

Influence of scent leaf (*Ocimum gratissimum*) powder supplementation on growth performance, serum biochemistry, organ weight, and organ histopathology of guinea fowls

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ABSTRACT

Background and Objectives: This study was designed to investigate the influence of scent leaf powder (SLP) supplementation on growth performance, serum biochemistry, organ weight, and organ histopathology of guinea fowls within a 56-day feeding trial.

Methodology: A total of 240 one-day-old guinea fowls were assigned based on weight equalization into four treatments consisting of basal diet (control; T1), diet containing antibiotic (enrofloxacin included at 1 g/kg; T2) and diet containing SLP (included at 0.5 g/kg; T3 and 1 g/kg; T4). Each treatment contained 60 birds and 6 replications of 10 birds each. Birds were fed and provided water *ad libitum* throughout the experiment. Blood samples were collected via the wing vein of the birds for serum biochemistry analyses. Data on growth performance, serum biochemistry, relative organ weights, and histopathological examination were collected. Data generated were subjected to analysis of variance at a 5% significance level.

Main Results: The final body weight and weight gain were significantly ($P < 0.05$) higher in guinea fowls fed a diet containing 1 g/kg SLP than other treatments. The lowest feed intake and best ($P < 0.05$) feed conversion ratio was shown in birds fed diet containing 1 g/kg SLP. Guinea fowls fed a diet containing 1 g/kg SLP recorded significantly ($P < 0.05$) reduced alanine aminotransferase (ALT). The heaviest ($P < 0.05$) relative bursa weight was recorded with birds fed a diet containing 1 g/kg SLP. Kidney samples from birds fed a diet containing antibiotics and 1 g/kg SLP revealed a mild diffuse degeneration of the epithelial linings.

Conclusions: Dietary supplementation with 1 g/kg SLP improved the body weight and activated the immune organs of guinea fowls. However, 1 g/kg SLP supplementation showed a mild health problem. Therefore, a dosage above 0.5 g/kg and below 1 g/kg of SLP is suggested for improved performance.

Keywords: Growth performance, organ histopathology, lymphoid organs, scent leaf powder, serum biochemistry

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INTRODUCTION

The poultry industry is constantly in search of new ways to improve the development, health, and general well-being of poultry species while also meeting the changing needs of consumers for products that are more sustainable and nutritionally superior (Uyanga *et al.*, 2023; Oni *et al.*, 2024). Incorporating natural dietary supplements has been a promising strategy in recent years for achieving sustainable poultry production. Medicinal plants, herbs, and spices have been widely used in animal nutrition as alternatives to antibiotic growth promoters due to bacteria resistance to antibiotics (WHO, 2017). These groups of plants and their products contain several bioactive compounds that exhibit antimicrobial, antioxidant, inflammatory, immune-modulatory, and digestive-stimulating properties (Khan *et al.*, 2012; Oke, 2018). Therefore, medicinal plants have been used in poultry production due to their health benefits (Amad *et al.*, 2011; Akbarian *et al.*, 2013). Intake of bioactive compounds as a result of dietary inclusion of phytogetic plants (León *et al.*, 2017) has been reported to have beneficial effects (Ahmadi, 2010; Akbarian *et al.*, 2013). Among the various phytogetic feed additives being considered, scent leaf has attracted a lot of attention due to its wide-ranging health benefits.

Scent leaf (*Ocimum gratissimum*) has been used traditionally for the treatment of various infections (Mann, 2012). The leaf extract is rich in eugenol, cinnamate, camphor, and thymol (Matasyoh *et al.*, 2007) and exhibits strong antimicrobial properties against most pathogenic bacteria (Prabhu *et al.*, 2009). According to Iwalokun *et al.* (2003) and Akinyemi *et al.* (2005), scent leaf has bioactive components that are effective against a variety of bacterial species. According to research by Oparaocha *et al.* (2010) and Pandey *et al.* (2017), the leaves, flowers, roots, and oils showed bactericidal, larvicidal, and antiviral effects. Anugom and Ofongo (2019) reported higher body weight gain and better feed conversion ratio (FCR) for broilers on scent leaf extract. Also, Olumide and Akintola (2020) reported that the inclusion of scent leaf in the diets of broiler chickens had no

detrimental effect on performance and carcass characteristics but improved the liveability of the birds. Literature on the use of scent leaf powder as a phytogetic feed additive in guinea fowl production is scarce. It is crucial to establish the potential advantages of supplementing guinea fowl with scent leaf powder, a bird species prized for its lean meat and unusual flavor.

The use of scent leaf powder in place of antibiotics has the potential to provide insightful information as the poultry industry navigates the combined challenges of satisfying customer desires for healthier and more sustainable poultry products. It is hypothesized that the growth performance, serum biochemistry, lymphoid organ development, and organ histology in guinea fowls supplemented with fragrance leaf powder will reveal possible benefits with the use of scent leaf. Therefore, this study aimed to promote the development of poultry farming practices that are in line with the current demand for nutritionally improved and environmentally friendly chicken products by deepening our understanding of the complex interaction between scent leaf powder and guinea fowl physiology.

MATERIALS AND METHODS

Preparation of Scent Leaf Powder and Composition

Mature fresh scent leaves that were still green and undamaged were harvested. The petioles and stalks were removed manually, and remnant leaves were rinsed in water to remove dirt spread evenly on a clean concrete floor in order to prevent fungal growth, the leaves were continuously turned over while being air dried during the day without exposure to direct sunlight. The leaves were ground into fine powder after 5 days of drying with a CSJ series rotary knife cutter mill (Changzhou Doing Machine Co., Ltd, Changzhou, Jiangsu, China). The rotary knife cutter is designed to cut corn, spices, roots, and other foods and grains into controlled sizes with little or no fines or dust in order to pass through a 2-mm sieve to yield scent leaf powder (SLP). Before use, the leaf meal was kept at 4°C in polythene plastic bags that were packed and sealed.

Qualitative Screening of Phytochemical Compounds

Qualitative analysis was carried out by a diagnostic pathologist to ascertain the presence of the different phytochemical compounds in the ethanol extract such as tannins, alkaloids, flavonoids, terpenes, saponins, and cyanogenic glycosides. This screening was conducted using the method described by Harborne (1973), and a preliminary study is shown in Table 2.

Phytogenic Screening

Alkaloids

Ten milligrams of extract was dissolved in 2 mL of 5% hydrochloric acid after mixing and filtering. Drops of Mayer reagents were added. A yellowish-white precipitate indicated the presence of alkaloids.

Flavonoids

Three drops of 10% sodium hydroxide were added to 1 mL of diluted extract in isopropyl alcohol. The formation of coffee-orange coloration indicated flavanols.

