

Journal of the Hellenic Veterinary Medical Society

Vol 76, No 3 (2025)



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doi: [10.12681/jhvms.40224](https://doi.org/10.12681/jhvms.40224)

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To cite this article:

Obakanurhe, O., Aberji, O., Onwumere-Idolo, O., & Gbayisomore, O. (2025). Does Goat weed and Scent leaf meals Influences Growth performance, Blood indices, Carcass traits and Gut Microbiota of broiler chicken?. *Journal of the Hellenic Veterinary Medical Society*, 76(3), 9763–9776. <https://doi.org/10.12681/jhvms.40224>

Does Goat weed and Scent leaf meals Influences Growth performance, Blood indices, Carcass traits and Gut Microbiota of broiler chicken?

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ABSTRACT: The search for a safer antibiotic of phytogetic origin using Goat weed (*Ageratum Conyzoides*) leaf meal (GWLM) and Scent leaf (*Ocimum Gratissimum*) meal (SLM) as additive was investigated on 240 unsex day old chicks of Ross 308 strain. The birds were allotted into six diets and replicate four times with 10 birds per replicate in a completely randomized design. D1 was the control, D2 had 10g of Antibiotics (Diaziprim-48% S), while D3-D6 have 0.6g + 0.6g, 0.9 + 0.9g, 1.2 + 1.2g and 1.5 + 1.5g (GWLM + SLM) per kg of the feed respectively. At 21 days old, D4 recorded significant values of 1116.22g on final weight, 1076.02g weight gains, 1544.06g feed intake and better feed conversion ratio of 1.43. The birds final weight (2571.35g) and weight gain (1462.35g) on D5 at 42days was significantly ($p>0.05$) different compared to the other diets. The result on feed intake revealed that D1 and D5 had superior (2607.45g) and least (2565.44g) value. The feed conversion ratio of birds showed significantly better value on D5 in comparison to other diets. Also, the additives affected the cost of diets and cost of weight gain of the birds across the diets. The result on haematology and serum biochemistry revealed significant differences on WBC where D5 and D6 recorded superior values compared to other treatments. The blood indices of the birds revealed no adverse effects cause by the diets. The results on AST and ALT recorded decrease values with higher supplementation of the additives. The MDA, SOD, T-AOC, GSH-Px and CAT were within the range of healthy birds. The microbial load of birds revealed a reduced microbial load of *Staphylococcus spp*, *Escherichia coli* and *Salmonella* were observed at higher inclusion of the additives. In conclusion, adding these additives in the diets of broiler chickens could enhance the growth performance as reflected on the blood profile, carcass qualities, oxidative status and the reduce microbial loads without any adverse effects on the birds.

Keyword: Broiler chickens; Blood profile; Growth performance; microbial loads and Phytogetic.

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Date of initial submission: 29-1-2025

Date of acceptance: 5-6-2025

INTRODUCTION

Poultry production stands as a cornerstone among animal enterprises; it is a vital pillar for ensuring food security as well as socio-cultural and economic growth across numerous countries (Wani et al., 2023). According to Akpodiete et al (2014), the provision of meat and eggs from poultry significantly contributes to the global protein food supply. However, there has been growing concern regarding the use of antibiotic growth promoters in poultry nutrition and this has resulted in the quest for safer and alternative feed additives in tropical poultry farming (Ofuoku et al., 2016; Gopal & Dhanasekaran, 2021; Sapsuha et al., 2021; Mnisi et al., 2022).

Feed additives are substances that are added to livestock diets to improve production efficiency, health, and reduce mortality (Onwumelu et al., 2022; Obakanurhe et al., 2024). Plant-based feed additives, also known as phytogenics, have recently been proposed for inclusion in broiler chicken diets as growth stimulating feed additives due to their abundance in our natural environment. Furthermore, its usage in poultry, their active elements are being absorbed in the intestine by enterocytes and promptly metabolized by the animals' body (Amad et al., 2011; Murugesan et al., 2015; Abdelli et al., 2021; Obakanurhe et al., 2025).

The development of phyto-additives as alternative possibilities in chicken production has been motivated by limits and limitations in some countries on the use of antibiotic-free animal products. The increased demand for chicken products, combined with the need for environmentally friendly and health-promoting measures has prompted interest in alternative feed additives (Golowczyc and Gomez-Zavaglia 2024, Hossain et al., 2024). Thus, several researchers have explored the inclusion of phyto-additives in numerous animals' diets such as neem leaf (*Azadirachta indica*) (Unigwe et al., 2016), Moringa leaf (*Moringa oleifera*) (Oghenebrorhie & Oghenesuvwe, 2016; Tekce et al., 2020), Duckweed leaf (*Lemna minor*) (Irabor et al., 2023), Sweet Potato leaf (*Ipomoea batatas*) (Obakanurhe & Akpodiete, 2021; Obakanurhe et al., 2024), Coontail (*Ceratophyllum demersum*) (Ade et al., 2024), Goat weed leaf (*Angeratum conyzoides*) (Gbadamosi & Olanipekun, 2020), Scent leaf (*Ocimum gratissimum*) (Adedoyin et al., 2023), bitter leaf (*Vernonia amygdalina*) (Onwumelu et al., 2022), and Tarragon (*Artemisia dracuncululus* L.) (Kaya et al., 2021) just

to mention a few.

In an effort to improve broiler chicken health and production, researchers and farmers are constantly searching for innovative dietary additives and supplements. Among these, the use of natural additives such as GWLM and SLM, has received considerable attention because of their nutritional (significant quantity of crude protein, crude fiber, ash, ether extract, minerals and vitamins) and medicinal properties (a wide range of chemical compounds including alkaloids, flavonoids, chromenes, benzofurans and terpenoids). These plants compounds have a variety of properties that make them effective therapeutic agents against diseases or pathogens, including antibacterial, anti-inflammatory, antioxidant, anticarcinogenic, anthelmintic, antiproliferative, and antigenotoxic effects. Furthermore, the wide range of phytochemicals and chemical components found in plants has a substantial impact on their therapeutic efficacy. In order to assess medicinal plants' therapeutic potential, it is essential to investigate their phytoconstituents, nutritional components, and mineral components. Also, some performance indicators such as weight gain, feed intake, and feed conversion ratio are pivotal in assessing the influence of these additives on overall productivity. Furthermore, the analysis of blood profiles could yield crucial insights into the additives' physiological effects, while the assessment of microbial loads could be significant in unravelling the potential antimicrobial properties in these additives (Boontiam et al., 2019; Ding et al., 2020).

