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Influence of *Telfairia occidentalis* Supplemented Diet on Growth Performance, Blood Profile, Immune Indices and Carcass Traits of Two Broiler Strains Raised Under Tropical Conditions

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ABSTRACT

This study evaluated the effects of strain and dietary supplementation with *Telfairia occidentalis* leaves (TOL) on growth performance, blood profile, immune indices, caeca microbial counts, and carcass traits of broilers raised under tropical conditions. A total of 288 male one-day-old chicks (144 Arbor Acres [AA] and 144 Cobb 500) were randomly assigned to 24 replicates (six birds per replicate) and allocated to four dietary treatments: negative control (NC, basal diet without supplementation), positive control (PC, basal diet + 0.4 g/kg oxytetracycline), basal diet + 2 g/kg TOL, and basal diet + 4 g/kg TOL for 35 days. The TOL-4 birds (1731 g) exhibited heavier body weight gain than other birds (1542-1549 g) except the PC birds ($P<0.05$). AA broilers showed higher ether extract digestibility (63.74% vs. 60.33%) and lower fiber digestibility (47.135 vs. 51.89%) than Cobb 500. The PC birds had the highest ether extract digestibility (66.85%), whereas TOL-4 birds had the highest fiber digestibility (53.30%) ($P<0.05$). TOL-2 and TOL-4 diets reduced Salmonella spp. counts compared to other groups. The PC birds had lower granulocyte concentration. AA broilers exhibited higher granulocyte, mean corpuscular hemoglobin, and platelet counts and lower IL-1β than Cobb 500. White blood cells were lower in TOL-2 and TOL-4 groups. NC birds had lower immunoglobulins and higher IL-1β than other birds. A significant strain-diet interaction was observed for serum lipids, TNF-α, and IL-10. Dietary supplementation with TOL-4 improved growth performance and fiber digestibility in broilers. However, the impact of TOL on cecal microbiota, and selected serum lipids, blood profile, and immune indices was strain-dependent.

1.Introduction

Given its rapid growth rate, efficient feed conversion, and relatively low production costs, the poultry industry plays a crucial role in meeting the global demand for animal protein (Erdaw and Beyene, 2022; Castro et al., 2023). However, the intensification of poultry production systems has increased the vulnerability of broilers to stress, diseases, and reduced performance (Hennessey et al., 2021; Gržinić et al., 2023). Antibiotics, such as oxytetracycline, have been extensively used as growth promoters and therapeutic agents to mitigate these challenges (El-Deek et al., 2012; Khadem et al., 2014; Agyare et al., 2019; Selaledi et al., 2020). Despite their effectiveness, the prolonged and indiscriminate use of antibiotics has raised significant concerns about the development of antimicrobial resistance, the presence of antibiotic residues in meat, and their potential impact on human health (Opatowski et al., 2021; Khmaissa et al., 2024; Pandey et al., 2024). These issues have spurred global regulatory actions (USFDA, 2013; EFSA, 2016; NAFDAC, 2017) and a shift towards reducing or eliminating antibiotics in animal feed, necessitating the search for natural and sustainable alternatives (Khadem et al., 2014; Adeyemi et al., 2023; Rassmidatta et al., 2024; Insawake et al., 2025a).

Telfairia occidentalis, commonly referred to as fluted pumpkin, is a tropical leafy vegetable widely consumed in Africa for its nutritional and medicinal properties (Okoye and Orakwue, 2019; Awoniyi et al., 2023). *T. occidentalis* leaves are rich in bioactive compounds such as flavonoids, alkaloids, tannins, and phenolic acids, which are known for their antioxidant, antimicrobial, anti-inflammatory, and immune-boosting effects (Okonwu et al., 2017; Awoniyi et al., 2023; Chijindu et al., 2024). These properties suggest its potential as a functional feed additive to improve broiler health and productivity. Unlike antibiotics, which may disrupt natural gut microbiota (Insawake et al., 2025b), plant-based alternatives such as *Telfairia occidentalis* leaves (TOL) can help maintain a balanced microbial population in the gastrointestinal tract, thereby promoting better nutrient absorption and overall performance.

In addition to diet, broiler performance, blood indices and carcass traits are influenced by genetic factors (Sola-Ojo et al., 2019; Sam and Okon, 2022; Tudorache et al., 2022). Different broiler strains exhibit variations in growth rate, feed efficiency, carcass composition, and resilience to environmental stressors (Sola-Ojo et al., 2019; Tudorache et al., 2022). Therefore, understanding the strain-specific responses to dietary interventions is critical for optimizing production outcomes. Furthermore, consumer demand for high-quality, residue-free meat has driven interest in strategies that not only enhance growth performance but also improve product quality. Previous studies have compared the growth performance and carcass characteristics (Kareem-Ibrahim et al., 2021; Kamporn et al., 2022) and blood profile (Sam et al., 2023) of Arbor Acres and Cobb 500 broilers. However, there is limited research on the gut microflora and immune indices of these broiler strains, particularly in response to dietary interventions. Examining the blood profile, gut microflora, and immune indices in broilers fed antibiotics or phytochemicals is essential for understanding their physiological responses and overall health (Adeyemi et al., 2022; Rassmidatta et al., 2024; Insawake et al., 2025a). Blood parameters provide insights into metabolic functions, organ health, and stress levels, helping to assess the safety and efficacy of dietary interventions (Odhaib et al., 2018; Sam et al., 2023). Gut microflora analysis is crucial in evaluating the impact of antibiotics or phytochemicals on microbial diversity, beneficial bacteria, and pathogen suppression, which directly influence gut health and nutrient absorption (Rassmidatta et al., 2024; Insawake et al., 2025a). In addition, assessing immune indices helps determine the birds' ability to resist infections and inflammation, offering a clearer picture of how these dietary additives modulate immune function (Adeyemi et al., 2022; Insawake et al., 2025a). Given growing concerns about antibiotic resistance, studying these parameters can support the development of effective and sustainable antibiotic alternatives, ensuring optimal broiler health and productivity. Therefore, this study aimed to investigate the effects of replacing oxytetracycline with *T. occidentalis* leaf powder in the diets on the growth performance, blood chemistry, cecal microbial counts, immune indices and carcass traits of two broiler strains. By assessing these parameters, this study sought to provide evidence for the feasibility of using TOL as a sustainable, natural alternative to antibiotics in broiler production. The findings are expected to contribute to the growing body of knowledge on phytogenic feed additives and their role in supporting antibiotic-free poultry systems, thereby aligning with global efforts to promote food safety and sustainable agriculture.