Saponins

One milliliter of distilled water was added to 10 drops of the extract dissolved in isopropyl alcohol (20 mg/mL) in a test tube, shaken vigorously to froth, and then allowed to stand for 10 min. Saponin content was measured in terms of the abundance of froth.

Tannins

Ten milligrams of extract was dissolved in 1 mL of ethanol, and 2 mL of distilled water was added, followed by 4 drops of ferric chloride aqueous solution 10% w/v. formation of green color indicated the presence of phenols.

Terpene

One milliliter of anhydrous acetic acid and 3 drops of concentrated sulphuric acid were added to 2 mL of the extract dissolved in alcohol. A red or magenta color indicated the presence of terpene.

Cyanogenic glycoside

One drop of water and 2 drops of toluene were added to 5 g of SLP in a test tube. The tube is then firmly corked, with a moistened picrate paper suspended inside from the cork, and left to incubate at 40°C for 2 h. A color change from yellow to reddish-brown indicates the presence of cyanogenic glycoside.

Phytate

The method of Latta and Eskin (1980) was adopted for the determination of phytate. This was based on discoloration of the Fe^{3+} sulfosalicylate complex by phytate.

Location of Experiment and Ethical Procedure

The feeding trial was carried out at the Poultry Unit of the Institute of Food Security, Environmental Resources and Agricultural Research (IFSERAR), Federal University of Agriculture, Abeokuta, Nigeria, following the guidelines of the Animal Care Committee of the Federal University of Agriculture, Abeokuta.

Experimental Management and Dietary Treatment

Two hundred and forty (240) one-day-old guinea fowls were allotted on a weight equalization basis into 24 (4 treatments with 6 replicates) floor pens in an open-sided poultry house. Each treatment consisted of 60 birds, with 10 birds per replicate. Before the commencement of the experiment, the pen was washed and disinfected, and wood shavings were used as litter materials. The brooding exercise was conducted for 21 days. The temperature and relative humidity during the experiment were $31 \pm 8^\circ\text{C}$ and $71 \pm 28\%$, respectively (CLIME, National Aeronautical and Space Administration). A standard diet (basal) was formulated to meet the NRC (1994) nutritional requirements of starter (0–8 weeks) guinea fowls (Table 1). Four experimental diets were formulated: basal diet (control; T1), diet containing enrofloxacin at 1 g/kg (T2), SLP at 0.5 g/kg (T3), and 1 g/kg (T4). The feed was mixed at a standard feed mill. No antimicrobial, anticoccidial drugs, and vaccinations were administered throughout the

study to avoid confounding, antagonistic, or interference effects. This study lasted for 8 weeks.

Experimental Layout

- Treatment 1: basal diet (control)
- Treatment 2: basal diet + 1 g/kg enrofloxacin (antibiotics)
- Treatment 3: basal diet + 0.5 g/kg SLP
- Treatment 4: basal diet + 1 g/kg SLP

Data Collection

Measurement of growth performance characteristics

The body weight of the guinea fowls per pen was measured, while weight gain was computed weekly. Daily feed intake was also measured as the difference between the feed offered and leftover, while the FCR was expressed as a ratio of the feed intake to weight gain.

Measurement of serum biochemistry

At the end of the study (day 56), blood was collected from the wing veins of the birds (2 guinea fowls per pen; n = 12 samples per treatment) into universal bottles containing no anticoagulant. Serum was collected by centrifugation and stored inside the freezer at -20°C until biochemical analysis. The method described by Varley *et al.* (1980), the bromocresol purple method, was used to determine total serum protein and albumin. The method described by Wootton (1964) was used to determine serum urea concentration. While alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT) concentrations were determined using the method described by Bonsnes and Tausky (1945).

Table 1 Gross composition of the experimental diets fed to guinea fowls

Composition	Starter (0–8 weeks)
Ingredient (g/kg)	
Maize	550.00
Soybean meal	258.00
Wheat offal	155.00
Limestone	10.00
Bone meal	18.00
Salt	3.50
Lysine	1.00
Methionine	2.00
Premix*	2.50
Total	1,000.00
Analyzed composition (%)	
Dry matter	84.57
Crude protein	24.00
Fat	2.18
Crude fiber	1.50
Ash	4.00
Metabolizable energy (kcal/kg)	2,850

Note: * Premix provides vitamins and minerals in the diet. Starter premix provides the following per kg of diet: vitamin A 10,000 IU, vitamin D₃ 2,000 IU, vitamin E 40 mg, vitamin K₃ 2 mg, vitamin B₁ 1.5 mg, vitamin B₂ 5 mg, vitamin B₆ 4 mg, vitamin B₁₂ 0.02 mg, calpan 1 mg, folic acid 1 mg, biotin 0.1 mg, antioxidants 100 mg, choline chloride 300 mg, manganese 80 mg, iron 40 mg, zinc 60 mg, copper 8 mg, iodine 0.8 mg, cobalt 0.3 mg, and selenium 0.2 mg.

Relative organ weights and histopathological examination

Two guinea fowls whose body weights were close to the average weight of each replication were picked, weighed, and slaughtered on the last day of the experiment. Examinations of carcasses were carried out, and internal organs were removed. The weights of the gizzard, lung, liver, heart, pancreas, kidney, spleen, thymus, and bursa were measured using a digital sensitive scale (Mettler, Mettler-Toledo, Leicester, UK) and expressed as relative weights (percentage of fasted live weight). Samples of the kidney, liver, bursa, and spleen from the slaughtered birds were used for the histopathological examination. The methods of Okur *et al.* (2022) were used for the assessment. Briefly, the samples were fixed in 10% neutral buffered formalin for at least 48 h, processed in graded levels of alcohol and cleared in xylene, embedded in paraffin wax, sectioned, then stained with hematoxylin and eosin prior to light microscopy and examined under an upright microscope coupled with a camera and NIS-D ver.4.0 software (Eclipse Ci-S, Nikon, Japan).

Statistical Analysis

Data generated were analyzed using the analysis of variance technique using the general linear model procedure contained in SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA). Significant mean values were separated using the Tukey test at a 5% probability level. The statistical model was $y_{ij} = \mu + T_i + e_{ij}$, where y_{ij} is the dependent variable, μ is the population mean, T_i is the effect of SLP supplementation, and e_{ij} is the random error.

