Acta fytotechn zootechn, 28, 2025(4): 324–335
http://www.acta.fapz.uniag.sk

Original Paper

Effects of Moringa and Garlic addition to Broiler's Diet on Growth, Internal Organs, and Gut Microbes

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Article Details: Received: 2025-03-05 | Accepted: 2025-08-12 | Available online: 2025-12-31

https://doi.org/10.15414/afz.2025.28.04.324-335

It has been found that some bioactive compounds discovered in herbs and spices can increase the number and quality of poultry products. Therefore, the purpose of this study was to examine the effects of adding *Moringa oleifera* leaf meal (MLM) and garlic bulb meal (GBM) to broiler diets on growth, internal organs, and gut microbes. In a completely randomized design, 240 broiler chickens, that were 8 days old, were divided into 5 nutritional regimens. Each treatment had 12 birds and 4 replicates. The treatments were T1: Control diet; T2: 1% MLM substitution for soybean meal; T3: 3% MLM substitution for soybean meal; T4: 1% MLM substitution for soybean meal along with 0.1% GBM; and T5: 3% MLM substitution for soybean meal along with 0.3% GBM correspondingly. Total phenols and flavonoids were high in both meals. On day 28, dietary treatment's (T2, T3, T4, and T5) final weights, average body weight gain, and total feed intake were all similar to those of T1 (Control). The liver in dietary treatments (T2, T3, T4, and T5) were smaller (P <0.05) to those in T1, while the spleen in T3 was larger (P <0.05). The *lactic acid bacteria* in T2, T3, T4, and T5 were found to be similar (P >0.05) to those in T1, while T1 had a higher value of *coilforms* (P <0.05) than T2, T3, T4, and T5. On the 56th day, T5's total feed intake and T3, T4, and T5 feed conversion ratio were lower (P <0.05) than T1. T1 and T4, kidneys were smaller in weight (P <0.05) than T3 kidneys. The total microbial count, *lactic acid bacteria* and *coilforms* in T3, T4, and T5 were considerably (P <0.05) lowered than those in T1 and T2. These results imply that because of the potential contribution of their phyto-supplements, MLM and GBM can be employed as feed additives in the formulation of broiler diets.

Keywords: broiler chicken, garlic bulb, growth performance, intestinal microflora, *Moringa oleifera*

1 Introduction

A significant loss of economically valuable birds as a result of some pathogenic microorganisms obstructing their gastrointestinal tracts and causing nutritional competition with the host animals is posing a challenge to the poultry industry's recent increase in production. Because of this situation, there are fewer nutrients available to support growth and maintain birds' usual activity (Abdelli et al., 2021). The inclusion of phytogenic plants as phyto-supplements in poultry feed for growth promotion and increased general activity has been the subject of expanded investigation due to earlier studies on natural growth promoters (NGPs) (Abdelli et al., 2021; Agbetuyi et al., 2023a). Through regulated pathogens, these phyto-supplements' stabilize feed hygiene and have positive effects on gastrointestinal bacteria (Agbetuyi et al., 2023a). These phyto-supplements were seen as environmentally beneficial, safe, and simple to use. Because they can enhance poultry products with natural antioxidants and antibacterial chemicals, phytogenic supplements are increasingly being employed by farmers to fight various diseases that have a detrimental impact on productivity (Agbetuyi et al., 2023a). Some studies in the field of poultry nutrition have demonstrated the appropriateness of phytogenic supplements (Chak et al., 2020; Abdelli et al., 2021) and plant products (Agbetuyi & Oloruntola, 2020). Researchers are now paying more attention to two phytogenic plants: *Moringa oleifera* leaf powder/meal and garlic bulb powder/meal (Agbetuyi et al., 2023b; Dhakad et al., 2024). The impact of the plant on broiler immune and digestive systems has been reported. By lowering serum and

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tissue levels of low-density lipoprotein, triglycerides, and cholesterol, adding garlic to broilers diets can enhance the birds' performance (Abdulkareem, 2024).

One plant that can be used in animal production to improve feed efficiency and nutrient content is *Moringa oleifera*, which can also be used as an inexpensive protein and mineral supplement for animals' diet (Agbetuyi et al., 2023a). In the composition of broiler diets, *Moringa oleifera*, a natural growth promoter (NGP), was used as an alternative supplement to antibiotic growth promoter (AGP) (Agbetuyi et al., 2024a). Because of its excellent nutritional content and use as a crop for cattle feed, the plant is being studied (Chaudhary et al., 2023). *Moringa oleifera* leaves and garlic bulbs have been found to be beneficial feed additions to broiler production (Agbetuyi et al., 2023b). The antioxidant-active phytonutrients such as carotenoids, tocopherols, phenolics, and ascorbic acid were abundant in *Moringa oleifera* leaves (Dhakad et al., 2024). Its leaves have been shown to have high levels of protein, minerals, β-carotene, vitamins A, C, and E, as well as antioxidants that are beneficial for human and animal nutrition (Agbetuyi et al., 2023a). According to a research by Adewumi et al. (2022), *Moringa oleifera* leaves contain 16–19 amino acids, 10 of which are essential amino acids with over 90 minerals, including a good amount α-linoleic acid. Niaziridin in *Moringa oleifera* has been shown by Mahfuz & Piao (2019) to enhance the absorption of several vitamins, minerals, and other micronutrients in the gastrointestinal tract of animals, improving the performance characteristics of broilers. According to the earlier studies, broiler chickens' growth performance significantly improved when *Moringa oleifera* leaf meal was added to their diet (Song et al., 2024). *Moringa oleifera* leaf supplementation increases body weight gain and improves intestinal morphology, according to a study by Moreno-Mendoza et al. (2021). Another study by Sugiharto et al. (2020) demonstrated that adding *Moringa oleifera* leaf meal to the broilers' diet enhanced their immunity against Newcastle disease (ND) and infectious bursal disease (IBD).

The common herb garlic (*Allium sativum*) has been used extensively for its therapeutic benefits. It has antibacterial, hypolipidemic, antihypertensive, anti-atherosclerosis, antidiabetic, antiviral, and antifungal qualities (Gupta et al., 2021). Along with a substantial amount of dietary fiber, vitamin C, and folic acid, it includes a number of phytogens, including flavonoids, saponins, tannins, alkaloids, steroids, hydrocyanide, and anthocyanin (Agbetuyi et al., 2024b). Iron, calcium, and a small amount of sodium are also included, along with high-quality protein. According to reports, broiler chickens who received *Allium sativum* as a feed additive at the rate of 20 g.kg^{-1} had superior weight gain, higher feed efficiency, lower mortality rate, and better health situation (Abd El-Ghany, 2024). Garlic added to feed at a rate of 3% significantly improves broiler chicken development performance without having any negative consequences (Hafeez et al., 2024). By adding 25 g.kg^{-1} of *Allium sativum* to the broiler's diet, the birds' live weight increased and their feed conversion ratio improved (Malematja et al., 2023). Broiler meat quality was enhanced with a better lipid profile when garlic and moringa were combined (Agbetuyi et al., 2024c). Garlic has been shown to improve digestion and absorption in broiler chicken by lowering intestine pathogenic bacteria loads (Abd El-Ghany, 2024). Additionally, it improves chicken's immune capacities by reducing *Escherichia coli* numbers and increasing *lactic acid bacteria* population (Abd El-Ghany, 2024). Broiler chicks benefit from the mutually stimulating impact of adding powdered *Allium sativum* bulbs in their diet (Agbetuyi et al., 2024a). Thus, examining how the growth performance, internal organs, and gut microbial load are affected by the addition of *Moringa oleifera* leaf meal and garlic bulb meal in broiler diets was the aim of this study.