2. Materials and Methods

2.1. Experimental site

The experiment was conducted at the Poultry Unit of the Teaching and Research Farm, University of Ilorin, Ilorin, Kwara State, which has a tropical climate. Ilorin is located at 8.4799° N latitude and 4.5418° E longitude, at an elevation of 305 m (1,001 feet) above sea level. The average annual rainfall ranges from 94 mm to 125 mm (NIMET, 2022). The birds were raised in open-sided pens. During the day (6:00–18:00 h), the average temperature ranged from 25°C to 38°C, with a relative humidity of 55–80%. At night (18:01–5:59 h), the average temperature ranged from 23 to 35°C, while the relative humidity ranged from 30 to 55%.

2.2. Preparation of *Telfairia occidentalis* leaf meal

Telfairia occidentalis leaves were obtained from a local market. The leaves were air-dried at 37 ^{o}C for 72 h and ground to pass through a 2 mm sieve. The *T. occidentalis* leaf powder was stored at -20 ^{o}C until analysis and utilization. The phytochemical constituent of TOL were determined using previously described protocols (Edeoga et al., 2005; Osuntokun et al., 2016). The TOL powder contained saponin (73.3 mg/kg), tannin (490 mg/kg), phenolics (302.8 mg/kg), steroids (1655 mg/kg), flavonoids (3580 mg/kg), coumarins (553.6 mg/kg), terpenoids (341.6 mg/kg), triterpenes (3036.8 mg/kg) and alkaloids (417.7 mg/kg).

2.3. Experimental birds and management

The findings are expected to contribute to the growing body A total of 288 male one-day-old chicks consisting of 144 Arbor acres (AA) and 144 Cobb 500 were obtained from a reputable hatchery. Each strain was randomly distributed into 24 pens of 6 birds each. The pens were randomly allotted to one of four dietary groups namely; basal diet without supplementation, negative control, NC; basal diet supplemented with 0.4 g/kg oxytetracycline, positive control, PC; basal diet supplemented with 2 g/kg TOL; or basal diet supplemented with 4 g/kg TOL for 35 days. The basal diet was a single straight diet (Table 1) and offered as a mash.

2.4. Growth performance

The body weights of all birds in each pen were recorded individually at 1, and 35 days of age. From these data, the body weight gain (BWG) of the birds was calculated. The feed offered and refused feed were recorded for each replicate to calculate feed intake (FI). Based on this data, the feed conversion ratio (FCR) was calculated.

2.5. Apparent nutrient digestibility

Nutrient digestibility was determined using the total collection method at the 3rd week of the trial. Weighed quantity of feed was supplied to the birds for 72 h. The excreta samples were collected from all the replicates, 300 g of excreta sample was weighed from each replicate and oven dried at 60 ^{o}C 24 h after which the samples were weighed and pulverized. The chemical composition of feed and digesta was determined in accordance to (AOAC, 2016) protocol.

2.6. Apparent nutrient digestibility

On day 34, 2 birds per pen were randomly selected for blood collection. Blood was collected via brachial venipuncture into plain and EDTA tubes. Hematological indices were determined using the Sysmex-K 1000 system (Sysmex Corporation, Kobe, Japan). Serum lipids were determined using an ELISA kit (Randox Laboratories, Kearneysville, WV, USA). Serum alanine transaminase (ALT) and aspartate transaminase (AST) levels were determined using test kits (Randox Laboratories, Kearneysville, WV, USA) according to the manufacturer's instructions.

Table 1. Ingredients and chemical composition of dietary treatments

	Diet[1]			
Ingredient (%)	NC	PC	TOL-2	TOL-4
Maize	50.00	50.00	50.00	50.00
Wheat offal	13.00	13.00	13.00	13.00
Soybean meal	20.00	20.00	20.00	20.00
Full-fat soybean	10.00	10.00	10.00	10.00
Fish meal (72%)	3.00	3.00	3.00	3.00
Oyster shell	1.00	1.00	1.00	1.00
Bone meal	2.00	2.00	2.00	2.00
Methionine	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
Mineral-vitamin premix[2]	0.25	0.25	0.25	0.25
Analyzed Chemical composition				
Dry matter	92.25	92.23	92.67	92.80
Crude protein	22.54	22.52	22.50	22.56
Ether extract	6.00	6.01	6.00	6.00
Ash	6.32	6.31	6.32	6.31
Crude fiber	6.50	6.50	6.51	6.52
Calculated analysis				
Metabolizable energy (kcal/kg)	3140	3140	3140	3140
Calcium (%)	1.06	1.06	1.06	1.06
Phosphorus (%)	0.65	0.65	0.65	0.65
Methionine (%)	0.75	0.75	0.75	0.75
Lysine (%)	1.18	1.18	1.18	1.18

[1]NC, basal diet without additive; PC, basal diet with 0.4 g/kg oxytetracycline; TOL-2, basal diet with 2 g/kg *Telferia occidentalis* leaves. TOL-2, basal diet with 4 g/kg *Telferia occidentalis* leaves.
[2]Supplied per kg of diet: Vitamin A, 11,494 IU; vitamin B1, 1.43 mg; vitamin B2, 3.44 mg; vitamin B3, 40.17 mg; vitamin B5, 6.46 mg; vitamin B6, 2.29 mg; vitamin B7, 0.05 mg; vitamin B9, 0.56 mg; vitamin B12, 0.05 mg; vitamin D, 1,725 IU; vitamin E, 40 IU; vitamin K3, 2.29 mg; Fe, 120 mg; Zn, 120 mg; Cu, 15 mg; Mn, 150 mg; Co, 0.4 mg; Se, 0.3 mg; I, 1.5 mg.

