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Supplementing Broiler Chicken Diet with *Uvaria chamae* Leaf Meal: Effects on Immune Response, Gut Microbial Population and Growth Performance

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Abstract

The purpose of this experiment was to determine how supplementing *Uvaria chamae* leaf meal (UCLM) affects growth performance and a few hematological markers of broiler chicken. For a 56-day study, 500 one-day-old (Ross 307) broiler chicks were randomly assigned to five treatments, each consisting of 100 birds. Standard feed was formulated according to the nutritional standards for broilers. Treatment 1 (T1) was fed a standard diet with 0.25 g/kg oxytetracycline while T2, T3, T4, and T5 were provided a standard diet supplemented with UCLM at 5g, 10g, 15g, and 20g, respectively. The treatments had a significant impact on body weight gain, feed conversion ratio and mortality. In contrast, the mortality rate and *Escherichia coli* counts were higher in T1 and T2 compared to other groups, whereas body weight gain was higher in T2, T3, T4, and T5 compared to T1. Regarding crude fiber digestibility and averagedaily feed intake, there was no statistically significant difference. The dry matter, crude protein, ether extract, ash digestibility, hemoglobin, packed cell volume, red blood cell, lymphocytes, monocytes, immunoglobulin A, Y, and M and *Lactobacillus* sp. counts of the birds supplemented with UCLM at 2 g/kg (T2), 4 g/kg (T3), 6 g/kg (T4), and 8 g/kg (T5) were similar but significantly greater than those of T1 (0.25 g/kg oxytetracycline). It was determined that broilers' diets can include up to 20 g/kg of UCLM supplementation without harming the birds' health.

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Introduction

Reducing the use of antibiotics is one of the goals of poultry production, which is also influenced by customer demand [1]. A significant percentage are still employed as antimicrobial growth promoters in addition to therapeutic applications [2, 3]. To avoid and reduce resistance and to support the sustainability of broiler production, it is crucial to reduce the use of antibiotics, particularly antibiotic growth boosters [4, 5]. Among the potential alternatives to antibiotics is the use of medicinal plants which have been reported to be eco-friendly, effective and safe for both human beings and animals [6].

Uvaria chamae is a member of the Annonaceae family of plants. It is a broad, aromatic shrub that grows extensively in West Africa, South America, Australia, and portions of Asia [7, 8]. Plant components with medicinal or therapeutic effects are abundant in phytochemicals, including leaves, seeds, stem bark, flowers, and roots [9]. Because of its significant role in traditional medicine, *U. chamae* is a plant of significance. There have been reports that *U. chamae* root extracts have been employed for treating fevers, gastrointestinal issues, abdominal aches and piles [10]. There have also been reports of using *U. chamae* leaves and stems to cure eczema, wounds, and malaria [7]. Phytochemicals like benzyl dihydrochalcone chamuvaritin, isolated from *U. chamae* are used to make antifungal remedies in the drug benzyl benzoate. Additionally, Oliveira et al. [11] discovered that the ethanolic extract from the stem bark of *U. chamae* had cytotoxic properties against lymphocytic leukemia *in vivo* as well as against human cancer cells from the nasopharynx *in vitro*. Because it contains essential minerals like calcium, magnesium, potassium, phosphorus, manganese, iron, and lead, all of which are crucial for the activation of enzymatic reactions and offer increased protection against oxidative stress. Further, *U. chamae* leaves possess both antioxidant and antimicrobial qualities [12, 13].

Previous studies have shown that the dietary supplementation of phytogenics such as *Moringa oleifera* leaves at 2 g/kg [14], *Phyllantus amarus* leaf at 5 g/kg [15], *Azadirachtica indica* at 3 g/kg [16], mango leaves 5 g/kg [17], garlic and ginger at 2 g/kg [18], amongst others optimizes the performance of broiler chickens. However, little or nothing is known about the *U. chamae* leaf meal supplementation in broiler chicken diet: effects on growth performance, hematological indices, intestinal microbial population and immune response. There is no reason to presume that components from *U. chamae* leaf meal (phytochemicals) must be safe. Carrying out this study will further promote livestock sustainability, and food safety, and unleash the potential of the test ingredient as well as their tolerable levels in broilers.