Understanding the ramification of introducing these phytogenic plants into the broiler diets is vital not only for improving production efficiency but also for maintaining the bird's health. Thus, this study aims to examine the efficacy and its potential benefits of Goat Weed Leaf Meal and Scent Leaf Meal on the growth performance, blood profile and microbial loads in broiler chicken.

MATERIAL AND METHODS

Ethical Standard

The present study was approved on 5th July, 2023 (-06-ANP-2023) by the Animal Welfare and Experimentation Ethics Committee in compliance with the Department of Animal Production, Dennis Osadebay University, Asaba, Delta State, Nigeria.

Experimental Site

The study was conducted from (September – October 2023) at the Poultry unit of the Teaching and

Research Farm, Department of Animal science, Denis Osadebay University, Anwai, Asaba. Asaba lies between longitude 6 ° 8' East and Latitudes 49°66' North of the equator. The mean annual rainfall ranges between 1500-1910 mm with its mean annual temperature and precipitation of 28°C±6°C and 117mm respectively (Asaba Metrological station, 2024).

Source and Processing of the Test ingredient

The GWLM and SLM were harvested and identified by the Departments of Agronomy and Environmental Toxicology. The leaves were cleaned to remove any soil and debris with water, and were allowed to air dry for 72 hours at room temperature (70 ° F) to

ensure adequate desiccation and crispy texture. The GWLM and SLM were ground to sizes between 2 and 3 mm using a hammer mill. Before being administered, the meals produced from the leaves were preserved in bags.

Proximate composition and phytochemical analysis of GWLM and SLM

The proximate composition was examined following (Suleiman et al., 2018) while the phytochemical properties were analysed according to procedures of (Morais et al., 2017).

Proximate composition of the test ingredients were analysed using these “formulas”

$$\% \text{ Moisture} = \frac{\text{weight of moisture}}{\text{weight of sample}} \times 100$$

$$\% \text{ Total ash} = \frac{\text{weight of ash alone}}{\text{weight of sample used}} \times 100$$

$$\% \text{ Ether extract} = \frac{\text{weight of flask and oil} - \text{weight of flask}}{\text{weight of sample}} \times 100$$

$$\% \text{ Crude fibre} = \frac{\text{weight of dish and fibre} - \text{weight of dish and silica dish}}{\text{weight of sample}} \times 100$$

$$\% \text{ Crude protein} = \frac{4.375 \times \text{Titre}}{\text{weight of sample}} \times 100$$

Preparation of the extracts

A Whatman filter paper was used to filter the powdered leaf sample after it had been steeped in ethanol for 72 hours. A rotary evaporator was used for concentrating the filtrate, producing a dark green material that was used as the extract.

Antioxidant Assays

The Folin-Ciocalteu technique was used to measure the total amount of phenolic compounds. Folin Ciocalteu reagent was used to homogenize various concentrations of ELEAC for five minutes, and then addition of aq. Na₂CO₃ (4 ml, 1 M). Phenols were quantified using a colorimetric (UV/Visible spectrophotometer; Perkin Elmer, Singapore) method at a wavelength of 765 nm after a 15-minute incubation time. With different amounts of gallic acid dissolved in ethanol:water (50:50, v/v) in µg/ml solutions, a suitable standard curve was produced. µg GAE (Gallic Acid Equivalent) per milligram of dry residue was used to express the total phenolic component concentrations.

Determination of DPPH radical scavenging activity

The DPPH radicals activity was measured using the method described by Morais et al., (2017) in order to evaluate the antioxidant capacity resulting from the extract's ability to hunt free radicals. Ethanol was used to create the extract in five distinct concentrations, ranging from 200 to 1000 µg/mL. A test tube was filled with 1 mL of each of the various doses and 1 mL of DPPH solution (0.3 mM). After 30 minutes, a UV/Visible spectrophotometer (Perkin Elmer, Singapore) was used to measure the absorbance at 517 nm. The formula below was used to calculate the percentage of DPPH radical hunting by ELEAC:

$$\% \text{ inhibition of DPPH radical} = \left(\frac{[Abb - Aba]}{Abb} \right) \times 100$$

In this case, Aba denotes the absorbance following the reaction, while Abb denotes the absorbance prior to the reaction (blank). The inhibition (%) plotted against the concentration of the extracted graph was used to calculate the IC₅₀ value, which represents the sample concentration thought to be required to

neutralize 50% of the DPPH free radical. Ferric Ion Reducing Antioxidant Power (FRAP) Determination. The extract was mixed with 2.5 milliliters of 1% potassium ferricyanide at different doses and 2.5 milliliters of phosphate buffer (20 mM). 2.5 ml of 10% trichloroacetic acid and 0.5 ml of 0.1% ferric chloride were added to the mixture after it had been incubated for 30 minutes at 50°C. After letting the solution remain for ten minutes, its absorbance was measured at 700 nm utilizing a UV/Visible spectrophotometer (Perkin Elmer, Singapore).

Experimental Animals

A total of 240 day old unsexed broiler chickens of Ross 308 strain were used for the experiment using the test ingredients as additives in their diets. The birds were randomly allotted into six dietary groups, each replicated four times, with 10 birds per replicate in a completely randomised design. The birds deter-

mine their intake of feed for 0 – 21 days and 22 - 42 days for starter and finisher phases (Table 1 and 2).