2 Material and methods

2.1 Gathering and Preparation of Moringa oleifera Leaves and Garlic Bulbs

Garlic bulbs were bought from a local market in Ado Ekiti, Ekiti State, Nigeria, while *Moringa oleifera* leaves were gathered from the moringa plantation set up at the experimental field, Crop Science Department, Teaching and Research Farm, Federal University, Oye Ekiti, Ikole Campus, Ekiti State, Nigeria. After being laid out on a clean and dry concrete floor, the moringa leaves were allowed to air dry for 7 days in a shaded, well-ventilated environment. Moringa leaf meal (MLM; Figure 1) was made by grinding the dried leaves with a mortar and pestle. Cloves were sliced into chips after the garlic

Figure 1 *Moringa oleifera* leaf meal/powder

Figure 2 Garlic bulb meal/powder

bulbs were de-segmented. For two weeks, the chips were carefully sun-dried until they were crispy. After using a mortar and pestle to grind the flake into garlic bulb meal (GBM; Figure 2), it was kept at room temperature for the feeding trial in a different plastic airtight container (Agbetuyi et al., 2023b; 2024a).

2.2 Quantitative Phyto-Constituents Analysis

Using the techniques outlined by Gupta et al. (2021), a quantitative analysis of a few secondary metabolites (alkaloids, saponnins total phenols, flavonoids, and tannins) was conducted to determine their proportion in the *Moringa oleifera* leaf meal and garlic bulb meal.

2.2.1 Alkaloids' Determination

After macerating the sample (1 g) with 20ml of ethanol and 20% sulfuric acid (H_2SO_4), or 1 : 1 v/v, it was filtered. 5 ml of 60% sulfuric acid were mixed with 1 ml of the filtrate. Five minutes later, the mixture (i.e., filtrate (1 ml) + 5 ml 60% H_2SO_4) was combined with 5 ml of 0.5% formaldehyde in 60% H_2SO_4 (sulphuric acid) and left to stand for 3 hours. At 565 nm, the absorbance was measured (Figure 3).

2.2.2 Saponins Determination

Ten milliliters (10 ml) of petroleum ether were used to macerate the sample (1 g), which was then decanted into a beaker. Six milliliters (6 ml) of ethanol were used to dissolve the residue after an additional 10ml of PET ether was added to the beaker, filtered, and the filtrate was dried off. After placing 2 ml of the solution in a test tube, 2 ml of chromagen solution were added, and the mixture was allowed to stand for half an hour. At 550 nm, the absorbance was measured (Figure 3).

2.2.3 Total Phenols Determination

After macerating the 1 g sample with 20 ml of 80% ethanol, it was filtered. After adding 5ml of the filtrated material to 0.5 ml of folicio calteus reagent (FCR), the mixture was left to stand for half an hour. After adding 2 ml of 20%

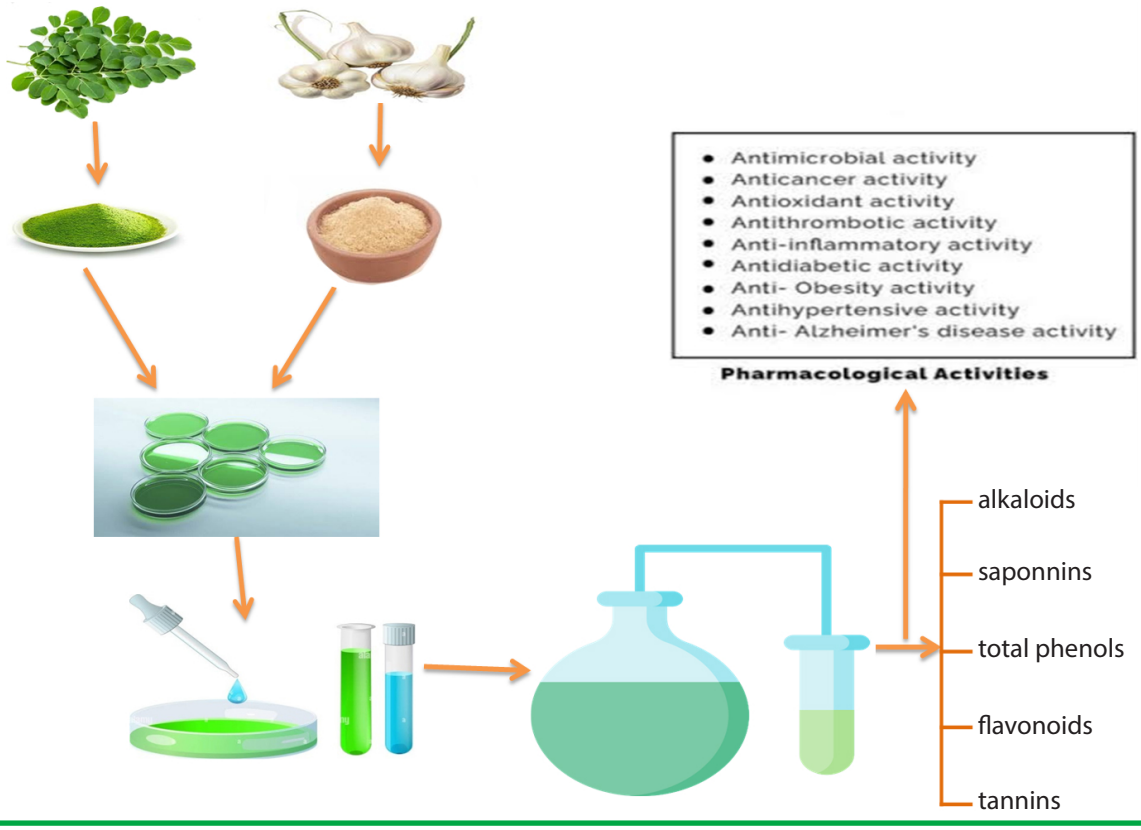

Figure 3 Systematic analysis of secondary metabolites from *Moringa oleifera* and garlic

sodium carbonate (Na_2CO_3), the absorbance at 650 nm was measured (Figure 3).

