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Growth performance, intestinal morphology, and carcass traits in broiler chicken fed *Conocarpus erectus* leaf meal

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ABSTRACT

This study evaluated the effects of adding *Conocarpus erectus* leaf meal to the diet on the performance, carcass traits, organ weights, and intestinal morphology of broiler chicken. A total of 396 one-day-old Ross 308 broilers were assigned to nine treatments, which included 0, 0.25%, 0.5%, 0.75%, 1%, 1.25%, 1.5%, 1.75%, and 2% *C. erectus* leaf meal addition to the broiler diet. Feed and bird weights were recorded weekly. On slaughter day, the weights of carcasses and organs were individually reported using a digital scale as well as the intestine samples were pooled for tissue analysis. High levels of *C. erectus* leaf meal reduced ($P<0.01$) body weight, body weight gain, and feed conversion ratio. The basal diet and 0.25% *C. erectus* leaf meal diet reported higher ($P<0.01$) body weight and body weight gain than did the other treatments. Birds fed 0.25% *C. erectus* leaf meal supplementation performed similarly to those fed the basal diet. Significantly, with increasing amounts of *C. erectus* leaf meal in the diets, there was a linear slope decrease in live weight and body weight gain as well as a linear slope rise in the values of feed intake and feed conversion ratio. Carcass trait and relative organ weights were not altered among the dietary treatments. Feeding 1% *C. erectus* leaf meal diet decreased ($P<0.01$) relative abdominal fat weight compared to birds fed the control diet. Birds fed dietary *C. erectus* treatments had higher ($P<0.01$) villus height, villus width, crypt depth, and lower villus height/crypt depth ratio than did birds fed the control diet. In conclusion, the study indicated that feeding 0.25% *C. erectus* leaf meal showed no deleterious effects on the growth performance of the broiler. Growth performance and intestinal morphology were linearly reduced when broilers were fed up 2% of *C. erectus* meal.

Keywords: Broiler, Conocarpus erectus, Intestinal morphology, Performance

INTRODUCTION

Phytogenic feed additives are increasingly being used in poultry diets after the use of antibiotics as growth promoters in animal feed was banned on January 1, 2006, by the European Parliament and the European Council. The effect of phytogenic feed additives added to poultry diets has been reported by many researchers (Al-Masari and Al-Himdany, 2022; Atiyah and Hamood, 2021). However, many plants have still not been studied for their effect as a feed additive in broiler diets.

Conocarpus erectus, called button mangrove and buttonwood (English), is a plant of the Combretaceae family and is native to the tropical and subtropical areas of the world. The plant is used as a folk remedy for many human diseases (Bashir *et al.*, 2015) as well as it is effective to get ride mites in poultry farms (Rajabpour *et al.*, 2018). Also, it is reported that numerous phytochemical compounds were isolated from *C. erectus* leaves such as gallic acid, ellagic acid, quercetin, tannin, and saponin (Ayoub, 2010; Nascimento *et al.*, 2016; Tawfeeq *et al.*, 2020). The phytochemicals are secondary metabolites that could be used as safe natural feed additive (Hashemi *et al.*, 2008); they possess a positive effect on bird bodies in terms of health, growth, and production (Abdel-Moneim *et al.*, 2020; Lipiński *et al.*, 2017; Tayeb *et al.*, 2019). Supplementation of plant leaf meals as feed additives in poultry diets due to their content of affective phytochemical compounds was reported (Basit *et al.*, 2020; Nkukwana *et al.*, 2014; Shiraze and Hassanabadi, 2019). The phytochemical compounds are capable to improve intestinal histomorphometry (Kamboh and Zhu, 2014; Oliveira *et al.*, 2018), and growth performance (Luo *et al.*, 2018). Information about the effect of *C. erectus* leaf meal in poultry research is rare. In farm animal studies, the replacement of berseem hay with up to 30% biologically treated *C. erectus* meal improved the body weight (BW) of rabbits (Ali *et al.*, 2017). The silage made from the Conocarpus plant could be cheaper than imported fodder and ease feed shortfall. Additionally, the performance of sheep growth was unaffected when corn silage was substituted with dried *C. erectus* leaves (Hoseini and Chaji, 2021). The digestion activity of Arabian sheep and some fermentation parameters could be improved by treating *C. erectus* leaves with the bacterium *Klebsiella pneumoniae* and *Acinetobacter sp* (Mohammadabadi *et al.*, 2020). Studies evaluating the effects of adding *C. erectus* leaves to broiler diets are scarce. Therefore, the objective of the current study is to evaluate the effect of dietary supplementation of *C. erectus* leaf meal in a broiler diet on the growth performance, carcass traits, organ weights, and intestinal morphology of broilers.

