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Impact of ginger (*Zingiber officinale*) and scent leaf (*Ocimum gratissimum*) on serum biochemistry and microbial load of broiler chickens

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ABSTRACT

The study was carried out to assess the effects of serum biochemistry and microbial load of broiler chickens fed diets containing ginger (*Zingiber officinale*) and scent leaf (*Occimum gratissimum*). 200-day-old Arbo acre chicks were divided into five groups at random and given different diets. Diet 1 served as the control group (0%) and diets 2, 3, 4, and 5 had an equal amount of ginger and scent leaf at 200g, 400g, 600g, and 800g. Each treatment was allocated to forty (40) birds in a completely randomized design (CRD), with ten (10) birds per replicate and four (4) replications for eight weeks. Microbial load and serum biochemical parameters were evaluated. The results showed significant ($P<0.05$) differences on biochemical indices such as total bilirubin, aspartate amino transferase, triglycerides, and alanine amino transferase. Significant ($P<0.05$) difference was also recorded for total heterotrophic bacteria across the test diet. The study showed that 200g inclusion of ginger and scent leaf should be adopted due to the positive influence on the parameters analyzed. Hence, this level is suitable to reduce the use of antibiotic growth promoters in broiler production.

Keywords: Antibiotics**,** blood, broiler production, ginger, microbial load, scent leaf

Résumé

Le chlorophytum, ou plante araignée (*Cleome gynandra* L.) est un légume africain indigène relativement peu étudié, qui a le potentiel de fournir des nutriments abordables et d'améliorer les moyens de subsistance et les revenus des petits agriculteurs. Une étude a été menée pour évaluer la variabilité génétique, la corrélation et le potentiel de sélection indirecte pour le rendement. Un total de 16 populations locales a été évalué dans un dispositif en lattices alpha 4x4 au parc agro-industriel de MUAST et à l'Institut de Recherche en Horticulture (HRI) pendant les saisons d'été 2022 et 2023. Cette étude a révélé une variation génotypique parmi les populations locales, avec des différences significatives ($p<0,05$) observées dans les jours jusqu'à 50 % de floraison (DF), la hauteur des plantes (PH), la longueur des feuilles (LL) et le rendement en feuilles fraîches comestibles (FY). Une héritabilité au sens large modérée à élevée et un pourcentage de progrès génétique ont été observés pour DF (0,74 ; 25,9 %), PH (0,32 ; 11,6 %) et FW (0,316 % ; 26,4 %), démontrant que ces caractères sont contrôlés par des effets génétiques additifs et peuvent être améliorés par sélection. De plus, des corrélations génotypiques significatives ($p<0,01$) fortes ont été détectées entre FY et PH ($rg = 0,84$) et entre FY et DF ($rg = 0,76$). En outre, les résultats de l'analyse des chemins pour le rendement en feuilles fraîches comestibles ont montré un effet direct fort de PH (0,63) et de DF (0,46), tandis qu'un effet direct modéré a été observé pour LL (0,20). En conclusion, les résultats de cette étude indiquent que PH et DF peuvent être utilisés pour améliorer le rendement en feuilles fraîches comestibles de la plante araignée. De plus, CGNPGRC353 et CGGURUVE ont montré une supériorité pour DF, PH et FY et ont le potentiel d'être utilisés comme donneurs ou directement. Les informations obtenues de cette étude peuvent être précieuses pour la sélection de la plante araignée pour le rendement en feuilles comestibles.

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Mots clés: antibiotiques, sang, charge microbienne, production de poulets de chair, gingembre, feuilles odorantes.

INTRODUCTION

Challenges over antibiotic resistance and the harmful effects of synthetic growth boosters have led to a renewed focus on the use of natural ingredients in chicken feed (Bartos *et al.*, 2019). Both ginger (*Zingiber officinale*) and scent leaf (*Ocimum gratissimum*) are well-known for their medicinal properties and potential benefits for animal health. According to Owen (2021), the well-known anti-inflammatory, antioxidant, and antibacterial qualities of ginger, can enhance the health and feed efficiency of chickens. Similarly, antibacterial properties of scent leaf have been documented; these properties may help regulate the quantity of microbes in the stomach, enhancing nutrient absorption and growth efficiency (Agholor *et al.*, 2018).

