

NUTRIENT DIGESTIBILITY AND CAECUM MICROBIAL POPULATION OF BROILER FED CONOCARPUS LEAF MEAL DIETS.

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ABSTRACT

The current study investigated the effect of *Conocarpus erectus* leaf meal on nutrient digestibility and cecal microbial quantification of broilers. A total of 48 Ross 308 chickens at 30 days old with similar body weights were randomly allocated into four treatments with 4 replicates of 3 birds each. The *Conocarpus erectus* leaf meal was supplemented to the basal diet at four levels (0%, 0.5%, 1%, and 2%). The diets were offered to the birds *ad libitum* for four days, after that the birds were slaughtered at 35 days old. Birds fed the diet containing 2 % *Conocarpus erectus* leaf meal reported significantly higher digestibility of crude protein, dry matter, and ash than other treatment diets. Also, crude fiber digestibility was increased significantly by 2% and 1% of *Conocarpus erectus* leaf meal. The digestibility of ether extract declined significantly by 0.5% *Conocarpus erectus* leaf meal. The number of *Lactobacillus spp.* was similar among birds fed dietary treatments. The 0.5% *Conocarpus erectus* leaf meal diet increased the number of *Bifidobacterium spp.* The lower number of *E. coli spp* was reported by the 2% *Conocarpus erectus* leaf meal. In conclusion, the *Conocarpus erectus* leaf meal has a potential nutritional value that can be used as feed additives in the broiler chicken diet.

Keywords: Bacteria; Bird; Button mangrove; Nutrient utilization.



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INTRODUCTION

Button mangrove or buttonwood (*Conocarpus erectus*) is classified under the Combretaceae family; the tropical and subtropical areas are the origin location for this plant. The pharmacognostic characteristics of the plant are reported (Bashir, Uzair, and Chaudhry, 2015) besides its uses in traditional medicine (Alsaraf, Mohammad, Al-Shammari, and Abbas, 2020; Asawer and Shawkat, 2023; Jasim, Shadhaan, Kadhim, and Almohsi, 2020; Mahdi and Al-Azawi, 2022; Rehman et al., 2019). The *Conocarpus erectus* leaf is rich in polyphenolic compounds known as nutritional factors like ellagic acid, gallic acid, quercetin, saponin, and tannin (Ayoub, 2010; Nascimento et al., 2016; Tawfeeq, Jasim, and Nasser, 2020). The leaf extract of *Conocarpus*

erectus possesses antimicrobial activity against the bacteria of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* due to their content of polyphenol compounds (do Nascimento Santos et al., 2018; Hajar and Gumgumjee, 2013; Yasin and Al-Azawi, 2019). Polyphenolic compounds are used as organic feed additives in animal feed after banning the European Parliament from using antibiotics as growth promoters in animal diets in 2006 (Mahfuz, Shang, and Piao, 2021; Naji, Al-Ganabi, and Al-qazzaz, 2010). Also, polyphenolic compounds can impact the digestion process in animals (Tahereh Mohammadabadi, Noruzi, Hoseini, and Direkvandi, 2023). There are few studies for using *Conocarpus erectus* leaf meal in broiler diets. Al-qazzaz, Humam, Al-

Mashhadani, Aljumaili, and Ezzat (2023) mentioned that the increasing levels of *Conocarpus erectus* leaf meal in the broiler diet by up to 2% led to a decline in the growth performance of the broiler. In large animal studies, the substituent of 22.6% of forage with *Conocarpus erectus* leaves and branches affect negatively nutrient digestibility of goats (Tahereh Mohammadabadi et al., 2023). However, Ehsan, 2020 reported improvement in vitro digestibility of *Conocarpus* leaves silage using sulfuric acid and molasses. Hosseini Asl and Chaji (2021) mentioned replacement of corn silage with silage or dried leaves of *Conocarpus* at 50% had no significant effect on nutrient digestibility in lambs. T Mohammadabadi (2020) mentioned the lack of a negative effect on nutrient

digestibility of Arabian sheep fed a diet containing 15% *Conocarpus* foliage. Tahereh Mohammadabadi, Jolazadeh, and Ghezi (2020) reported improvement in digestion activity in Arabian sheep fed fermented *Conocarpus erectus* leaf with two tannin-degrading bacteria *Klebsiella pneumoniae* and *Acinetobacter*. The nutrient utilization and microbial quantification of broiler fed *Conocarpus erectus* leaf meal is yet to be evaluated, and it was hypothesized that *Conocarpus erectus* leaf meal might positively affect the intestinal microbiota and nutrient digestibility in broilers. Therefore, this study was conducted to evaluate the effect of different levels of *Conocarpus erectus* leaf meal in diets on the nutrient digestibility and cecal microbial quantification of broiler.