MATERIALS AND METHODS

Experimental Diets

The fresh leaves of *C. erectus* were collected daily from trees in Baghdad city, Al-Mansour Street, and immediately dried inside a shadow room for three days. The dried leaves were stored in polyethylene bags before vacuum sealing. The dried leaves were ground using an electric grinder (Model No. TG-1678) before these were added to the treatment diets at numerous levels to choose the best level that could achieve the top result. Regarding the treatments, the first treatment consisted of a basal diet (Table 1). The second until the ninth treatment consisted of increasing levels (0.25%, 0.5%, 0.75%, 1%, 1.25%, 1.50%, 1.75%, and 2%) of *C. erectus* added to the basal diet, respectively.

Management of Birds

A total of 396 one-day-old Ross 308 broilers were randomly assigned to nine treatments after individual weighing using a digital scale. Each treatment consisted of four groups with 11 birds each. The birds of each group were housed in a single-floor cage. The experiment was performed in a closed house provided with artificial light using electric bulbs. Continuous *ad* libitum feeding and water were provided during the experimental days. A completed vaccination program was likewise applied for the birds according to standard veterinary practice. The experiment was performed according to the committee approval of the University of Baghdad 111/19/10/2021.

Growth Performance

Body weight (BW) and feed intake (FI) of the birds were measured weekly using a digital scale, and thereafter body weight gain (BWG)

Table 1. Composition of Experimental Diets

Ingredients (%)	Starter Period (0-14 d)	Grower Period (15-25 d)	Finisher Period (26-35 d)
Yellow corn	48.00	48.00	48.00
Wheat	9.70	12.80	15.00
Soybean meal	33.00	29.10	25.50
Protein concentrate [A]	5.00	5.00	5.00
Corn oil	2.00	3.10	4.50
Limestone	1.10	1.10	1.10
Dicalcium phosphate	0.80	0.50	0.50
Salt	0.20	0.20	0.20
Vitamin and mineral premix [B]	0.20	0.20	0.20
Calculated analysis			
Crude protein (%)	23.10	21.60	20.04
Metabolic energy (kcal/kg)	3001.00	3101.00	3208.50
Methionine (%)	0.56	0.48	0.46
Lysine (%)	1.30	1.21	1.11
Calcium (%)	0.94	0.87	0.86
Phosphorus (%)	0.50	0.44	0.44

[A] Provided per kilogram of diet: crude protein 40%, crude fat 5%, crude fiber 2.26%, calcium 5%, phosphorus 4.68%, lysine 3.85%, methionine 3.7%, methionine and cystine 4.12% ,sodium 2.4%, energy 2107 kcal/kg, vit A 200000 I.U., vit D 60000 I.U., vit E 600 mg., vit K 50 mg, vit B1 60mg, vit B2 140 mg, vit B6 80 mg, folic acid 20 mg, biotin 100 mg, iron 1 mg, copper 200 mg, manganese 1.6 mg, zinc 1.6 mg, niacin 700 mg/kg, pantothenic acid 147 mg/kg, vit b12 400 mg/kg, choline, Iodine 20 mg, Selenium 5 mg, antioxidant (BHT) 900 mg.

[B] Supplied per kg diet: Vitamin A 4000 I.U., Vitamin D3 750I.U., Vitamin E 500 mcg, Vitamin k3 500 mcg, Vitamin B1 HCl 250 mcg, Vitamin B2 250 mcg, Vitamin B6 HCl 100 mcg, Vitamin B12 4 mcg, Calcium-D- Pantothenate 2 mcg, Niacin 3 mcg, Folic Acid 25 mcg, Manganese Sulphate 200 mcg, Zinc Sulphate 75 mcg, Ferrous Sulphate 250 mcg, Copper Sulphate 20 mcg, Cobalt Sulphate 5 mcg.

and feed conversion ratio (FCR) were calculated. The proximate composition (moisture, crude fiber, crude protein, ash, and ether extract) of *C. erectus* leaf meal was determined according to standard procedures (George, 2016).

Carcass Traits and Organ Weights

The treatment birds that underwent treatments (two birds from each replicate) were selected for being slaughtered at the end of the experiment. Carcass weights of the slaughtered birds were recorded using digital scales. The dressing percentage was calculated according to the formula: (carcass weight / BW) × 100. Relative breast muscle weight was calculated according to the formula: (breast weight / BW) × 100. Relative organ weights of the tract, heart, liver, gizzard, spleen, abdominal fat, and bursa of fabricius were calculated by dividing the organ weight individually over the BW(Alqazzaz *et al.*, 2019).

Intestinal Morphology

Samples (5 cm/sample) were collected from the birds' duodenum, jejunum, and ileum of the intestine. The samples were immediately rinsed with phosphate buffer saline and then placed in boxes containing 10% formalin. The samples were washed with distilled water after, and the morphology analysis was performed according to the method described by Bancroft and Gamble (2008). They were tested using a light microscope (Future Win Joe microscopic imaging program). Five replicate slides per intestine were evaluated as part of the treatment. Villus height (VH) of the sample referred to the distance between the tip of the villus and the villus crypt junction. Crypt depth (CD) referred to the depth of the invagination between the two villi. Measurements were conducted using a Winjoe ocular micrometer(Al-Rubaee *et al.*, 2020).

