gizzard	2.39	2.45	0.09	2.24	2.50	2.43	2.50	0.13
Small intestine	2.98	2.93	0.13	3.29^{a}	3.03^{ab}	2.92^{ab}	2.58^{b}	0.16
Large intestine	0.24	0.24	0.01	0.24	0.23	0.25	0.26	0.01
Caeca	0.80	0.79	0.03	0.70^{b}	0.80^{a}	0.82^{a}	0.86^{a}	0.03
Liver	1.55	1.53	0.05	1.49	1.55	1.58	1.54	0.07

SEM: Standard error of mean; ^{a,b,}: Means in the same row not sharing common superscript by factor are significantly (P<0.05) different

phytochemicals in the root compared to leaf. However, Sobayo et al. (2018a, p. 305; 2018b, p. 65) and Muhammad et al. (2019, p. 530) found no difference in serum cholesterol of *Petiveria alliacea* treated and non-treated chickens, while Odetola et al. (2019, p. 48) reported that inclusion of 2500 g/100kg feed of *Petiveria alliacea* root meal increased serum cholesterol level in broiler chickens. This contradiction may result from differences in plant source and certain agronomical factors (Rehman and Munir, 2015, p. 32) and processing methods which might affect concentration and stability of active biochemical (Poojary et al. 2017, p. 30).

Increase in proventriculus weight at 30 g/l leaf extract could suggest increase in physiological activities within this organ thus increased secretion of digestive enzymes although it was not reflected in weight gain. According to Birger (2014, p. 309), interpretation of changes in weight of small intestine is often difficult. Nevertheless, concentration dependent reduction in weight of small intestine of *Petiveria alliacea* treated pullets in this study could be attributed to thinner intestinal wall. Cross et al. (2007, p. 500) indicated that accumulation of lymphocytes during pathogenic bacteria infection causes inflammation and increase in muscular thickness. Antibacterial activity of *Petiveria alliacea* might have prevented bacteria invasion in the small intestine thereby preventing thickening of the intestinal wall and eventual weight increase. This is similar to the finding of Visek (1978, p. 1461) who reported that dietary inclusion of antibiotic herbal materials reduced intestinal weight by thinning of the intestinal wall. The caeca is noted as a site for microbial action and production of immunoglobulins (Clench & Mathias 1995, p. 114). The administered extracts of *Petiveria alliacea* might have stimulated a rise in physiological activities within the caeca due to their immunomodulatory and antimicrobial activities thus resulting in the observed increase in caeca weights.

Table VIII. Interactive effect of aqueous extract of different parts (root and leaf) and concentration of extraction of *Petiveria alliacea* on intestinal organ weights of growing pullets at 18 weeks of age (Efecto interactivo del extracto acuoso de diferentes partes (raíz y hoja) y concentración de extracción de Petiveria alliacea en los pesos de órganos intestinales de pollitas en crecimiento a las 18 semanas de edad).

Plant part	Root				Leaf				
Concentration of extraction	0 g/l	15 g/l	30 g/l	45 g/l	0 g/l	15 g/l	30 g/l	45 g/l	SEM
Organs (% of body weight)									
Oesophagus	0.33	0.37	0.32	0.34	0.32	0.34	0.33	0.35	0.02
Crop	0.63	0.64	0.56	0.57	0.58	0.63	0.62	0.59	0.03
Proventriculus	0.50 ^{ab}	0.52 ^{ab}	0.53 ^{ab}	0.54 ^{ab}	0.48 ^{b}	0.51 ^{ab}	0.56^{a}	0.54 ^{ab}	0.02
Empty gizzard	2.25	2.55	2.29	2.47	2.24	2.46	2.57	2.53	0.19
Small intestine	3.33	3.09	2.90	2.62	3.25	2.97	2.95	2.55	0.23
Large intestine	0.23	0.24	0.24	0.26	0.24	0.21	0.27	0.26	0.02
Caeca	0.71b^{c}	0.82^{abc}	0.80^{abc}	0.85^{ab}	0.68^{c}	0.78^{abc}	0.83^{ab}	0.89^{a}	0.04
Liver	1.51	1.58	1.54	1.57	1.48	1.52	1.61	1.51	0.10

SEM: Standard error of mean; $^{a,b,c;}$ Means in the same row not sharing common superscript are significantly (P<0.05) different

CONCLUSIONS

The study concluded that aqueous extract of *Petiveria alliacea* root or leaf can be administered at 45 g/l concentration to improve health and immune status of growing pullets while maintaining standard growth.