Experimental Design and Diet Formulation

D1 – Control (0%)

D2 – 1.0 g of Antibiotics (Diaziprim-48% S) per Kg of feed

D3 - 0.6 g GWLM + 0.6 g SLM per Kg of feed

D4 - 0.9 g GWLM + 0.9 g SLM per Kg of feed

D5 - 1.2 g GWLM+ 1.2 g SLM per Kg of feed

D6 - 1.5 g GWLM + 1.5 g SLM per Kg of feed

DATA COLLECTION

Growth performance

The feed intake of birds were calculated by the total amount of feed administered minus the leftover feed weighed and deducted from the total amount fed per replicate. The weight gains of the birds were estimated by subtracting the initial weight from the

Table 1. Ingredients composition of broiler starter chickens diets

Feedstuff (kg)	D1	D2	D3	D4	D5	D6
GWLM (g)	-	-	0.6	0.9	1.2	1.5
SLM (g)	-	-	0.6	0.9	1.2	1.5
Maize	52.00	52.00	52.00	52.00	52.00	52.00
Soyabean meal	20.00	20.00	20.00	20.00	20.00	20.00
Groundnut cake	14.00	14.00	14.00	14.00	14.00	14.00
Wheat bran	8.00	8.00	8.00	8.00	8.00	8.00
Bone meal	2.50	2.50	2.50	2.50	2.50	2.50
Limestone	1.75	1.75	1.75	1.75	1.75	1.75
Lysine	0.50	0.50	0.50	0.50	0.50	0.50
Methionine	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Premix	0.50	0.50	0.50	0.50	0.50	0.50
	100.00	100.00	100.00	100.00	100.00	100.00
Calculated values						
Crude protein (%)	23.12	23.03	23.00	23.10	23.07	23.05
ME (Kcal/kg)	3192.25	3194.30	3203.21	3201.67	3203.81	3205.11
Available phosphate (%)	0.76	0.72	0.78	0.81	0.84	0.82
Calcium (%)	1.72	1.73	1.71	1.76	1.80	1.84
Fat (%)	2.58	2.54	2.52	2.58	2.51	2.52
Crude fibre (%)	5.41	5.39	5.45	5.58	5.42	5.42

* Composition of vitamin/mineral premix broiler starter per kg: Vitamin E, 25mg; Vitamin A, 6250 IU; Vitamin D3,1250 IU; Vitamin K3, 25mg; Vitamin B1, 25mg; Vitamin B2, 60mg; Vitamin B6, 40mg; Vitamin B12, 2mg; Elemental calcium, 25mg; Elemental phosphorus, 9mg; Elemental magnesium, 300mg; Iron, 400mg; Selenium 1.0mg, Iodine 20mg, Copper 60mg, Magnesium 100mg, cobalt 10mg, Zink, 150mg; Sodium Chloride, 1.5mg; Choline Chloride, 500mg; Live *Lactobacillus* spore, 0.2 million cfu; Niacin, 40mg; Folic Acid, 10mg; d-Biotin, 5mcg.

Table 2. Ingredients composition of broiler finisher chickens diets

Feedstuff (kg)	D1	D2	D3	D4	D5	D6
GWLM (g)	-	-	0.6	0.9	1.2	1.5
SLM (g)	-	-	0.6	0.9	1.2	1.5
Maize	56.00	56.00	56.00	56.00	56.00	56.00
Soyabean meal	16.00	16.00	16.00	16.00	16.00	16.00
Groundnut cake	10.00	10.00	10.00	10.00	10.00	10.00
Wheat bran	12.00	12.00	12.00	12.00	12.00	12.00
Bone meal	2.70	2.70	2.70	2.70	2.70	2.70
Limestone	1.50	1.50	1.50	1.50	1.50	1.50
Lysine	0.50	0.50	0.50	0.50	0.50	0.50
Methionine	0.30	0.30	0.30	0.30	0.30	0.30
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Premix	0.50	0.50	0.50	0.50	0.50	0.50
	100.00	100.00	100.00	100.00	100.00	100.00
Calculated values						
Crude protein (%)	20.12	20.03	20.00	20.10	20.07	20.05
ME (Kcal/kg)	3192.25	3194.30	3203.21	3201.67	3203.81	3205.11
Available phosphate (%)	0.76	0.72	0.78	0.81	0.84	0.82
Calcium (%)	1.72	1.73	1.71	1.76	1.80	1.84
Fat (%)	2.58	2.54	2.52	2.58	2.51	2.52
Crude fibre (%)	5.41	5.39	5.45	5.58	5.42	5.42

* Composition of vitamin/mineral premix broiler finisher per kg: Vitamin E, 25mg; Vitamin A, 6250 IU; Vitamin D3, 1250 IU; Vitamin K3, 25mg; Vitamin B1, 25mg; Vitamin B2, 60mg; Vitamin B6, 40mg; Vitamin B12, 2mg; Elemental calcium, 25mg; Elemental phosphorus, 9mg; Elemental magnesium, 300mg; Iron, 400mg; Selenium 1.0mg, Iodine 20mg, Copper 60mg, Magnesium 100mg, cobalt 10mg, Zinc, 150mg; Sodium Chloride, 1.5mg; Choline Chloride, 500mg; Live *Lactobacillus* spore, 0.2 million cfu; Niacin, 40mg; Folic Acid, 10mg; d-Biotin, 5mcg.

final weight of birds per replicate. On the feed conversion ratio of birds, the total feed consumed per replicate divided by the weight gain of birds per same replicate. All growth parameters were done weekly (Obakanurhe 2024).

Economics of Production of birds fed experimental diets

The economic evaluation of feeds was based on the current market pricing of ingredients utilized during the investigation. The feed cost per kilogram of diet and feed cost per kilogram of weight increase served as the foundation for the economic study. The meal cost per kg diet and feed conversion ratio for each dietary treatment were multiplied to determine the feed cost per kg per live weight gain.