Materials and Methods

Research location and *Uvaria chamae* leaf meal preparation

The experiment was carried out in the Sumitra Research Institute Teaching and Research farm, which is situated in Gujarat, India, between 15°N and 30°E. The leaves of *U. chamae* were taken from the farm. The taxonomy department received the harvested leaves and certified them (HD-0888T-2023). The leaves were cleaned using distilled water after being rinsed with running tap water, spread out on a plastic tray and brought to a shade for 12 days. Samples were checked every day to make sure the drying process. A high-power electric blending machine was used to ground the dried leaves into powder. The powdered samples were then labeled and placed into transparent polythene bags before being sent to the laboratory.

Animal housing, feeding and experimental design

A total of five treatment groups, each consisting of 100 birds with five repetitions (20 birds per replicate), were created from 500 one-day-old Ross 307 broilers that were acquired from a respectable commercial farm in Gujarat as approved by the ethical committee of the Sumitra Research Institute (APP/08E/2023). The birds were housed in 200 cm (length), 150 cm (width) and 95 cm (height) galvanized battery cages that were semi-closed, well-sanitized, and fitted with an automatic nipple drinker and a manual aluminum feeder. The study's diet, based on a corn-soya meal diet, was prepared using additional ingredients under the 1994 guidelines [19], as shown in Table 1. Two nutritional phases of feeding were implemented for the birds: starter mash (0–4 weeks) and finisher mash (5–8 weeks). The birds were given unlimited access to food and clean, fresh water, and were weighed both upon arrival and every week after that. Following each injection, birds were given vitamins (Vitamix wsp® at 5 g in 5 liters of water) in strict accordance with the vaccination schedule. The trial was conducted with a fully randomized design for

56 days. In treatment 1 (T1), antibiotics (oxytetracycline) were added at a rate of 0.25 g/kg to the standard feed, and in T2, T3, T4, and T5, *U. chamae* leaf meal (UCLM) was added at a rate of 5 g, 10 g, 15 g, and 20 g/kg to the standard feed.

Table 1 Ingredient and chemical composition of basic experimental diet.

Ingredients	Phase 1 (0 - 28 d)	Phase 2 (29 - 56 d)
Yellow maize	52.00	56.05
Soybean meal	40.00	32.00
Fish meal (72%)	2.00	2.00
Oyster shell	2.00	2.40
Bone meal	4.50	6.00
Lysine	0.21	0.21
Methionine	0.22	0.22
*Premix	0.25	0.20
Salt	0.35	0.40
Toxin binder	0.02	0.02
Total	100.00	100.00
Determined analysis (percentage dry matter)		
Crude protein	23.06	21.04
Crude fibre	4.00	5.38
Ether extract	4.89	5.00
Calcium	1.60	1.80
Phosphorus	0.65	0.77
Lysine	1.43	1.52
Energy (Kcal/kg)	2995.8	3100.5

*Premix starter: Min/vitamin premix given per kilogram of feed: vitamin A, 10,000 I.U., vitamin E, 28.0 mg, vitamin D, 4,000 I.U., vitamin K, 5.00 mg, vitamin B2, 5.0 mg, niacin, 80 mg, vitamin B12, 25 mg, choline chloride, 100 mg, manganese, 10.0 mg, zinc, 40.1 mg, copper, 8.0g,folic acid, 4.5 mg, iron, 5.1g, pantothenic acid, 30 mg, biotin, 31.5 mg, and antioxidant, 70 mg
*Premix for finisher: Mini/Vitamin premix given per kilogram of feed: -7,800 I.U. of vitamin A; 20.0 mg of vitamin E; 2,500 I.U. of vitamin D; 10.00 mg of vitamin K; 8.0 mg of vitamin B2; 80 mg of niacin; 30 mg of vitamin B12; 80 mg of choline chloride; 3.5 mg of manganese; 30.2 mg of zinc; 5.0g of copper; folic acid; 2.0 mg of iron; 20 mg of pantothenic acid; 30.0 mg of biotin; antioxidant, 65 mg.