ACKOWLEGMENTS

This study was funded by the first author. Contributions from other authors are duly acknowledged.

BIBLIOGRAPHY

Adesipo, AT, Lajide, L, Owolabi, BJ, & Adejoro, F 2017, 'Phytochemical Screening and Antimicrobial Activity of the Aerial Part of Three Selected Plants', *Journal of Natural Sciences Research,* vol. 7, no. 16, pp. 21-27.

Alabi, RA & Samuel, KD 2002, 'An economic analysis of poultry production system in Ondo State, Proceedings of 7th Annual Conference of Animal Science Association (ASAN), Abeokuta, pp. 316-318.

Awosanya, B, Joseph, JR, Apata, DF, & Agboola, MA 1999, 'Performance, blood chemistry and carcass quality attribute of rabbits fed raw and processed pueraria seed meal', *Tropical Journal of Animal Science,* vol. 2, no. 2, pp. 89-96.

Ayodele, OD, Oyegbade, O, & Oseni, SR 2015, 'Phytochemical Analysis and Antioxidant Activities of Dry and Fresh Leaves *of Petivera alliacea* and *Ocimum gratissimum'*, *International Journal of Sciences: Basic and Applied Research*, vol. 24, no. 3, pp. 1-13.

Bamiro, OM, Phillip, DOA, & Momoh, S 2006, 'Vertical Integration and Technical Efficiency in Poultry (Egg) Industry in Ogun and Oyo State, Nigeria', *International Journal of Poultry Science*, vol. 5, no. 12, pp. 1164-1171.

Benson, HJ, Gunstream, SE, Talaro, A, & Talaro, KP 1989, Anatomy and Physiology Laboratory Texbook, Win. C. Brown Publisher, Dubuque IOWA.

Birger, S 2014, 'Function of the digestive system', *The Journal of Applied Poultry Research,* vol. 23, no. 2, pp. 306-314.

Castanon, JIR 2007, 'History of the use of antibiotic as growth promoters in European poultry feeds', *Poultry Science,* vol. 86, no. 11, pp. 2466-2471.

Clench, MH & Mathias, JR 1995, 'The Avian Caecum: A Review', *The Wilson Bulletin*, vol. 107, no. 1, pp. 93-121.

Cross, DE, McDevit, RM, Hilman, K, & Acamovic, T 2007, 'The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7 to 28 days of age', *British Poultry Science*, vol. 48, no. 4, pp. 496–506.

Dayyal, GG 2016, Cyanmethemoglobin (Hemoglobin-Cyanide) Method for Estimation of Hemoglobin, viewed 12 December 2019, <https://www.bioscience.com.pk/topics/cell-biology/item/167-cyanmethemoglobin-hemoglobin-cyanide-method-for-estimation-of-hemoglobin>

Devegowda, G 1996, 'Herbal medicines, an untapped treasure in poultry production', Proceedings 20th World's Poultry Congress New Delhi, India.

Dhama, K, Latheef, SK, Mani, S, Samad, HA, Karthik, K, Tiwari, R, Khan, RU, Alagawany, M, Farag, MR, Alam, GM, Laudadio, V, & Tufarelli, V 2015, 'Multiple Beneficial Applications and Modes of Action of Herbs in Poultry Health and Production-A Review', *International Journal of Pharmacology,* vol. 11, no. 3, pp. 152-176.

Diarra, MS & Malouin, F 2014, 'Antibiotics in Canadian poultry productions and anticipated alternatives', *Frontiers in Microbiology,* vol. 5, pp. 1-15.

Doumas, BT & Biggs, HG 1971, 'Determination of serum albumin', in GR Cooper (ed.), Standard methods of clinical chemistry, New York, USA., pp. 7-175.

Ekunseitan, DA, Yusuf, AO, Olayinka, OA, Ayoola, A, & Adedotun, A 2016, 'Comparative study of two plants (Lagenaria breviflora and *Petiveria alliacea*) and their phytobiotic potential in poultry health', *Nigerian Journal of Animal Production,* vol. 43, pp. 289-298.