2.7. Immune indices

Serum samples were used to quantify cytokines, including interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), and tumor necrosis factor-alpha (TNF-α), and immunoglobulins (IgA, IgG, and IgM). Analyses were performed using ELISA kits (Cusabio Technology, Houston, TX, USA) according to the manufacturer's instructions.

2.8. Carcass analysis

On day 35, the birds were fasted overnight with ad libitum access to water. Three birds were randomly selected per replicate, humanely slaughtered, de-feathered and eviscerated. Carcass analysis was conducted as described by Adeyemi et al. (2021a).

2.9. Caeca microbial profile

Ceca digesta sample were obtained from three birds per pen. Digesta were aseptically sampled from the right and left ceca into sterile plain bottle, and immediately transferred into a cooler filled with ice to the laboratory for microbial analysis. Samples were cultured and enumerated for total bacterial count (TBC) and Salmonella spp. One gram of digesta was placed in a test tube containing 9 mL of sterile phosphate-buffered saline. The mixture was vortexed and diluted to 1010. One millimeter of the mixture was introduced into a petri dish containing sterile molten agar. Plates were incubated for 48 h at 37 °C, and total aerobic bacteria were cultured on nutrient agar whereas Salmonella spp were cultured on Salmonella shigella agar. The agar plates were prepared according to the manufacturer's instruction.

2.9. Statistical analysis

The trial was laid out in a 2 × 4 factorial arrangement using a completely randomized design. Data were analyzed using the MIXED procedure of the Statistical Analysis System (SAS 9.2; SAS Institute Inc., Cary, NC, USA). The model included strain, diet and their interaction as fixed effects, whereas pens were treated as random effects. A significance level of P<0.05 was used, and least-square means were compared using the PDIFF option.

3. Results

3.1. Growth performance

The strain had no significant effect on broiler growth performance (Table 2). Birds in the TOL-4 group exhibited a higher BWG than all other groups, except for PC birds. Feed intake was greater in TOL-4 birds than in NC birds. The feed intake of the PC and TOL-2 birds was similar to that of the other birds. Strain and diet had no significant effects on feed efficiency in broilers.

Table 2. Growth performance of two strains of broilers supplemented with *T. occidentalis* leaves

Factor		Total BWG (g/bird)	Average BWG (g/bird)	Total feed Intake (g/bird/d)	Average feed intake (g/bird/d)	Feed conversion ratio
Strain	Abor Acres	1618.3	46.22	2846.44	81.32	1.75
	Cobb 500	1607.5	45.91	2797.41	79.91	1.74
	SEM	87.22	4.21	121.3	3.45	0.34
	P value	0.215	0.200	0.183	0.116	0.216
Diet[1]	PC	1616.70^{ab}	46.20^{ab}	2861.60^{bb}	81.74^{ab}	1.77
	NC	1549.17^{b}	44.26^{b}	2747.20^{b}	78.48^{b}	1.77
	TOL-2	1542.17^{b}	44.06^{b}	2878.10^{ab}	82.37^{ab}	1.86
	TOL-4	1731.67^{a}	49.48^{a}	3100.80^{a}	88.88^{a}	1.79
	SEM	110.46	3.07	234.67	6.52	0.23
	P value	0.021	0.043	0.034	0.033	0.122
Strain × Diet		0.154	0.211	0.100	0.109	0.321

[a,b] Means in a column with different superscripts differ significantly ($P < 0.05$). [1]NC, basal diet without additive; PC, basal diet with 0.4 g/kg oxytetracycline; TOL-2, basal diet with 2 g/kg *Telferia occidentalis* leaves. TOL-2, basal diet with 4 g/kg *Telferia occidentalis* leaves. SEM, standard error of the mean.

3.2. Nutrient digestibility

Neither strain nor diet significantly influenced crude protein digestibility in broilers (Table 3). However, AA broilers showed greater ether extract digestibility and lower fiber digestibility than Cobb 500 broilers (P<0.05). The PC birds had higher ether extract digestibility than other groups (P<0.05), whereas the TOL-2 birds had the lowest ether extract digestibility. No significant differences in ether extract digestibility were observed between NC and TOL-4 groups. Fiber digestibility was the highest in TOL-4 birds, whereas NC and TOL-2 birds exhibited the lowest fiber digestibility. The diet-strain interaction did not significantly affect nutrient digestibility.

Table 3. Nutrient retention in two strains of broilers supplemented *T. occidentalis* leaves

		Nutrient retention (%)		
Factor		Crude protein	Ether extract	Crude fiber
Strain	Abor acres	65.60	63.74^{a}	47.13^{b}
	Cobb 500	65.61	60.33^{b}	51.89^{a}
	SEM	3.02	2.44	2.98
	P value	0.213	0.034	0.025
Diet[1]	PC	66.27	66.85^{a}	51.81^{b}
	NC	66.99	60.68^{b}	47.61^{c}
	TOL-2	67.73	58.14^{c}	49.31^{c}
	TOL-4	66.03	62.49^{b}	53.30^{a}
	SEM	3.98	2.09	2.15
	P value	0.162	0.001	0.023
Strain × Diet	P value	0.140	0.312	0.198

[a,b] Means in a column with different superscripts differ significantly ($P < 0.05$). [1]NC, basal diet without additive; PC, basal diet with 0.4 g/kg oxytetracycline; TOL-2, basal diet with 2 g/kg *Telferia occidentalis* leaves. TOL-2, basal diet with 4 g/kg *Telferia occidentalis* leaves. SEM, standard error of the mean

3.3. Caeca microbial counts

A significant diet-strain interaction was observed for TBC and Salmonella spp. counts (Table 4). Cobb 500 broilers fed the NC diet had higher TBC than other groups (Table 5). AA broilers supplemented with PC, TOL-2, or TOL-4 diets had TBCs similar to Cobb 500 broilers receiving the same treatments. However, Cobb 500 birds fed the NC diet had higher TBC than their AA counterparts. Both AA and Cobb 500 broilers fed TOL-2 or TOL-4 diets had lower Salmonella spp. counts than the other groups (Table 5).

