

Growth Performance, nutrient digestibility, cecal microbial population, and histomorphology of broiler fed *Conocarpus erectus* leaf extract

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ABSTRACT

A feeding trial investigated the effects of *C. erectus* leaf extract supplementation on growth performance, nutrient digestibility, microbial population, and intestinal histomorphology of broiler chickens. A total of three hundred one-day-old broilers were allocated in a completely randomised design to evaluate five dietary treatments with five replicates of twelve broilers each. The experimental diets were formulated by supplementing the basal diet with *C. erectus* leaf extract at 0%, 0.25%, 0.50%, 0.75%, and 1.0%. Feed and body weight were recorded weekly; digesta samples were collected on the slaughter day. Results showed that dietary supplementation with *C. erectus* leaf extract did not affect ($P > 0.05$) growth performance and nutrient digestibility. The 0.75% and 1% *C. erectus* leaf extract enhanced ($P < 0.05$) relative ileum length, whereas 0.5% increased ($P < 0.01$) relative ileum weight. The relative gizzard weight was higher ($P < 0.05$) in birds fed 1%, whereas the lowest in birds given 0.25%. Birds receiving 0.25% and 0.5% *C. erectus* leaf extract showed a significant reduction ($P < 0.01$) in *Escherichia coli* numbers, whereas dietary inclusion of 0.75% resulted in an increase ($P < 0.01$) in *Salmonella* numbers and improved ($P < 0.05$) the gut morphology. In conclusion, *C. erectus* leaf extract at 0.25 % could be supplemented to the broiler diet, resulting in improved intestinal morphology, reduced *Escherichia coli* numbers without any detrimental impacts on the growth performance and nutrient digestibility.

Keywords: Broiler, *Conocarpus erectus*, Leaf extract, Nutrient digestibility

INTRODUCTION

The widespread use of growth promoters, including antibiotics in broiler feed, poses a risk to human health, and the emergence of antibiotic resistance has become a subject of increasing global concern (Izah *et al.*, 2025). Antibiotic growth promoters (AGPs) have been banned in the European Union since 2006, and researchers have been exploring alternative feed additives that should be safe, cheap, available, and sustain-

able (Biswas *et al.*, 2024; Ibeagha-Awemu *et al.*, 2025; Manuelian *et al.*, 2021). *Conocarpus erectus* (*C. erectus*), commonly known as buttonwood mangrove (Bashir *et al.*, 2015), inhabits tropical and subtropical regions (Abdel-Hameed *et al.*, 2012; Schoener, 1988). It has long been recognized for its use in traditional medicine (Raza *et al.*, 2016; Raza *et al.*, 2018) and possesses diverse bio-therapeutic uses (Hussein and El-Dawwy, 2024); it could be a potential phyto-genic supplementation. The *C. erectus* leaf con-

tains bioactive compounds that support having antimicrobial, free radical scavenger, anti-inflammatory, and growth-promoting activities (Bashir *et al.*, 2015; Khalil *et al.*, 2020; Rehman *et al.*, 2019). The leaves of *C. erectus* have been utilized in large animal diets. However, the use of fresh leaves has been reported to be more effective than silage (Al-Koaik *et al.*, 2014). Also, the leftovers from its pruning could be utilized as fodder in the cattle industry (Direkvandi *et al.*, 2020). In addition, inclusion of biologically treated *C. erectus* biomass in the diet of Najdi dairy goats led to enhanced milk production, improved fatty acid profile, increased antioxidant activity, and reduced microbial load (Mohammadabadi *et al.*, 2023). The treatment of *C. erectus* leaf with *Klebsiella pneumoniae* and *Acinetobacter* improved digestive activity in Arabian sheep (Mohamadabadi *et al.*, 2023). In contrast, feeding studies reported that *Conocarpus* products had no significant effect on body dimensions in Holstein calves and on the performance of sheep (Shershab and Al-Galbi, 2025). Although many studies have investigated the effect of *Conocarpus* as a feed ingredient in ruminant diets, data on broilers remain extremely scarce. The leaf meal of *C. erectus* has been supplemented at different levels to broiler diets, with results indicating that *C. erectus* leaf meal at 0.25% did not affect growth performance, whereas supplementation of 1% decreased live body weight, abdominal fat percentage, and increased feed conversion ratio (Al-qazzaz *et al.*, 2023). Also, the 2% *C. erectus* leaf meal enhanced nutrient digestibility and lowered the number of the *E. coli* (Al-qazzaz *et al.*, 2026). Limited information is available on the dietary use of *C. erectus* leaf extract in broiler feed. Based on these observations, graded levels of *C. erectus* leaf extract were evaluated for their impact on growth performance, nutrient digestibility, cecal microbiota, and intestinal histomorphology in Ross 308 broilers. The present study hypothesizes that using the ethanolic extract of *C. erectus* leaf instead of the leaf meal reduces the levels of anti-nutritional factors (tannins, saponins, and glycosides), while maintaining the bioactive phenolic and flavonoid compounds.