Table 1. Ingredient composition of the experiment diet

Items (%)	Period (30-35 days)
Yellow corn	48
Wheat	15
Soybean meal	25.5
Protein concentrate ^a	5
Corn oil	4.5
Limestone	1.1
Dicalcium phosphate	0.5
Salt	0.2
Vitamin and mineral premix	0.2
<u>Calculated analysis ^b</u>	
Crude protein (%)	20.04
Metabolic energy (kcal/kg)	3208.5
Methionine (%)	0.46
Lysine (%)	1.11
Calcium (%)	0.86
Phosphorus (%)	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 based on National Research Council (1994) table of feed composition.	

MATERIALS AND METHODS

Management of birds

The current trial was conducted at the College of Agricultural Engineering Sciences, University of Baghdad. A total of 48 homogenized weighted Ross 308 broilers were

randomly divided into four dietary treatments (4 groups per treatment) at 30 days old. All the birds were located in floor cages. The electric bulbs were used to provide artificial light for the birds in the semi- closed house. Water and

feed were offered *ad libitum* for the birds during the experimental days.

Dietary treatments.

The basal diet was formulated using conventional ingredients, and then *Conocarpus erectus* leaf meal was added at 0%, 0.5%, 1%, and 2% to form four dietary treatments (Table 1), respectively. The titanium dioxide (TiO₂) at 5 g/kg were mixed in the diets. The diets and water were continuously provided *ad libitum* for 4 days.

Samples preparation

On slaughter day (35 days old), the birds were slaughtered without scalding, and then the ileal contents between the Merckel's diverticulum to the ileal-cecal-colon junction were collected. The feed and digesta samples were dried using an oven-dried method at 60 °C for 48 hours and analyzed for crude protein, ether extract, moisture, ash, and crude fiber as described in AOAC (2007). Titanium dioxide content was determined following the protocol mentioned by Short, Gorton, Wiseman, and Boorman (1996). The sample (0.1g) of digesta was ashed in a porcelain crucible at 580 °C for 13, then the sample was cooled by adding 10 ml Sulphuric acid (7.4M). Gently, the sample was heated for 60 minutes or until it dissolved completely. The sample was put into a small beaker including 25 ml distilled water. The sample was filtered using filter paper (Whatman 541) in a volumetric flask (100 ml), the flask was filled with distilled water after adding 20 ml of hydrogen peroxide (30%). The sample was read on a spectrophotometer at 410 nm. The calibration curve was performed by pipetting 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 ml of standard titanium dioxide (0.5mg ml⁻¹ TiO₂) in 100 ml volumetric flasks individually. 20 ml H₂O₂ was added to each flask after adding 10 ml of 7.4 M sulfuric acid, and then fill the flask up to 100 ml with distilled water. The sample without titanium

was the blank reading of the spectrophotometer. The standard curve was obtained by the relation between the sample absorbance and the TiO₂ concentration. The following formula was used to measure the apparent nutrient digestibility = 100-[100×(%TiO₂ in feed / % TiO₂ in digesta) × (%nutrient in digesta / % nutrient in feed)].(Maynard and Loosli, 1969) Digesta samples of caeca from each treatment were pooled in plastic boxes on slaughter day and then stored at – 20 °C for bacteria quantification. The DNA of samples was extracted using gSYNC™ DNA Extraction Kit. A small piece of digesta sample was placed in a microcentrifuge tube and then centrifuged for 5 minutes at 300 x g. After discarding the supernatant, the pipette was used to resuspend the cells in 200 µl of PBS. Add 20 µl of Proteinase K and pipette to combine. Incubate for five minutes at 60°C. The samples were subjected to follow the procedure of the product.