Haematological parameters

On day 42, following a night of starvation at the conclusion of the feeding session, blood samples were taken from one bird per replicate for haematological

and serum biochemical examination. A sterile disposable syringe and needle were used to draw blood from each bird's vein beneath its wings between 6.30 and 7.30 am. To prevent infection and widen the vein before bleeding, a cotton swab soaked in 70% ethanol was employed. Using sterile universal bottles labeled with Ethylene-Diamine-TetraAcetic acid (EDTA) as an anticoagulant, a 3.0 ml blood sample was first drawn. Within an hour after sample collection, this was used to identify the haematological components. The blood samples Haematological parameters such as red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), haemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin Concentration (MCHC) were determined by routine procedures previously described by (Livinston *et al.*, 2020). While MCV, MCH and MCHC were calculated as follows:

$$\text{Mean corpuscular volume (MCV \%)} = \frac{\text{PCV}}{\text{RBC}} \times 100$$

$$\frac{Hb}{RBC} \times 100 = \text{MCHC} = \frac{HB}{PCV} \times 100$$

Serum biochemistry and Antioxidants

Following coagulation, the blood samples were centrifuged at 3500 rpm (2328.24 G) for 15 minutes, and serum was stored for analysis at -20°C. We measured the following biochemical parameters: Alanine, protein, and its portions. A specific kit (diagnostic tools from Biodiagnostic Co., Giza, Egypt) was used to measure the following: alanine transaminas (ALT), creatinine, urea, aspartate aminotransferase (AST), uric acid, triglycerides, cholesterol, malondialdehyde (MDA), superoxide dismutase (SOD), total antioxidant capacity (TAC), glutathione peroxidase (PSH), catalase (CAT) according to Keyhani et al. (2024).

Carcass qualities of birds fed experimental diets

At 42 days, two birds were selected to represent each replicate at the end of the feeding trial, fasted throughout the night, weighed, and then sacrifice by dislocation of the cervical vertebrae. The eviscerated weight is calculated as the weight of a carcass after all internal organs (such as the gastrointestinal tract, respiration, and reproduction organs) were completely evacuated. The dressed weight percentage was determined by dividing the derived carcass weight by the bird's final live weight after the head, feathers, and internal organs were removed. Each bird's breast, thigh, and abdominal fat were weighed and represented as a proportion of the carcass weight and some vital organs mean weights were also recorded.

Cecal Microbiota

The birds' microbial weights were examined on day forty. After being labelled and fed for two days, ten birds per replicate were selected depending on how much they weighed corresponding to the average group weight. Following their random selection, the ceca of the same ten birds from each treatment were gathered. The cecal tissue from each group was mixed and homogenized. Ten grams of this mixture were then mixed with 90 milliliters of sterile water peptone, and the mixture underwent stimulation for half an hour. The liquid portion was collected to create a 1:10 dilution (10⁻¹ dilution). Additional dilutions were carried out in tenfold stages up to a dilution (10⁻⁷) (Abd-El-Hack et al., 2021). Almonella species, coliforms, Escherichia coli, lactic acid bacteria, total bacterial count (TBC), and total yeast and

mold count (TYMC) were measured (Helen et al., 2020, Abd El-Hack et al., 2021 and Abou-Kassem et al., 2021).

Statistical Analysis

The statistical programme GenStat (Release 4.24) was used to perform an analysis of variance on the data. For the mean separation, Duncan's multiple range tests were employed.

RESULTS

Proximate compositions and photochemical analysis of the additives

The proximate analysis and phytochemical composition of the test ingredients showed significant quantities of crude protein, ether extract and crude fibre, and tannin, flavonoids (anthocyanins), anthraquinones and phenolic acids respectively Table 3.

Growth performance and economics of production of birds fed experimental diets

The growth performance of broiler starter birds showed that D4 recorded significant superior values in final weight (1116.22 g) and weight gain (1076.02 g). A better feed conversion ratio value of 1.43 was recorded compare to the other diets (Table 4). The economics of production at the starter phase (Table 4) showed that the cost of diets, cost of feed intake and cost of body weight gain/birds on D2 recorded significantly ($p < 0.05$) superior values compared to the other diets respectively. It was observed that, the test diets D3 – D6 showed a progressive decline on the various economics of production parameters as the inclusion levels increased.

In the finisher phase, the diets fed on the birds have significant effects on the growth performance the diets (Table 5). It was observed that birds on D5 showed significant ($p > 0.05$) mean values in final weight (2571.35 g), weight gain (1462.35 g) and a better feed conversion ratio of 1.76. The economics of production of the birds showed that the cost of diets on D2 was significantly different in comparison to the other diets. Similar trend of progressive decreased of values across the test diets (D3 – D6) in the starter phase same was observed during the finisher phase. The values recorded on the cost of body weight gain showed that D1 was significantly ($p > 0.05$) superior in comparison to the other diets.

Blood profile and Serum Antioxidants activities

The results of the haematological and serological analysis are presented in Table 6. There was a sig-

Table 3. Proximate compositions and phytochemicals analysis of the additives

Parameters (%)	Goat Weed Leaf Meal	Scent Leaf Meal
Moisture	9.43±1.05	8.16±0.33
Crude Protein	13.42±0.52	8.27±0.21
Crude Fibre	13.87±0.45	11.46±0.59
Ether Extract	4.80±0.08	3.42±0.14
Ash content	10.96±1.13	12.88±0.89
Nitrogen Free Extract	47.52±0.72	45.11±0.05
Alkaloids	28.64±0.28	22.22±0.80
Tannins	7.44±0.28	5.21±0.81
Saponins	61.31±0.15	42.61±0.72
Flavonoids	21.52±0.61	25.60±1.21
Phenol	6.14±0.15	3.62±0.04
Ascorbic Acids	12.43±0.12	8.23±0.07
Anthraquinons	10.53±0.25	14.30±1.14
Anthocyanin	15.65±0.56	10.41±0.09
Cadiac glycosides	-	-
Trypsin inhibitor	4.32±0.04	1.05±0.09
Oxlate	5.02±0.08	2.55±0.47
Phytate	2.18±0.04	0.12±0.08