MATERIALS AND METHODS

Ethical Statement

All animal care and experimental procedures were approved by the Animal Production Department Committee, University of Baghdad.

Extract Preparation

Leaves of *Conocarpus erectus* were collected from the Al-Jadriya area, Baghdad, during July, then air-dried in a well-ventilated room. The dried leaf was milled into a powdered form using a laboratory grinder (RRH-1000). The leaf was soaked in 70% ethanol at a ratio of 1:10 (w/v) for 72 hours. The obtained extract was filtered and evaporated using a rotary evaporator, then dried at room temperature before being stored at 5°C until use.

Management of Birds

Three hundred one-day-old Ross 308 broiler chicks were randomly allotted to twenty-five cages. Each cage contained twelve birds to serve as one replicate, with five replicates for each treatment. The control group received a basal diet (Table 1) without an additive, whereas the remaining treatment groups received the basal diet supplemented with *C. erectus* leaf extract at 0.25, 0.50, 0.75, or 1.00%. The feeding trial was conducted inside a closed-sided house equipped with continuous lighting for 24 h. The birds had free access to feed and water.

Growth Performance and Organ Measurements

Body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were determined at 1, 10, 20, and 35 days of age. Birds in each treatment were weighed together to determine the average weight of the treatment, after which five birds from each treatment with body weight corresponding to the mean were selected for slaughter. At 35 days old, the weights (g) of flushed gastrointestinal components (gizzard, duodenum, jejunum, and ileum), bursa of Fabricius, spleen, heart, and liver were taken using a digital scale. The relative weights of organs were calculated using the following formula: [Relative weight

Table 1. The Chemical Compositions of the Experimental Diet

Ingredients (%)	Starter Period (0-14 d)	Grower Period (15-25 d)	Finisher Period (26-35 d)
Yellow corn	48	48	48
Wheat	9.7	12.8	15.6
Soybean meal	33	29.1	25.3
Protein concentrate ^a	5	5	5
Corn oil	2	3.1	4.3
Limestone	1.2	1.1	1
Dicalcium phosphate	0.7	0.5	0.4
Salt	0.2	0.2	0.2
Vitamin and mineral premix ^B	0.2	0.2	0.2
Calculated analysis			
Crude protein (%)	23.1	21.6	20.04
Metabolic energy (kcal/kg)	3001	3101	3208.5
Methionine (%)	0.56	0.48	0.46
Lysine (%)	1.3	1.21	1.11
Calcium (%)	0.94	0.87	0.86
Phosphorus (%)	0.5	0.44	0.44

^a Protein Concentrate (per kilogram of diet) contained 40% cp, 5% fat, 2.26% fiber, 5% calcium, 4.68% phosphorus, 3.85% lysine, 3.7% methionine, 4.12% methionine + cystine, 2.4% sodium, and 2107 kcal/kg ME. Also, it included fat-soluble vitamins (A: 200k IU; D3: 60k IU; E: 600 mg; K: 60 mg) and a comprehensive B-complex blend (B2, B6, B12, niacin, biotin, and folic acid). Trace elements were supplied as Cu (200 mg), Se (5 mg), Zn/Mn (1.6 mg), and I (20 mg), with BHT (900 mg) added as an antioxidant."