RESULTS AND DISCUSSION

Qualitative screening of SLP shown in Table 2 revealed the presence of phytate, tannins, alkaloids, flavonoids, and saponins. Scent leaf has been reported to be rich in alkaloids, tannins, phytates, flavonoids, and saponins (Ladipo *et al.*, 2010; Oladosu-Ajayi *et al.*, 2017), thus justifying its use as a medicinal plant in poultry. Phytochemical analysis of scent leaf showed the presence of 5.47% tannin, 9.87% saponin, 16.75% flavonoid, 8.97% phenol, and 6.81% alkaloid (Olobatoke

and Okaragu, 2021). A quantitative phytochemical analysis of scent leaf was also carried out by Adewole (2014), who reported in percentages that scent leaf contains tannin ($10.90 \pm 0.06\%$), saponin ($12.87 \pm 0.19\%$), flavonoid ($8.20 \pm 0.06\%$), phenol ($7.50 \pm 0.06\%$) and alkaloid ($11.43 \pm 0.09\%$).

Alkaloids present in medicinal plant leaves have been reported to exhibit analgesic (Uhegbu *et al.*, 2012) and antimicrobial (Kin *et al.*, 2018) activities. Aziba *et al.* (1999) studied the pharmacological activity of aqueous scent leaf extracts in isolated rabbit jejunum and their analgesic qualities in mice. The analgesic investigation indicated an extended reaction time of 85% throughout a 20-minute observation period with no evident signs of toxicity. Scent leaf exhibited the potential to reduce pain perception in an analgesic experiment with mice (Ajayi *et al.*, 2017a). The presence of alkaloids in SLP in the present study suggests its potential antimicrobial properties. A couple of studies have confirmed the antimicrobial activities of scent leaf (Nweze and Eze, 2009; Prakash *et al.*, 2011; Melo *et al.*, 2019). Chimnoi *et al.* (2018) showed that the essential oil extract of scent leaf caused rapid inhibition of *Escherichia coli* and *S. typhimurium*. An *in vitro* study by Ohimain *et al.* (2015) reported the antibacterial effect of scent leaf extract against *Salmonella* sp. and *E. coli* isolated from the ileum of broiler birds. Anugom and Ofongo (2019) reported a significant reduction of *E. coli* in the ileum and caecum of the group fed scent leaf diet compared to the control group. Also, Ishiwu *et al.* (2014) found that an increase in the concentration of scent leaf extract reduces the number of viable *E. coli* from 36 to 5 cfu/mL.

Flavonoids act as free radical scavengers (Ezeabara *et al.*, 2013). The occurrence of flavonoids in SLP might be responsible for its use as a cure for both acute and chronic inflammation (Ajayi *et al.*, 2017b). In addition, scent leaf has antioxidant properties that can help to reduce oxidative stress (Abd Rani *et al.*, 2018). Saponin in medicinal plants has been found to interfere with intestinal absorption of cholesterol, exhibiting hypocholesterolemic and anti-diabetic effects (Ezeabara *et al.*, 2013). Therefore, the presence of saponins in SLP used in the current study serves as a potential antimicrobial agent.

Table 2 Qualitative screening of scent leaf powder

Phytochemical compounds	Presence
Phytate	+
Tannin	+
Alkaloids	+
Flavonoids	+
Terpenes	-
Saponins	+
Cyanogenic glycosides	-

Note: (+) = present, (-) = absent.

The effect of dietary inclusion of SLP on growth performance and serum biochemistry of guinea fowl (Table 3) showed that guinea fowls fed a diet containing 1 g/kg SLP had higher ($P < 0.05$) final body weight and weight gain than its other counterparts fed the control diet, diet containing antibiotics and those fed a diet containing 0.5 g/kg SLP. Birds fed a diet containing antibiotics, 0.5 and 1 g/kg SLP had lower ($P < 0.05$) feed intake and better ($P < 0.05$) FCR than the control group, but the least feed intake was recorded with birds fed a diet containing 1 g/kg SLP. The reduced weight gain observed in the group fed a 0.5 g/kg SLP diet could be attributed to the low dosage, which was not enough to stimulate improvement in nutrient utilization.

The increased final body weight and weight gain recorded for guinea fowls fed a diet containing 1 g/kg SLP when compared with the other treatments, could infer that SLP (at 1 g/kg inclusion) showed antimicrobial potential to replace antibiotics without compromising body weight gain. Improved body weight gain following the dietary inclusion of phytochemicals has been reported in previous studies (Brenes and Roura, 2010; Olobatoke and Okaragu, 2021). Despite the reduced feed intake, improved FCR obtained for guinea fowls fed diets supplemented with 1 g/kg SLP suggested better feed utilization. This agreed with the report of Egbeyale *et al.* (2021)

that improvement in FCR following supplementation of phytochemical feed additives is mostly linked with reduced feed intake and improved feed utilization. The presence of anti-nutrients such as saponin and tannin in feed containing SLP may result in a reduction in the feed intake of chicks, and this can be attributed to reduced acceptability of the feed (Olobatoke and Okaragu, 2021). A report by Anugom and Ofongo (2019) and Olobatoke and Okaragu (2021) recorded those birds fed diets supplemented with scent leaf meal showed significantly higher body weight, better efficiency of feed utilization, and improved performance index than birds in the control group both at the chick and grower phases. The phytochemical properties and the mineral composition of scent leaf meal may have been responsible for the enhanced feed utilization and improved performance of the birds (Nte *et al.*, 2017). Alkaloids and saponins have antibiotic potentials that may have acted as growth promoters and thus could be responsible for the improvement in growth performance (Olobatoke and Okaragu, 2021). Anugom and Ofongo (2019) similarly noted enhanced final body weight, weight gain, and FCR of broiler chickens administered with an aqueous extract of scent leaf. The mechanism of action of SLP in this study indicates that birds were able to convert minimal feed into meat, suggesting better feed utilization, thereby leading to improved feed efficiency, as observed by Nte *et al.* (2017).

Table 3 Effect of dietary inclusion of scent leaf powder (SLP) on growth performance and serum biochemistry of guinea fowl

Parameters	Control	Antibiotic	SLP (0.5 g/kg)	SLP (1.0 g/kg)	SEM	P-value	Avian reference range
Growth performance							
Initial body weight (g)	30.50	30.49	30.51	30.50	0.01	0.0864	
Final body weight (g)	925.71 ^c	992.91 ^b	920.66 ^d	1,000.77 ^a	11.15	0.0001	
Body weight gain (g)	895.21 ^c	962.42 ^b	890.15 ^d	970.22 ^a	11.10	0.0001	
Feed intake (g)	2,100.79 ^a	1,763.75 ^b	1,662.75 ^c	1,654.76 ^d	54.72	0.0001	
Feed conversion ratio	2.35 ^a	1.83 ^c	1.87 ^b	1.71 ^d	0.07	0.0001	
Serum biochemistry							
Total protein (g/dL)	5.50	5.55	5.62	6.02	0.08	0.0836	2.58–5.22 ¹
Albumin (g/dL)	3.15	3.05	3.20	3.30	0.04	0.2758	1.17–2.74 ¹
Uric acid (mg/dL)	9.45 ^b	9.22 ^b	9.42 ^b	10.02 ^a	0.09	0.0001	1.9–12.5 ²
GGT (U/L)	21.45 ^a	15.40 ^b	15.25 ^b	14.90 ^b	0.82	0.0001	9–17 ³
AST (U/L)	36.62 ^a	35.70 ^b	34.65 ^c	36.20 ^{ab}	0.23	0.0002	70–220 ¹
ALT (U/L)	27.40 ^a	26.72 ^a	22.95 ^b	21.45 ^c	0.76	0.0001	9.5–37.2 ⁴

Note: GGT = gamma-glutamyl transferase, AST = aspartate aminotransferase, ALT = alanine aminotransferase, SEM = standard error of mean. Means on the same row with different superscripts are different ($P < 0.05$). ¹ Meluzzi *et al.* (1992), ² Clinical Diagnostic Division (1990), ³ Borsa *et al.* (2006), ⁴ Roa *et al.* (2020).