Chemical Analysis

Total Phenolic Content. The Folin-Ciocalteu method described by Singleton and Rossi (1965) was performed with a slight modification to determine the total phenolic content in the *C. erectus* leaf samples. At 50°C–55°C, samples of *C. erectus* leaf were extracted with 300 ml ethanol using a Soxhlet extractor for 3–4 h. The samples were filtered using No. 1 filter paper before drying using an evaporator and then kept in storage at 4°C. A sodium carbonate solution of 20% was prepared for the next step. In a 5 ml tube, the aliquot extract sample (150 µl) was mixed with a Folin-Ciocalteu reagent (500 µl) and sodium carbonate (1.5 ml) using a vortex mixer. The mixture was diluted up to 10 ml with distilled water. The tubes were allowed to stand for 2 h before the absorbance was scanned at 765 nm. A standard calibration curve of gallic acid (Sigma-Aldrich, Germany) was used as the standard to estimate the phenolic amount in *C. erectus* leaf meal, as expressed in mg gallic acid equivalent per g dry weight.

Total Flavonoid Content. The aluminum chloride colorimetric technique was applied to determine the total flavonoid content in *C. erectus* leaf samples according to the method described by Laouini and Ouahrani (2017).

Tannin Content. Tannin content in the *C. erectus* leaf samples was determined according to the method described by Abdelkader *et al.* (2014) with slight modifications. Briefly, 2 g of extract was blended with ethanol (80%) before heating in a water bath. The process was followed by filtering before ferric chloride was added to the filtrate. Tannin content in the sample was inferred from the indicator of dark-green color. The filter process was repeated after mixing 1 ml of the extract with 2 ml of sodium chloride (2%). The final volume was mixed with 5 ml of 1% gelatin solution before the absorption was scanned at 540 nm.

Saponin Content. The double extraction gravimetric method was applied to determine saponin content in the *C. erectus* leaf samples according to the procedure described by Harborne (1973) with a slight amendment. Briefly, the samples (5 g/sample) of *C. erectus* leaf meal were added to the flask containing 50 ml of ethanol (20%) with mixing. The mixture was held in a water bath at 55°C for 90 min and then filtered through Whatman filter paper (No. 42). Afterwards, the residue was mixed with 50 ml of 20% ethanol and heated at 90°C until the volume was reduced to approximately 40 ml. The samples were vigorously shaken with 40 ml of diethyl

Table 2. Result of Phenolic Compounds and Proximate Analysis of *C.erectus* Leaf Meal

Phenolic Compounds	Content
Total phenolic (mg gallic / gm)	271.80
Total flavonoid (mg rutin / gm)	68.00
Tannin (%)	58.50
Saponin (%)	9.45
Glycoside (%)	11.90
Gallic acid (ppm)	235.80
Apigenin (ppm)	98.70
Catechin (ppm)	104.80
Quercetin (ppm)	217.90
Proximate analysis	
Moisture (%)	5.23
Crude protein (%)	6.31
Ether extract (%)	4.30
Fiber (%)	13.05
Ash (%)	70.45

ether in a separate funnel. Re-extraction was applied until the aqueous layer color became clear. Saponins were extracted using 60 ml of normal butanol. After the samples were washed with 5% aqueous sodium chloride solution, these were dried in a pre-weighed evaporation dish using an evaporator and then held in the oven at 60°C and reweighed after cooling in a desiccator. Saponin content in the samples was determined according to the following formula:

Saponin content (%) = (W2 – W1 / Wt.) × 100

W1 = weight of the dried dish

W2 = weight of the dried dish + sample

Wt.= weight of the sample

Glycoside Content. The method of Solich *et al.* (1992) with a slight amendment was applied to determine glycoside content in the *C. erectus* leaf samples. The samples (10 g/sample) were macerated repeatedly in methanol (80%) at room temperature for 24 h. The extracted samples were concentrated under a vacuum after mixing with 10 ml of Baljet's reagent, which was freshly prepared from 95 ml of 1% picric acid and 5 ml of 10% NaOH. After 1 h, 20 ml of distilled water was added to each sample. The samples were scanned at 495 nm using the Shimadzu UV/VIS spectrophotometer model 1600A (Kyoto, Japan). The standard curve was made with different concentrations (12.5–100 mg/L) of 10 ml of securidaside. Glycoside content in the sample was expressed as mg of securidaside per gm of dried extracts.