Table 2 Phytochemical composition of *Uvaria chamae.*

Phytochemicals	Composition (%)
Alkaloids	2.47
Terpenoids	5.60
Flavonoids	9.40
Tannins	3.06
Saponins	1.24
Phenols	9.93
Steroids	1.45

Assessment of performance standards

Body weight growth (kg/b) was calculated as follows: final weight (kg) - initial weight (kg). The average daily weight growth (kg/b) was calculated by dividing the study time (that is, 56 days) by the difference between the final and initial body weights (kg); total feed intake divided by the testing duration (56 days) yields the average daily feed intake (kg/b); The feed conversion ratio was calculated by dividing the daily average weight increase by the daily average feed intake. Mortality (%) was the overall number of birds multiplied by 100 divided by the number of bird deaths.

Apparent nutrient digestibility trial

The apparent digestibility of nutrients was measured on day 56 of the experiment when three birds per replication were randomly chosen and moved to metabolism cages with feeders and drinkers. The birds were fed a predetermined amount of food, and after five days, their droppings were collected and oven-dried at 68^{o}C until a consistent weight was obtained. The duplicates from each treatment were then bulked and utilized for proximate analysis. A 500 ml sample volume was used, and the near-infrared (NIR) analyzer (InfraLUM-FT-12, Netherlands) was calibrated to a spectral range of 760 to 1150 nm and power consumption of 110V to examine the approximate composition of the feed and droppings. Samples are placed on the collecting tray and after 1.5 mins, the results are shown on the monitor. The digestibility coefficient (DC) was calculated as follows: nutrition in feed intake divided by nutrients in droppings then multiplied by 100.

Analysis of blood parameters

For hematological investigations, blood samples were taken on the morning of the 56th day of the trial from three randomly chosen birds per replication. Two milliliters of blood were drawn from the wing veins of particular birds and refrigerated until they were sent to the Sumitra Research Laboratory in Gujarat, India, for additional study. The SMT-50 hematology analyzer, which uses the multi-angular laser scattering electric impedance technology and flow cytometry as its working principles, was used to evaluate blood samples. Following the manufacturer's instructions, the kit was calibrated using particular reagents (built-in diff lyse, LH lyse, and external diluent) and set to an operating temperature (10°C to 35°C) and relative humidity (20% to 85%). After 120 s, the results were obtained.

Immunity analysis

Two milliliters of blood were extracted from three randomly chosen broilers per replicate after the experiment (the identical birds were utilized for hematological research). Collected samples were analyzed using a commercial kit (Dynex, DS2 automated with a patented multi-plate carrier and

standard filters; 630 nm) and adjusted to a reading (4.000 A) and wavelength range (850 nm) before the results were displayed via the output unit.

Intestinal microbial profile examination

At the end of the experiment, a swap of the intestinal content was carried out using a cotton bud from 2 randomly selected birds per replicate. Samples were collected into a sterile sample bottle and sent to the laboratory for further investigation. Analysis was carried out using a commercial diagnostic kit (Milliflex® quantum reader) adjusted at an input power supply of 50-60 Hz, temperature of 40°C and detection area within the area of 55 mm diameter [32].

Phytochemical evaluation

Flavonoids analysis

The total flavonoid content was determined using the aluminum chloride procedure, with catechin serving as a reference. 0.5 grams of *U. chamae* leaf meal (UCLM), 0.1 mL of aluminum chloride and 0.2 ml of 5% sodium nitrite were added. The reaction mixture was allowed to sit at room temperature for six minutes before being supplemented with two milliliters of 1M sodium hydroxide. The final volume was immediately increased with 10 ml of distilled water. The absorbance of the reaction mixture at 410 nm was measured with a spectrophotometer and compared to a blank [32].

Total phenolic content analysis

The total phenolic content of UCLM was ascertained using the Folin-reagent Ciocalteu's. 0.4 ml of 1:10 v/v diluted FCR and 0.5 g of UCLM were mixed in the procedure. After five minutes, 4 ml of sodium carbonate solution was added. After adding 10 ml of distilled water to the tubes to the fullest, they were allowed to stand at room temperature for ninety minutes. The sample's absorbance at 850 nm was measured using a spectrophotometer in relation to the blank. By expressing the oil's phenolic content in milligrams of catechol per dry gram of dry weight, a calibration curve was made with the standard graph and catechol solution as the standard [21].