Quantitative real-time PCR

Specific primers with a concentration of 10 ng/µl from several bacterial populations were used to quantify the number of bacteria (Table 2).The amplification reactions were performed using Exicycler™ 96 (Ver.4) Real-Time Quantitative Thermal Block. The template of the qPCR assay was the extracted DNA of the samples. The final volume (25 µl) of each reaction consisted of 9.5 µl RNase-free water, 12.5 SYBR Green, 1 µl forward primer, 1 µl reverse primer, and 1 µl of DNA. The amplification condition was 94°C for 5 min, afterward 40 cycles of 94 °C × 20s, primer annealing at 50, 50, 60, and 58°C × 30 s for *Salmonella spp*, *Escherichia coli spp*, *Bifidobacterium spp*, and *Lactobacillus spp* respectively, followed by final extension at 72°C × 20 s.

Table 2. The sequence of primers used to target *Lactobacillus spp*, *Escherichia coli spp*, *Bifidobacterium spp*, *Salmonella spp*.

References	Bacteria	Sequences
Wang, Cao, and Cerniglia (1996)	<i>Lactobacillus spp</i>	F-5'-CATCCAGTGCAAACCTAAGAG-3' R-5'-GATCCGCTTGCCCTTCGCA-3'
Frahm and Obst (2003)	<i>Escherichia coli spp</i>	F-5'-GTGTGATATCTACCCGCTTCGC-3' R-5'-GAACGCTTTGTGGTTAATCAGGA-3'
Bartosch, Fite, Macfarlane, and McMurdo (2004)	<i>Bifidobacterium spp</i>	F-5'-GGGTGGTAATGCCGGATG-3' R-5'-TAAGCCATGGACTTTCACACC-3'
Rezaei et al. (2015)	<i>Salmonella spp</i>	F-5'-TCGTCATTCCATTACCTACC-3' R-5'-AAACGTTGAAAACTGAGGA-3'

Statistical analysis

A completely randomized design (CRD) was used to allocate the treatments. The Statistical Analysis System's general linear model (GLM) was used to analyze the data that were pooled using the Statistical Analysis System (version 9.4). The comparison among means was performed using Tukey's honestly significant difference (HSD).

RESULTS AND DISCUSSION

The composition findings of the chemical and phytochemicals of the *Conocarpus erectus* leaf meal are given in Table 3. Leaves of *Conocarpus erectus* were found to have a low amount of ether extract (4.3%) and crude protein (6.31%); a high amount of fiber (13.05%), and ash (70.45%). Also, the findings of the total phenolic content analysis revealed that the *Conocarpus erectus* leaf meal contained 271.8 mg/g total phenolic, 68 mg/g total flavonoid, 58.5% tannin, 11.9% glycoside, 9.45% saponin, 235.8ppm gallic acid, 217.9 ppm quercetin, 104.8 ppm catechin, and 98.7ppm apigenin. The Se content was 106.8 ppm. It's crucial to formulate broiler feeds containing phytogenic ingredients. Evaluations of *Conocarpus erectus* leaf meal in broiler diets are few. In the current study, the phytochemical analysis's findings disagreed with (Hosseini Asl and Chaji, 2021) who found that *Conocarpus erectus* leaf meal had lower levels of tannin (54%), ash (13.3%), and ether extract (0.95%), as well as high levels of crude fiber

(26.1%) and crude protein (10.5%) than reported in this study.

Table 3. Nutrient contents of *Conocarpus erectus* leaf meal.

Phytochemicals compounds	Amount
Total phenolic (mg gallic / gm)	271.8
Total flavonoid (mg rutin / gm)	68
Tannin (%)	58.5
Glycoside (%)	11.9
Saponin (%)	9.45
Gallic acid (ppm)	235.8
Quercetin (ppm)	217.9
Catechin (ppm)	104.8
Apigenin (ppm)	98.7
Chemical composition	
Crude protein (%)	6.31
Ether extract (%)	4.3
Fiber (%)	13.05
Ash (%)	70.45
Moisture (%)	5.23
Mineral	
Se (ppm)	106.8

This could be because of the differences in soil characteristics, and the environmental changes in the planted area. Environmental influences have been shown to affect the active compounds of identical plant kinds (Florou-Paneri, Christaki, and Giannenas, 2019). The active compounds of *Conocarpus erectus* leaf meal (phenolic compounds, flavonoids, tannins, saponins, quercetin, apigenin, gallic acid, catechin, and, glycoside) were found in this study in comparable amounts to those found in other studies (Afifi, Al Marzooqi, Tabbaa, and Arran, 2021; Nascimento et al., 2016), which they identified the components of the aqueous and ethanolic extract of