Broiler hens, which are raised primarily for meat production, are particularly susceptible to diseases due to their intensive farming practices. The microbial load in an animal's environment can influence its growth and productivity, potentially leading to various health problems (Martin and Ferasyi, 2019). Microbial load refers to the number of microorganisms (such as bacteria, fungi, viruses, or protozoa) present in a particular environment, tissue, or organism (Froebel *et al.*, 2019). In poultry, microbial load typically pertains to the concentration of bacteria, fungi, and other microbes found in the digestive system, on the skin, or in meat products (Deng *et al.*, 2020). Bouchama *et al.* (2024) observed that in broiler birds supplemented with *Curcuma longa* and *Zingiber officinale*, there was a complete absence of coliforms, *Salmonella sp.*, *Clostridium sp.*, and lactic acid bacteria (LAB) in both the treated and control groups. However, coliforms were detected in the control batch, highlighting the potential benefits of these spice supplements in reducing harmful microbial presence. In addition to positively influencing blood biochemical indicators, such as metabolites, electrolytes, and liver enzymes, natural herbs may also help reduce the microbial load in animals' diets (Akinmoladun and Oloyede, 2017). Umit *et al.* (2011) highlighted that biochemical parameters refers to the chemical analysis of blood serum and crucial for assessing the physiological and metabolic status of animals, providing valuable insights into overall health, growth performance, and meat quality in poultry. In Daramola *et al.*'s (2018) study on herbal supplementation in poultry diets, key parameters like blood glucose, albumin and globulins, liver enzymes, urea, and creatinine were used to evaluate the effects of dietary changes on blood health, nutrient metabolism, and the overall well-being of the birds. This was confirmed by the works of Yildirim *et al.*, (2011) who stated that the levels of enzymes such as aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) serve as bioindicators of liver function and potential damage. To support this assertion, Lumeij, (2008) also confirmed that elevated enzyme levels are often linked to liver or muscle damage caused by the body's stress response. Hence, the works of Bouchama *et al.* (2024) confirmed that adding 0.5% ginger to broiler diets reduced triglyceride concentrations, cholesterol, aspartate aminotransferases (ASAT), and alanine aminotransferases (ALAT), suggesting that ginger promotes better health and metabolism in broiler chickens, potentially leading to healthier livestock and improved production outcomes. Since microbial load and serum parameters are considered reliable indicators of an animal's physiological condition, monitoring changes in these factors is vital for evaluating how animals respond to various physiological and nutritional conditions. Notwithstanding, there is limited information on the use of a ginger and scent leaf blend as poultry feed. Therefore, the combined properties of ginger and scent leaf inspired this study on their effects on broiler chickens. The outcome of the study would be valuable for evaluating the health status of the birds and understanding the impact of these dietary interventions on their well-being. This would also reduce the need for synthetic antibiotics while encouraging the production of healthier chickens.

MATERIALS and METHODS

The study was carried out in the University of Port Harcourt Teaching and Demonstration Farm, Choba, Port Harcourt, Rivers State, Nigeria.

Source and processing of test ingredients. Ginger (*Zingiber officinale* Roscoe) and Scent Leaf (*Ocimum gratissimum*, Lamiaceae) were sourced from commercial vendors in the study area. The fresh ginger was thoroughly cleaned and sliced to facilitate effective dehydration. The sliced ginger roots were then oven-dried at 65°C for four days to reduce spoilage, enhance shelf life, and subsequently ground using an Attrition Milling Machine. The scent leaves were washed, separated from their stems, and oven-dried at 55°C until they became crispy in order to preserve their greenish-brown colour. The dried leaves were pulverized using a laboratory blender

Experimental treatments and feeding. Two hundred unsexed day-old broiler chicks (Arbo Acre Plus) were obtained from CHI Hatcheries in Oyo State, Nigeria for this study. The chicks were randomly selected and divided into five dietary treatment groups, with each group consisting of 40 chicks. These groups were then assigned into four replicates, each containing 5 chicks. Five treatment diets were formulated to meet the minimum nutritional requirements of the experimental birds. Diet T1 served as the control, while diets T2, T3, T4, and T5 included 200 g, 400 g, 600 g, and 800 g of the mixture of ginger and scent leaf, respectively, in equal proportions. The study was conducted over a period of 8 weeks.

The empirical model: The Statistical Model is stated as; $Y_{ij} = U + T_i + E_{ij}$
Where; Y_{ij} = Single Observation
U = Population mean
T_i = Effect where i^{th} treatment where i = 1, 2…5
E_{ij} = Random error

Data collection and analysis. In the fourth week of the experiment, 20 faecal samples were collected from each replicate and analyzed for microbial load. At the end of the study, samples from the trachea and large intestine were also collected for microbial culture to determine both the load and types of bacteria present at University of Port Harcourt Animal Science Laboratory. Microbial identification followed standard laboratory procedures (Valarmathy *et al*., 2010). The analysis involved serial dilution, microscopic examination, media preparation, and culturing of microorganisms. Smears of the samples were prepared and examined under a microscope to detect parasites and protozoans. Various media, including Mac Conkey, Nutrient, and Blood Agar, were sterilized, prepared, and cultured following manufacturer guidelines. After incubation, Gram staining was performed on bacterial isolates for further identification through biochemical characterization. The total bacteria viable count (CFU/ml) was expressed as total colony count divided by the dilution factor and multiplied by ml of the sample.