Saponins analysis

Saponin was measured by use of a colorimetric method employing vanillin and concentrated sulfuric acid. 0.5 ml of freshly prepared vanillin solution, 0.2 ml of UCLM and 0.4 ml of 77% sulfuric acid were mixed. After allowing the mixture to settle to ambient temperature, it was heated for 15 minutes at 60°C in a water bath. Utilizing a spectrophotometer, the absorbance at 545 nm was measured.

Steroids analysis

Ten ml of volumetric flask were filled with 0.5 g of UCLM, as per the reported previously [22]. Then, 2 ml of potassium hexacyanoferrate solution (0.5% w/v) was added. Before being diluted to the appropriate concentration with distilled water, the material was heated in a steam bath for 20 minutes at 40-50°C while being constantly shaken. At 380 nm, the absorbance was computed and contrasted with a blank for the reagent.

Alkaloids complex analysis

Using the gravimetric technique, the alkaloid content of UCLM was determined [32]. Alkaloids were precipitated by mixing 20 ml of acetic acid solution in ethanol (10% w/v) with 0.5 g of UCLM and placing the mixture in a water bath. The alkaloids were then precipitated by adding drops of extremely concentrated ammonium hydroxide. After the precipitate reached a constant weight, it was transferred to desiccators and reweighed.

Total tannins analysis

The Folin-Ciocalteau technique was employed to determine the total tannin concentration [24]. 0.5 ml of metaphosphoric acid, 1.5 ml of 90% ethanol, 1.5 ml of 1.0 mol/ml Na_2CO_3, and 1.5 ml Folin-Ciocalteau were added to 1.0 g of UCLM to dilute it (100 ml). The combination was thoroughly blended and then left to cool for 20 mins at room temperature. Then, using a spectrophotometer, the absorbance of the standard curve and UCLM were compared to a blank at 880 nm.

Terpenoids analysis

Terpenoid contents were determined using anion exchange techniques as outlined previously [25]. 0.5 g of UCLM was measured in a beaker and mixed with 1.4 ml of distilled water, 1.5 ml of ferric aluminum sulfate solution and 1 ml of ammonium thiocyanate solution and thoroughly mixed, and then measured using a spectrophotometer at 465 nm.

Statistical analysis

The statistical program for social sciences (SPSS version 21.0) was used to perform a randomized

design analysis of variance on the collected data. The same software's Duncan multiple range test was performed to assess the significance of the mean difference at the $P \leq 0.05$ level. This study utilized the following model: $Yxy = \mu + \alpha x + \beta xy$, where x is the overall mean, αx is the influence of the xth treatment (1=5), and βxy is the random error term for each estimate.

Results and Discussion

Phytochemical composition of *Uvaria chamae* leaf meal

The phytochemical composition of U. chamae was determined by looking at Table 2, which indicates that saponins had the lowest content (1.24 %) and phenols or phenolic compounds had the highest concentration (9.93 %). There were also significant amounts of flavonoids, terpenoids, alkaloids, tannins, and steroids at 9.40%, 5.60%, 2.47 %, 3.06 %, and 1.45 %, respectively. Every phytochemical compound found in this investigation has different pharmacological or therapeutic qualities [26]. The outcomes, however, agreed with the report of Olumese et al. [10]. *Alchornea laxiflora*, *Sida acuta*, *Prosopis africana*, *Rhamnus californica*, and *Umbellularia californica* leaves have all been reported to have comparable phytoconstituents [27-30]. Owing to its antioxidant qualities, *U. chamae* leaf meal may scavenge reactive oxygen species, as evidenced by the high quantity of phenolic compounds it contains [31, 32]. Phytochemicals shield plants against insects, birds, and microbes that prey on them [33, 34]. Many different compounds are produced by plants, including terpenoids, which give them their smell; quinones and tannins, which give them their color; flavonoids and alkaloids, which give them their flavor. It has been shown that phenols have antibacterial effects against bacteria such as *Escherichia coli* and *Staphylococcus aureus* [36, 37] as well as viruses [35]. They are additionally acknowledged to supply stable free radicals, which combine with protein amino acids to create irreversible complexes [38]. Flavonoids can attach to bacterial cell proteins, which results in the inhibition and deactivation of enzymes [39, 40]. Alkaloids have been shown to block the synthesis of RNA and proteins and to have antibacterial properties against a variety of pathogenic species [41, 42]. According to reports, terpenoids are involved in the rupture of the bacterial membrane and have antioxidant effects [43]. A class of phytochemicals known as saponins protects plants from microbial, fungal, and insect attacks [44, 45]. On the other hand, tannins have been linked to the creation of metal-ion complexes, which can damage membranes [42].