^bVitamin–Mineral Premix Composition (per kilogram of diet)

The diet was fortified with a vitamin-mineral premix providing fat-soluble vitamins (A: 4000 IU; D3: 750 IU; E: 500 mcg; K3: 500 mcg), B-complex vitamins (B1, B2, B6, B12, niacin, folic acid, and pantothenate), and essential trace minerals (sulfates of Cu, Zn, Mn, and Co).

(g/100g BW)= {organ weight (g)/ BW (g)} × 100]. Additionally, the following formula was used for calculating the relative lengths of the small intestinal segments (duodenum, jejunum, and ileum). [relative length (cm/100g BW)= {organ length (cm)/ BW (g)} × 100].

Intestinal Histomorphology

The Intestinal segments (2cm each) from each replicate were trimmed as cross-sections and fixed in buffered formalin saline (10%) immediately after slaughtering. The tissue samples were dehydrated through a graded series of ethanol, cleared in xylene, and embedded in paraffin wax before sectioned at 3–4 µm thickness, then stained with haematoxylin and eosin (De Maesschalck *et al.*, 2015). Morphological parameters,

including villus height (VH), crypt depth (CD), and the VH/CD ratio, were measured using an Olympus microscope equipped with a digital camera (Future Win Joe) and analyzed with the Fiji image analysis system.

Nutrient Digestibility

Fifteen uniform birds at the age of 31 days old were taken from each treatment then kept in clean cages (one bird each cage), with feed and water available to the birds *ad libitum*. Titanium dioxide (TiO₂), an indigestible marker, was mixed into all experimental diets at a rate of 5 g/kg for four days preceding slaughter. At 35 days old, birds without scalding were slaughtered to pool the ileal digesta samples from the intestinal segment extending between Meckel's diverticu-

lum and the ileocecal junction. The digesta samples were dried in an oven at 60 °C for 48 h and then kept at -20 °C until analysis. The samples of the diet and dried ileal digesta were analyzed for chemical composition (AOAC, 1995) and for TiO₂ content (Short *et al.*, 1996). Apparent nutrient digestibility (AD) was determined according to the following equation:

$$\text{AD (\%)} = 100 - [100 \times (\% \text{ TiO}_2 \text{ in feed} / \% \text{ TiO}_2 \text{ in digesta}) \times (\% \text{ nutrient in digesta} / \% \text{ nutrient in feed})].$$

Proximate and Nutrient Analysis

Samples of basal diet were analyzed for their proximate composition, including moisture, crude fiber, crude protein, ash, and ether extract (AOAC, 1995). Phytochemical screening of the *C. erectus* leaf extract was conducted using several established protocols. The total phenols were determined by the Folin-Ciocalteu technique (Ainsworth and Gillespie, 2007). Total flavonoids were estimated using a colorimetric assay based on aluminum chloride (Laouini and Ouahrani, 2017). The saponin content was quantified using the double extraction gravimetric technique (Tiwari *et al.*, 2015). The tannin content was measured following the protocol of Hagerman and Butler (1978) as modified by Lau *et al.* (1989). Total glycoside content was measured using a modified colorimetric method described by Solich *et al.* (1992). The samples were macerated in methanol (80%) for 24 h at room temperature, then mixed with 10 ml of Baljet's reagent before concentrating under a vacuum. The fresh reagent consisted of 95 mL of 1% picric acid mixed with 5 mL of 10% NaOH. Each sample received twenty milliliters of distilled water after an hour. The absorbance of the

samples was measured at 495 nm using a UV/VIS spectrophotometer (model 1600A, Kyoto, Japan). Ten milliliters of securidaside at varying concentrations (12.5–100 mg/L) were used to create the standard curve. The glycoside content was quantified and reported in terms of milligrams of securidaside per gram of dried extract.