The uric acid level was higher ($P < 0.05$) in guinea fowls fed 1 g/kg SLP diet compared with other treatment groups (Table 3). The lowest ($P < 0.05$) ALT concentration was obtained with guinea fowls fed a diet containing 1 g/kg SLP. While GGT and AST was significantly ($P < 0.05$) higher in birds fed the control diet. Total serum protein and albumin were not affected ($P > 0.05$) by dietary treatments. Serum enzyme concentrations exist at low concentrations in a normal healthy animal but increase under stress conditions, hepatotoxic situations, and inhibition of protein synthesis (Busari *et al.*, 2021). The reduced ALT and GGT concentration recorded with guinea fowls fed diets containing 1 g/kg SLP compared with other dietary treatments suggests the absence or mild abnormalities in liver functioning, as also recorded by Uhegbu *et al.* (2012) and Chiu *et al.* (2014).

The relative weights of the kidney, lung, heart, liver, and spleen of the guinea fowls were not affected ($P > 0.05$) by SLP inclusion (Table 4). All birds fed diets containing antibiotics, 0.5 and 1 g/kg SLP had higher ($P < 0.05$) relative thymus weights than the control group. Guinea fowls fed a diet supplemented with 1 g/kg SLP had the highest

($P < 0.05$) relative bursa weight, while those in the control group recorded the lowest ($P < 0.05$) relative thymus and bursa weights. The relative weights of lymphoid organs have been measured as indicators of health and immune status in animals (Elmore, 2018). The bursa and thymus are responsible for recruiting B and T lymphocytes, which constitute vital components of humoral and cellular immunity. Reduced relative weights of thymus and bursa weight recorded for birds fed the control diet suggested reduced immune status and a tendency of reduced productivity in case of any sanitary challenge (Eyng *et al.*, 2015). The highest relative bursa weight obtained with birds fed a diet supplemented with 1 g/kg SLP suggested an improved gut-associated immune system of guinea fowls (Guo *et al.*, 2003). Previous studies also corroborate the fact that dietary inclusions of phytogetic feed additives significantly influenced the weights of lymphoid organs (Toghyani *et al.*, 2010). The increased relative weights of bursa and thymus following SLP inclusion in the present study could indicate or suggest that SLP confers an activation of the immune response of guinea fowls.

Table 4 Effect of dietary inclusion of scent leaf powder (SLP) on organ weight of guinea fowl

Parameters	Control	Antibiotic	SLP (0.5 g/kg)	SLP (1.0 g/kg)	SEM	P-value
Lymphoid organs						
Kidney	0.36	0.35	0.38	0.38	0.018	0.1233
Lungs	0.48	0.54	0.52	0.52	0.026	0.3830
Heart	0.42	0.45	0.44	0.44	0.013	0.0634
Liver	1.41	1.36	1.48	1.48	0.050	0.0931
Thymus	1.82 ^b	2.80 ^a	2.57 ^a	2.57 ^a	0.101	0.0120
Bursa	0.04 ^c	0.11 ^b	0.12 ^b	0.21 ^a	0.009	0.0101
Spleen	0.63	0.68	0.67	0.67	0.005	0.2429

Note: SEM = standard error of mean. Means within the same row with different superscripts are different ($P < 0.05$).

Organ histology revealed that kidney samples from birds fed the control diet and those fed a diet containing 0.5 g/kg SLP showed no visible lesions (Figures 1A and 1C). Kidney samples from birds fed a diet containing antibiotics (Figure 1B) and 1 g/kg SLP (Figure 1D) revealed a mild diffuse degeneration of the epithelial linings. Liver samples of guinea fowls fed the

control diet (Figure 2A) and antibiotics (Figure 2B), and those fed a diet containing 0.5 g/kg SLP (Figure 2C) showed normal liver configuration with no visible lesions. Meanwhile, liver samples from guinea fowls fed a diet containing 1 g/kg SLP (Figure 2D) showed proliferation of the bile duct with the periportal area of necrosis having prominent sinusoid and mild hepatic necrosis.