Performance of 56-day-old broilers fed *Uvaria chamae* leaf meal

The growth performance of broilers treated with *U. chamae* leaf meal (UCLM) at 56 days of age is displayed in Table 3. The birds fed treatment 2 (T2) and treatment 3 (T3) had body weights that were considerably ($P<0.05$) greater than those fed T1 (0.25 g/kg oxytetracycline), but similar ($P>0.05$) to those fed in T4 and T5. Every treatment showed identical average daily weight gain ($P>0.05$). The values of the average daily feed intake and feed conversion ratio varied from 1.73 to 1.90 and from 0.0782 to 0.0790 kg, respectively. In comparison to other treatments, the birds in T3, T4, and T5 had the best feed conversion ratios ($P<0.05$). T1 had a higher mortality rate (0.25 g/kg oxytetracycline), T2

Table 3 Performance of broilers at 56 days of age supplemented with *Uvaria chamae* leaf meal.

Treatments	IW (kg)	FBW (kg)	BWG (kg)	ADWG (kg)	FI (kg)	ADFI (kg)	FCR	Mortality (%)
Standard diet + 0.25 g oxytetracycline/kg (T1)	0.0524	2.300^{b}	2.2476^{b}	0.0401^{b}	4.381	0.0782	1.90^{a}	1.74^{a}
Standard diet + 2.00 g UCLM/kg (T2)	0.0521	2.560^{a}	2.5079^{a}	0.0447^{a}	4.402	0.0786	1.76^{b}	0.36^{b}
Standard diet + 4.00 g UCLM/kg (T3)	0.0519	2.594^{a}	2.5421^{a}	0.0454^{a}	4.411	0.0787	1.73^{c}	0.04^{c}
Standard diet + 6.00 g UCLM/kg (T4)	0.0510	2.607^{a}	2.5560^{a}	0.0456^{a}	4.420	0.0789	1.73^{c}	0.00
Standard diet + 8.00 g UCLM/kg (T5)	0.0520	2.608^{a}	2.5560^{a}	0.0456^{a}	4.428	0.0790	1.73^{c}	0.00
Standard error of mean (SEM)	0.02	36.79	32.10	0.66	61.70	0.03	0.02	0.01

Duncan's test reveals a 5% significant difference between means denoted by various letters in the same column. IW = Initial weight; FBW = Final bird weight; BWG = Bird weight gain; ADWG = Average daily weight gain; FI = Feed intake; ADFI = Average daily feed intake; FCR = Feed conversion ratio.

had an intermediate mortality rate, and T3 had the lowest mortality rate (P<0.05). T4 and T5 had no mortality at all. Due to the presence of phytochemicals, particularly phenols and flavonoids, which are found in abundance in the phytogenic feed additive, UCLM is thought to have growth-promoting properties, as evidenced by the higher body weights recorded in birds fed with UCLM in T2 (2.0 g/kg), T3 (4.0 g/kg), T4 (6.0 g/kg), and T5 (8.0 g/kg) [44]. The phytochemicals can increase saliva and digestive enzyme secretion, which enhances the overall digestibility of nutrients [45]. This outcome is consistent with that of another report [46], when probiotics (B-Act) were added as a supplement to the broiler chickens' diet. It is evident from the non-statistical difference (P>0.05) in ADFI that UCLM was unable to increase the palatability of the birds under any of the treatments. This observation runs counter to earlier research conducted earlier when broiler diets were supplemented with *Daniellia oliveri* extracts [47]. When broiler diets were supplemented with phytogenic feed additives instead of antibiotics, comparable outcomes were observed [47, 48]. These differences may be explained by differences in the phytochemical concentrations in the test ingredients, the processing technique and the dose used [49]. The feed intake result indicates that, in comparison to other treatments, raising UCLM above 8.00 g/kg may further modify the feed retention time. Birds fed 6 g/kg (T4) and 8 g/kg (T5) did not die, suggesting that UCLM could prevent infections with pathogens by enhancing the intestinal barrier, secreting antimicrobial compounds, and activating the immune system [3].