2.2.4 Flavonoids Determination

After macerating the 1 g sample for 5 minutes with 20 ml of ethyl acetate, it was filtered. 5ml of diluted ammonia (NH_3) was added to 5 ml of the filtrate, and the mixture was agitated for an additional 5minutes. After gathering the top layer, the absorbance at 490 nm was measured (Figure 3).

2.2.5 Tannins Determination

Fifty millilters (50 ml) of methanol were used to macerate the 1 g sample before it was filtered. After adding 0.3 ml of 0.0008 M potassium ferro cyanide and 0.30 ml of 0.1 N ferric chloride in 0.1 N HCl to the filtrate (5 ml), the absorbance was measured at 720 nm (Figure 3).

2.3 Management of the Birds, Experimental Site, and Ethical Permission

The Faculty Board of the Faculty of Agriculture, Federal University Oye Ekiti, Ekiti State, Nigeria, provided the ethical permission for the use of animals and animal procedure prior to the start of this project. The Poultry Research Unit, Animal Production and Health Department, Ikole Campus of Federal University Oye Ekiti, was the site of the experiment. The average annual temperature at the site is 24.2 °C (Agbetuyi et al., 2023b). The 1.83 × 1.22 m (length × width) experimental pens were cleaned, fumigated, and disinfected with potassium permanganate before the chicks' arrival. The birds' bedding consisted of wood shavings that were scattered 6 inches (15.24 cm) deep on the floor and replaced every two weeks. To disinfect the footwear of individuals entering the pen, a foot dip was placed at the entrance of the bird's house. On the day of arrival, a glucose anti-stress pack was administered via water to combat the stress of transportation. At 7 and 14 days of age, the birds received vaccinations against Gumboro disease, and at 21day, they received vaccinations against Newcastle disease. All of the replicates underwent standard management procedures. Throughout the experiment, biosecurity, cleanliness, and good hygiene were maintained (Agbetuyi et al., 2024c). The brooding house was kept at a temperature of 35 °C. Later, this was changed to 33 °C at the second week, 31 °C at the third, and 27 °C at the fourth week.

2.4 Feed and Supplementation Process

To produced the diet that was used for the study, five experimental diets were formulated, which were manually blended into homogenous meals by adding *Moringa oleifera* leaf meal and garlic bulb meal. Prior to the phyto-supplements addition to the predetermined feed ingredients, the phyto-supplements were thoroughly mixed with other microminerals for one minute. This mixture was ater mixed with one kilogram of crushed maize, and it was homogenized by turning them over for another minute. To create 100 kg of each experimental diet, these were then combined on the floor using a spade (Agbetuyi et al., 2023b).

2.5 Experimental Design and Diets

Two hundred and forty broiler chickens ($n = 240$) that were 8 days old, unsexed (Cobb 500), and having average weight of 119.75 ±1.66 g were randomly assigned to 5 nutritional regimens, with 4 replicates, each with 12 birds. To satisfy the nutritional needs of broiler chicken, a basic diet was produced. For days 1–7 of their lives, the birds were given a base diet *ad libitum*. Five experimental diets were produced, and MLM and GBM were used to replace the soybean meal in each experimental diet as follows: Treatment 1 (T1): Control diet; Treatment 2 (T2): 1% MLM substitution for soybean meal; Treatment 3 (T3): 3% MLM substitution for soybean meal; Treatment 4 (T4): 1% MLM substitution for soybean meal along with 0.1% GBM; and Treatment 5 (T5): 3% MLM substitution for soybean meal along with 0.3% GBM (Tables 1 and 2). From week two to eight, the experimental diets and water were provided *ad libitum*. The experiment was designed using a completely randomized design (CRD).

2.6 Measurements

2.6.1 Growth performance

The feed intake was monitored by feeding a weighed amount of feed each week and deducting the amount of feed leftover from the amount of feed served for the week. The weekly feed consumption (g.bird^{-1}) was calculated by dividing the total amount of feed consumed by the number of birds per replicate. The body weight was also measured each week, and the body weight gain (BWG) was calculated by dividing the weekly weight gain by the number of weighed birds. The feed conversion ratio was calculated by dividing the feed intake by the weight gained.

$$\text{feed intake (g.bird}^{-1}) = \frac{\frac{\text{feed given}}{\text{week}} - \frac{\text{feed leftover}}{\text{week}}}{\frac{\text{number of birds}}{\text{replicate}}}$$

$$\text{body weight gain (g.bird}^{1}) = \frac{\text{total body weight gain}}{\text{number of birds}}$$

$$\text{feed conversion ratio (FCR)} = \frac{\text{feed intake}}{\text{weight gained}}$$

Table 1 Composition and nutrient content of the grower diets (g.100 g^{-1})

Ingredients (%)	T1	T2	T3	T4	T5
MLM	–	0.36	1.07	0.36	1.07
GBM	–	–	–	0.10	0.30
Maize	50.00	50.00	50.00	50.00	50.00
Soybean meal	35.50	35.14	34.43	35.04	34.13
Fish meal 72%	1.00	1.00	1.00	1.00	1.00
Wheat offal	10.00	10.00	10.00	10.00	10.00
Bone meal	1.00	1.00	1.00	1.00	1.00
Limestone	1.90	1.90	1.90	1.90	1.90
Methionine	0.15	0.15	0.15	0.15	0.15
Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.20	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00	100.00
Calculated values					
ME (Kcal.kg^{-1})	2,891.10	2,882.12	2,864.40	2,882.12	2,864.40
Crude protein (%)	23.04	22.94	22.75	22.94	22.75
Crude fiber (%)	4.17	4.17	4.17	4.17	4.17
Crude Fat (%)	3.63	3.63	3.62	3.63	3.62
Lysine (%)	1.25	1.24	1.22	1.24	1.22
Methionine (%)	0.49	0.49	0.49	0.49	0.49

1 kg of vitamin premix contains: vitamin A –10,000,000 IU; vitamin D3 – 2,000,000 IU; vitamin E – 20,000 IU; vitamin K – 2,250mg; thiamine B1 – 1,750mg; riboflavin B2 – 5,000 mg; pyridoxine B6 – 2,750mg; niacin: 27,500 mg; vitamin B12 – 15 mg; pantothenic acid – 7,500mg; folic acid – 7 ,500mg; biotin – 50 mg; choline chloride – 400 g; antioxidant – 125 mg; magnesium – 80 g; zinc – 50 g; iron – 20 g; copper – 5 g; iodine – 1.2 g; selenium – 200 mg; cobalt – 200 mg; ME – metabolizable energy; MLM – *Moringa oleifera* leaf meal; GBM – garlic bulb meal; T1 – control diet; T2 – 1% MLM substituted for soybean meal (SBM); T3 – 3% MLM substituted for SBM; T4 – 1% MLM substituted for SBM + 0.1% GBM; T5 – 3% MLM substituted for SBM + 0.3% GBM