Elson, CE & Qureshi, AA 1995, 'Coupling the cholesterol-and tumor-suppressive actions of palm oil to the impact of its minor constituents on 3-hydroxy-3-methylglutaryl coenzyme A reductase activity', *Prostaglandins, Leukotrienes & Essential Fatty Acids Fatty Acids,* vol. 52, no. 2, pp. 205-208.

Evans, WC 2000, Trease and Evans Pharmacology', 14th edn., WB Saunders Company Ltd., UK, pp. 224-293.

Food and Agricultural Organization (FAO) 2003, Egg marketing, A guide for the production and sale of eggs, viewed 12 December 2019, <http://www.fao.org/3/y4628e/y4628e03.htm>

Google Earth. 2020.

Harborne, JB 1973, Photochemical Methods: A Guide to Modern Techniques of Plant Analysis, Chapman A. and Hall, London. pp. 279.

Jahan ZA, Ahsan, UH, Muhammad, Y, Tanveer, A & Sarzamin, K 2008, 'Evaluation of different medicinal plants as growth promoters for broiler chicks', *Sarhad Journal of Agriculture*, vol. 24, no. 2, pp. 323-329.

Khan, SH, Sardar, R, & Anjum, MA 2007, 'Effects of Dietary Garlic on Performance and Serum and Egg Yolk Cholesterol Concentration in Laying Hens', *Asian Journal of Poultry Science,* vol. 1, no. 1, pp. 22-27.

Kim, S, Kubec, R & Musah, RA 2006, 'Antibacterial and antifungal activity of sulfur-containing compounds from *Petiveria alliacea* L.', *Journal of Ethnopharmacology,* vol. 104, no. 2, pp. 188-192.

Lamb, GM 1981, Manual of Veterinary Laboratory Rabbit, Technique, Ciba- Geigy, Kenya.

Liu, RH 2013, 'Health-Promoting Components of Fruit and Vegetables in the Diet', *Advances in Nutrition,* vol. 4, no. 3, pp. 3845–3925.

Lopes-Martins, RA, Pegoraro, DH, Woisky, R, Penna, SC & Sertié, JA 2002, 'The anti-inflammatory and analgesic effects of a crude extract of *Petiveria alliacea* L. (Phytolaccaceae)', *Phytomedicine,* vol. 9, no. 3, pp. 245-248.

McDonald, S 1996, Complete blood count. Avian quarterly, viewed 20 July 2019 <http://www.scottemcdonald.com/pdfs/CBC.pdf>

Muhammad, SB, Sobayo, RA, Oso, AO, Sogunle, OM, Ayoola, AA, Adeyemo, YO & Basiru, YT 2019, 'Effects of dosage and plant parts of Petiveria alliacea used as phytobiotics on growth, nutrient digestibility and blood profile of Pullet chicks', *Archivos de Zootecnia*, vol. 68, no. 264, pp. 524-533.

Nodu, MB, Okpeku, M, Akpoveta, ZA & Iroegbu, DO 2016, 'Evaluation of *azadirachta indica* leave extract on hematology and biochemical profiles, organs weight and growth parameters of broiler chickens', *Journal of New Sciences,* vol. 32, no. 5, pp. 1879-1884.

Odetola, OM, Adejinmi, OO, Owosibo, OA, Banjo, OT & Awodola-Peters, OO 2019, 'Growth Response, Serum Biochemistry and Organ Histopathology of Broilers Fed Diets supplemented with Graded levels of *Petiveria alliacea* Root Meal', *International Journal of Poultry Science,* vol. 18, no. 1, pp. 45-50.

Olawumi, SO 2011, 'Study on Pre-laying Characteristics of Three Breeds of Commercial Layers in the Derived Savannah Zone of Nigeria', *Pakistan Journal of Biological Sciences,* vol. 14, no. 23, pp. 1061-1065.

Oni, OO, Alabi, JO, Adewole, MA & Adegbenjo, AA 2018, 'Effect of phytobiotics (mixture of garlic, ginger and chaya leaf) on growth performance, haematological and biochemical indices of pullet chicks', *Slovak Journal of Animal Science,* vol. 51, no. 2, pp. 69–78.