Apparent digestibility of broilers at 56 days supplemented with Uvaria chamae leaf meal

As revealed in Table 4, on the apparent digestibility (%) of broilers at 56 days of age supplemented with *U. chamae* leaf meal (UCLM). Dry matter, crude protein, ether extract and ash digestibility of birds supplemented with UCLM at 2.0 g/kg (T2), 4.0 g/kg (T3), 6.0 g/kg (T4) and 8.0 g/kg (T5) were similar (P>0.05) but significantly (P<0.05) greater than those in T1 (0.25 g/kg oxytetracycline). No statistical differences (P>0.05) were observed during the study in crude fibre digestibility. The higher digestibility recorded among birds fed diets supplemented with UCLM (T2 to T5) in this research reflects that it has the ability to increase the permeability of the intestinal wall of birds leading to an improved absorption of nutrients [5]. The result also signifies that phytochemicals in UCLM were within the permissible or non-toxic level for birds, thus modulating bile flow in the gut [50]. Extreme levels of crude fibre could harm the growth of broilers; however, the results showed that the experimental diet met the nutritional standards for birds according to NRC [19]. The outcome of this experiment aligns with another report on the apparent digestibility of broilers fed diets containing *Gliricidia sepium* leaf meal at 100 g/kg [51]. Similar results were also recorded by Alagbe et al. [48]

Table 4 Apparent digestibility (%) of broilers at 56 days of age supplemented with *Uvaria chamae* leaf meal.

Treatments	Dry matter	Crude protein	Crude fibre	Ether extracts	Ash
Standard diet + 0.25 g oxytetracycline/kg (T1)	77.60^{b}	79.72^{b}	48.14	55.60^{b}	40.80^{b}
Standard diet + 2.00 g UCLM/kg (T2)	87.45^{a}	78.00^{a}	46.09	63.90^{a}	52.15^{a}
Standard diet + 4.00 g UCLM/kg (T3)	87.08^{a}	78.12^{a}	46.27	64.56^{a}	52.90^{a}
Standard diet + 6.00 g UCLM/kg (T4)	87.11^{a}	78.50^{a}	47.55	65.08^{a}	53.74^{a}
Standard diet + 8.00 g UCLM/kg (T5)	87.15^{a}	78.95^{a}	47.89	65.79^{a}	53.76^{a}
Standard error of mean (SEM)	0.73	0.43	0.33	0.48	0.51

Duncan's test reveals a 5% significant difference between means denoted by various letters in the same column; DM = Dry matter; CP = Crude protein; CF = Crude fibre; EE = Ether extract.

Table 5 Hematological indices of broilers at 56 days of age supplemented with *Uvaria chamae* leaf meal.

Treatments	PCV (%)	Hb (g/dL)	RBC (10)^{12}/L	WBC (×10)^{9}/L	LYM (%)	MON (%)
Standard diet + 0.25 g oxytetracycline/kg (T1)	29.45^{c}	10.76^{c}	1.95^{c}	12.66^{b}	58.96^{b}	1.03
Standard diet + 2.00 g UCLM/kg (T2)	30.80^{b}	12.22^{b}	2.00^{b}	12.70^{b}	59.10^{b}	1.06
Standard diet + 4.00 g UCLM/kg (T3)	30.71^{b}	12.60^{b}	2.15^{b}	19.81^{a}	70.95^{a}	1.50
Standard diet + 6.00 g UCLM/kg (T4)	35.60^{a}	15.74^{a}	2.89^{a}	19.90^{a}	71.22^{a}	1.55
Standard diet + 8.00 g UCLM/kg (T5)	35.66^{a}	15.89^{a}	3.00^{a}	19.97^{a}	75.94^{a}	1.63
Standard error of mean (SEM)	0.06	0.21	0.11	0.27	0.82	0.01
Standard Range	27-36	8.01 – 20.0	1.20 – 6.00	11.00 – 25.00	51 – 80	0.05 – 1.50

Duncan's test reveals a 5% significant difference between means denoted byvarious letters in the same column. PCV = Pack cell volume; Hb = Hemoglobin; RBC = Red blood cell; WBC = White blood cell; LYM = Lymphocytes; MON = Monocytes.