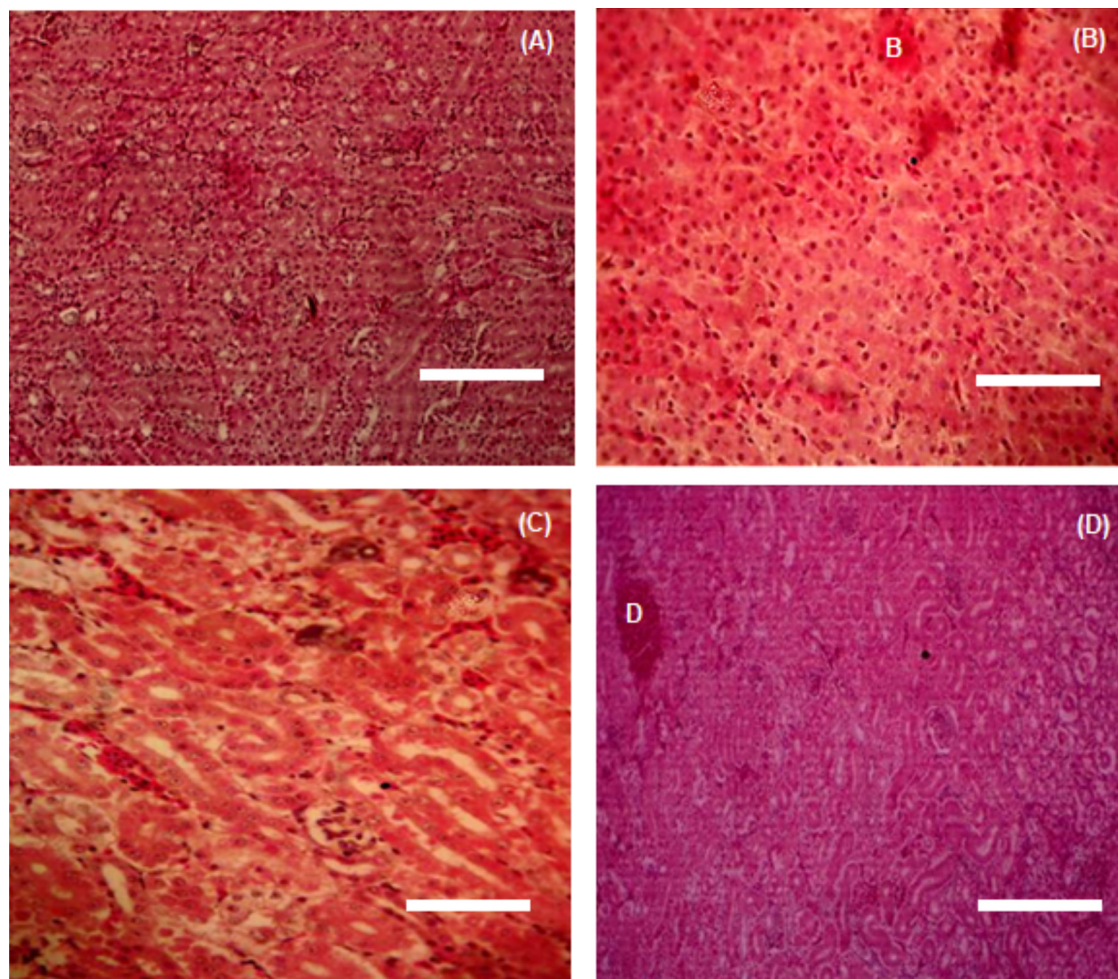


Figure 1 Kidney samples of birds fed scent leaf powder. (A) Kidney sample from birds fed the control diet showing no visible lesion H&E bar = 150 μ , (B) kidney sample from bird fed diet containing 0.5% SLP showing no visible lesion H&E bar = 150 μ ; B = diffuse degeneration, (C) kidney sample from birds fed diet containing antibiotic revealing mild diffuse degeneration of the epithelial linings H&E bar = 150 μ , and (D) kidney sample from bird fed diet containing 1% SLP showing mild diffuse degeneration of the epithelial linings H&E bar = 250 μ ; D = diffuse degeneration.

The normal configuration with no visible lesions observed in kidney samples from birds fed the control diet and diet containing 0.5 g/kg SLP suggested that SLP inclusion up to 0.5 g/kg poses no health threat to the birds. Meanwhile, mild diffuse degeneration of the epithelial linings of the kidney observed with birds-fed diets containing antibiotics and 1 g/kg SLP suggested early signs of possible

kidney problems. The degeneration of the epithelial linings observed in the liver could be associated with the phenolic compound contained in SLP. The liver is the primary site of nutrient metabolism in the body (Rahman Alizadeh *et al.*, 2017), and medicinal plants have been known to be the chief source of most dietary polyphenols (León *et al.*, 2017).

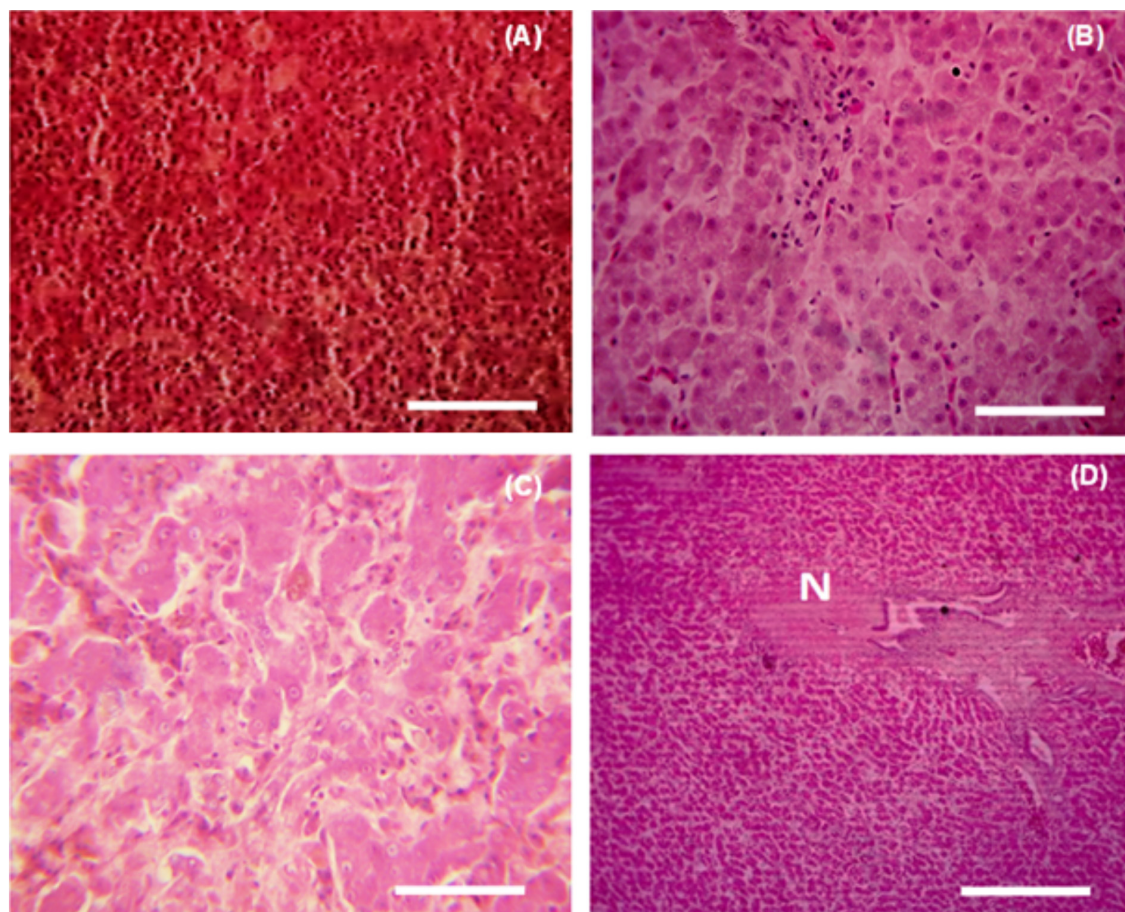


Figure 2 Liver samples of a bird-fed diet containing scent leaf powder. (A) Liver samples of birds fed the control diet showing no visible lesion H&E bar = 200 μ , (B) liver samples from birds fed diet containing antibiotics showing no visible lesion H&E bar = 150 μ , (C) liver sample from bird fed diet containing 0.5% SLP showing no visible lesion H&E bar = 200 μ , and (D) liver sample from bird fed diet containing 1% SLP revealed proliferation of bile duct, peri-portal area of necrosis with prominent sinusoid and mild hepatic necrosis H&E bar = 200 μ .; B = proliferation of bile duct, N = hepatic necrosis.