Table 4. Caeca microbial counts in two strains of broilers supplemented with *T. occidentalis* leaves

		Log_{10} CFU	
		Total bacteria count	*Salmonella spp.*
Strain	Abor acres	5.14[b]	10.08
	Cobb 500	5.89[a]	10.50
	SEM	0.58	1.46
	P value	0.007	0.487
Diet[1]	NC	7.30[a]	16.85[a]
	PC	4.52[b]	14.50[b]
	TOL-2	5.20[b]	5.03[c]
	TOL-4	5.05[b]	4.76[c]
	SEM	0.44	0.69
	P value	0.001	0.001
Strain × Diet	P value	0.001	0.023

[a,b] Means in a column with different superscripts differ significantly (P < 0.05). [1]NC, basal diet without additive; PC, basal diet with 0.4 g/kg oxytetracycline; TOL-2, basal diet with 2 g/kg *Telferia occidentalis* leaves. TOL-2, basal diet with 4 g/kg *Telferia occidentalis* leaves. SEM, standard error of the mean.

Table 5. Detail of interactions between strain and *T. occidentalis* leaf supplementation on total bacteria count and *Salmonella spp.* in caecum of broilers

Strain	Diet[1]	Total bacteria count	*Salmonella spp.*
Abor acres	NC	6.37[b]	18.20[a]
	PC	5.13[cbd]	15.00[b]
	TOL-2	4.57[cd]	3.57[c]
	TOL-4	4.50[cd]	3.53[c]
Cobb 500	NC	8.23[a]	15.50[b]
	PC	3.90[d]	14.00[b]
	TOL-2	5.83[cb]	6.50[c]
	TOL-4	5.60[c]	6.00[c]

[a,b,c,d] Means in a column with different superscripts differ significantly (P < 0.05). [1]NC, basal diet without additive; PC, basal diet with 0.4 g/kg oxytetracycline; TOL-2, basal diet with 2 g/kg *Telferia occidentalis* leaves. TOL-2, basal diet with 4 g/kg *Telferia occidentalis* leaves.

3.4. Hematology

Granulocyte concentration was lower in PC birds than in TOL-2 and TOL-4 birds, whereas NC birds showed no significant difference from the other groups (Table 6). AA broilers exhibited higher granulocyte counts, mean corpuscular hemoglobin, and platelet counts than Cobb 500 broilers (P<0.05). A significant strain-diet interaction was observed for white blood cell (WBC), and lymphocytes, (P<0.05). Regardless of the strain, broilers supplemented with TOL-2 or TOL-4 diets had lower WBC counts than those on the NC and PC diets (Table 7). In addition, AA broilers supplemented with TOL-2 or TOL-4 had lower WBC counts than Cobb 500 broilers fed the same diets. The lymphocyte concentration was higher in AA broilers supplemented with TOL-4 group than in the other groups.

Table 6. Hematology indices of two strains of broilers supplemented with *T. occidentalis* leaf

Factors		WBC (10^9/l)	RBC (10^{12}/l)	LYM (%)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/l)	RDW (%)	GRAN (%)	PLT (10^9/l)	MPV (fl)
Diet (D)[1]	NC	104.75	2.39	76.56	35.67	146.45[a]	48.68	337.2	35.25	9.50[ab]	519	6.10
	PC	103.25	2.30	77.77	31.61	145.97[a]	47.65	328.2	36.35	8.87[b]	533	6.15
	TOL-2	61.73	2.59	75.40	34.26	137.55[b]	47.53	339.5	32.53	10.55[a]	527	6.15
	TOL-4	60.03	2.21	84.12	32.45	139.92[ab]	48.27	337.3	33.58	10.72[a]	534	6.12
	SEM	0.03	0.33	1.20	0.89	1.36	0.88	2.33	1.03	0.59	2.64	0.17
	P value	<.0001	0.291	0.014	0.101	0.031	0.809	0.645	0.210	0.023	0.584	0.688
Strain	Abor acres	79.02	2.34	79.61	30.97	146.88[a]	49.30[a]	337.6	34.70	10.77[a]	538.2[a]	6.13

(S)	Cobb 500	85.87	2.39	77.31	31.02	138.07[b]	46.77[b]	335.5	34.15	9.05[b]	518.8[b]	6.12
	SEM	2.00	0.58	2.08	1.54	2.36	1.52	4.04	1.79	1.02	4.54	0.29
	P value	0.001	0.714	0.212	0.952	0.001	0.016	0.550	0.684	0.001	0.035	0.817
D × S	P value	0.012	0.324	0.010	0.033	0.310	0.471	0.312	0.110	0.101	0.210	0.130

[a,b,c,d] Means in a column bearing different superscripts differ significantly ($P < 0.05$). [1]NC, basal diet without additive; PC, basal diet with 0.4 g/kg oxytetracycline; TOL-2, basal diet with 2 g/kg *Telferia occidentalis* leaves. TOL-2, basal diet with 4 g/kg *Telferia occidentalis* leaves. SEM, standard error of the mean.
WBC: white blood cell, RBC: red blood cell, LYM: lymphocyte, PCV: packed cell volume, MCV: mean corpuscular volume, MCHC: mean cell hemoglobin concentration, PLT: platelets, MPV: mean platelet volume.