Poojary, MM, Putnik, P, Kovacevic, DB, Barba, FJ, Lorenzo, JM, Dias, DA & Shpigelman, A 2017, 'Stability and extraction of bioactive sulfur compounds from Allium genus processed by traditional and innovative technologies', *Journal of Food Composition and Analysis*, vol. 61, pp. 28-39.

Quadros, MR, Souza-Brito, ARM and Queiroz, MLS 1999, '*Petiveria alliacea* L. extracts protects mice against Listeria monocytogenes infection-effects on bone marrow progenitor cells', *Immunopharmacology and Immunotoxicology,* vol. 21, no. 1, pp. 109-124.

Randle, MM, Riley, CK, Williams, LAD &Watson, CT, 2018, 'A Systematic Review of the Traditional and Medicinal Uses of *Petiveria alliacea* L.

In The Treatment of Chronic Diseases', *Journal of Plant Science and Research,* vol. 5, no. 1, pp. 179-185.

Rehman, SU, Durrani, FR, Chand, N, Khan, RU & Rehman, FU 2011, 'Comparative efficacy of different schedules of administration of medicinal plants infusion on hematology and serum biochemistry of broiler chicks', *Research Opinions in Animal and Veterinary Sciences*, vol. 1, no. 1, pp. 8-14.

Rehman, Z & Munir, MT 2015, 'Effect of garlic on the health and performance of broilers', *Veterinaria,* vol. 3, no. 1 pp. 32-39.

Reinhold, JG 1953, Standard Methods of Clinical Chemistry, Academic Press, New York.

Rosner, H, Williams, L, Jung, A & Kraus, W 2001, 'Disassembly of microtubules and inhibition ofneurite outgrowth, neuroblastoma cell proliferation, and map kinase tyrosine depho-sphorylation by dibenzyl trisulphide', *Biochimica et Biophysica Acta,* vol. 1540, no. 2, pp. 166–177.

Santander, SP, Hernandez, JF, Baretto, CC, Masayuki, A, Moins-Teisserenc, H & Fiorentino, S 2012, 'Immunomodulatory Effects of Aqueous and Organic Fractions from *Petiveria alliacea* on Human Dendritic Cells', *The American Journal of Chinese Medicine,* vol. 40, no. 4, pp. 833-844.

Statistical Analysis System Institute Inc, 2010, SAS. STAT. Program, Cary, NC: SAS Institute International.

Sobayo, RA, Okonkwo, IJ, Muhammad, SB, Sanwo, KA, Oso, OA & Sogunle, OM 2018a, 'Haematological and serum indices of finishing broiler chickensfed graded levels of guinea hen weed (*Petiveria alliacea*) parts', *Bulletin of Animal Health and Production in Africa,* vol. 66, no. 2, pp. 299-311.

Sobayo, RA, Muhammad, SB, Oso, AO, Sogunle, OM, Ayoola, AA, Oke, EO & Abotinde, RO 2018b, 'Influence of Guinea hen weed (*Petiveria alliacea*) on growth performance, nutrient digestibility and blood indices of growing pullets', *Malaysian Journal of Animal Science,* vol. 2, no. 1, pp. 53-71.

Sobayo, RA, Okonkwo, IJ, Sanwo, KA, Oso, OA, Eruvbetine, D, Oguntona, EB & Muhammed, SB 2017, 'Effect of dietary supplementation of guinea hen weed (*Petiveria alliacea*) leaf and root meals on nutrient utilization and intestinal morphology of finishing broiler chicken', *Nigerian Journal of Animal Science,* vol. 19, no. 1, pp. 144-156.

Sofowora, A 1993, Medicinal Plants and Traditional Medicines in Africa, Chichester John Wiley and Sons, New York, pp. 97-145.

Visek, WJ 1978, 'The mode of growth promotion by antibiotics', *Journal of Animal Science,* vol. 46, no. 5, pp. 1447–1469.

Williams, LAD, Rosner, H, Levy, HG & Barton, EN 2007, 'A critical review of the therapeutic potential of dibenzyl trisulphide isolated from *Petiveria alliacea* L (Guinea hen weed, anamu)', *West Indian Medical Journal,* vol. 56, no. 1, pp. 17–21.

Windisch, W, Schedle, K, Plitzer, C & Kroismayr, A 2008, 'Use of phytogenic products as feed additives for swine and poultry', *Journal of Animal Science,* vol. 86, no. 14, pp. 140-148.