Table 2 Composition and nutrient content of the finisher diets (g/100g)

Ingredients (%)	T1	T2	T3	T4	T5
MLM	–	0.29	0.87	0.29	0.87
GBM	–	–	–	0.10	0.30
Maize	52.00	52.00	52.00	52.00	52.00
Soybean meal	29.00	28.71	28.13	28.61	27.83
Wheat offal	12.40	12.40	12.40	12.40	12.40
Bone meal	1.80	1.80	1.80	1.80	1.80
Limestone	4.00	4.00	4.00	4.00	4.00
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.20	0.20	0.20	0.20	0.20
Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
Calculated values					
ME (Kcal.kg^{-1})	2,830.56	2,823.32	2,808.85	2,823.32	2,808.85
Crude protein (%)	20.07	19.99	19.83	19.99	19.83
Crude fiber (%)	3.98	3.98	3.98	3.98	3.98
Crude Fat (%)	3.53	3.52	3.52	3.52	3.52
Lysine (%)	1.05	1.05	1.03	1.05	1.03
Methionine (%)	0.50	0.49	0.49	0.49	0.49

1 kg of vitamin premix contains: vitamin A – 10,000,000 IU; vitamin D3 – 2,000,000 IU; vitamin E – 20,000 IU; vitamin K – 2,250mg; thiamine B1 – 1,750mg; riboflavin B2 – 5,000 mg; pyridoxine B6 – 2,750mg; niacin: 27,500 mg; vitamin B12 – 15 mg; pantothenic acid – 7,500 mg; folic acid – 7,500 mg; biotin – 50 mg; choline chloride – 400 g; antioxidant – 125mg; magnesium – 80 g; zinc – 50 g; iron – 20 g; copper – 5 g; iodine – 1.2 g; selenium – 200 mg; cobalt – 200 mg; ME – metabolizable energy; MLM – *Moringa oleifera* leaf meal; GBM – garlic bulb meal; T1 – control diet; T2 – 1% MLM substituted for soybean meal (SBM); T3 – 3% MLM substituted for SBM; T4 – 1% MLM substituted for SBM + 0.1% GBM; T5 – 3% MLM substituted for SBM + 0.3% GBM

2.6.2 Internal Organ Weights

Twenty birds that weighed almost the same as the average weight of birds in each feeding experiment phase were chosen at random, tagged, fasted for the whole night, and sacrificed by severing their jugular veins at the 28th and 56th day of life. The sacrificed birds were manually de-feathered after being scalded for two minutes at 80 °C. The gastrointestinal tracts were meticulously removed from the carcass by splitting it apart and eviscerating it. A digital Camry scale (Sensitivity = 0.01 g) was used to weight the organs (lung, gizzard, liver, kidney, spleen, heart, and pancreas), and the results were reported grams.

2.6.3 Gut Microbial Counts

The ceca's microbiological concentration count was determined using the pour plate method (Terrones-Fernandez et al., 2023). For the analysis, a portion of the ceca was excised. Nine milliliters (9 ml) of an appropriate diluents, such as sterile buffer, were combined with about 1ml of the intestinal scrapping sample (ceca) and homogenized for 3 minutes each. Three-fold serial dilutions were made in sterile pre-reduced dilution blank solution for total coliforms, *lactic acid bacteria* (LABs), and total microbial count from the first sample (Dilution 1), which was concentrated (number of microorganisms per ml), or one-tenth (1/10th) of the original sample. 1 ml of each dilution was injected in growth media, which included MacConkay agar for coliforms and MRS agar for LABs. Following inoculation, each plate was incubated for 24 hours at 35 °C, which is the ideal growth temperature for the bacteria under investigation. To find out how many bacteria were in the original sample, the agar plates were taken out of the incubator and the colonies were counted and documented.

2.7 Statistical Analysis

The SAS (SAS Institute Inc., 2020) software's General Linear Model Procedure was used to perform one-way analysis of variance (ANOVA) on the obtained datasets. Tukey's Honestly Significant Difference (HSD) test was used to explain differences in treatment means. Disparities were deemed significant at the 5% probability level. The experiment's model is as follows:

$$X_{ab} = \mu + \alpha_a + \beta_{ab}$$

where: X_{ab} – response in a^{th} replicate and b^{th} treatment; μ – general means; α_a – effect of treatments (T = 1 to 5); β_{ab} – Random error

3 Results and Discussion

3.1 Phyto-Constituents of Moringa oleifera Leaf and Garlic Bulb

In terms of quantity, the most prevalent phyto-constituents in *Moringa oleifera* leaves were total phenols, which had 22.36 mg GAE.g^{-1}. There is a substantial amount of flavonoids present, 18.63 mg QE.g^{-1}. Tannins are the next (6.95 mg TAE.g^{-1}) in the series, followed by alkaloids, and the least are the saponins (0.13 mg.g^{-1} and 0.12 mg.g^{-1}), respectively (Figure 4). In garlic bulbs, tannins, saponins, total phenols, and flavonoids were quantified, with total phenols found to be a maximum 8.75 mg GAE.g^{-1}. Next are flavonoids (2.62 mg QE.g^{-1}), followed by tannins (0.52 mg TAE.g^{-1}), and saponins (0.07 mg.g^{-1}). Plants frequently contain secondary metabolites, which are chemical substances that give rise to biological functions. They are substances that are not required for an organism or cell to survive, but they are important in how the organism or cell interacts with its surroundings and are frequently involved in protecting both plants and animals from biotic and abiotic stressors (Ahlawat et al., 2024). This investigation on phyto-constituents found that *Moringa oleifera* and garlic contain certain secondary metabolites, with total phenols and flavonoids having the highest concentrations of these secondary metabolites. This high total phenol and flavonoid content suggests a wealth of antioxidant and antibacterial compounds that can be used to treat a variety of poultry diseases. Flavonoids found in phytogenic plants are free scavengers and water-soluble antioxidants that have potent anticancer properties and stop oxidative cell damage (Muhammad & Idris, 2019). The presence of saponins, tannins, flavonoids, and terpenoids in garlic bulb powder has been shown to have a wide range of antibacterial action against both bacteria and fungi. Additionally, it was noted that because of their interactions with bio-molecules, phenols have a potent regulating influence on enzyme systems (Chak et al., 2020). According to Gupta et al. (2021), quantitative investigation showed that tannins and alkaloids were essential for plant oxidative resistance as well as their antibacterial, anti-malaria, anticancer, vasodilator, analgesic, and anti-hyperglycemic properties. These therapeutic plants demonstrated their abundance of secondary metabolites, which are used extensively in traditional medicine to treat and prevent a variety of diseases: bactericidal, pesticidal, or fungicidal in nature, thus confirming the anti-bacterial property to plants (Agbetuyi et al., 2024a). The phyto-constituents in *Moringa oleifera* and garlic have significant effects on disease regulation such as *Staphylococcus* spp., *Streptococcus pneumonia*, *Enterococcus* spp., and the overall performance of broiler chickens (Agbetuyi et al., 2024a).