Table 7. Details of interaction between strain and *T. occidentalis* supplementation on hematological indices in broilers

Diet[1]	NC		PC		TOL-2		TOL-4	
Strain	Abor acres	Cobb 500	Abor acres	Cobb 500	Abor acres	Cobb 500	Abor acres	Cobb 500
WBC (10^9/l)	105.9[a]	100.6[a]	107.9[a]	101.6[a]	52.67[c]	70.8[b]	49.6[c]	70.47[b]
LYM (%)	75.23[b]	80.3[b]	72.57[b]	80.53[b]	76.03[b]	74.77[b]	94.6[a]	73.63[b]

[a,b] Means in a row with different superscripts differ significantly ($P < 0.05$). [1]NC, basal diet without additive; PC, basal diet with 0.4 g/kg oxytetracycline; TOL-2, basal diet with 2 g/kg *Telferia occidentalis* leaves. TOL-2, basal diet with 4 g/kg *Telferia occidentalis* leaves. WBC: white blood cell, LYM: lymphocyte, PCV: packed cell volume

3.5. Serum biochemistry

Strain and diet did not affect AST, total protein, urea, uric acid, albumin or globulin in broiler chickens (Table 8). The TOL-4 group had higher HDL-cholesterol levels than the other dietary groups. The serum ALT levels were higher in the AA group than in the Cobb 500 group. A significant strain-diet interaction was observed for total cholesterol, triglycerides, and LDL-cholesterol in broilers (Table 9). The total cholesterol concentrations in AA broilers supplemented with NC, PC, or TOL-2 diets were comparable to those of their Cobb 500 counterparts. However, AA broilers supplemented with TOL-4 had higher total cholesterol than Cobb 500 broilers on the same diet (P<0.05). Cobb 500 broilers supplemented with PC, NC, or TOL-2 diets had triglyceride levels similar to those of AA broilers fed on the same diets. However, Cobb 500 broilers on the TOL-4 diet had lower triglycerides levels than AA broilers on TOL-4. LDL cholesterol levels did not differ between AA and Cobb 500 broilers fed NC or PC diets. However, AA broilers supplemented with TOL-2 or TOL-4 had higher LDL cholesterol levels than Cobb 500 broilers on the same diets.

3.6. Immune indices

Dietary treatments had no significant effect on serum IL-6 levels in broilers (Table 10). However, serum IgM concentrations were higher in TOL-4 birds than in the NC and TOL-2 birds (P<0.05). The PC birds exhibited IgM levels that were similar to those of the other groups.

Table 8. Serum biochemical indices in two strains of broilers supplemented with *T. occidentalis* leaves

	Diet[1]						Strain				
	NC	PC	TOL-2	TOL-4	SEM	P value	Abor acres	Cobb 500	SEM	P value	D × S
AST (u/l)	230.39	244.3	248.24	235.52	4.63	0.057	236.07	243.15	3.27	0.146	0.33
ALT (u/l)	87.75	95.10	84.77	92.32	8.24	0.062	96.69[a]	83.27[b]	1.59	<.0001	0.126
TP (g/dl)	8.24	7.85	6.915	8.23	0.25	0.006	8.137	7.48	0.18	0.021	0.051
ALB (mg/dl)	4.53	4.25	3.99	4.62	0.46	0.778	3.66	5.04	0.33	0.093	0.358
GLO (mg/dL)	3.75	3.94	3.53	3.64	0.32	0.071	4.13	3.79	0.22	0.305	0.139
ALB/GLO	1.02	1.15	1.19	1.46	0.19	0.44	0.92	1.48	0.13	0.011	0.082
Urea (mg/dL)	5.63	5.58	5.85	5.93	0.27	0.78	5.95	5.54	0.19	0.163	0.939
Uric acid (mm/L)	1.65	1.64	1.72	1.74	0.08	0.77	1.75	1.63	0.06	0.169	0.943
TCHL (mg/dL)	237.51	251.68	175.11	226.46	6.83	<.0001	233.42	211.96	4.83	0.006	<.0001
TRIG	131.99	210.13	198.06	185.74	7.24	<.0001	171.62	191.39	5.10	0.015	0.001

(mg/dL)											
HDLC (mg/dL)	106.33^{b}	108.18^{b}	107.94^{b}	123.08^{a}	2.90	0.003	111.23	117.71	4.21	0.793	0.078
LDLC (mg/DL)	107.46^{b}	108.55^{b}	111.71^{b}	130.38^{a}	5.06	0.018	129.17^{a}	99.89^{b}	3.58	<.0001	<.0001

^{a,b} Means in a row with different superscripts differ significantly (*P* < 0.05). [1]NC, basal diet without additive; PC, basal diet with 0.4 g/kg oxytetracycline; TOL-2, basal diet with 2 g/kg *Telferia occidentalis* leaves. TOL-2, basal diet with 4 g/kg *Telferia occidentalis* leaves. SEM, standard error of the mean.
AST- Aspartate aminotransferase, ALT- Alanine transaminase, ALB- Albumin, GLO- Globulin**,** TCHL- Total cholesterol, TRIG, Triglyceride, HDLC, High density lipoprotein cholesterol, LDLC, Low density lipoprotein cholesterol.

Table 9. Details of interaction between strain and *T. occidentalis* leaf supplementation on serum lipids in broilers

Strain	Diet[1]	Total cholesterol (mg/dL)	Triglycerides (mg/dL)	LDL-Cholesterol (mg/dL)
Abor acres	NC	221.74^{bc}	118.30^{c}	93.80^{cd}
	PC	242.93^{ab}	196.64^{ab}	96.67^{cd}
	TOL-2	183.04^{cd}	162.91^{bc}	152.05^{ab}
	TOL-4	285.98^{a}	208.63^{ab}	174.15^{a}
Cobb 500	NC	253.28^{ab}	145.68^{c}	121.12^{bc}
	PC	260.43^{ab}	223.62^{a}	120.44^{bc}
	TOL-2	167.18^{d}	233.21^{a}	71.38^{d}
	TOL-4	166.95^{d}	162.84^{bc}	86.62^{cd}

^{a,b} Means in a column with different superscripts differ significantly (*P* < 0.05). [1]NC, basal diet without additive; PC, basal diet with 0.4 g/kg oxytetracycline; TOL-2, basal diet with 2 g/kg *Telferia occidentalis* leaves. TOL-2, basal diet with 4 g/kg *Telferia occidentalis* leaves.