Individual Phenolic Compounds. The phenolic compounds (gallic acid, apigenin, catechin, and quercetin) of *C. erectus* leaf samples (10.0 g) were extracted by ethanol (70%) using theBrason B-220 Ultrasonic Bath (Smith-Kline Company, USA) at room temperature for 1 h (Mladenovic *et al.*, 2011). The samples were dried at 40°C after the solvent was removed by a rotary evaporator under a vacuum (Slovenia). The extract samples were stored at 4°C in glass bottles to protect them from oxidation until analysis. Reversed phase HPLC analysis was conducted on the samples using a Sykamn HPLC chromatographic system equipped with a UV detector for quantification of individual phenolic compounds. The temperature of the column (Zorbax Eclipse Plus-C18-OSD 0.25 cm, 4.6 mm) was 30°C with a mobile phase involving eluent A (methanol) and eluent B (1% formic acid in water (v/v)). The conditions (initial 0–13 min, 40% B; 14–20 min, 50% B; and flow rate of 0.7 ml/min) of the mobile phase were performed using the gradient system. The volume of the injected samples and the standard were both 100 µl. The photodiode array absorption spectrum was scanned at 280 nm.

Statistical Analysis

The treatments were assigned using a completely randomized design, and the collected data were subjected to analysis using the general linear models of the Statistical Analysis System (version 9.4). The differences among means were compared using Tukey's honestly significant difference (HSD). The simple linear regression model was used to describe the relationship between independent variables of growth performance with dependent variables of *C. erectus* levels in the diets.

RESULTS

The results of proximate analysis of the *C. erectus* leaf meal showed that the moisture, crude protein, ether extract, fiber, and ash contents were 5.23%, 6.31%, 4.3%, 13.05%, and 70.45%, respectively (Table 2). In the same table, the results of quantitative phytochemical analysis of *C. erectus* leaf meal were total phenolic (271.8 mg/g), total flavonoid (68 mg/g), tannin (58.5%), saponin (9.45%), glycoside (11.9%), gallic acid (235.8%), apigenin (98.7ppm), catechin (104.8 ppm), and quercetin (217.9ppm). The growth performance of birds fed dietary treatments of *C. erectus* meal is revealed in Table 3.

In the starter period, FI, BWG, FCR, and BW were not affected significantly by the addition of different levels of *C. erectus* in broiler diets. In the grower period, the dietary treatments of *C. erectus* meal did not affect FI. Significantly, BWG was lowered ($P<0.01$) when broiler

Table 3. Growth Performance of Birds Fed Dietary Treatments of *C. erectus* Leaf Meal

Variables	Dietary Treatments (%)										
	0	0.25	0.5	0.75	1	1.25	1.5	1.75	2	SEM	*P*-value
					Starter period (0-15 d)						
FI (g)	272.30	304.33	285.70	277.88	293.79	289.70	300.85	293.27	305.46	20.81	0.33
BWG (g)	250.75	264.14	261.72	260.81	266.32	273.35	263.11	252.38	257.66	9.76	0.08
FCR	1.08	1.15	1.09	1.06	1.10	1.06	1.15	1.16	1.18	0.07	0.18
BW (g)	291.03	304.42	302.00	301.09	306.60	313.63	303.39	292.66	297.94	9.76	0.08
					Grower period (16-25 d)						
FI (g)	1271.76	1329.34	1279.21	1328.42	1262.79	1309.70	1363.22	1311.09	1428.10	79.78	0.15
BWG (g)	955.24^{abc}	1006.00^{a}	952.97^{abc}	920.76^{bcd}	931.12^{bcd}	966.64^{ab}	920.00^{bcd}	896.82^{cd}	868.27^{d}	28.88	<0.01
FCR	1.33^{b}	1.32^{b}	1.34^{b}	1.44^{ab}	1.36^{b}	1.35^{b}	1.48^{ab}	1.46^{ab}	1.64^{a}	0.10	<0.01
BW (g)	1246.27^{abc}	1310.42^{a}	1254.97^{abc}	1221.85^{bcd}	1237.73^{abcd}	1280.27^{ab}	1223.39^{bcd}	1189.49^{cd}	1166.21^{d}	33.08	<0.01
					Finisher period (26-35 d)						
FI (g)	1359.13	1387.77	1361.25	1337.03	1374.20	1417.53	1368.30	1337.26	1353.13	45.32	0.31
BWG (g)	859.85	832.85	792.42	756.81	853.61	829.85	825.61	783.85	745.16	52.04	0.03
FCR	1.58	1.67	1.74	1.79	1.61	1.71	1.65	1.70	1.82	0.12	0.12
BW (g)	2106.06^{ab}	2143.27^{a}	2047.39^{abc}	2018.86^{bcd}	2091.33^{abc}	2110.12^{ab}	2049.00^{abc}	1973.33^{dc}	1911.37^{d}	51.87	<0.01
					Overall period 0-35d						
FI (g)	2903.33	3021.44	2926.16	2943.34	2930.77	3016.92	3032.37	2941.63	3086.68	81.88	0.04
BWG (g)	2075.85^{a}	2113.06^{a}	2017.18^{ab}	1988.65^{abc}	2061.12^{ab}	2079.91^{a}	2018.79^{ab}	1943.12^{bc}	1881.16^{c}	55.65	<0.01
FCR	1.39^{c}	1.43^{bc}	1.45^{bc}	1.48^{bc}	1.42^{bc}	1.45^{bc}	1.50^{b}	1.51^{b}	1.64^{a}	0.04	<0.01
Mortality rate (%)	0.0	0.0	0.0	2.2	0.0	0.0	0.0	0.0	0.0	1.5	0.45