Conocarpus erectus. The results of supplementing *Conocarpus erectus* leaf meal in broiler diets on nutrient digestibility are presented in Table 4. The birds fed the 2% *Conocarpus erectus* leaf meal diet had the highest ($P<0.01$) crude protein digestibility than birds fed the other treatment diets. An increase in *Conocarpus erectus* leaf meal in the diets was associated with an incline ($P<0.01$) in the digestibility of dry matter. The birds fed the 2% *Conocarpus erectus* leaf meal diet reported the highest dry matter digestibility. Also, ash digestibility was higher ($P<0.05$) in birds fed the 2% *Conocarpus erectus* leaf meal diet than in birds fed the 0.5% *Conocarpus erectus* leaf meal diet and control diet. The 1% of *Conocarpus erectus* leaf meal had comparable effect ($P>0.05$) on the ash digestibility. The increased supplementation (2% and 1%) of *Conocarpus erectus* leaf meal had a similar effect ($P>0.05$) on the digestibility of ether extract while the 0.5% of *Conocarpus erectus* leaf meal lowered ($P<0.05$) digestibility of ether extract compared to basal diet. In addition, the digestibility of crude fiber was higher ($P<0.01$) by 1% and 2% *Conocarpus erectus* leaf meal compared to 0.5% *Conocarpus erectus* leaf meal. However, the crude fiber digestibility was comparable ($P>0.05$) between the birds

fed the control diet and birds fed *Conocarpus erectus* leaf meal diets. In the current study, the fiber content of the *Conocarpus erectus* leaf meal may have contributed to the 2% improvement in the digestibility of crude protein, dry matter, and ash. The content of fiber (soluble fiber vs. insoluble fiber) plays a significant impact on nutrient digestibility (Sebola, Mlambo, Mokoboki, Hugo, and Muchenje, 2018). Also, the Se content in the *Conocarpus erectus* leaf meal could affect the nutrient digestibility. Elnaggar, Ghazalah, Elsayed, and Abdelalem (2020) reported improvement in the digestibility of dry matter and crude protein in birds fed 100 ppm of organic selenium. Proper processing of *Conocarpus erectus* leaf meals is essential to optimize ash digestibility. Grinding of *Conocarpus erectus* leaf meals could increase the surface area of leaf meals, facilitating better digestion and absorption of minerals. Kareem et al. (2022) reported a decline in the digestibility of ash and crude fiber when the particle size of the broiler diet rose from 3 to 4 mm. The decrease in fat digestibility may result from the tannin content in the *Conocarpus erectus* leaf meal which could inhibit the action of pancreatic lipase, the enzyme that breaks down dietary fats into absorbable fatty acids and glycerol.

Table 4. Effect of different levels of *Conocarpus erectus* leaf meal on broiler nutrient digestibility

Parameters (%)	Dietary treatments				SEM	P- value
	Control	0.5%	1%	2%		
Crude protein	63.20 ^b	55.41 ^b	61.04 ^b	78.81 ^a	4.67	**
Dry matter	49.42 ^c	49.83 ^c	67.54 ^b	74.35 ^a	0.875	**
Ash	23.74 ^b	19.06 ^b	36.38 ^{ab}	55.60 ^a	10.79	*
Ether extract	84.74 ^a	58.80 ^b	73.91 ^{ab}	72.24 ^{ab}	9.76	*
Crude fiber	85.27 ^{ab}	81.13 ^b	94.35 ^a	93.03 ^a	4.41	**
^{abc} Means in the same raw followed by different superscript letters are significantly different. * ($P<0.05$); **($P<0.01$)						

The results of the microbial populations in the caeca contents of broiler chickens fed *Conocarpus erectus* leaf meal are shown in Table 5. The *Conocarpus erectus* leaf meal

had comparable effect ($P>0.05$) on the number of *Lactobacillus spp.* The 0.5% *Conocarpus erectus* leaf meal diet increased ($P<0.01$) the number of *Bifidobacterium spp.* compared to