$$TBC = \frac{TCC}{DF} X\ ML$$

Where TBC = Total Bacteria Count
TCC = Total Colony Count
CFU = Colony forming Unit
DF = Dilution Factor or Factor of Serial Dilution Prepared (10-5)
ML = Milliliter of Sample or ml of sample.
At eight weeks, blood samples were obtained from five randomly selected broiler birds from each replicate using a syringe through the brachial wing veinApproximately 5 ml of blood were collected per bird into glass tubes and stored at -20°C for the analysis of serum glucose, total protein, cholesterol, and triglycerides, using Randeox and Biomerinx kits as outlined by Bahman *et al*. (2011).

Data collected from the study were analyzed using analysis of variance (ANOVA) procedure of Statistical Package for the Social Sciences (SPSS). Treatment means were compared using the Duncan multiple range test within the same software.

RESULTS AND DISCUSSION

Table 1 shows the impact of ginger and scent leaf on the microbial load of broiler chickens. The results indicated a significant ($P<0.05$) difference in the activity of total heterotrophic bacteria. Total heterotrophic in the droppings was highest in T4 (146.25±20.99) and lowest in T1 (60.00±20.99). Although, not significant ($P>0.05$) total coli form counts was highest in T4 (52.50±15.17) and lowest in T3 (50.25±15.17).

Total heterotrophic in the colon was significantly higher (P<0.05) in T4 (168.00±17.98) and lowest in T1 (72.25±17.98). Total coli form counts in the colon was significantly higher (P<0.05) in T4 (37.50±8.65) and lowest in T1 (35.00±8.65). The notable reduction in total heterotrophic bacteria in the colon compared to the droppings of birds fed 0g, 200 g, and 400 g demonstrates the effectiveness of the test diet in inhibiting pathogen growth which consequently reduces the availability of nitrogen and fermentable energy to intestinal microbes, as noted by Yang *et al.* (2022) and Cherian *et al.* (2023). This is in agreement with studies of Kirkpinar *et al.* (2011) and Moses *et al.* (2017) who reported that plant herbs inhibits the proliferation of harmful microbes in broiler chickens as compared to those administered synthetic antibiotics in the control group. However, the lack of significant difference in total coliform count (TCC) across the treatment and control groups indicates the antimicrobial properties of the plant extracts, as confirmed by Liu *et al.* (2019). This contributes to a balanced gut microflora, thereby enhancing the preservation of intestinal integrity (Akbarian *et al.*, 2019).

Table 2 shows the impact of ginger and scent leaf on serum biochemical indices of broilers. The values from the serum lipids and enzymes showed that triglyceride, aspartate amino-transferase, alanine aminotransferase, and total buliburin were significantly (p<0.05) affected by the test diets, while total cholesterol, high density lipoprotein, low density lipoprotein, alkaline phosphatise and conjugated bilirubin were unaffected by the diet. The values of these enzymes in the present study showed significant differences (p<0.05) between the control and the treated group with the highest values of AST at 0g, and 400g, while, ALT was highest among birds fed 400g,600g and 800g of ginger and scent leaf. Although Hassan *et al.* (2023) found no significant differences in uric acid, creatine kinase, glucose, and AST levels between the control group and the herbal groups. However, these biomarkers were significantly improved in the herbal groups compared to the antibiotic group. Therefore, the reduction in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) due to ginger and scent leaf at 200g inclusion level may be deduced as an indication of better liver function (Aikpitanyi and Egweh, 2020). This indicates that the herbal treatment was more effective in improving these biomarkers than the antibiotic treatment. The decreased levels of triglyceride at 0g and 200g inclusion levels from the current study was in tandem with Mohamed *et al.* (2022) and Rafiee *et al.* (2022) that had similar result in serum triglyceride with supplementation of ginger powder in broiler diets. Also, Kirkpinar *et al.* (2011) reported that broilers fed with diets supplemented with herbs had significantly lower triglycerides compared to those given antibiotics in the control. This shows the hypolipidemic effect of the plant extract in the liver as stated by Shewita and Taha, (2018) and Ciftci *et al.* (2020). Thus, the highest triglycerides at 600g inclusion level of ginger and scent leaf is suggestive of an increased protein catabolism and increased lipid availability for fat deposition (Hossein *et al.*, 2020). The lower levels of total bilirubin, recorded in ginger and scent leaf extract at 200g inclusion levels, agrees with the work of Ogbuewu *et al.* (2018). This suggests that the 200g dietary treatment had no adverse effects on the liver (Ubiogoro *et al.*, 2017), indicating that this specified amount appears to be safe for liver health, with no signs of liver damage or negative impact observed during the study.