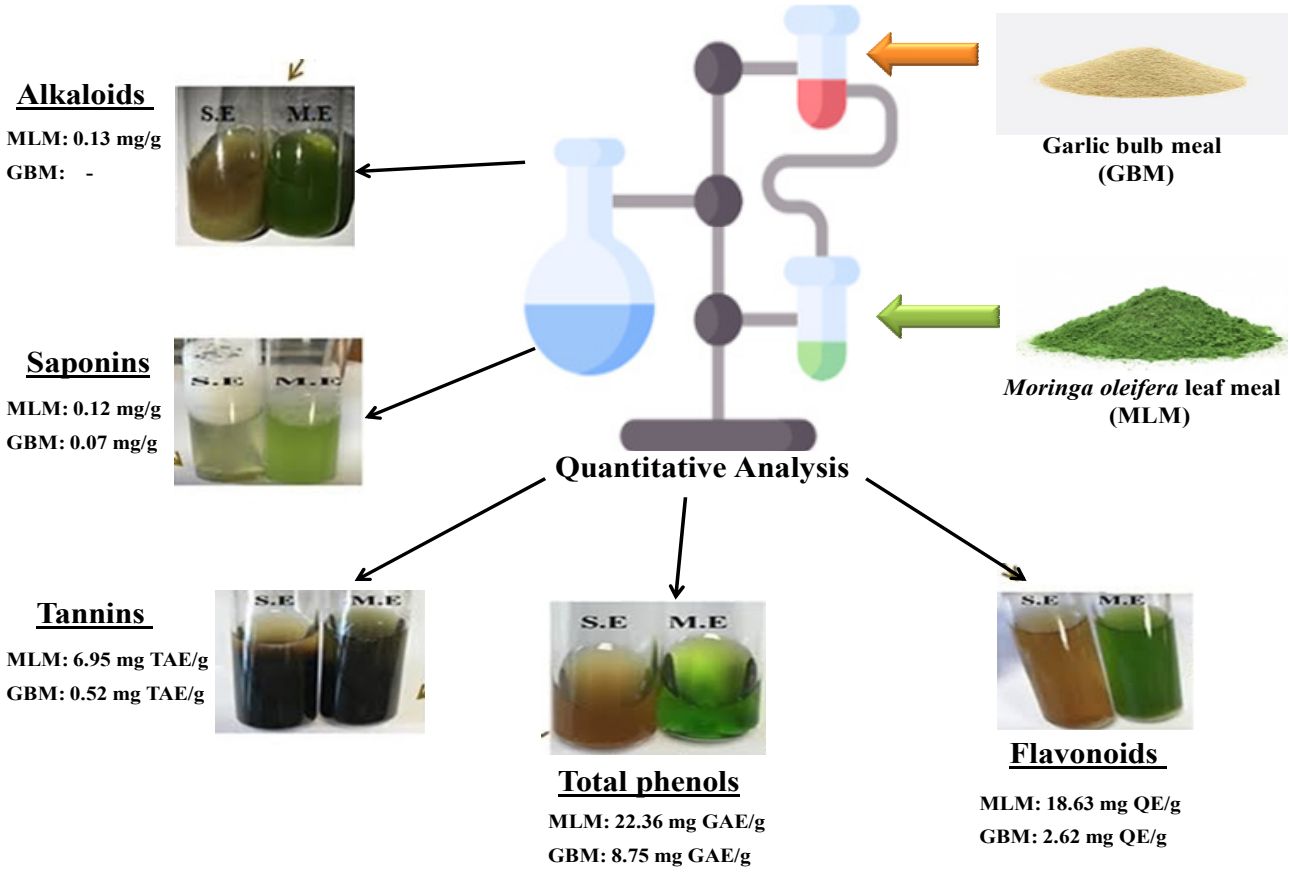

Figure 4 Phyto-constituents of *Moringa oleifera* leaf and garlic bulb

3.2 Growth Performance of Broiler Chickens (Age 7–56 days)

The growth performance between days 7 and 28 indicates that the final weights, average body weight gain, and total feed intake of the birds in T2, T3, T4, and T5 were comparable to those in T1 (Control), while the feed conversion ratio (FCR) was not affected by the dietary treatments (Table 3). On days 29th to 56th, the total feed intake of the birds in T5 was significantly (P <0.05) lowered than those in T1 and T2, but comparable to those in T3 and T4. The FCR in T3, T4, and T5 were significantly (P <0.05) decreased than those in T1, but similar (P >0.05) to those in T2. On days 7th to 56th (overall), the total feed intake of the birds in T5 was significantly (P <0.05) reduced when compared to T1 and T2, but similar (P >0.05) to those in T3 and T4. The FCR in T5 was significantly (P <0.05) improved than in T1, but similar (P >0.05) to those in T2, T3 and T4. At the 28th day of age, there was a similarity effect on the average body weight gain, feed intake, and feed conversion ratio in the dietary treatments and control diet, indicating that feeding *Moringa oleifera* and garlic to broiler chickens had no negative effect on its performance characteristics. In comparison to the control group, broiler fed *Moringa oleifera* leaf powder inclusion in baseline diet showed no changes in body weight gain or feed conversion ratio (Akib et al., 2024). Despite this, the average body weight growth was unaffected by the considerable decrease in feed intake in T5 and the feed conversion ratios in T3, T4, and T5 at day 56. Therefore, by improving nutrient utilization and digestibility, giving antioxidants and phytobiotics to broiler chickens may gradually boost their average body weight gain and feed conversion ratio as they get older. Agbetuyi et al. (2023a; b) found that the phyto-constituents in the powdered leaves of *Moringa oleifera* and the bulbs of *Allium sativum* improved the performance of broiler chicken. It has been proposed that plant species including *Moringa oleifera* leaf powder (Agbetuyi et al., 2024a) and *Allium sativum* (Hafeez et al., 2024) can enhance the performance triats of broiler chickens. This work is consistent with Nantapo et al. (2024), who found that broiler chickens fed *Moringa oleifera* leaf meal gained more body weight at 35 days of age without changing their feed consumption or feed conversion ratio.