Means within the same row with different superscripts (a,b,c,d) are significantly different; means within the same row with no superscripts are not significantly different. FI, feed intake; BWG, body weight gain, BW, body weight; FCR, feed conversion ratio; SEM, standard error of the mean.

diets were accompanied by rising levels of *C. erectus* meal. Also, the birds receiving 2% *C. erectus* showed the lowest BWG, whereas the birds receiving 0.25% *C. erectus* showed the highest BWG. In addition, birds fed 0.25% *C. erectus* meal diet had higher BWG compared with birds fed 0.75%,1%, 1.5%,1.75%, and 2% of *C. erectus* meal diets. There were no significant differences among BWG of birds fed 0%, 0.25%, 0.5%, and 1.25% *C. erectus* meal diets. Also, the birds fed 0%, 0.5%, 0.75%, 1%, 1.25%, and 1.5% *C. erectus* meal diets had a similar BWG. In the same line, the BWG was comparable among birds fed 0%,0.5%, 0.75%, 1%, 1.25%, 1.5%, and 1.75% *C. erectus* meal diets. Moreover, there were no significant differences among BWG of birds fed 0.75%, 1%, 1.5%, 1.75%, and 2% *C. erectus* meal diets. Significantly, the effect of the dietary treatments on BW was as similar to their effect on BWG in this period. The poorer (P<0.01) FCR was accompanied by enhancement levels of *C. erectus* in broiler diets. The birds fed 2% of *C. erectus* reported the worst FCR among dietary treatments. Also, the FCR was similar among birds fed 0%,0.25%, 0.5%, 0.75%, 1%, 1.25%, 1.5%, and 1.75% *C. erectus* diets. Also, no significant differences were observed among the FCR of birds fed 0.75%, 1.5%, 1.75%, and 2% of *C. erectus* meal. In the finisher period, dietary treatments did not affect (P>0.05) FI, BWG, and FCR. However, BW was lowered (P<0.01) in birds that received high levels of *C. erectus* meal compared to birds that received low levels of *C. erectus* meal in their diet; the birds fed 2% of *C. erectus* meal had the lowest BW. In the overall period, the dietary treatments had no impact on FI and mortality rate. Significantly, higher BWG (P<0.01) was observed in birds fed 0%, 0.25%, and 1.25% of *C. erectus* meal diets compared with birds fed 1.75% and 2% of *C. erectus* meal diets. There were no significant differences among BWG of birds fed 0%, 0.25%, 0.5%, 0.75%, 1%, 1.25%, and 1.5% *C. erectus* meal in the diet. Also, similar differences were noted in BWG of birds fed 0.5%, 0.75%, 1%, 1.5%, and 1.75% *C. erectus* meal diets. Moreover, the BWG was comparable in birds fed 0.75%, 1.75%, and 2% *C. erectus* meal diets. The birds fed high levels of *C. erectus* meal had poor (P<0.01) FCR compared with birds fed low levels of *C. erectus* meal in the diet. No significant differences were noted among FCR of birds fed 0.25%, 0.5%, 0.75%, 1%, 1.25%, 1.5%, and 1.75% of *C. erectus* diets. Also, the levels 0%, 0.25%, 0.5%, 0.75%, 1%, and 1.25% of *C. erectus* meal had a similar FCR values. The birds fed 2% of *C. erectus* meal reported the highest FCR value whereas the birds fed basal diet had the lowest value.

The FI, WG, LW, and FCR depended on *C. erectus* meal levels in the diet were studied (Table 4). There was a negative linear regression (P<0.01) for WG and LW with an increase of *C. erectus* levels in the diets. The value of the slope coefficient of WG was -79.60, and the same value for LW. Also, There was a positive linear regression (P<0.01) for FI and FCR values with an increase of *C. erectus* levels in the diets. The value of the slope coefficient of FI was 51.99, and 0.08 for FCR.