Key: - : Absent

Table 4. Growth performance and economics of broiler chickens fed starter diets

Parameters	D1	D2	D3	D4	D5	D6	SEM	P value
Initial weight (g)	40.06	40.20	40.50	40.20	40.15	40.09	0.00	0.10
Final weight (g)	1072.18 ^d	1075.37 ^d	1085.22 ^c	1116.22 ^a	1109.00 ^b	1103.39 ^{bc}	27.04	0.02
Weight gain (g)	1032.12 ^d	1035.17 ^c	1044.72 ^c	1076.02 ^a	1068.85 ^b	1063.30 ^{bc}	37.02	0.01
Feed intake (g)	1547.04 ^b	1544.14 ^a	1528.48 ^d	1544.06 ^d	1552.94 ^c	1556.11 ^c	42.16	0.04
FCR	1.50 ^a	1.49 ^a	1.46 ^b	1.43 ^c	1.45 ^b	1.46 ^b	0.04	0.00
Cost of Diet/Kg (₦)	920.00 ^c	934.25 ^a	925.40 ^b	906.64 ^d	884.45 ^c	867.05 ^f	27.02	0.02
Cost of feed intake/ bird/g (₦)	1442.28 ^a	1442.61 ^b	1414.46 ^c	1399.91 ^d	1373.50 ^e	1349.23 ^f	25.32	0.01
Cost of body weight gain/bird (₦)	1380.00 ^b	1392.03 ^a	1351.08 ^c	1296.50 ^d	1282.45 ^e	1265.89 ^f	20.83	0.03

a-f: Treatment means with different superscripts within the same row are significantly not different at $p > 0.05$; SEM = Standard error of mean, Feed Conversion Ratio (FCR), ₦ = Naira

nificantly influenced ($p < 0.05$) of the diet on the WBC, Uric acid, Total Protein, glucose, AST, ALT, CAT), GPx, SOD, and MDA. However, Hb, PCV, MCV, MCH, MCHC, Creatinine, Urea, Cholesterol, Albumin, and Globulin were not affected ($p > 0.05$) by the diets.

Carcass qualities of experimental birds fed experimental diets

The result on carcass qualities showed that the additives significant ($P < 0.05$) affected the dressed weight, plucked weight, eviscerated and breast (Table 7). However, the organ weight were not negatively affected as inclusion increase.

Table 5. Growth performance characteristics and economics of broiler chickens fed finisher diets

Parameters	D1	D2	D3	D4	D5	D6	SEM	P value
Initial weight (g)	1072.18 ^d	1075.37 ^d	1085.22 ^c	1116.22 ^a	1109.00 ^b	1103.39 ^{bc}	27.04	0.02
Final weight (g)	2492.29 ^d	2528.41 ^c	2542.18 ^c	2562.48 ^b	2571.35 ^a	2561.92 ^a	87.44	0.04
Weight gain (g)	1420.11 ^b	1453.04 ^c	1456.96 ^c	1446.26 ^b	1462.35 ^a	1458.53 ^a	35.16	0.02
Feed intake (g)	2607.45 ^a	2604.21 ^b	2598.92 ^c	2582.54 ^d	2565.44 ^d	2589.81 ^c	82.05	0.01
FCR	1.84 ^a	1.79 ^c	1.78 ^b	1.79 ^{ab}	1.76 ^d	1.78 ^c	0.04	0.00
Cost of Diet/Kg (₦)	840.15 ^b	853.63 ^a	830.16 ^c	804.82 ^d	784.21 ^e	769.03 ^f	15.44	0.02
Cost of feed intake/ bird/g (₦)	2190.65 ^b	2223.03 ^a	2157.52 ^c	2078.48 ^d	2011.84 ^e	1991.64 ^f	81.37	0.04
Cost of body weight gain/bird (₦)	1545.88 ^a	1528.00 ^b	1477.68 ^c	1440.63 ^d	1380.21 ^e	1368.87 ^f	32.02	0.03

a,b,c,d: Treatment means with different superscripts within the same row are significantly not different at $P > 0.05$; SEM = Standard Error of Mean, Feed Conversion Ratio (FCR), ₦ = Naira

Table 6. Blood profile and antioxidant activities of broiler finisher chickens

Parameters	D1	D2	D3	D4	D5	D6	SEM	P value
Hb (g/dL)	8.10	10.27	10.02	10.06	10.46	10.92	8.33	0.74
PCV (%)	22.68	23.11	22.97	23.42	23.05	23.47	11.27	0.10
RBC ($\times 10^6/\text{mm}^3$)	15.82	12.02	12.22	13.27	13.03	11.96	7.43	0.24
WBC ($\times 10^3/\text{mm}^3$)	98.05 ^d	105.21 ^c	97.32 ^d	104.14 ^c	112.65 ^a	108.41 ^b	27.82	0.02
MCV (fl)	134.15	136.89	130.74	130.42	132.54	130.55	13.44	0.33
MCH (pg)	68.62	71.34	72.08	67.83	70.22	72.04	10.42	0.19
MCHC (g/dL)	59.71	58.67	57.89	56.83	54.41	52.82	12.41	0.61
Creatinine (mg/dL)	0.45	0.46	0.45	0.44	0.42	0.40	0.03	0.45
Uric acid ($\mu\text{mol/L}$)	414.10 ^a	404.5 ^c	407.32 ^b	403.19 ^d	400.13 ^e	399.51 ^e	22.83	0.04
Urea (mmol/L)	5.36	5.34	5.42	5.33	5.37	5.28	0.07	0.25
Cholesterol (g/dL)	6.98	3.40	4.80	3.20	2.96	2.49	4.00	0.03
Total protein (g/dL)	5.28 ^b	5.80 ^a	5.77 ^a	5.28 ^b	4.75 ^c	4.87 ^c	0.08	0.04
Glucose (g/dL)	2.56 ^a	1.96 ^b	1.82 ^c	1.76 ^d	1.60 ^f	1.64 ^e	0.38	0.01
Albumin (g/dL)	2.46	2.41	2.46	2.48	2.50	2.56	0.04	0.51
Globulin (g/dL)	2.90	3.39	3.31	3.00	3.28	3.21	0.05	0.08
AST (U/L)	123.06	121.11	122.52	122.33	121.68	121.25	21.41	0.15
ALT (U/L)	22.23 ^a	20.50 ^b	21.01 ^{ab}	19.14 ^b	17.62 ^c	17.74 ^c	0.55	0.04
CAT ($\mu\text{mol/L}$)	114.32 ^d	114.12 ^d	114.38 ^d	116.64 ^c	120.08 ^b	123.27 ^a	1.68	0.01
GPx (U/g Hb)	120.07 ^e	123.72 ^d	122.15 ^d	125.02 ^c	128.62 ^b	135.33 ^a	1.23	0.02
SOD (U/g Hb)	58.69 ^c	65.44 ^b	63.91 ^c	60.27 ^d	65.10 ^b	68.06 ^a	1.54	0.01
MDA ($\mu\text{mol/L}$)	2.10 ^a	2.02 ^c	1.97 ^b	1.68 ^c	1.72 ^d	1.65 ^e	0.16	0.02