3.3 Internal Organ Weights of Broiler Chickens

The internal organ weights of broiler chickens on the 28th and 56th days of age were presented in Table 4. On the 28th day of age, the lung of the bird in T2

Table 3 Growth performance of broiler chickens (age 7–56 days)

Parameters	T1	T2	T3	T4	T5	SEM (±)	*P*-value
Age 7–28 days							
IW (g.bird^{-1})	120.16	120.34	118.75	118.75	118.62	0.37	0.16
FW (g.bird^{-1})	703.53^{ab}	753.87^{a}	721.43^{ab}	668.93^{b}	716.07^{ab}	8.31	0.01
ABWG (g.bird^{-1})	583.17^{ab}	633.33^{a}	602.68^{ab}	550.18^{b}	622.85^{ab}	9.77	0.03
TFI (g.bird^{-1})	1,187.50^{ab}	1,212.46^{b}	1,215.18^{b}	1,193.75^{ab}	1,179.47^{a}	4.50	0.02
FCR	2.04	1.96	2.02	2.17	1.90	0.03	0.08
Age 29–56 days							
FW (g.bird^{-1})	1,959.25	2,083.33	2,005.00	1,989.25	1,992.09	15.05	0.08
ABWG (g.bird^{-1})	1,255.72	1,329.47	1,283.57	1,320.32	1,276.02	10.38	0.10
TFI (g.bird^{-1})	3,724.17^{b}	3,727.09^{b}	3,519.80^{ab}	3,539.59^{ab}	3,464.58^{a}	33.42	0.01
FCR	2.97^{b}	2.81^{ab}	2.75^{a}	2.68^{a}	2.71^{a}	0.03	0.01
Age 7–56 days							
FW (g.bird^{-1})	1,959.25	2,083.33	2,005.00	1,989.25	1,992.09	15.05	0.08
ABWG (g.bird^{-1})	1,838.89	1,962.80	1,886.25	1,870.50	1,898.87	15.82	0.14
TFI (g.bird^{-1})	4,911.67^{b}	4,939.54^{b}	4,734.98^{ab}	4,733.34^{ab}	4,644.05^{a}	34.72	0.01
FCR	2.67^{b}	2.52^{ab}	2.51^{ab}	2.53^{ab}	2.45^{a}	0.02	0.01

a, b – values within the same line with common superscripts are not significantly different at $P > 0.05$; IW – initial weight; FW – final weight; ABWG – average body weight gain; FCR – feed conversion ratio; TFI – total feed intake; SEM – standard error of the mean; MLM – *Moringa oleifera* leaf meal; GBM – garlic bulb meal; T1 – control diet; T2 – 1% MLM substituted for soybean meal (SBM); T3 – 3% MLM substituted for SBM; T4 – 1% MLM substituted for SBM + 0.1% GBM; T5 – 3% MLM substituted for SBM + 0.3% GBM

and T3 were significantly ($P < 0.05$) reduced compared to T1. The gizzard and liver weights of birds in T1 were comparable ($P > 0.05$) to those in T2, T3, T4, and T5. The weight of spleen in birds fed T3 was higher ($P < 0.05$) than in T1, but similar to the rest of the treatment groups. The bird's heart weight was significantly ($P < 0.05$) lower in T3 and T5 than in T1, but similar ($P > 0.05$) to those in T2 and T4. On the 56th day, the gizzard, and pancreas weights in T1 were similar ($P > 0.05$) to those in treatment groups (T2, T3, T4, and T5). Kidney weight in T1 and T4 were similar but significantly ($P < 0.05$) lowered than the rest of the treatments. Observably, the spleen weight of the bird was significantly ($P < 0.05$) impacted across the treatments, with T5 having the highest (2.27 g) value. The heart weight of the bird in T1 was higher ($P < 0.05$) than in T3, but comparable ($P > 0.05$) to T2, T4 and T5. On the 28th day, the weights of the lungs and heart decreased, while the weight of the spleen increased. This result indicated that the nutritional treatments had different effects on the internal organs of the bird. The observed rise in spleen weight of bird fed 3% MLM substitution for soybean meal, suggests that broiler chicken's immune systems are functioning better, which may have been made possible by the presence of phyto-supplement components of the dietary treatments. As a lymphoid organ that filters out pathogens to maintain the population of mature lymphocytes, which always boost the bird's immunity, the spleen weight was found to be related to immune function (Li et al., 2024). On day 56, the weight of kidney showed a discernible increase in bird fed 3% MLM substitution for soybean meal, while the weights of the heart and spleen decreased. These situations suggest that as the bird ages, the spleen's immunological activity was suppressed, with a decrease in the kidney's blood acid-base balance promotion, which affect its ideal production performance and health status. According to a prior study by Araujo et al. (2022), broiler chicken kidney weight can rise as a result of acid-base balance. The current study's findings regarding the reduction in the heart's oxygen and blood supply are inconsistent with those of (Rizwan et al., 2022), which found no discernible effect of adding phyto-constituents to broiler chickens' diets.

3.4 Gut Microbial Load of Broiler Chickens

The ceacal microbiota content of broiler chickens on the 28th day of age is presented in Table 5. The result revealed that the total microbial count in T1 and T4 was ($P < 0.05$) more than those in T5, but comparable to those in T2 and T3. The *lactic acid bacteria* in T2, T3, T4, and T5 were found comparable ($P > 0.05$) to those in T1. The value of *coilforms* in T1 was significantly ($P < 0.05$) higher than those found in the treatment groups (T2, T3, T4, and T5). On the 56th day, the total microbial count, *lactic acid bacteria*, and *coilforms* in T3, T4, and T5 were significantly ($P < 0.05$) reduced compared to those in T1

Table 4 Internal organ weights of broiler chickens

Organs	T1	T2	T3	T4	T5	SEM (±)	*P*-value
Day 28th							
Lung (g)	7.32^{a}	6.56^{b}	6.43^{b}	7.03^{ab}	7.08^{ab}	0.10	0.01
Gizzard (g)	30.72^{ab}	31.55^{a}	28.17^{ab}	25.34^{b}	27.13^{ab}	0.72	0.01
Liver (g)	23.85^{ab}	24.81^{a}	21.32^{ab}	19.95^{b}	20.88^{ab}	0.58	0.01
Kidney (g)	4.87	4.37	4.06	4.28	4.48	0.10	0.12
Spleen (g)	0.75^{b}	0.87^{ab}	1.00^{a}	0.83^{ab}	0.84^{ab}	0.03	0.01
Heart (g)	4.99^{a}	4.79^{ab}	4.24^{b}	4.75^{ab}	4.18^{b}	0.10	0.02
Pancreas (g)	2.18	1.95	2.11	2.01	2.07	0.03	0.21
Day 56th							
Lung (g)	12.97	12.70	13.50	12.81	13.28	0.16	0.55
Gizzard (g)	56.76^{ab}	50.70^{b}	60.66^{a}	60.67^{a}	58.30^{a}	1.03	0.01
Liver (g)	40.56	42.20	39.16	40.32	40.21	0.41	0.21
Kidney (g)	8.87^{b}	9.33^{ab}	10.51^{a}	8.77^{b}	9.19^{ab}	0.21	0.04
Spleen (g)	2.01^{ab}	1.52^{c}	1.73^{bc}	1.65^{bc}	2.27^{a}	0.07	0.03
Heart (g)	10.64^{a}	9.34^{ab}	9.03^{b}	10.04^{ab}	9.61^{ab}	0.18	0.01
Pancreas (g)	5.10^{ab}	4.28^{b}	5.15^{a}	5.64^{a}	5.07^{ab}	0.13	0.03