The effect of dietary treatments of *C. erectus* meal on carcass traits and relative organ weight is revealed in Table 5. Carcass weight and relative weight of organs were similar among treatments of *C. erectus* meal supplementation,

Table 4. Linear Regression of Growth Performance on *C. erectus* Levels in Broiler Diets

	Intercept	Slop			
Parameters	Estimate	Estimate	SE	*P*-value	*R*-Square
FI (g)	2926.07	51.99	22.80	0.02	0.13
WG (g)	2089.40	-79.60	17.18	0.01	0.38
LW (g)	2129.68	-79.60	17.18	0.01	0.38
FCR	1.39	0.08	0.01	0.01	0.53

FI = feed intake; BWG = body weight gain, BW = body weight; FCR = feed conversion ratio

Table 5. Relative Carcass Traits and Organ Weights of Birds Fed Dietary Treatments of *C. erectus* Leaf Meal

Variables	Dietary Treatments (%)										
	0	0.25	0.5	0.75	1	1.25	1.5	1.75	2	SEM	*P*-value
Carcass (gm)	1667.25	1654.75	1587.25	1710	1627.25	1699.25	1569.25	1659.25	1635.25	79.49	0.24
Breast (%)	29.77	28.78	29.96	31.06	30.29	32.33	28.8	29.65	29.87	1.49	0.06
Dressing (%)	75.25	74.25	74.75	75.75	75	77	74	76	76.25	2.05	0.52
Tract (%)	4.53	5.93	4.7	4.17	4.98	4.35	4.02	4.19	4.75	1.06	0.49
Heart (%)	0.56	0.52	0.5	0.45	0.45	0.46	0.51	0.49	0.51	0.05	0.11
Liver (%)	2.19	1.72	2.01	1.85	2.05	1.83	1.85	2.01	1.91	0.24	0.26
Gizzard (%)	1.66	1.81	1.94	1.71	1.56	1.43	1.62	1.6	1.66	0.23	0.19
Spleen (%)	0.11	0.12	0.1	0.09	0.1	0.07	0.11	0.09	0.12	0.02	0.39
Abdominal fat (%)	0.98^{a}	0.64^{ab}	0.88^{ab}	0.77^{ab}	0.56^{b}	0.66^{ab}	0.88^{ab}	0.83^{ab}	0.76^{ab}	0.14	<0.01
Bursa of fabricius(%)	0.06	0.06	0.07	0.12	0.11	0.07	0.07	0.07	0.08	0.04	0.40

Means within the same row with different superscripts (a, b) are significantly different; means within the same row with no superscripts are not significantly different; SEM, standard error of the mean.

excluding abdominal fat (P<0.01). Birds fed 1% of the *C. erectus* diet had lower (P<0.01) relative abdominal fat weight than did birds fed the basal diet. There was a lack of differences because the dietary treatments of *C. erectus* meal supplementation excluded the treatment supplemented with 1% *C. erectus* meal in terms of relative abdominal fat weight. The intestinal morphology of birds fed dietary treatments are shown in Table 6. Dietary treatments of *C. erectus* meal increased VH in the intestines of birds. Birds fed 1.25% and 2% C. erectus meal diets revealed a significant (P<0.01) rise in VH of the birds' intestine compared to birds fed the control diet. Birds fed a 2% C. erectus meal diet had higher VH compared to birds fed diets containing 0%, and 1.75% of C. erectus meal. There were no significant differences among VH of birds fed 0.25%, 0.5%, 0.75%, 1%, 1.25%, and 2% *C. erectus* meal diets. Furthermore, VH was similar in birds fed levels of 0%, 0.25%, 0.5%, 0.75%, 1%, 1.5%, and 1.75 % of *C. erectus* meal. Birds fed dietary treatments of *C. erectus* meal also had significantly increased (P<0.01) CD compared with birds fed the control diet. No significant differences among CD of birds fed 0.5%, 0.75%, 1%, 1.5%, and 1.75% of % *C. erectus* meal diets. Also, the birds fed 0.25%, and 2% of *C. erectus* meal diets had similar CD. In addition, the intestine of birds fed 0.25%, and 1% of *C. erectus* diets had higher (P<0.01) VW compared with birds fed 0%, 0.5%, 1.25%, 1.75%, and 2% of *C. erectus* meal diets. There was a lack of differences among VW of birds fed 0.25%, 0.75%, 1%, and 1.5% C. erectus diets. Furthermore, the birds fed levels of 0.5%, 0.75%, 1.25%, 1.5%, and 2% of *C. erectus* meal had similar VW. In addition, the birds fed 0%, 0.5%, 1.25%, 1.75%, and 2% of *C. erectus* diets had the same VW. Dietary treatments of *C. erectus* meal significantly lowered (P<0.01) the VH/CD ratio compared with the control diet. There were no significant differences between birds fed 0% *C. erectus* meal and birds fed 1.25% C. erectus meal on VH/CD ratio. Also, a similar VH/CD ratio was observed in birds fed 0.75%, 1%, 1.25%, 1.5%, and 1.75% of *C. erectus* meal diets. In addition, birds fed diets supplemented with *C. erectus* meal at 0.5%, 0.75%,1%, 1.5%, and 1.75% had the same VH/CD ratio. No significant differences among the VH/CD ratio of birds fed 0.25%, 0.5%, 1.75%, and 2% *C. erectus* meal.