Table 6 Immune response of broilers at 56 days of age supplemented with *Uvaria chamae* leaf meal.

Treatments	[1]IgA (µg/mL)	[2]IgY (µg/mL)	[3]IgM (µg/mL)
Standard diet + 0.25 g oxytetracycline/kg (T1)	2.41[b]	1.80[b]	4.00[b]
Standard diet + 2.00 g UCLM/kg (T2)	3.00[a]	1.86[b]	4.16[b]
Standard diet + 4.00 g UCLM/kg (T3)	3.10[a]	2.00[a]	5.30[a]
Standard diet + 6.00 g UCLM/kg (T4)	3.18[a]	2.12[a]	5.60[a]
Standard diet + 8.00 g UCLM/kg (T5)	3.30[a]	2.85[a]	5.72[a]
Standard error of mean (SEM)	0.02	0.01	0.08

Duncan's test reveals a 5%significant difference between means denoted by various letters in the same column.
IgA = Immunoglobulin A; IgY = Immunoglobulin M; IgM = Immunoglobulin M.

Table 7 Caecal microbial population of broilers at 56 days of age supplemented with *Uvaria chamae* leaf meal.

Treatments	*Escherichia coli* (cfu/g)	*Lactobacillus sp.* (cfu/g)
Standard diet + 0.25g oxytetracycline/kg (T1)	4.11[a]	5.33[c]
Standard diet + 2.00 g UCLM/kg (T2)	3.84[b]	8.70[b]
Standard diet + 4.00 g UCLM/kg (T3)	3.06[b]	8.75[b]
Standard diet + 6.00 g UCLM/kg (T4)	2.58[c]	9.00[a]
Standard diet + 8.00 g UCLM/kg (T5)	2.50[c]	9.76[a]
Standard error of mean (SEM)	0.03	0.28

Duncan's test reveals a 5% significant difference between means denoted by various lettersin the same column.

when broilers were given *Microdesmis puberula* leaf meal at 200 g/kg.

Hematological indices of broilers at 56 days supplemented with *Uvaria chamae* leaf meal

As shown in Table 5, hematological indices of broilers at 56 days of age supplemented with *U. chamae* leaf meal (UCLM). Pack cell volume, hemoglobin and red blood cells of birds supplemented with 2 g/kg UCLM (T2) and 6g/kg UCLM (T3) were similar (P>0.05) to those fed in T4 (6g/kg UCLM) and T5 (8g/kg UCLM) but greater than those of fed with 0.25 g/kg oxytetracycline (T1). White blood cell and lymphocyte counts follow a similar trend, though their values ranged from 12.66-19.97 $(\times 10)^9$/L and 58.96-75.94%, respectively. However, their values were maximum in T3 to T5 relative to other treatments (T1 and T2). Monocyte values were not affected (P>0.05) by the treatments. Pack cell volume, red blood cell, hemoglobin, white blood cell, lymphocytes and monocytes values investigated in this study were within the established range for healthy broilers [51-53]. This result reflects the anti-inflammatory, immuno-modulatory and antimicrobial physiological effects of UCLM [54]. Elevation in values of pack cell volume (PCV) values could result from polycythemia, hypoxia conditions and dehydration [55] while a decrease in PCV suggests kidney or renal disease, infection and anemia [56]. Hemoglobin is a conjugated protein that is capable of transporting oxygen from the lungs to tissues and carbon (iv) oxide from tissues to the lungs [53]. Hemoglobin also acts as a buffer and aids in maintaining a constant pH [57]. A low count of red blood cells indicates bone marrow disorder or kidney dysfunction [58]. White blood cells are a vital part of the body's immune defense system that are involved in protecting the body against both infections and foreign invaders [42]. Having higher or lower numbers of white blood cells may indicate an underlying health condition, for instance, bone marrow damage or tumor, aplastic anemia, or infectious diseases amongst others [58]. The lymphocytes of the birds fed T3, T4 and T5 were similar (P>0.05) but significantly higher than T1 and T2 (P<0.05). The primary function of lymphocytes is to produce antibodies. Monocyte count was not significantly influenced (P>0.05) by the treatments. However, values were within the range reported by the Merck Veterinary manual [59]. Monocytes metamorphose into macrophages in the tissue where they clean up cells by phagocytosis [60]. They are also responsible for killing foreign invaders or alerting other blood cells to destroy them and prevent infection [57].