a, b c – values within the same line with common superscripts are not significantly different at $P > 0.05$; MLM – *Moringa oleifera* leaf meal; GBM – garlic bulb meal; T1 – control diet; T2 – 1% MLM substituted for soybean meal (SBM); T3 – 3% MLM substituted for SBM; T4 – 1% MLM substituted for SBM + 0.1% GBM; T5 – 3% MLM substituted for SBM + 0.3% GBM

and T2. The study's findings demonstrated that feeding broiler chickens *Moringa oleifera* leaf meal and garlic bulb meal increases the number of helpful bacteria (*lactic acid bacteria*) while lowering the number of harmful bacteria (Coliform) by the 28th day of age. This suggests that *Moringa oleifera* leaf meal and garlic bulb meal have a good impact on the development of both hazardous and helpful microorganisms. This result is consistent with a similar research finding by Attia et al. (2023), which found that feeding garlic to broiler chickens can lower the numbers of coliform bacteria in their ileal digesta. Moreover, Khukhodziinai et al. (2024) report that by raising *Lactobacillus* loads, dietary supplementation of essential oils and benzoic acid changed microbiome populations. Additionally, it was shown that *Moringa oleifera* leaf (Agbetuyi et al., 2024a) and garlic (Abd El-Ghany, 2024) have antibacterial qualities that could help reduce the numbers of potentially harmful bacteria in broiler chicks' digestive tracts. With the exception of T2, the observed decrease in the numbers of *coilform*, *lactic acid bacteria*, and total microbial count at day 56 showed that the detrimental bacteria were more impacted by reducing their numbers in the digestive tract than the helpful ones. The addition of *Moringa oleifera* leaf meal and garlic bulb meal to the broiler chicken's diet increased the feed's antibacterial activity, which is responsible for this beneficial microbial influence. Beneficial bacteria in the gut are therefore essential for

Table 5 Ceacal microbiota content of broiler chickens

Parameters	T1	T2	T3	T4	T5	SEM (±)	*P*-value
Day 28th							
TMC	320.00^{a}	252.50^{ab}	180.00^{ab}	342.50^{a}	136.25^{b}	24.73	0.01
LAB	252.50^{ab}	172.50^{ab}	151.25^{ab}	300.00^{a}	107.50^{b}	21.98	0.01
Coliforms	175.00^{a}	132.50^{b}	116.75^{bc}	170.00^{ab}	82.00^{c}	9.37	0.01
Day 56th							
TMC	302.50^{a}	260.00^{a}	178.50^{b}	160.00^{b}	143.75^{b}	16.07	0.01
LAB	268.00^{a}	227.50^{a}	168.75^{b}	127.50^{b}	138.50^{b}	16.85	0.01
Coliforms	242.00^{a}	211.75^{a}	111.75^{b}	99.50^{b}	68.75^{b}	16.79	0.01

a, b, c – values within the same line with common superscripts are not significantly different at $P > 0.05$; MLM – *Moringa oleifera* leaf meal; GBM – garlic bulb meal; T1 – control diet; T2 – 1% MLM substituted for soybean meal (SBM); T3 – 3% MLM substituted for SBM; T4 – 1% MLM substituted for SBM + 0.1% GBM; T5 – 3% MLM substituted for SBM + 0.3% GBM; TMC – total microbial count; LAB – *lactic acid bacteria*

the uptake and utilization of nutrients and serve as a sign of a bird's healthy physiological condition. Furthermore, these bacteria generate organic acids and other bactericidal compounds that can prevent harmful germs from colonizing the intestine (Agbetuyi et al., 2024a).

4 Conclusions

As a prospective feed supplement because of their phyto-constituents, the significance of *Moringa oleifera* leaf meal and garlic bulb meal in the diet of broiler chickens was emphasized. It was shown that these phyto-supplements were beneficial in modulating the digestive tract of broiler chicken; hence enhance the microbial ecosystem and improve the general performance. This facilitates small intestine's effective use and absorption of nutrients. Broiler feed intake was reduced between day 28 and 56 when *Moringa oleifera* leaf meal and garlic bulb meal were added, but the average body weight gain and feed conversion ratio remained same. These data are just a few of the many indications that adding *Moringa oleifera* leaf meal and garlic bulb meal to broilers diets has improved the quality of chicken feed. In conclusion, using these plant-based chemicals may be the simplest method for farmers to improve the quality of the feed they produce, which invariably promote the betterment of the heath of their birds when fed. Therefore, in order to increase performance, gut health, and microbial balance, it is advised that broiler basal diets be supplemented with 3% *Moringa oleifera* leaf meal for soybean meal with 0.3% garlic bulb meal.

Acknowledgments

Not applicable.

Conflict of Interest

The authors declare that they have no conflict of interest to disclose about this research article.

Author Contributions

Oluwafemi Abel Agbetuyi: conceptualization, data curation, formal analysis, funding acquisition, Investigation, methodology, resources, writing – original draft, and writing – review & editing. Anthony Henry akeocha: project administration, supervision, validation, and writing – review & editing. Ademiju Adeolu Aganaga: project administration, supervision, validation, visualization and writing – review & editing.

AI and AI-Assisted Technologies Use Declaration

The following AI tools-AI-assisted technology was used during the preparation of the manuscript: QuillBot was engaged to improve the readability and language of the manuscript.

References

Abdelli, N. et al. (2021). Phytogenic feed additives in poultry: achievements, prospective and challenges. *Animals*, 11(12),3271. https://doi.org/10.3390/ani11123471

Abd El-Ghany, W. A. (2024). Potential effects of garlic (*Allium sativum*) on the performance, immunity, gut health, anti-oxidant status, blood parameters, and intestinal microbiota of poultry: an updated comprehensive review. *Animals*, 14(3),498. https://doi.org/10.3390/ani14030498

Abdulkareem, Z. A. (2024). Effect of dietary supplementation of wild leek (*Allium tricoccum*) and garlic (*Allium sativum*) leaves on production, egg quality, serum lipid profile, intestinal morphology and nutrient digestibility of laying quails. *Tropical Animal Health and Production*, 56(7), 224. https://doi.org/10.1007/s11250-024-04090-z

Adewumi, O. O. et al. (2022). Functional properties and amino acids profile of Bambara groundnut and *Moringa oleifera* leaf protein complex. *Processes*, 10(2), 205. https://doi.org/10.3390/pr10020205

Agbetuyi, O. A. et al. (2024a). *Moringa oleifera* and *Allium sativum* in broiler nutrition: carcass traits, biochemical characterization, and economic analysis. *Discover Food*, 4, 86. https://doi.org/10.1007/s44187-024-00155-y

Agbetuyi, O. A. et al. (2024b). Assessing the impact of moringa and garlic supplementation on egg production and quality in laying hens. *Dysona-Applied Science*, 5(2), 41–51. http://dx.doi.org/10.30493/DAS.2024.451601

Agbetuyi, O. A. et al. (2024c). Modulation of physic-chemical traits and lipid profile in broiler meat through moringa and garlic supplementation. *Dysona-Applied Science*, 5(2), 52–61. http://dx.doi.org/10.30493/DAS.2024.451989

Agbetuyi, O. A. et al. (2023a). The use of *Moringa oleifera* and *Allium sativum* and funding of poultry production in Ekiti State, Nigeria. *Journal of Agriculture and Food Environment*, 10(3), 11–23.