abcd- Means within the row with different superscripts are different at $P < 0.05$. SEM: Standard error of the mean Hb: Haemoglobin, PVC: Packed cell volume, RBC: Red blood cell counts, WBC: White blood cell counts, Hb: haemoglobin, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration. MDA, malondialdehyde; SOD; superoxide dismutase; GSH-Px, glutathione peroxidase

Caecal Microbiota

The results of the effect of the GWLM and SLM on the caecal microbial loads in the broiler chicken are

summarised in Table 8. The results showed that the control (D1) recorded significantly ($p < 0.05$) higher levels of the microbial load compared with other

Table 7. Carcass Characteristics of Broilers Finisher fed Experimental Diets at 42 days

Parameters	D1	D2	D3	D4	D5	D6	SEM	P value
Live wt (g)	2492.29 ^d	2528.41 ^c	2542.18 ^c	2562.48 ^b	2571.35 ^a	2561.92 ^b	87.44	0.04
Plucked wt (g)	2467.35 ^e	2483.35 ^d	2497.12 ^c	2517.32 ^b	2526.24 ^a	2516.88 ^b	86.30	0.01
Dress Weight (DW)	2221.43 ^c	2221.07 ^d	2242.34 ^c	2254.12 ^b	2274.25 ^a	2267.08 ^b	86.62	0.03
Dress Carcass (%LW)	89.13	87.84	88.21	87.97	88.45	88.49	8.12	0.51
Eviscerated Carcass wt (g)	1789.23 ^c	1827.03 ^d	1842.84 ^c	1838.45 ^c	1895.74 ^a	1852.06 ^b	5.02	0.02
Eviscerated weight (%LW)	71.79	72.26	72.49	71.74	73.73	72.29	80.24	0.42
Head (g)	6.08	6.18	6.87	5.98	6.12	6.09	0.24	0.11
Shank (g)	74.05	76.31	75.04	77.12	77.24	75.24	9.22	0.09
Breast (g)	636.03 ^e	644.04 ^d	653.21 ^c	658.55 ^b	680.11 ^a	661.74 ^b	3.07	0.02
Back (g)	545.07	553.43	552.28	581.18	557.51	551.06	52.01	0.64
Drumstick (g)	177.09	179.44	180.81	178.32	182.71	179.92	15.44	0.82
Thigh (g)	195.81	194.88	190.75	192.03	195.11	192.04	16.71	0.25
Neck (g)	56.08	52.94	54.21	54.50	55.01	53.20	6.38	0.64
Wing (g)	202.11	201.21	204.43	205.10	203.64	200.11	12.05	0.04
Abdominal Fat (g)	2.80 ^a	2.54 ^b	2.20 ^c	2.11 ^c	1.78 ^d	1.60 ^d	0.52	0.00
Organ evaluation								
Heart (%DW)	14.20	14.49	14.31	14.06	14.28	14.32	0.15	0.44
Liver (%DW)	44.02	44.80	43.69	42.22	39.25	40.17	0.22	0.12
Bursa of Fabricius	0.19	0.17	0.16	0.18	0.19	0.20	0.16	0.12
Gizzard (%DW)	50.11	50.45	51.65	52.09	52.03	51.93	1.09	0.07
Pancrease (%DW)	4.72	4.55	4.47	4.87	4.67	4.88	3.44	0.06
Spleen (%DW)	4.04	4.32	4.22	4.06	4.11	4.65	4.14	0.52
Colon (cm/100gDW)	19.93	19.37	20.11	21.44	20.01	19.94	0.55	0.10
Small Intestine (cm/100gDW)	215.02	216.07	216.22	215.98	216.12	215.94	1.22	0.07
Caecum (cm/100gDW)	20.58	20.43	20.44	20.46	20.34	20.28	5.37	0.33
Proventriculus (cm/100gDW)	10.05	10.08	11.82	11.44	11.07	10.83	1.33	0.09

a,b,c,d: Treatment means with different superscripts within the same row are significantly ($P < 0.05$) different; SEM = Standard error of mean; DW = Dressed Weight, EW = Eviscerated Weight, LW = Live Weight, and DC=Dressed Carcass

Table 8. Cecal microbiota load of broiler finisher fed experimental diets

Parameters	D1	D2	D3	D4	D5	D6	SEM	P value
Total bacterial count	8.25 ^a	7.54 ^c	7.72 ^b	7.21 ^d	6.96 ^e	6.71 ^f	0.04	0.02
Lactobacilli	9.81 ^a	7.57 ^c	8.63 ^b	8.06 ^d	8.27 ^e	8.42 ^f	0.03	0.04
Coliform	6.52 ^a	6.27 ^c	6.21 ^c	6.34 ^b	6.08 ^e	5.94 ^f	0.06	0.02
Escherichia coli	7.85 ^a	7.44 ^c	7.52 ^b	7.11 ^d	6.55 ^f	6.95 ^e	82.05	0.01
Salmonella	4.85 ^a	4.32 ^c	4.55 ^b	4.06 ^d	3.85 ^e	3.84 ^e	0.04	0.00

* a,b,c,d: Treatment means with different superscripts within the same row are significantly ($P < 0.05$) different; SEM = Standard error of mean

diets. The level of microbes in the broilers chickens reduces on the increase of the experimental diets across the groups.