Table 1. Influence of ginger and scent leaf on microbial load (droppings /colons) of broiler chickens

Samples	Parameters	T_1(0g)	T_2(200g)	T_3(400g)	T_4(600g)	T_5(800g)
Droppings	THB($X10^3$)	60.00±20.99^{b}	92.00±20.99^{ab}	101.00±20.99^{ab}	151.00±20.99^{a}	146.25±20.99^{a}
	TCC($X10^3$)	51.58±15.17^{a}	50.25±15.17^{a}	52.00±15.17^{a}	50.75±15.17^{a}	52.50±15.17^{a}
Colon	THB($X10^3$)	72.25±17.98^{b}	93.75±17.98^{b}	119.00±17.98^{b}	127.25±17.98^{ab}	168.00±17.98^{a}
	TCC($X10^3$)	35.00±8.65^{a}	35.75±8.65^{a}	36.00±8.65^{a}	36.25±8.65^{a}	37.50±8.65^{a}

$^{a, b, ab}$ Means within the same row with different superscripts are significantly different (P<0.05). THB- Total heterotrophic bacteria, TCC- Total coli form counts

Table 2. Influence of ginger and scent leaf on serum lipids and enzymes on broilers

Parameters	T 1 (0g)	T 2 (200g)	T 3 (400g)	T 4 (600g)	T 5 (800g)
Total Cholesterol(mm/l)	3.25±0.27 [a]	3.33±0.27 [a]	3.45±0.27 [a]	3.73±0.27 [a]	3.85±0.27[a]
Triglyceride (mg/dl)	0.98±0.11[b]	0.90±0.11[b]	1.04±0.11[ab]	1.36±0.11[a]	1.17±0.11[ab]
High density lipoprotein(mm/l)	1.10±0.09 [a]	1.05±0.09 [a]	1.15±0.09 [a]	0.99±0.09 [a]	1.02±0.09 [a]
Low density lipoprotein(mm/l)	1.87±0.17 [a]	1.96±0.17 [a]	1.82±0.17 [a]	1.86±0.17 [a]	1.90±0.17 [a]
Aspartate amino-transferase (u/l)	66.50±4.63[a]	55.75±4.63[ab]	69.25±4.63[a]	43.75±4.63[b]	56.75±4.63[ab]
Alanine amino-transferase **(**u/l)	14.00±1.54[ab]	9.75±1.54[b]	14.75±1.54[a]	16.00±1.54[a]	17.00±1.54[a]
Alkaline phosphatase (u/l)	57.00±0.92 [a]	56.25±0.92 [a]	54.25±0.92 [a]	56.50±0.92 [a]	56.00±0.92 [a]
Total Biluribin (µmol/L)	7.10±0.38[ab]	6.00±0.38[b]	7.48±0.38[a]	6.35±0.38[ab]	6.85±0.38[ab]
Conjugated bilirubin. (µmol/L)	4.18±0.63 [a]	3.90±0.63 [a]	3.90±0.63 [a]	3.85±0.63 [a]	4.05±0.63 [a]

[a, ab, b] Means within the same row with different superscripts are significantly different (P<0.05) TC- Total cholesterol, TG- Triglyceride, HDL-High density lipoprotein, LDL-Low density lipoprotein, AST - Aspartate amino-transferase, ALT-Alanine amino-transferase, ALP- Alkaline phosphatase,TB-Total Biluribin, CB-conjugated bilirubin.

CONCLUSION

The study on the effects of Ginger (*Zingiber officinale*) and Scent Leaf (*Ocimum gratissimum*) on the biochemical serum parameters and microbial load of broiler chickens yielded promising results. The incorporation of these herbs into the diets of broiler chickens significantly impacted on biochemical indicators such as total bilirubin, aspartate amino transferase, triglycerides, and Alanine amino transferase. Importantly, the addition of ginger and scent leaf resulted in a notable reduction in total heterotrophic bacteria in the intestines, indicating their effectiveness in enhancing gut health and potentially lowering the risk of pathogenic infections. Therefore, supplementation with ginger and scent leaf at a rate of 200 g/kg may serve as a viable alternative to synthetic antibiotics in poultry production.

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DECLARATION OF NO CONFLICT OF INTEREST

The authors declare no conflict of interest in the paper.

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