Cecal Digesta Collection and DNA Extraction

Digesta samples of each treatment were pooled from the cecum of birds on slaughter day, then kept at -20°C before use. The digesta samples (200 mg each) were placed in bead tubes, and the tubes were placed on ice. A total of 300 µl SDE1 Buffer was added to each tube before adding 20 µl of proteinase K. DNA was extracted according to the manufacturer's instructions using the FavorPrep™ Stool DNA Isolation Mini Kit.

Quantitative Real-Time Amplification

Quantitative real-time PCR (qPCR) amplification was performed using specific primers listed in Table 2. A total reaction volume of 25 µL contained 1 µL of DNA template, 1 µL forward primer, 1 µL reverse primer, 12.5 µL of SYBR Green master mix, and 9.5 µL of RNase-free water. Amplification reaction was run on a Qiagen real-time PCR system under the following thermal cycling conditions: The PCR reactions were incubated at 94°C for 5 min, subsequently subjected to 40 thermal cycles at 94°C for 20 s, primer-specific annealing for 30 seconds at 58°C for *Lactobacillus*, 50°C for *Escherichia coli*, 60°C for *Bifidobacterium*, 50°C for *Salmonella*, and extension at 72°C for 20 seconds. Absolute quantification of cecal bacteria was achieved using standard curves constructed by amplification of known amounts of target

Table 2. Primer Sequence of Target Microbes

References	Bacteria	Sequences
Wang <i>et al.</i> (1996)	<i>Lactobacillus</i>	F-5'-CATCCAGTGCAAACCTAAGAG-3' R-5'-GATCCGCTTGCCTTCGCA-3'
Bartosch <i>et al.</i> (2004)	<i>Bifidobacterium</i>	F-5'-GGGTGGTAATGCCGGATG-3' R-5'-TAAGCCATGGACTTTTCACACC-3'
Rezaei <i>et al.</i> (2015)	<i>Salmonella</i>	F-5'-TCGTCATTCCATTACCTACC-3' R-5'-AAACGTTGAAAACTGAGGA-3'
Frahm and Obst (2003)	<i>Escherichia coli</i>	F-5'-GTGTGATATCTACCCGCTTCGC-3' R-5'-GAACGCTTTGTGGTTAATCAGGA-3'

bacterial DNA. The obtained Cq values were converted to bacterial copy numbers using the corresponding calibration equations, and the results were expressed as log₁₀ copies/g of digesta.

The experiment was conducted using a completely randomized design, with a general linear model of SAS (Statistical Analysis System, version 9.4 was used for data analysis. Mean differences were considered significant at $P \leq 0.05$ and were compared using Tukey's multiple range test.

RESULTS

Phytochemical Composition

The analysis of phytochemical content of *C. erectus* leaf extract revealed that the major constituents (Table 3) were total phenolic content (278.9mg GAE/mg), total flavonoid content (98.2 mg RE/g), total tannin content (2.6%), total saponin content (0.45), and total glycoside content (1.00%). Also, the phenolic constituents included (ppm) were gallic acid (210.6), apigenin (85.6), catechin (74.1), quercetin (88.6), ferulic acid (68.0), and kaempferol (44.9).

Growth Performance

The effects of dietary supplementation with *C. erectus* leaf extract on growth performance are represented in Table 4. BW, BWG, FI, and FCR were comparable ($P > 0.05$) among all treatments during the starter, grower, and finisher periods. Also, comparable effects were noted in WG, FI, and FCR among broilers across the overall period of the experiment.

Nutrient Digestibility

The effects of dietary supplementation on

nutrient digestibility are presented in Table 5. The dietary inclusion of *C. erectus* leaf extract had no impact ($P > 0.05$) on the digestibility of crude protein, ether extract, crude fiber, dry matter, and ash.

Organ Weights and Lengths

The relative organ weights of broilers in response to dietary *C. erectus* leaf extract supplementation are presented in Table 6. No significant differences ($P > 0.05$) were observed with *C. erectus* leaf extract supplementation on the relative length and weight of the duodenum and jejunum. The high levels (0.75 % and 1%) of *C. erectus* leaf extract increased ($P < 0.05$) the relative length of the ileum of broilers, whereas supplementation with 0.50% increased ($P < 0.01$) the relative ileum weight. The relative weight of the gizzard increased ($P < 0.05$) by 1% *C. erectus* leaf extract compared to the 0.25% level.