These dietary compounds from medicinal plants have been reported to exert hepato-protective effects in reducing liver toxicity (Al-Okbi *et al.*, 2014; El-Hadary and Ramadan Hassanien, 2016) or induce slight proliferation and necrosis of the liver (Ali *et al.*, 2014). The proliferation of bile duct, peri-portal area of necrosis, and prominent sinusoid with mild hepatic necrosis and a reduction in serum enzymes observed in the liver samples of birds fed diets supplemented with SLP at 1 g/kg suggested hepato-protective effects of the liver following ingestion of biological compound in SLP (Uhegbu *et al.*, 2012; Ajayi *et al.*, 2017a).

CONCLUSIONS

Dietary supplementation with 1 g/kg SLP improved the body weight and feed conversion ratio and activated the immune organs of guinea fowls. However, with 1 g/kg SLP supplementation, there was a mild diffuse degeneration of the epithelial linings of the kidney. Since there was no pathological evidence of toxicity in the birds when SLP was included in the diet at 0.5 g/kg, a dosage above 0.5 g/kg and below 1 g/kg of SLP is suggested for improved growth performance.

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REFERENCES

- Abd Rani, N.Z., K. Husain and E. Kumolosasi. 2018. *Moringa* genus: A review of phytochemistry and pharmacology. *Front. Pharmacol.* 9: 108. <https://doi.org/10.3389/fphar.2018.00108>.
- Adewole, E. 2014. Proximate and phytochemical constituents of *Ocimum gratissimum*. *J. Phys. Chem. Sci.* 1(1): 1–3.
- Ahmadi, F. 2010. Effect of turmeric (*Curcumin longa*) powder on performance, oxidative stress state and some of blood parameters in broiler fed on diets containing aflatoxin B1. *Glob. Vet.* 5(6): 312–317.
- Ajayi, A.M., D.T. de Oliveira Martins, S.O. Balogun, R.G. de Oliveira, S.D. Ascêncio, I.M. Soares, R. Dos Santos Barbosa and O.G. Ademowo. 2017a. *Ocimum gratissimum* L. leaf flavonoid-rich fraction suppress LPS-induced inflammatory response in RAW 264.7 macrophages and peritonitis in mice. *J. Ethnopharmacol.* 204: 169–178. <https://doi.org/10.1016/j.jep.2017.04.005>.
- Ajayi, A.M., S. Umukoro, B. Ben-Azu, B. Adzu and O.G. Ademowo. 2017b. Toxicity and protective effect of phenolic-enriched ethylacetate fraction of *Ocimum gratissimum* (Linn.) leaf against acute inflammation and oxidative stress in rats. *Drug Dev. Res.* 78(3-4): 135–145. <https://doi.org/10.1002/ddr.21384>.
- Akbadian, A., A. Golian, H. Kermanshahi, R. Farhoosh, A.R. Raji, S. De Smet and J. Michiels. 2013. Growth performance and gut health parameters of finishing broilers supplemented with plant extracts and exposed to daily increased temperature. *Span. J. Agric. Res.* 11(1): 109–119. <https://doi.org/10.5424/sjar/2013111-3392>.
- Akinyemi, K.O., U.E. Mendie, S.T. Smith, A.O. Oyefolu and A.O. Coker. 2005. Screening of some medicinal plants used in south-west Nigerian traditional medicine for anti-*Salmonella typhi* activity. *J. Herb. Pharmacother.* 5(1): 45–60.

- Ali, S., R. Prasad, A. Mahmood, I. Routray, T.S. Shinkafi, K. Sahin and O. Kucuk. 2014. Eugenol-rich fraction of *Syzygium aromaticum* (clove) reverses biochemical and histopathological changes in liver cirrhosis and inhibits hepatic cell proliferation. *J. Cancer Prev.* 19(4): 288–300. <https://doi.org/10.15430%2FJCP.2014.19.4.288>.
- Al-Okbi, S.Y., D.A. Mohamed, T.E. Hamed and A.E. Edris. 2014. Protective effect of clove oil and eugenol microemulsions on fatty liver and dyslipidemia as components of metabolic syndrome. *J. Med. Food.* 17(7): 764–771. <https://doi.org/10.1089/jmf.2013.0033>.
- Amad, A.A., K. Männer, K.R. Wendler, K. Neumann and J. Zentek. 2011. Effects of a phytogetic feed additive on growth performance and ileal nutrient digestibility in broiler chickens. *Poult. Sci.* 90(12): 2811–2816. <https://doi.org/10.3382/ps.2011-01515>.
- Anugom, Y.O. and R.T.S. Ofongo. 2019. Impact of aqueous *Ocimum gratissimum* (Lyn) leaf extract on growth performance, gut pH and bacterial counts in broiler chickens. *Int. J. Poult. Sci.* 18(7): 309–316. <https://doi.org/10.3923/ijps.2019.309.316>.
- Aziba, P.I., D. Bass and Y. Elegbe. 1999. Pharmacological investigation of *Ocimum gratissimum* in rodents. *Phytother Res.* 13(5): 427–429. [https://doi.org/10.1002/\(sici\)1099-1573\(199908/09\)13:5%3C427::aid-ptr467%3E3.0.co;2-t](https://doi.org/10.1002/(sici)1099-1573(199908/09)13:5%3C427::aid-ptr467%3E3.0.co;2-t).
- Bonsnes, R.W. and H.H. Taussky. 1945. On the colorimetric determination of creatinine by the Jaffe reaction. *J. Biol. Chem.* 158: 581–591.
- Borsa, A., A. Kohayagawa, L.P. Boretti, M.E. Saito and K. Kuibida. 2006. Serum levels of hepatic enzyme function in clinically healthy broiler chickens. *Arq. Bras. Med. Vet. Zootec.* 58(4): 675–677.
- Brenes, A. and E. Roura. 2010. Essential oils in poultry nutrition: Main effects and modes of action. *Anim. Feed Sci. Technol.* 158(1–2): 1–14. <https://doi.org/10.1016/j.anifeedsci.2010.03.007>.
- Busari, A.O., F.O. Lawal, K.A Adedokun and S. Bashir. 2021. Effect of aqueous extract of *Ocimum gratissimum* (scent leaf) on hepatic profile of male wistar rats. *Al-Hikmah Journal of Health Sciences.* 1(1): 36–42.
- Chimnoi, N., N. Reuk-Ngam, P. Chuysinuan, P. Khlaychan, N. Khunnawutmanotham, D. Chokchaichamnankit, W. Thamniyom, S. Klayraung, C. Mahidol and S. Techasakul. 2018. Characterization of essential oil from *Ocimum gratissimum* leaves: Antibacterial and mode of action against selected gastroenteritis pathogens. *Microb. Pathog.* 118: 290–300. <https://doi.org/10.1016/j.micpath.2018.03.041>.
- Chiu, Y.W., P.Y. Chao, C.C. Tsai, H.L. Chiou, Y.C. Liu, C.C. Hung, H.C. Shih, T.J. Lai and J.Y. Liu. 2014. *Ocimum gratissimum* is effective in prevention against liver fibrosis *in vivo* and *in vitro*. *Am. J. Chin. Med.* 42(4): 833–852. <https://doi.org/10.1142/s0192415x14500530>.
- Clinical Diagnostic Division. 1990. Veterinary Reference Guide. Eastman Kodak Company, Rochester, New York, USA.
- Egbeyale, L.T., O.O. Adeleye, A.V. Adegoke, A.A. Ayoola, C. Oluitan and J.I. Ogunsakin. 2021. Growth performance and carcass characteristics of broiler chicken on administration of *Ocimum gratissimum* (scent leaf) leaf extract. *Nigerian J. Anim. Sci.* 23(3): 199–206.
- El-Hadary, A.E. and M.F. Ramadan Hassanien. 2016. Hepatoprotective effect of cold-pressed *Syzygium aromaticum* oil against carbon tetrachloride (CCl₄)-induced hepatotoxicity in rats. *Pharm. Biol.* 54(8): 1364–1372. <https://doi.org/10.3109/13880209.2015.1078381>.