Serum IgG concentrations were significantly higher in PC birds than in the other groups (P<0.05). NC broilers had lower IgA levels than PC and TOL-4 birds (P<0.05), while IgA levels in TOL-2 birds did not differ from those in other groups. The NC birds had higher IL-1β compared with the other birds. The strain had no significant effect on serum IgG, IgM, or IL-6 concentrations. However, Cobb 500 broilers exhibited higher serum IgA and IL-1β levels than AA broilers (P<0.05). A significant strain-diet interaction was observed for TNF-α and IL-10 (Table 11). In addition, AA broilers supplemented with TOL-4 had higher serum TNF-α and IL-10 levels than other birds.

Table 10. Immune indices in two strains of broilers supplemented with *T. occidentalis* leaves

		Parameter (ng/mL)						
Factors		IgM	IgG	IgA	IL-1β	TNF-α	IL-6	IL-10
Diet[1]	NC	0.22^{b}	2.15^{b}	28.86^{b}	82.36^{a}	35.78	14.25	100.55
	PC	0.51^{ab}	3.14^{a}	31.26^{a}	66.15^{b}	43.98	15.64	103.57
	TOL-2	0.27^{b}	2.07^{b}	30.77^{ab}	66.76^{b}	30.88	15.94	110.37
	TOL-4	0.78^{a}	1.97^{b}	31.62^{a}	46.68^{c}	60.39	14.89	126.23
	SEM	0.11	0.12	0.47	2.04	2.95	0.46	2.68
	P value	0.010	<.0001	0.046	<.0001	<.0001	0.086	<.0001
Strain	Abor acres	0.49	2.23	29.41^{b}	53.31^{b}	49.53^{a}	15.06	112.56
	Cobb 500	0.39	2.43	31.84^{a}	77.67^{c}	35.99^{b}	15.29	107.80
	SEM	0.08	0.28	1.34	1.44	2.08	0.33	1.89
	P value	0.433	0.140	0.002	<.0001	0.001	0.639	0.095
D × S	P value	0.784	0.126	0.500	0.062	0.001	0.260	0.001

^{a,b} Means in a column with different superscripts differ significantly (*P* < 0.05). [1]NC, basal diet without additive; PC, basal diet with 0.4 g/kg oxytetracycline; TOL-2, basal diet with 2 g/kg *Telferia occidentalis* leaves. TOL-2, basal diet with 4 g/kg *Telferia occidentalis* leaves. SEM, standard error of the mean.

Table 11. Details of interaction between strain and *T. occidentalis* leaf supplementation on immune indices in broilers

Strain	Diet[1]	TNF-α	IL-10
Abor acres	NC	41.40[b]	98.16[b]
	PC	45.47[b]	100.66[b]
	TOL-2	30.47[b]	109.83[b]
	TOL-4	80.78[a]	141.61[a]
Cobb 500	NC	30.16[b]	102.93[b]
	PC	42.50[b]	106.49[b]
	TOL-2	31.30[b]	110.92[b]
	TOL-4	40.23[b]	110.86[b]

[a,b] Means in a column with different superscripts differ significantly ($P < 0.05$). [1]NC, basal diet without additive; PC, basal diet with 0.4 g/kg oxytetracycline; TOL-2, basal diet with 2 g/kg *Telferia occidentalis* leaves. TOL-2, basal diet with 4 g/kg *Telferia occidentalis* leaves.

3.7. Carcass traits

Neither diet nor strain influenced carcass weights, dressing percentage or the percentage of carcass cuts in broilers (Table 12). The TOL-2 and TOL-4 birds had a higher percentage of crop weight than the PC and NC birds (P<0.05). Crop weight was greater in Cobb 500 broilers than in AA broilers (P<0.05).

4. Discussion

The supplementation of TOL-4 enhanced feed intake in broilers compared with the NC diet. This finding may be due to the bioactive compounds of TOL that support gut health, regulates appetite-related hormones, stimulates digestive enzymes and improve the overall metabolic efficiency. This may explain the improvement in BWG in the TOL-4 birds with performance comparable to that of the PC group. In a similar manner, dietary supplementation with *Launaea taraxacifolia* and *Crescentia cujete* leaves (Adeyemi et al., 2021a), Capsicum annum (El-Deek et al., 2012) and *Pulicaria jaubertii* powder (Al-Baadani et al., 2025) improved feed intake and BWG in broilers. The lack of significant strain effect suggested that both AA and Cobb 500 broilers responded similarly in terms of growth. Consistently, no significant differences in FI and BWG have been observed between Marshall and Anak broilers (Amao et al., 2011). In contrast, Adedokun et al. (2022) observed that Cobb 500 and Ross 308 broilers fed a single straight diet had greater BWG and FI than AA broilers. In addition, Sam and Okon (2022) and Kamporn et al. (2022) observed significant differences in BWG of Cobb 500, Ross 308 and AA broilers. The strain-dependent growth performance suggests that TOL supplementation can be applied across different commercial broiler strains. However, environmental factors, such as tropical heat stress, should be considered when

Table 12. Carcass traits and internal organs in two strains of broilers supplemented with *T. occidentalis* leaves