Enumerations of Cecal Microbiota

Dietary supplementation with *C. erectus* leaf extract influenced the cecal microbiota, as presented in Table 7. The birds supplemented with *C. erectus* leaf extract had similar ($P > 0.05$) numbers of *Bifidobacterium* and *Lactobacillus*. The copy number was *Escherichia coli* was lower ($P < 0.01$) in birds given 0.25 % and 0.50% *C. erectus* leaf extract, whereas a higher number was reported in 0% and 0.75% supplementation. The birds given a diet supplemented with 0.75% *C. erectus* leaf extract exhibited higher ($P < 0.01$) *Salmonella* numbers in comparison with birds given 0%, 0.25%, and 1% diets.

Table 3. Phenolic Compounds of *C. Erectus* Leaf Extract

No	Name	Content
1	Total phenolic content (mg GAE/g)	278.9
2	Total flavonoid content (mg RE/g)	98.2
3	Total tannin content %	2.6
4	Total saponin content %	0.45
5	Total glycoside content %	1.00
6	Gallic acid (ppm)	210.6
7	Apigenin (ppm)	85.6
8	Catechin (ppm)	74.1
9	Quercetin (ppm)	88.6
10	Ferulic acid (ppm)	68.0
11	Kaempferol (ppm)	44.9

Table 4. Response of Broilers to Dietary Supplementation with *C. Erectus* Leaf Extract

Parameters	<i>C. erectus</i> leaf extract (%)					SEM	P-value
	0	0.25	0.5	0.75	1		
1–10 days							
BW (g)	234.31	231.92	240.08	234.80	214.03	23.92	ns
BWG (g)	195.01	234.79	193.51	201.16	196.13	23.03	ns
FI (g)	203.27	214.60	215.28	211.84	194.90	21.46	ns
FCR	1.04	1.11	1.07	1.08	1.11	0.052	ns
10–20 days							
BW (g)	1012.97	974.03	984.08	945.58	937.90	64.51	ns
BWG (g)	778.65	742.11	744.00	710.78	723.87	48.88	ns
FI (g)	749.00	738.11	742.43	608.55	689.83	59.81	ns
FCR	0.96	0.99	0.99	0.86	0.95	0.054	ns
20–35 days							
BW (g)	1988.25	2025.55	1980.11	2010.12	1972.92	98.03	ns
BWG (g)	975.28	1051.51	996.02	1064.54	1035.02	72.65	ns
FI (g)	1497.98	1552.38	1556.43	1527.65	1502.30	54.59	ns
FCR	1.55	1.47	1.56	1.44	1.45	0.11	ns
1–35 days							
BWG (g)	1948.95	1987.13	1941.20	1971.46	1934.50	97.71	ns
FI(g)	2450.25	2505.10	2514.14	2348.04	2387.03	141.56	ns
FCR	1.18	1.19	1.21	1.12	1.17	0.07	ns

ns= non-significant; SEM= standard error of means; FI= feed intake; BW= body weight; BWG= body weight gain

Table 5. Effects of *C. Erectus* Leaf Extract Levels on Nutrient Digestibility of Broiler

Nutrient (%)	<i>C. erectus</i> leaf extract (%)					SEM	P-value
	0	0.25	0.5	0.75	1		
Dry matter	71.42	72.10	72.86	71.95	72.34	1.12	ns
Crude protein	78.55	79.12	80.04	79.30	78.90	1.48	ns
Ether extract	74.08	75.20	75.68	74.91	74.33	1.36	ns
Crude fiber	54.21	53.87	55.02	54.66	54.10	1.95	ns
Ash	64.33	65.11	64.92	65.37	64.70	1.73	ns

ns = non-significant; SEM= standard error of means

Table 6. Effects of *C. Erectus* Leaf Extract Levels on Relative Organ Weights of Broilers