DISCUSSION

The formulation of broiler diets with phytogenic additives is critical. Few studies have evaluated *C. erectus* meals in animal diets. In this study, the results of the phytochemical analysis

Table 6. Morphology Indicators of Birds Fed Dietary Treatments of *C. erectus* Leaf Meal

Dietary Treatments (%)	VH (μm)	VW (μm)	CD (μm)	VH/CD ratio $(\mu m.\mu m)^{-1}$
0	336.99^{c}	56.92^{c}	62.08^{c}	5.53^{a}
0.25	395.13^{abc}	77.83^{a}	137.40^{a}	2.95^{d}
0.50	382.36^{abc}	63.25^{bc}	102.13^{b}	3.74^{cd}
0.75	398.60^{abc}	69.55^{ba}	92.84^{b}	4.39^{bc}
1.00	393.53^{abc}	79.37^{a}	95.72^{b}	4.26^{bc}
1.25	424.56^{ab}	60.01^{bc}	91.08^{b}	5.08^{ab}
1.50	365.77^{bc}	69.17^{ab}	88.64^{b}	4.39^{bc}
1.75	343.51^{c}	57.74^{c}	87.10^{b}	3.99^{bcd}
2.00	433.62^{a}	60.76^{bc}	149.12^{a}	2.95^{d}
SEM	56.85	9.57	20.27	0.95
P-value	<0.01	<0.01	<0.01	<0.01

Means within the same row with different superscripts (a,b,c,d) are significantly different; SEM = standard error of mean; VH = villus height; VW = villus width; CD = crypt depth; VH/CD = villus height/ crypt depth.

did not agree with Hoseini and Chaji (2021) who reported higher contents of crude protein (10.5%) and crude fiber (26.1%) and lower contents of ether extract (0.95%), ash (13.3%), and tannin (54%) in *C. erectus* meal than did the results of the current study. This discrepancy could result from different soil properties, as well as climate and environmental changes in the planted area.

In this study, the active compounds (phenolic compounds, flavonoid, tannin, saponin, glycoside, gallic acid, apigenin, catechin, and quercetin) of *C. erectus* leaf meal were similar to previous studies that detected the phenolic compounds, saponins, flavonoids, and tannins in the aqueous and ethanolic extract of *C. erectus* (Afifi *et al.*, 2021; Nascimento *et al.*, 2016). Studies showed that various secondary metabolite components, such as saponins, tannins, alkaloids, phenolics, and flavonoids, are available in many parts of plants, particularly medicinal plants (Amal *et al.*, 2009; Cutter, 2000), and are known to be antioxidants, antimicrobials, and anti-inflammatory (Ibrahim *et al.*, 2006; Lo and Chung, 1999; Thompson and Collins, 2013; Wang *et al.*, 2015; You *et al.*, 2014). It has been reported that environmental factors influence the active compound of the same species of plant (Florou-Paneri *et al.*, 2019). The supplementation of natural feed additives such as phenolic compounds may essentially affect production performance in poultry (Mahfuz *et al.*, 2021). In the present study, diets supplemented with increasing levels of *C. erectus* meal decreased the growth performance of broilers without significant effect on FI for 0–35 days. The similar FI among treatments indicated that adding *C. erectus* meal up to 2% did not affect palatability. Phytogenic compounds that were added to the diet may affect the animal FI negatively (Greathead, 2003). Hoseini and Chaji (2021) observed no adverse effects on FI in lamb-fed diets containing 50% silage or dried leaf of *C. erectus*.