- Elmore, S.A. 2018. Enhanced histopathology evaluation of lymphoid organs, pp. 147–168. *In*: J.C. DeWitt, C.E. Rockwell and C.C. Bowman, (Eds.), Immunotoxicity Testing. Methods in Molecular Biology 1803. Humana Press, New York, USA.
- Eyng, C., A.E. Murakami, T.C. Santos, T.G.V. Silveira, R.B. Pedroso and D.A.L. Lourenco. 2015. Immune responses in broiler chicks fed propolis extraction residue-supplemented diets. *Asian-Australas. J. Anim. Sci.* 28(1): 135–142. <https://doi.org/10.5713/ajas.14.0066>.
- Ezeabara, C.A., C.U. Okeke and B.O. Aziagba. 2013. Flavonoid content of *Citrus* species grown in Awka, Anambra State, Southeastern Nigeria. *Inter. J. Agri. Biosci.* 2(3): 103–107.
- Guo, Y., R.A. Ali and M.A. Qureshi. 2003. The influence of beta-glucan on immune responses in broiler chicks. *Immunopharmacol. Immunotoxicol.* 25(3): 461–472. <https://doi.org/10.1081/iph-120024513>.
- Harborne, J.B. 1973. *Phytochemical Methods*. Chapman and Hall Ltd., London, UK.
- Ishiwu, C.N., C.P. Umenwanne, J.E. Obiegbuna and N.N. Uchegbu. 2014. *In vitro* assessment of antibacterial effect of extracts *Ocimum gratissimum* and *Carica papaya* leaves. *Int. J. Appl. Sci. Technol.* 4(1): 171–177.
- Iwalokun, B.A., G.O. Gbenle, T.A. Adewole, S.I. Smith, K.A. Akinsinde and E.O. Omonigbehin. 2003. Effects of *Ocimum gratissimum* L. essential oil at subinhibitory concentrations on virulent and multidrug-resistant *Shigella* strains from Lagos, Nigeria. *APMIS.* 111(4): 477–482. <https://doi.org/10.1034/j.1600-0463.2003.1110405.x>.
- Khan, R.U., Z. Nikousefat, V. Tufarelli, S. Naz, M. Javdani and V. Laudadio. 2012. Garlic (*Allium sativum*) supplementation in poultry diets: Effect on production and physiology. *Worlds Poult. Sci. J.* 68(3): 417–424. <https://doi.org/10.1017/S0043933912000530>.
- Kin, A., L.M. Yaki, I. Abubakar, L.F. Olusola and R. Zubairu 2018. Antibacterial activity of *Ocimum gratissimum* (scent leaf) on some pathogenic gastrointestinal bacteria. *Afr. J. Microbiol. Res.* 12(40): 923–929. <https://doi.org/10.5897/AJMR2018.8847>.
- Ladipo, M.K., V.F. Doherty and U.C. Kanife. 2010. Phytochemical screening and antibacterial investigation of the extract of *Ocimum gratissimum* (scent leaf) on selected Enterobacteriaceae. *PAT.* 6(2): 75–84.
- Latta, M. and M. Eskin. 1980. A simple and rapid colorimetric method for phytate determination. *J. Agric. Food Chem.* 28(6): 1313–1315. <https://doi.org/10.1021/jf60232a049>.
- León, J.M., S.E. López Medina, H. Yabar and J. De La Cruz Castillo. 2017. Preserving traditional botanical knowledge: The importance of phytogeographic and ethnobotanical inventory of peruvian dye plants. *Plants.* 6(4): 63. <https://doi.org/10.3390/plants6040063>.
- Mann, A. 2012. Phytochemical constituents and antimicrobial and grain protectant activities of clove basil (*Ocimum gratissimum* L.) grown in Nigeria. *Int. J. Plant Res.* 2(1): 51–58. <https://doi.org/10.5923/j.plant.20120201.08>.
- Matasyoh, L.G., J.C. Matasyoh, F.N. Wachira, M.G. Kinyua, A.W.T. Muigai and T.K. Mukiyama. 2007. Chemical composition and antimicrobial activity of the essential oil of *Ocimum gratissimum* L. growing in Eastern Kenya. *Afr. J. Biotechnol.* 6(6): 760–765.