DISCUSSION

Growth performance and economics of birds fed experimental diets

Broiler chicken, like other livestock, requires an adequate supply of nutrient-rich feed to sustain their health and productivity (Kaya, 2023). Due to their abilities to enhance broiler chicken growth performance and feed utilization, Additives of plant origin are currently regarded as a promising major cure for antibiotic-free animal nutrition. These have been identified as an abundant protein source which promotes growth and health due to its phytochemical constituents (Popescu et al., 2021; Akpodiete et al., 2023)

Table 4 showed the effect of GWLM and SLM on the growth performance of the broiler chicken at starter phase. The growth parameters (final weight, weight gain, feed intake, feed conversion ratio) of the broiler birds were significantly influenced ($p > 0.05$) by the graded inclusion of both GWLM and SLM in their diet and this is in line with the findings of Adeyeye et al. (2020) who reported that there was significant effect of wild sunflower and goat weed leaf meal on the growth performance of broiler chicken. The highest final weight and weight gain were at D4 which corresponds with the findings of several authors who reported that increased supplementation of (*Ocimum gratissimum*) did not reduce final weight and body weight gain (Chima 2016; Anugom & Ofongo 2019). Feed conversion ratio values decrease with increase in GWLM and SLM across the dietary treatment and this conforms to the findings of Essien & Udoh (2021) that evaluate the effect of *Ocimum gratissimum* (Scent leaf) on the performance, haematological and serum biochemical indices, and carcass characteristics of broiler chicken. The present experiment revealed that D4 (0.9 g of both GWLM and SLM) at the starter phase, utilized the nutrients in the diet efficiently to produce the highest final weight compared to other dietary treatment. This findings are similar to the earlier study of Adedeye et al. (2020) that recorded superior growth performance on birds fed wild sun flower and goat weed.

Table 5 showed the effect of GWLM and SLM on the growth performance of the broiler chicken at finisher phase. The result on body weight gain showed significant difference across the treatment

Also, D5 and D1 recording the highest and lowest value for final weight. The finding are similar to the results reported by researchers who documented that supplementation of feed additives influenced the body weight in broiler chickens (Chima 2016; Anugom & Ofongo 2019). The spike in body weight caused by the inclusion of feed additive could be attributed to the immediate effect of both GWLM and SLM, which meets the phenol, flavonoids, and saponins deficits while also protecting the birds from a variety of adverse environmental conditions. Regarding the feed conversion ratio, D5 recorded the optimum value for FCR and thus had the highest total weight gain. This is in close agreement to the report of Qorbanpour *et al.* (2018) that reported that additive supplement in broiler chicken diet improved growth performance, nutrient utilization and feed conversion ratio of the birds.

The nutrient requirement of birds are largely influenced by the cost of feed ingredients at various production phases. Feed formulations which is the computation of the various quantity and quality of the different ingredients also depends on the nutrients requirement of the animal. The production of these diets varies in corresponding to the production phase. The production of feeds for poultry is significant to their growth and performance. Recently, most farms operating on large production scale often procured feeds from commercial feed mills at high cost in other to curb the issue of feed formulation. The quantity and quality of these feeds produced are often sub-standard and could affects the animal's growth and performance. The differences recorded on the cost of diets on D2 was due to the cost of the antibiotic used in the experiment. Several authors have reported the increase in the cost of diets produce with synthetic antibiotics compared to phytogetic plants and probiotics in poultry production (Chima 2016; Anugom & Ofongo 2019; Helen et al., 2020)

Carcass qualities of experimental birds fed experimental diets

The addition of additives did yield a significant ($P < 0.05$) impact on the relative weights of dressing, plucked weight, eviscerated and breast (Table 7). Nevertheless, the inclusion of the additives reduces the mean abdominal fat as inclusion levels increase ($P < 0.05$) significantly.

Our study found that the mean percentage of abdominal fat was significantly lower in the additives groups, aligning with the findings of Gakuya et al. (2014), which indicated that high levels of

Moringa Leaf Extract supplementation (exceeding 7.5 %) reduced abdominal fat. Three potential methods could be used by SLM and GWM leaves to decrease fat deposition. First, by suppressing genes involved in fat production, lipid biosynthesis is decreased (Jeon et al. 2023). Second, by encouraging the growth of advantageous gut bacteria such *Lactobacillus* species, which can decrease the activity of the acetyl-COA carboxylase enzyme involved in fatty acid production, the additions may indirectly decrease lipid biosynthesis (Abu Hafsa et al. 2020). Thirdly, these leaves contain polyunsaturated fatty acids (PUFA), which may stimulate the fatty acid β -oxidation process, improve lipid metabolism, and decrease fat accumulation (Naseer et al. 2023). We discovered that broilers supplied with additives, the weights of the other organs—the heart, liver, pancreas, spleen, proventriculus, gizzard, intestine, and bursa were not altered.

This implies that the carcass and organ weight of the broiler chicken significantly increase by the inclusion of these additives. Nonetheless, the current findings are in consistent with those of Glamoclija et al. (2016) and Oloruntola et al. (2018), who discovered that broiler chickens' dressing % and relative internal organ weights were greatly increased by the addition of phyto-additives.

The values recorded on the internal organs are similar with earlier studies that noted that the small intestine is the main location for nutritional breakdown and absorption, it plays a critical role in digestion. Changes in its gross architecture and histology can therefore change the region of digestion and absorption, which in turn can affect growth performance (Chaiyasing et al., 2024). Several studies have showed that the internal systems are essential for various operations; such as metabolism, which influence performance in birds (Çapar et al., 2024; Chaiyasing et al., 2024).