In the current study, low performance was recorded in birds fed a high level of *C. erectus* meal in the diet. The increase of *C. erectus* meal in the diet linearly declined the BW and WG of broiler chicken. This could be due to the high polyphenol content in *C. erectus* meal may play a role in reducing nutrient utilization, thus affecting negatively BW and WG. Brenes *et al.* (2008) reported a negative effect on poultry performance when using a high concentration of polyphenol compounds in the diet. The inhibition of digestive enzymes because of polyphenol compounds were reported (McDougall *et al.*, 2005; Yilmazer-Musa *et al.*, 2012; You *et al.*, 2011), and this could be by the capability of polyphenol compounds in forming complexes with proteins in the digestive system (Horigome *et al.*, 1988). This complexation led to a decline in the protein and amino acid digestibility thus negatively effect on BW and WG (Ortiz *et al.*, 1993). An earlier study mentioned that decreasing the activity of the digestive enzyme may be due to the capability of polyphenol compounds to form insoluble complexes by binding the nutrients of feed and endogenous proteins in the gut (Horigome *et al.*, 1988). In another study, Cengiz *et al.* (2017) reported that high tannin dosage may cause antigrowth in broilers, which could be attributed to the protein-binding capacity, and can reduce nutrient digestibility in birds fed a diet containing a high dose of polyphenol compounds. The type and dosage of the polyphenol compounds as well as the combination with other compounds could affect the absorption and assimilation the nutrients in the bird intestine (Martel *et al.*, 2010). Also, Chamorro *et al.* (2013) mentioned that the content of polyphenol compounds in grape seed extract added to a diet at 5% decreased WG in the birds. . A similar study carried out by Goliomytis *et al.* (2014) observed that adding 0.5–1 g/kg of dietary quercetin in the diet did not affect the BW of the broiler. In the current study, an increase of *C. erectus* meal by up to 2% in the diet led to a decrease in the FCR. The present findings are in agreement with those of Goliomytis *et al.* (2014) who reported increasing levels of dietary quercetin in the diet from 0.5 g/kg to 1 g/kg led to a decrease in the FCR of the broiler. Feeding birds a 1% *C. erectus* meal diet lowered the relative abdominal fat weight compared to birds fed the basal diet. This could be due to the polyphenol compounds in *C. erectus* meal (Krogdahl, 1985) hindering the digestive enzyme. Researchers reported that a high level of gallic acid and quercetin inhibited pancreatic enzymes such as lipase and α-amylase (Ganjayi *et al.*, 2017). Also, it could be due to the high tannin content that could bind biliary salts and be a barrier to effective fat digestion in poultry (Krogdahl, 1985), with a decline in fat absorption. In addition, investigators reported that broiler diets supplemented with leaf meal as a source of polyphenol compounds led to a decrease in the relative abdominal fat weight of broilers (Santoso and Sartini, 2001). In vitro and in vivo studies likewise mentioned that phenolic compounds have been revealed to possess anti-obesity effects (Hsu and Yen, 2008). By contrast, Goliomytis *et al.* (2014) were unable to detect significant effects among birds fed diets supplemented up to 1 g/kg of dietary quercetin. It has been proven that dietary polyphenol compounds have exhibited anti-obesity properties (Liu *et al.*, 2019).

Villus height, CD, and VW can be used to evaluate the integrity and nutrient absorption of the gastrointestinal system (Wright, 2008; Xu *et al.*, 2003). Feeding *C. erectus* diets increased VH, CD, and WH in the intestine of birds. The long VH led to an increase in the expression enzymes of brusher border, nutrient transport systems, and absorptive surface area, thus improving the digestive and absorptive function (Caspary, 1992). The polyphenolic compounds of *C. erectus* meal could stimulate epithelial cell mitosis resulting in longer VH in the intestine of birds. The relation between VH and activated cell mitosis was reported by Kamboh and Zhu (2014). Also, the proper villus structure refers to better digestion and absorption(Bai *et al.*, 2020). . The increase in the CD of birds fed *C. erectus* diets referred to a decrease in the number of intestinal epithelial mature cells thus an acceleration of villus renewal, which led to a decline in the upper function of the small intestine. The influence of intestinal mucosa integrity on CD value was reported by Sayrafi *et al.* (2011). The intestinal mucosa damage could be due to the presence of toxic agents in *C. erectus* meal. Nascimento *et al.* (2016) reported low acute toxicity in Swiss albino mice treated with an aqueous extract of *C. erectus* leaf. Shallower VH and CD have been associated with the presence of toxins in the diet (Girgis *et al.*, 2010). Contrast results were reported by Moreno-Mendoza *et al.* (2021) who mentioned that diets supplemented with 1.5% moringa leave meal improved the villus traits of the broiler. Omar *et al.* (2020) reported high VH, VW, and CD in birds-fed diets supplemented with phenolic-rich onion (Allium cepa L.) extract. In addition, the decreasing VH/CD ratio in birds that received *C. erectus* meal led to a decrease in the digestive capacity of the nutrients, and poorer growth performance in the birds. This could be due to the negative influence of polyphenol compounds on mucus secretion (Akbarian *et al.*, 2013). The VH/CD ratio is a morphological indicator of intestinal digestive capacity, and a higher ratio refers to superior gut health and a greater capability for absorption in broiler chicken (Abolfathi *et al.*, 2019). Unlike results that indicated dietary polyphenol-rich grape products effectively increased the VH, and VH/CD ratio in broiler jejunum (Viveros *et al.*, 2011).

CONCLUSION

Dietary treatment of 0.25% *C. erectus* meal had no negative effect on growth performance. By contrast, high levels of *C. erectus* negatively influenced growth performance and, intestinal morphology. Dietary treatments of *C. erectus* decreased the relative weight of abdominal fat but did not affect carcass traits and organ weights. This study, therefore, suggests that 0.25% *C. erectus* meal could be supplemented in broiler diets without deleterious effects.

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