- Melo, R.S., A.M.A. Azevedo, A.M.G. Pereira, R.R. Rocha, R.M.B. Cavalcante, M.N.C. Matos, P.H.R. Lopes, G.A. Gomes, T.H.S. Rodrigues, H.S. Dos Santos, I.L. Ponte, R.A. Costa, G.S. Brito, F.E.A.C. Júnior and V.A. Carneiro. 2019. Chemical composition and antimicrobial effectiveness of *Ocimum gratissimum* L. essential oil against multidrug-resistant isolates of *Staphylococcus aureus* and *Escherichia coli*. *Molecules*. 24(21): 3864. <https://doi.org/10.3390/molecules24213864>.
- Meluzzi, A., G. Primiceri, R. Giordani and G. Fabris. 1992. Determination of blood constituent's reference values in broilers. *Poult. Sci.* 71(2): 337–345. <https://doi.org/10.3382/ps.0710337>.
- NRC (National Research Council). 1994. Nutrient Requirements of Poultry. 9th Edition. National Academy Press, Washington, D.C., USA.
- Nte, I.J., V.U. Oleforuh-Okoleh and L.B. Fakae. 2017. Responses of broiler chicks to scent leaf (*Ocimum gratissimum*) aqueous extracts during different stages of growth. *Delta Agriculturists*. 9(1): 99–107.
- Nweze, E.I. and E.E. Eze. 2009. Justification for the use of *Ocimum gratissimum* L. in herbal medicine and its interaction with disc antibiotics. *BMC Complement. Altern. Med.* 9: 37. <https://doi.org/10.1186/1472-6882-9-37>.
- Ohimain, E.I., R.T.S. Ofongo-Abule and D.V. Zige. 2015. *In-vitro* antibacterial effect of *Ocimum gratissimum* on broiler gut microflora. *Bulletin of Advanced Scientific Research*. 1(1): 37–41.
- Oke, O.E. 2018. Evaluation of physiological response and performance by supplementation of *Curcuma longa* in broiler feed under hot humid tropical climate. *Trop. Anim. Health Prod.* 50(5): 1071–1077. <https://doi.org/10.1007/s11250-018-1532-8>.
- Okur, N., S.A. Eratalar, A.A. Yiğit, T. Kutlu, R. Kabakçı and S.Y. Özsoy. 2022. Effects of incubator oxygen and carbon dioxide concentrations on hatchability of fertile eggs, some blood parameters, and histopathological changes of broilers with different parental stock ages in high altitude. *Poult. Sci.* 101(2): 101609. <https://doi.org/10.1016/j.psj.2021.101609>.
- Oladosu-Ajayi, R.N., H.E. Dienye, C.T. Ajayi and O.D. Erinle. 2017. Comparative screening of phytochemical compounds in scent leaf (*Ocimum gratissimum*) and bitter leaf (*Vernonia amygdalina*) extracts. *J. Fish. Aquac. Dev.* 2017(2): 112. <http://doi.org/10.29011/JFAD-112/100012>.
- Olobatoke, R.Y. and B. Okaragu. 2021. Scent leaf (*Ocimum grtissimum*) meal improved the growth performance and lowered blood cholesterol level of cockerels. *Int. J. Vet. Sci. Anim. Husb.* 6(1): 23–27. <https://doi.org/10.22271/veterinary.2021.v6.i1a.319>.
- Olumide, M.D. and A.S. Akintola. 2020. Effect of scent leaf meal (*Ocimum gratissimum*) supplementation on performance, carcass and meat quality of broiler chicken. *Nig. J. Anim. Prod.* 45(3): 228–236. <https://doi.org/10.51791/njap.v45i3.436>.
- Oni, A.I., O.O. Adeleye, T.O. Adebawale and O.E. Oke. 2024. The roles of phytogenic feed additives in stress mitigation in broiler chickens. *J. Anim. Physiol. Anim. Nutr.* 108: 81–98. <https://doi.org/10.1111/jpn.13869>.
- Oparaocha, E.T., I. Iwu and J.E. Ahanakuc. 2010. Preliminary study on mosquito repellent and mosquitocidal activities of *Ocimum gratissimum* (L.) grown in eastern Nigeria. *J. Vector Borne Dis.* 47(1): 45–50.

- Pandey, S., S.K. Singh, N. Kumar and R. Manjhi. 2017. Antiviral, antiprotozoal, antimalarial and insecticidal activities of *Ocimum gratissimum* L. AJPRD. 5(5): 1–9.
- Prabhu, K.S., R. Lobo, A.A. Shirwaikar and A. Shirwaikar. 2009. *Ocimum gratissimum*: A review of its chemical, pharma-cological and ethnomedicinal properties. Open Complement. Med. J. 1: 1–15. <http://doi.org/10.2174/1876391X00901010001>.
- Prakash, B., R. Shukla, P. Singh, P.K. Mishra, N.K. Dubey and R.N. Kharwar. 2011. Efficacy of chemically characterized *Ocimum gratissimum* L. essential oil as an antioxidant and a safe plant based antimicrobial against fungal and aflatoxin B₁ contamination of spices. Food Res. Int. 44(1): 385–390. <https://doi.org/10.1016/j.foodres.2010.10.002>.
- Rahman Alizadeh, M., A.H. Mahdavi, H.R. Rahmani and E. Jahanian. 2017. Clove bud (*Syzygium aromaticum*) improved blood and hepatic antioxidant indices in laying hens receiving low n-6 to n-3 ratios. J. Anim. Physiol. Anim. Nutr. 101(5): 881–892. <https://doi.org/10.1111/jpn.12502>.
- Roa, M.L., J.R. Corredor and M.C. Hernandez. 2020. Physiological behavior of broilers using diets with *Tithonia diversifolia* and probiotics. Arch. Zootec. 69(268): 406–417. <https://doi.org/10.21071/az.v69i268.5388>.
- SAS. 2002. SAS/STAT Statistical Analytical Systems User's Guide (Release 9.1 Edition). SAS Institute Inc., Cary, North Carolina, USA.
- Toghyani, M., M. Tohidi, A.A. Gheisari and S.A. Tabeidian. 2010. Performance, immunity, serum biochemical and haematological parameters in broiler chicks fed dietary thyme as alternative for an antibiotic growth promoter. Afr. J. Biotechnol. 9(40): 6819–6825. <https://doi.org/10.5897/AJB09.1998>.
- Uhegbu, F.O., I. Elekwa, E.I. Akubugwo, G.C. Chinyere and E.E.J. Iweala. 2012. Analgesic and hepatoprotective activity and methanolic leaf extract of *Ocimum gratissimum* (L.). Res. J. Med. Plant. 6(1): 108–115. <https://doi.org/10.3923/rjmp.2012.108.115>.
- Uyanga, V.A., T.H. Musa, O.E. Oke, J. Zhao, X. Wang, H. Jiao, O.M. Onagbesan and H. Lin. 2023. Global trends and research frontiers on heat stress in poultry from 2000 to 2021: A bibliometric analysis. Front. Physiol. 14: 1123582. <https://doi.org/10.3389/fphys.2023.1123582>.
- Varley, H., A.H. Gowenlock and M. Bell. 1980. Practical Clinical Biochemistry. 5th Edition. William Heinemann Medical Books Ltd, London, UK.
- WHO (World Health Organization). 2017. WHO Guidelines on Use of Medically Important Antimicrobials in Food-Producing Animals. Web Annex A: Evidence Base. World Health Organization, Geneva, Switzerland.
- Wootton, I.D.P. 1964. Microanalysis in Medical Biochemistry. 4th Edition. Churchill, London, UK.