Agbetuyi, O. A. et al. (2023b). Organoleptic parameters, tibia bone growth and mineral retention of broiler chicken fed *Moringa oleifera* and *Allium sativum*. Slovak *Journal of Animal Science*, 56(3),3–12. https://doi.org/10.36547/sjas.819

Agbetuyi, O. A., & Oloruntola, O. D. (2020). Effects on performance of broiler chickens of feeding Shea butter as replacement for vegetable oil in their diet. *Livestock Research for Rural Development*, 32(6). http://www.lrrd.org/lrrd32/6/oloru32094.html

Ahlawat, Y. K. et al. (2024). Plant phenolics: neglected secondary metabolites in plant stress tolerance. *Brazilian Journal; of Botany*, 47, 703–721. https://doi.org/10.1007/s40415-023-00949-x

Akib, M. G. et al. (2024). Effects of *Moringa oleifera* leaf powder on the growth performance, meat quality, blood parameters, and cecal bacteria of broilers. *Veterinary Science*, 11(8),374. https://doi.org/10.3390/vetsci11080374

Araujo, A. C. et al. (2022). Effects of dietary electrolyte balance on performance, energy balance, and expression of genes related to acid-basic balance, absorption, and transport

of nutrients in broilers. *Tropical Animal Health and Production*, 54(3),165. https://doi.org/10.1007/s11250-022-03165-z

Attia, Y. A. et al. (2023). Effects of *Spirulina platensis* and/or *Allium sativum* on antioxidant status, immune response, gut morphology, and intestinal Lactobacilli and Coliforms of heat-stressed broiler chicken. *Veterinary Sciences*, 10(12), 678. https://doi.org/10.3390/vetsci10120678

Chak, P. et al. (2020). Phytochemical and Antioxidant Studies of *Moringa oleifera* powder and *Allium sativum* Flower Extracts. *Research Journal of Pharmacy and Technology*, 13(10),4579–4585. DOI: 10.5958/0974-2020.00806.9

Chaudhary, M. N. et al. (2023). Effect of feeding *Moringa oleifera* replacing TMR on nutrients intake, rumen fermentation and economics in adult cattle. *International Journal of Bio-resources Stress Management*, 14(11),1518–1522. https://doi.org/10.23910/1.2023.4877a

Dhakad, A. K. et al. (2024). Proximate composition, mineral profiling, and antioxidant potential in genotypes affected with leaf maturity stage. *South African Journal of Botany*, 168, 227–235. https://doi.org/10.1016/j.sajb.2024.03.023

Gupta, S. et al. (2021). Quantitative analysis of tannins, alkaloids, phenols, and flavonoids in *Ficus semicordata* leaf, stem, stem bark, root, and fruit powder. *Journal of Indian Systemtics Medicine*, 9,171–174. DOI: 10.4103/jism.jism_16_21

Hafeez, A. et al. (2024). Impact of coriander (*Coriandrum sativum*), garlic (*Allium sativum*), fenugreek (*Trigonella foenum-graecum*) on zootechnical performance, carcass quality, blood metabolites and nutrient digestibility in broilers chickens. *Veterinary Quarterly*, 44(1), 1–7. https://doi.org/10.1080/01652176.2023.2300948

Khukhodziinai, J. S. et al. (2024). Effect of dietary benzoic acid and oregano essential oil as a substitute for an anti-coccidial agent on growth performance and physiological and immunological responses in broiler chickens challenged with Eimeria Species. *Animals*, 14(20),3008. https://doi.org/10.3390/ani14203008

Li, Y. et al. (2024). Spleen contributes to chronic restraint stress-induced lung injury through splenic CD11b^{+} cells. *International Immunopharmacology*, 126,111258. https://doi.org/10.1016/j.intimp.2023.111258

Mahfuz, S., & Piao, X. S. (2019). Application of Moringa (*Moringa oleifera*) as natural feed supplement in poultry diets. *Animals*, 9(7),431. https://doi.org/10.3390/ani9070431

Malematja, E. et al. (2023). Effects of onion extracts (*Allium cepa*) inclusion in diets on growth performance, carcass characteristics, and bone morphometric of broiler chickens. *Animal Bioscience*, 36(7),1075–1082. https://doi.org/10.5713/ab.22.0399

Moreno-Mendoza, Y. et al. (2021). Effect of moringa leaf powder and agave inulin on performance, intestinal morphology, and meat yield of broiler chickens. *Poultry Science*, 100(2),738–745. https://doi.org/10.1016/j.psj.2020.11.058

Muhammad, A., & Idris, S. I. (2019). Phytochemical screening and proximate analysis of garlic (*Allium sativum*). *Archives of Organic and Inorganic Chemical Sciences*, 4(1). DOI: 10.32474/AOICS.2019.04.000180

Nantapo, C. W. T. et al. (2024). Effect of phytogenic *Moringa oleifera* leaf powder on performance, carcass characteristics, immune indicators, gut microbial population and economic viability of broiler chickens. *Discover Agriculture*, 2(1),1–18. https://doi.org/10.1007/s44279-024-00104-4

Rizwan, H. M. et al. (2022). A new insight in immunomodulatory impact of botanicals in treating avian coccidiosis. *Journal of Parasitic Diseases*, 46(4), 1164–1175. https://doi.org/10.1007/s12639-022-01519-w

Song, Q. et al. (2024). Effect of *Moringa oleifera* leaf powder supplementation on growth performance, digestive enzyme activity, meat quality, and ceacum microbiota of Ningdu Yellow Chickens. *Agriculture*, 14(9),1523. https://doi.org/10.3390/agriculture14091523

Sugiharto, S. et al. (2020). Effect of feeding fermented mixture of cassava pulp and *Moringa oleifera* leaf meal on immune responses, antioxidative status, biochemistry indices, and intestinal ecology of broilers. *Veterinary World*, 13(2), 392–399. https://doi.org/10.14202/vetworld.2020.392-399

Terrones-Fernandez, I. et al. (2023). Improvement of the pour plate method by separates sterilization of Agar and other medium components and reduction of the Agar concentration. *Microbiological Spectrum*, 11(1), 1–12. DOI: 10.1128/spectrum.03161-22.