Immune response of broilers at 56 days supplemented with *Uvaria chamae* leaf meal

As presented in Table 6, the immune response of broilers at 56 days of age supplemented with *U. chamae* leaf meal (UCLM). Immunoglobulin A (IgA) of birds fed T2 (2g/kg UCLM), T3 (4 g/kg UCLM) were similar (P>0.05) to T4 (6 g/kg UCLM) and T5 (8 g/kg UCLM) but greater than T1 (0.25 g/kg oxytetracycline). Immunoglobulin Y (IgY) and M (IgM) were significantly influenced by the treatments, values were higher in T3, T4 and T5

relative to other groups (P<0.05). Immunoglobulins are globular proteins produced by plasma cells of B-cells that react specifically with antigens that stimulate their production [3]. IgA, Y and M play an active role in protecting pathogens from attaching themselves to the epithelial cells and other body tissues [2]. These activities are due to the presence of phytogenic in UCLM, especially phenolic compounds which are capable of stabilizing free radicals and neutralizing harmful effects in the body of animals [1]. Research has also shown that the synergy between phytochemicals can prevent the risk of cardiovascular and other degenerative diseases in animals [3]. UCLM has immune-modulatory properties, thus reducing the need for antimicrobials [2]. Results obtained in this trial align with other reports that reported that the dietary supplementation of broiler feed with *Ocimum gratissimum* leaf extract at 2.5 g/kg had a positive impact on the immune response of birds [47]. Alagbe [60] also observed a numerical increase in IgA and IgM values of animals fed diet supplemented with *Litsea cubeba* essential oil at both 200 mg and 400 mg/kg.

Caecal microbial population of broilers at 56 days supplemented with Uvaria chamae leaf meal

As presented in Table 7, the caecal microbial population of broilers at 56 days of age was supplemented with *U. chamae* leaf meal (UCLM). The population of *Escherichia coli* count was higher in T1 (0.25 g/kg oxytetracycline). Conversely, *Lactobacillus sp.* count in T4 (6 g/kg UCLM) and T5 (8 g/kg UCLM) were similar (P>0.05), T2 (2 g/kg UCLM) and T3 (4 g/kg UCLM) also follow a similar pattern but values were lower than those of T1. This result suggests that UCLM is capable of balancing the microbial gut flora through competitive exclusion [2, 3]. Optimal microbiota lowers the population of pathogenic bacteria and increases the number of beneficial bacteria [1]. Lactobacilli produce lactic acid, which lowers the pH in the gut and acts against pathogenic bacteria [4], this means that *U. chamae* leaf meal can effectively repress the pathogenicity of intestinal microorganisms and treat infectious diseases in birds [60]. The result recorded in T1 simply shows that apart from antibiotic resistance, environmental pollution and other health associated with the use of conventional antibiotics, oxytetracycline could also affect both pathogenic and beneficial bacteria in the animal's gut. This process will alter the population of microorganisms in the system of the birds [57]. The results obtained in this study are in agreement with the findings of Kritas [61] where probiotics were supplemented in the diets of broilers during subclinical necrotic enteritis. Alagbe [62] also recorded a positive result when fish oil and green tea were added at different levels in the diets of broilers.

Conclusions

In conclusion, *U. chamae* leaf meal is rich in phytochemicals (phenols, alkaloids, terpenoids, flavonoids, steroids, amongst others) which have the potential to improve growth performance, nutrient digestibility, immune response and gut health of animals, including broiler chickens due to their pharmacological properties (anti-inflammatory, immune-stimulatory, antioxidant, hepato-protective, antimicrobial, antiviral, anti-helminthic and antifungal activities). To promote livestock sustainability and food safety, *U. chamae* leaf meal can be included up to 8g/kg without any deleterious effect on the general performance of birds. The dangers of environmental pollution, antimicrobial resistance, or multiple drug resistance are also averted.

Conflict of interest

The authors have declared that no competing interests exist.

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