	<i>C. erectus</i> leaf extract (%)					SEM	P-value
	0	0.25	0.5	0.75	1		
Relative length (cm/100g BW)							
Duodenum	1.36	1.46	1.54	1.52	1.52	0.16	ns
Jejunum	3.63	3.61	4.14	4.11	4.06	0.45	ns
Ileum	3.23 ^c	4.01 ^{ab}	3.87 ^{ab}	4.29 ^a	4.35 ^a	0.50	*
Relative weight (g/100g BW)							
Duodenum	0.62	0.64	0.69	0.66	0.74	0.06	ns
Jejunum	1.27	1.29	1.49	1.30	1.39	0.11	ns
Ileum	0.98 ^b	0.92 ^b	1.32 ^a	0.99 ^b	1.06 ^{ab}	0.14	**
Heart	0.64	0.56	0.58	0.64	0.55	0.05	ns
Liver	1.82	1.84	1.95	1.76	1.99	0.22	ns
Spleen	0.26	0.09	0.12	0.10	0.13	0.13	ns
Bursa	0.12	0.08	0.07	0.11	0.10	0.04	ns
Gizzard	1.79 ^{ab}	1.54 ^b	1.82 ^{ab}	1.60 ^{ab}	1.95 [?]	0.19	*

* = within a row, means differ significantly at $P < 0.05$; SEM = standard error of means; ns = non-significant

Table 7. Quantification of Cecal Microbiota (Log₁₀ Copies/g Digesta) in Broilers Fed Different Levels of *C. Erectus* Leaf Extract

	<i>C. erectus</i> leaf extract (%)					SEM	P-value
	0	0.25	0.50	0.75	1		
<i>Lactobacillus</i>	6.02	6.08	5.97	6.13	6.10	0.13	ns
<i>Bifidobacterium</i>	5.03	4.88	4.84	4.48	4.40	0.28	ns
<i>Escherichia coli</i>	7.64 ^a	7.25 ^b	7.26 ^b	7.62 ^a	7.40 ^{ab}	0.14	**
<i>Salmonella</i>	6.48 ^c	6.48 ^{bc}	6.76 ^{ab}	6.89 ^a	6.51 ^{bc}	0.12	**

* = within a row, means differ significantly at P < 0.05; SEM = standard error of means; ns = non-significant

Table 8. Intestinal Histomorphology of Broilers Fed *C. Erectus* Leaf Extract

	<i>C. erectus</i> leaf extract (%)					SEM	P-value
	0	0.25	0.50	0.75	1		
Duodenum							
VH (μm)	720.34 ^b	802.90 ^{ab}	754.36 ^b	885.83 ^a	842.69 ^a	45.51	**
CD (μm)	71.66 ^d	117.46 ^b	162.36 ^a	82.99 ^{cd}	95.86 ^c	7.78	**
VH/CD ratio	10.11 ^{ab}	6.84 ^c	4.65 ^d	10.77 ^a	8.84 ^b	0.87	**
Jejunum							
VH (μm)	545.72 ^b	685.89 ^a	591.87 ^b	607.54 ^b	582.42 ^b	41.34	**
CD (μm)	92.64 ^b	107.11 ^a	94.72 ^{ab}	77.15 ^c	81.72 ^{bc}	7.27	**
VH/CD ratio	5.94 ^b	6.42 ^{ab}	6.29 ^b	7.96 ^a	7.15 ^{ab}	0.87	*
Ileum							
VH (μm)	545.72 ^b	685.89 ^a	591.87 ^b	607.54 ^b	582.42 ^b	41.34	**
CD (μm)	454.02 ^c	506.60 ^b	407.34 ^d	635.30 ^a	365.78 ^c	19.66	**
VH/CD ratio	67.70 ^b	68.11 ^b	55.90 ^b	110.30 ^a	68.70 ^b	10.68	**
	6.83 ^{ab}	7.54 ^a	7.48 ^a	5.84 ^{ab}	5.41 ^b	1.06	*

* = within a row, means differ significantly at P < 0.05; SEM = standard error of means; ns = non-significant