Factors		Carcass weight (g)	Dressing %	Carcass cuts (% carcass weight) Breast	Back	Thigh	Drumstick	Organ (% of body weight) Heart	Crop	Gizzard	Liver
Diet[1]	NC	1187.77	72.53	35.08	24.62	17.62	14.34	0.78	0.43[b]	3.73	1.89
	PC	1172.59	73.68	36.26	24.56	16.64	14.21	0.65	0.39[b]	2.75	1.90
	TOL-2	1083.56	70.27	35.02	25.56	15.10	14.44	0.71	0.51[a]	3.11	1.93
	TOL-4	1211.70	70.00	35.95	24.29	16.42	14.99	0.72	0.55[a]	2.94	1.90
	SEM	78.75	3.63	2.19	3.56	1.13	2.78	0.04	0.04	0.17	0.20
	P value	0.053	0.393	0.210	0.213	0.261	0.310	0.230	0.033	0.101	0.119
Strain	Abor Acres	1147.16	70.92	30.64	26.23	15.94	14.34	0.70	0.43[b]	2.98	1.96
	Cobb 500	1169.41	72.77	32.02	26.67	16.95	14.04	0.73	0.51[a]	3.19	1.98
	SEM	30.23	2.57	1.55	4.00	0.79	2.78	0.03	0.020	0.12	0.21
	P value	0.478	0.615	0.540	0.324	0.091	0.126	0.411	0.040	0.250	0.191
D × S	P value	0.121	0.383	0.650	0.111	0.600	0.214	0.120	0.140	0.831	0.100

[a,b] Means in a column with different superscripts differ significantly ($P < 0.05$). [1]NC, basal diet without additive; PC, basal diet with 0.4 g/kg oxytetracycline; TOL-2, basal diet with 2 g/kg *Telferia occidentalis* leaves. TOL-2, basal diet with 4 g/kg *Telferia occidentalis* leaves. SEM, standard error of the mean.

formulating diets with plant-based additives to optimize their efficacy.

The improved ether digestibility in the PC birds may be attributed to the optimal fat metabolism, enzyme activity, and bile salt function. The reduced ether extract digestibility in the TOL-2 birds was likely due to fiber-lipid interactions, antinutritional factors, and bile salt binding effects. The similarity in ether extract digestibility between the NC and TOL-4 group suggests that a higher level of TOL may have led to metabolic adaptations mitigating negative effects on fat digestion. The enhanced fiber digestibility in TOL-4 could indicate improved gut microbiota composition or enhanced fermentation capacity due to the bioactive compounds in *T. occidentalis*. Abor Acres had greater ether extract digestibility likely due to enhanced lipid absorption, higher lipase activity, and increased energy metabolism. The lower fiber digestibility of AA may be due to faster intestinal transit time, or reduced ceca microbial fermentation.

Strain-diet interactions significantly influenced TBC and Salmonella spp. count. Cobb 500 broilers on the NC diet had higher TBC, which suggests that supplementation (with either PC or TOL) helped regulate bacterial populations. TOL-2 and TOL-4 reduced the Salmonella counts in both strains, highlighting the potential antimicrobial benefits of *T. occidentalis* as an alternative to antibiotics. Consistently, dietary supplementation with *Morinda lucida* leaves reduced caeca Salmonella counts in broilers (Adeyemi et al., 2023). The ability of TOL to improve gut microbiota balance and reduce pathogenic bacteria, such as Salmonella spp., highlights its antimicrobial properties, which could be beneficial in antibiotic-free poultry production systems.

The lower granulocyte concentration in the PC birds compared to the TOL groups was possibly due to reduced immune activation from antibiotic supplementation (El-Deek et al., 2012; Khadem et al., 2014). The lower WBC counts in the TOL-2 and TOL-4 birds, indicates anti-inflammatory or immunomodulatory effect of *T. occidentalis*. Lower WBC was reported in broilers supplemented with *Morinda lucida* leaf (Adeyemi et al., 2023) and Capsicum annum (El-Deek et al., 2012). The lowering effect of TOL-based diets on WBC count was more pronounced in AA than in Cobb 500 broilers. AA broilers exhibited higher granulocyte, MCH, and platelet counts than Cobb 500 broilers, indicating inherent strain differences in blood cell profiles. AA broilers supplemented with TOL-4 had the highest lymphocyte concentration, suggesting enhanced adaptive immunity.

The lack of dietary effects on aspartate aminotransferase, alanine aminotransferase, total protein, urea, uric acid, albumin, and globulin in broiler chickens suggests that neither TOL supplementation nor oxytetracycline significantly influence liver function, protein metabolism, or kidney function. This indicated that all diets provided adequate nutrition without causing hepatic or renal stress. In addition, the phytochemicals in TOL and the antibiotic did not induce significant metabolic changes in protein synthesis or degradation, suggesting that broilers efficiently utilized the dietary components without adverse physiological effects. This finding reflects the similarity in protein digestibility across the diets. This finding aligns with those of Adeyemi et al. (2023) in which dietary supplementation of Morinda lucida leaf did not alter serum AST, ALT, urea and total protein in broilers. However, the supplementation with Persicaria odorata leaves reduced serum AST, ALT and urea and increased serum total protein in broilers (Abdul Basit et al., 2020). The higher serum ALT levels in AA broilers than in Cobb 500 broilers may be due to genetic differences affecting liver metabolism, lipid deposition, and oxidative stress. AA broilers may experience greater hepatic stress, fat accumulation, or metabolic load, leading to increased ALT release. Variation in the immune responses could also contribute to these differences. The effect of TOL supplementation on serum cholesterol levels in broilers was strain-dependent. While both the TOL-2 and TOL-4 diets reduced serum cholesterol in Cobb 500 broilers, the TOL-2 diet decreased the total serum cholesterol in AA broilers, whereas the TOL-4 diet increased it. Nonetheless, the TOL-4 birds had higher HDL-cholesterol levels than other birds. Cobb 500 broilers on the TOL-4 diet had lower triglycerides levels than AA broilers on TOL-4. However, AA broilers supplemented with TOL-2 or TOL-4 had higher LDL cholesterol than Cobb 500 broilers on the same diets. This finding may be ascribed to the phytochemicals in TOL, which are capable of altering cholesterol metabolism. Similar alterations in serum lipids were observed in broilers supplemented with *Launaea taraxacifolia* and *Crescentia cujete* leaves (Adeyemi et al., 2021a).