Blood profile of broiler finisher fed diets supplemented graded levels of GWLM and SLM

Blood indices are important instruments for determining the nutritional, pathological and physiological status of an animal. Analysis of haematological constituents in livestock, such as haemoglobin, red blood cells, white blood cells, packed cell volume, among others, aids in the diagnosis and monitoring of illnesses related with feed toxicity or infections (Hernandez et al., 2020; Neethirajan, 2020). Table 6 displayed the varying inclusion level of GWLM

and SLM on the blood profile of broiler chicken at finisher stage. The range of Hb values obtained (8.10 to 10.92 g/dl) fell slightly lower than the range of (11.60 to 13.68 g/dl) and (13.00 to 14.86 g/dl) reported by Wikivet, 2019 and Adeyeye et al. (2020) respectively. Thus, the haemoglobin was a sign of variation in carbon dioxide and oxygen transportation capacity of the birds fed varying levels of GWLM and SLM. The PCV values obtained from this research (22.68 to 23.47) fell within the standard range of 22 - 35 % reported by Etim et al. (2014) for healthy birds. The WBC, uric acid, total protein, glucose, AST, ALT, CAT, GPx, SOD and MDA were significantly affected by the dietary treatment. However, CAT, GPx, SOD and MDA values increase at higher inclusion levels across the treatment group. AST is widely distributed in numerous organs, including the heart, liver and skeletal muscles, while ALT is primarily found in the liver. Any abnormally high level of serum enzyme AST could be an indicator of liver injury and necrosis. The non-significant values recorded in AST values across the diets demonstrated that GWLM and SLM could be fed to broiler chicken without causing deleterious effect. However, low AST values suggest the presence of toxins in the liver and heart as a result from the feed.

Antioxidant enzymes such as GsPx, CAT, and SOD remove free radicals and reactive species from tissues, protecting the organism from oxidative stress. CAT reacts immediately with free radical species, while GPx recovers antioxidants and converts hydrogen peroxide to water, alcohols and lipid peroxides in the tissue (Delles et al., 2014). Birds supplemented with GWLM and SLM had higher levels of catalase and glutathione peroxidase contrasted to control and antibiotic medication, indicating that the phyto-additive enhances antioxidant status by scavenging free radicals and reactive species. Several authors have reported that adding phyto-additives to broiler diets significantly increased GPx and CAT concentrations compared to the control group (Kostadinović et al., 2015; Adeyeye et al., 2020).

Cecal Microbiota level of broiler finisher fed diets supplemented with graded levels of GWLM and SLM

The study's ingredients include bioactive substances including polyphenols and flavonoids that have antimicrobial qualities. The decrease in total bacterial counts, *Salmonella* and *Escherichia coli* counts seen in the treated groups could be attributed to these substances (Manso et al., 2022).

Polyphenols of plant origin demonstrate antibacterial abilities against a variety of bacteria, including Gram-positive and Gram-negative bacteria Manso et al., 2022. A large number of bacterial strains are resistant to numerous treatments, posing a unique risk to people working in care facilities. Polyphenols are prospective for studying compounds that are novel antibiotics opening the route for successful innovative antibacterial agents' development, considering its advantages and disadvantages. Studies have demonstrated that polyphenols improve the gut microbiota by decreasing harmful bacteria and encouraging the development of helpful bacteria (Fang et al, 2018; Wang *et al.* 2022). The synthesis of amino acids and B vitamins, which are vital for the sustenance and well-being of broiler chickens, as well as digestion, especially with regard to dietary fibers, are facilitated by the gut microorganisms (Rifat et al. 2024). In contrast to the control group and the antibiotic diet, we found that the broiler caeca of the additives groups had substantially reduced levels of *E. coli* and total bacteria counts. Similar results were noted when adding powdered *Moringa oleifera* leaves and *Moringa oleifera* leaf extracts to bird's meals and water significantly decreased the gastrointestinal microbial load and *E. coli* counts when opposed to the control group and the antibiotic-supplemented groups (Divya et al. 2014 and Rifat et al. (2024).

Furthermore, several authors have reported the efficacy of some medicinal plants added to poultry diets to have significant effects in reducing the microbial population such as *E. coli* colonies (Ardiansyah et al., 2024, Oliveira et al. 2024). Several chemical compounds and essential oils found in these leaves are thought to have the potential to lower coliform counts and the microbial load (Hassan et al. 2024; Oliveira et al 2024).

The gastrointestinal tract of an animal can be described a micro-ecosystem that provides specialized niche for intestinal bacteria communities to habit, and in turn the bacteria act in an intraorganismal mutualism with the host animal for optimal advantage of both (Fijabi *et al.*2018). The results of

microbial level in the ceacum of birds fed varying levels of GWLM and SLM reduced as inclusion level increased.

CONCLUSION

Generally, the present study showed that birds on D5 had significant weight gain, improved FCR with a better economics of production. The additives significantly improves the carcass qualities, haematology and serum oxidative status of the chicken without any negative effect. Also, impacting positively by reducing *E. coli* and total bacterial counts. It was therefore recommended that birds on D5 should be incorporated in broiler chickens diets for optimum performance without residual effects. Therefore, the inclusion of these additives could be used as a natural feed additive for organic broiler production, particularly when in-feed antibiotics are not available. However, based on these findings, some variables including dosage, length, management, and the diet's overall nutritional quality, the advantages of SLM and GWLM supplementation may differ. The ideal dosage and duration for the effective interaction of the bioactive components in these additives and the main nutrients in the diet require more investigation. This will make it easier to understand how they could affect meat quality, health status, and resistance to disease threats.

Author Contributions All authors contributed equally to this work.

Funding No external funding received

Data availability: Relevant data will be made available by the corresponding author upon reasonable request.

DECLARATIONS

Ethical Approval The Experiment was approved on 5th July, 2023 (-06-ANP-2023) by the Animal Welfare and Experimentation Ethics Committee in compliance with the Department of Animal Production, Dennis Osadebay University, Asaba, Delta State, Nigeria.

Competing Interest The authors declare no competing of interests.

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