Intestinal Morphology

The effects of dietary *C. erectus* leaf extract on intestinal morphology are displayed in Table 8. In the duodenum, the addition of 0.75% and 1% *C. erectus* leaf extract increased (P<0.01) VH compared to birds receiving 0% and 0.50% supplementation. Birds receiving 0.50% *C. erectus* leaf extract showed the highest CD (P < 0.01), while the control group had the lowest. Birds fed a diet supplemented with 0.75% *C. erectus* leaf extract had a higher (P<0.01) VH/CD ratio compared to other diets, excluding the control diet. In the jejunum, the 0.25% *C. erectus* leaf extract diet increased (P<0.01) VH and CD, whereas the 0.75% diets had a lower CD value. Birds fed a diet supplemented with 0.75% *C. erectus* leaf extract showed a higher (P < 0.05) VH/CD ratio compared to birds fed 0% and 0.50% diets. In the ileum, the highest (P<0.01) VH was reported in birds fed 0.75% *C. erectus* leaf extract, whereas the lowest was found in birds fed 1% diet. The birds given 0.75% *C. erectus* leaf extract revealed higher (P<0.01) CD compared with other dietary treatments. The VH/CD ratio was re-

duced in birds fed 1% *C. erectus* leaf extract compared with those fed 0.25% and 0.50% levels.

DISCUSSION

Chemical Analysis

The supplementation of natural extracts to broiler diets is critical for improving bird health (Urban *et al.*, 2025). The tree of *C. erectus* has been the subject of attention as a valuable non-conventional feed additive due to its richness in bioactive secondary metabolites (Nasr *et al.*, 2025). These compounds have nutritional and health benefits for the birds (Afifi *et al.*, 2021). The outcomes of this study contribute to novel insight into broiler nutrition, as the influence of *C. erectus* leaf extract in bird diets has not been previously reported. The phytochemical screening results showed that *C. erectus* leaf extract had high content of total phenolics (278.9 mg GAE/g) and flavonoids (98.2 mg RE/g), indicating a high abundance of bioactive compounds. The results of this study aligned with previous findings for *C. erectus* leaf meal, which reported

the total content of phenolics and flavonoids of 271.8mg/g and 68mg/g, respectively (Al-qazzaz *et al.*, 2023). Likewise, de Santana *et al.* (2019) noted a range from 17.29 to 72.52 ± 5.12 µg/ml QE for the flavonoid content in *C. erectus* leaf meal. The bioactive compounds of the most therapeutic plants (Roufunnesa *et al.*, 2025), including phenolics, flavonoids, tannins, saponins, and alkaloids, as well as specific constituents such as conocarpan, gallic acid, ellagitannins, quercetin, and myricetin. (de Santana *et al.*, 2019; Rehman *et al.*, 2019) act as antimicrobials, anti-inflammatories, and antioxidants (Al Mamari, 2021).

The results of the current study showed that the addition of *C. erectus* leaf extract to broiler diets had no significant effect ($P>0.05$) on growth performance and nutrient digestibility. The *C. erectus* leaf extract exhibited no anti-nutritional effects; it can be safe to add up to 1% in broiler diets. Also, similar nutrient digestibility in birds fed *C. erectus* leaf extract indicated that there was no detrimental effect on digestive enzymes. Al-qazzaz *et al.* (2026) observed that inclusion of *C. erectus* leaf meal at levels up to 2% to the broiler diet had no adverse effect on nutrient digestibility.

No notable changes in the relative weight and relative length of the duodenum and jejunum were observed by adding the extract of *C. erectus* leaf, which may explain the absence of significant effects on BW and WG. The improvement in intestinal histomorphology observed in broilers fed *C. erectus* leaf extract may be attributed to its content of polyphenols and flavonoids, which possess potent antioxidant and anti-inflammatory properties. These bioactive compounds protect intestinal epithelial cells from oxidative damage, promote epithelial cell renewal, and maintain intestinal barrier integrity, thereby enhancing villus development and the villus height-to-crypt depth ratio (Abdel-Latif *et al.*, 2021; Paredes-Lopez *et al.*, 2025). The dietary supplementation with 1% *C. erectus* leaf extract increased ($P<0.05$) the relative weight of the gizzard compared with the 0.25 %inclusion level, thus leading to enhanced mechanical digestive capacity. The absence of a significant effect of dietary treatments on the relative weight of the heart, spleen, liver, and bursa indicated that there

was no toxic effect or adverse physiological stress by the extract of *C. erectus* leaf (up to 1%) on broilers. Despite evidence that flavonoids improve gut health, and performance indices of broiler chickens (Paredes-Lopez *et al.*, 2025), their impact in the current study focused on maintaining systemic health instead of altering organ weights.