IgM levels were the highest in TOL-4 birds, indicating enhanced humoral immunity with higher levels of inclusion. The PC birds had the highest IgG levels, reinforcing the role of oxytetracycline in immune stimulation. IgA levels were higher in the TOL-4 and PC groups, suggesting that *T. occidentalis* enhances mucosal immunity. As a broad-spectrum antibiotic, oxytetracycline can reduce the gut microbial load and the burden of harmful pathogens. By minimizing infections and systemic inflammation, the immune system can allocate more resources towards antibody production rather than fighting active infections (El-Deek et al., 2012; Khadem et al., 2014). The bioactive compounds in TOL can exert antioxidant or anti-inflammatory effect (Okonwu et al., 2017; Awoniyi et al., 2023; Chijindu et al., 2024), which may enhance gut health and immune function. Improved gut integrity leads to improved nutrient absorption, which supports immune cell function. In addition, phytochemicals in the TOL can directly stimulate immune cells and enhance the production of immunoglobulins.

Dietary PC and TOL lowered serum IL-1β in broilers. Oxytetracycline can reduce the population of gram-negative bacteria, which are a major source of lipopolysaccharides, a potent IL-1β inducer. At lower LPS exposure, macrophages and other immune cells produce less IL-1β, leading to reduced systemic inflammation. It can also reduce oxidative stress, indirectly lowering the production of inflammatory cytokine (Khadem et al., 2014). Phytochemicals in TOL may support beneficial gut microbiota, enhancing immune balance and reducing inflammatory signaling. With improved gut integrity, there is less translocation of bacterial antigens into the circulation, leading to lower IL-1β levels. A consistent reduction in IL-1β level was observed in broilers supplemented with *Pulicaria jaubertii* powder (Al-Baadani et al., 2025) and 5 g/kg onion leaf (Adeyemi et al., 2021b). The strain-diet interaction revealed that AA broilers supplemented with TOL-4 had higher levels of TNF-α and IL-10 than other treatment combinations. TNF-α is a key cytokine involved in the immune response, signaling inflammation and helping the body combating infections (Kaiser and Stäheli, 2013). The elevated TNF-α levels in AA broilers suggest that TOL supplementation may stimulate an immune response, possibly owing to the presence of bioactive compounds. These compounds can activate the immune cells and trigger cytokine production. IL-10 is an anti-inflammatory cytokine that plays a crucial role in immune homeostasis by counteracting excessive inflammation (Kaiser and Stäheli, 2013). The simultaneous increase in IL-10 levels suggests that while TNF-α was upregulated, the immune system also activated regulatory mechanisms to prevent excessive inflammation. This balance between pro-inflammatory (TNF-α) and anti-inflammatory (IL-10) responses indicates that TOL may have an immunomodulatory role by fine-tuning immune activity rather than causing chronic inflammation. The differential response in AA broilers compared to Cobb 500 suggests that genetic factors influence the metabolism and response of broilers to phytochemicals in the TOL. The immunomodulatory effects of TOL, demonstrated by increased IgM and IL-10 levels, indicate its role in supporting adaptive immunity, potentially reducing the incidence of disease-related performance losses.

TOL supplementation did not affect carcass weight, carcass yield or relative carcass cuts of broiler chickens. This suggests that diets did not significantly alter muscle deposition and distribution in broilers. Consistently, supplementation with *Persicaria odorata* leaves did not affect dressing percentage in broilers (Abdul Basit et al., 2020). TOL supplementation increased the crop weight of broilers. *Telfairia occidentalis* is rich in phytochemicals such as flavonoids, saponins, and alkaloids, which can influence gut motility and secretion. These compounds may increase crop muscular activity or enhance glandular function, contributing to higher crop weight. Since TOL modulates the gut microbiota, it may alter the fermentation dynamics in the upper gastrointestinal tract. Some fermentable fibers encourage microbial activity in the crop, potentially leading to thicker crop walls owing to increased mucosal cell turnover or immune cell infiltration. The greater crop weight in Cobb 500 broilers than in AA broilers may be linked to their higher fiber digestibility, which may lead to crop enlargement. In addition, differences in gut motility between the two strains may have contributed to variations in crop size.

One potential limitation of this study is the influence of tropical environmental conditions on broiler performance, which was not explicitly accounted for in the analysis. High ambient temperatures and humidity typical of tropical climates can negatively impact feed intake, nutrient utilization, and overall growth performance (Adeyemi et al., 2022; Insawake et al., 2025b), potentially contributing to the lower-than-expected body weights observed in both strains compared to their standard breed recommendations (Aviagen, 2018; Cobb-vantres, 2022). Heat stress can also alter immune responses and gut microbiota composition, which may have influenced some of the measured parameters (Adeyemi et al., 2022; Insawake et al., 2025b).

5.Conclusion

This study demonstrated that strain had no significant effect on the growth performance or carcass characteristics of broiler chickens. Cobb 500 broilers exhibited greater fiber digestibility and heavier crops whereas AA broilers showed higher ether extract digestibility and serum ALT levels while dietary supplementation with TOL at 4 g/kg improved body weight gain and fiber digestibility. A significant strain-diet interaction was observed for serum lipids. TOL-4 supplementation also enhanced HDL-cholesterol and immune responses, as indicated by increased IgM levels. In addition, birds fed TOL-2 or TOL-4 diets exhibited reduced Salmonella spp. count and higher crop weight. Strain differences were evident in immune parameters, with Cobb 500 broilers having higher serum IgA and IL-1β, while AA broilers supplemented with TOL-4 had higher TNF-α and IL-10 levels. The results highlight *T. occidentalis* as a potential natural alternative to antibiotics for broiler chickens. These findings provide poultry producers with a natural feed additive that can improve broiler health and performance while aligning with the growing demand for antibiotic-free poultry production. While this study was conducted under practical rearing conditions, future research should incorporate detailed environmental monitoring and physiological stress markers to better assess the extent to which tropical conditions affect broiler performance and the efficacy of dietary interventions. Future research should explore the long-term effects of *T. occidentalis* supplementation on poultry health and productivity under commercial tropical farming conditions. In addition, studies evaluating cost-effectiveness, optimal inclusion levels across different production phases, and potential interactions with other dietary components could further support its practical application in antibiotic-free poultry feeding programs.

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