Dietary supplementation with 0.50% and 0.75% *C. erectus* leaf extract increased *Salmonella* numbers, whereas supplementation at 0.25% and 0.50% reduced *E. coli* numbers. The lower susceptibility of *Salmonella* numbers to *C. erectus* leaf extract may be attributed to differences in outer membrane permeability and the presence of efficient multidrug efflux systems, such as AcrAB–TolC, which reduce the intracellular accumulation of phenolic compounds (Akshay *et al.*, 2023; Punchihewage-Don *et al.*, 2024). In contrast, *E. coli* appeared to be more susceptible to the antimicrobial activity of the phenolic constituents of the extract (Cueva *et al.*, 2010; Liu *et al.*, 2022). These results agreed with Al-qazzaz *et al.* (2026), who reported a reduction in *E. coli* numbers with 2% *C. erectus* leaf meal. Likewise, the essential oil of *C. erectus* inhibited *E. coli* numbers (Salim *et al.*, 2025).

In this study, the 0.75% *C. erectus* leaf extract improved the gut morphology by increasing VH and the VH/CD ratio. Increased VH refers to increased nutrient absorption, whereas the higher VH/CD ratio reflected shallower crypts, less morphological damage to the intestinal wall, and lower energy and protein requirements for tissue renewal (Adeleye *et al.*, 2018; Izadi *et al.*, 2013). These findings were partially in agreement with Al-qazzaz *et al.* (2023), who observed that *C. erectus* leaf meal increased villus width, VH, CD, and a low VH/CD ratio. Previous studies on plants belonging to the *Combretaceae* family reported an increase in villus height, a higher villus height to crypt depth ratio, and reduced crypt depth in broilers fed *Terminalia chebula* extract up to 600 mg/kg (Cheng *et al.*, 2024). Also, Hidayati *et al.* (2022) observed that supplementation of drinking water with 30 mL/L nanoencapsulated *Terminalia catappa* leaf extract led to improved histomorphology of the broiler intestine. As observed in the present

study, the bioactive compounds stimulated the intestinal tissue growth by influencing gut morphology (Oni and Oke, 2025). Previous studies showed that the plants rich in polyphenols may increase the VH and VH/CD ratio in the broiler intestine (Paredes-López *et al.*, 2024; Wu *et al.*, 2024). Also, the quercetin supplementation at levels from 200 to 400 ppm enhanced gut development and the intestinal environment of broiler chickens (Abdel-Latif *et al.*, 2021). Similarly, ellagic acid improved the intestinal barrier function and flora structure of broiler chickens challenged with *E. coli* K88 (Li *et al.*, 2024).

Despite the favorable intestinal morphological responses observed in birds receiving *C. erectus* supplementation, no corresponding improvement in growth performance was observed. The enhanced absorptive surface was not the limiting factor for bird growth under the present experimental conditions because no improvement in nutrient digestibility was observed among treatments fed *C. erectus* supplementation. Yasmin *et al.* (2022) mentioned that *Catharanthus roseus* extract did not affect growth performance but improved morphological histomorphometric parameters of broilers. Similar results were reported in broilers fed phyto-genic feed additives (Ahsan *et al.*, 2018; Othman *et al.*, 2022).

CONCLUSION

Based on the present findings, dietary supplementation of *C. erectus* leaf extract at 0.25% to the broiler diet improved selected intestinal histomorphological parameters and reduced *Escherichia coli*, without any detrimental effects on growth performance and nutrient digestibility.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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