the control diet and the 2% *Conocarpus erectus* leaf meal diet. The 2% *Conocarpus erectus* leaf meal diet decreased the number of *E. coli* spp. compared to the control diet and 1% *Conocarpus erectus* leaf meal diet. No numbers of *Salmonella* were reported in the broiler. In the current study, the 2% *Conocarpus erectus* leaf meal decreased the number of *Bifidobacterium* spp. compared to 0.5% *Conocarpus erectus* leaf meal. Also, the highest level of *Conocarpus erectus* leaf meal lowered the number of *E. coli* spp compared to the control diet which is the most frequent cause of diarrhea in underdeveloped countries (Gaucher et al., 2018). This could be because of the content of phenolic compounds in the *Conocarpus erectus* leaf meal. According to Min, Pinchak, Merkel, and Tomita (2008), tannins can prevent bacterial growth by interfering with microbial metabolism directly or by sequestering the substrates necessary for microbial growth. They can also do this by destabilizing and permeabilizing the cytoplasmic membrane, and by inhibiting

extracellular microbial enzymes. Pacheco-Ordaz et al. (2018) mentioned that phenolic compounds inhibit pathogenic bacteria such as *E. coli* and probiotic bacteria. Also, Pacheco-Ordaz et al. (2018) reported the antimicrobial activity of the methanolic extract of *Conocarpus lancifolius* against the *E. coli* bacteria. It is reported that the *Conocarpus erectus* possesses antimicrobial properties due to its phytochemical and biological composition (Abdel-Hameed, Bazaid, Shohayeb, El-Sayed, and El-Wakil, 2012; do Nascimento Santos et al., 2018). Also, Rehman et al. (2019) mentioned that the crude extract of different parts of *Conocarpus erectus* inhibited the *S. cerevisiae* using agar disc diffusion method. In addition, Adonizio (2008) reported inhibition of *P. aeruginosa* pathogenicity by an aqueous extract of *Conocarpus erectus*. In the vitro study, Yasin and Al-Azawi (2019), reported inhibition of bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa* by leaf extract of *Conocarpus erectus*.

Table 5. Effect of different levels of *Conocarpus erectus* leaf meal on cecal microflora (log10 copy n/ml DNA extract) in broilers

Bacteria	Dietary treatments					
	Control	0.5%	1%	2%	SEM	
<i>Lactobacillus</i> spp.	7.61	7.06	7.18	7.36	1.42	ns
<i>Bifidobacterium</i> spp.	7.17 ^b	8.73 ^a	7.59 ^{ab}	7.34 ^b	0.58	*
<i>E. coli</i> spp.	6.72 ^a	5.73 ^{ab}	6.38 ^a	5.19 ^b	0.48	**
<i>Salmonella</i> spp.	--	--	--	--	--	--

^{ab} Different superscript alphabets in the same raw means differ significantly; ns= Means with similar superscripts in the same raw do not differ significantly; * (P<0.05); ** (P<0.01)

CONCLUSION

The supplementing of 2% *Conocarpus erectus* leaf meal in the broiler diet improved the nutrient digestibility of crude protein, ash, dry matter, and crude fiber as well as the decline in the number of *E. coli*.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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معامل هضم العناصر الغذائية واعداد ميكروبات اعورين فروج اللحم المغذى على علائق مسحوق أوراق الكونوكاريس

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المستخلص

بحثت هذه الدراسة تأثير مسحوق أوراق الكونوكاريس في معامل هضم العناصر الغذائية والعد الميكروبي لعورين فروج اللحم. وزعت طيور التجربة (48 فروج لحم) المتماثلة الوزن بعمر 30 يوم على أربع معاملات واحتوت كل معاملة على أربع مكررات بواقع 3 طيور لكل مكرر. اضيف مسحوق أوراق نبات الكونوكاريس الى العليقة الاساسية بنسبة 0%، 0.5%، 1% و2%. غذيت الطيور بشكل حر لمدة 4 ايام ثم ذبحت بعمر 35 يوم. بينت النتائج: حققت الطيور التي تغذت على 2% مسحوق أوراق الكونوكاريس اعلى نسبة معامل هضم للبروتين الخام، المادة الجافة والرماد. في حين ارتفع معامل هضم الالياف الخام باضافة 2% و1% مسحوق أوراق الكونوكاريس وانخفض معامل هضم مستخلص الايثر باضافة 0.5% مسحوق أوراق الكونوكاريس الى العليقة. لم يلاحظ أي تأثير معنوي لمعاملات الإضافة في عدد بكتريا *Lactobacillus spp*. سُجلت اقل عدد بكتريا *E. coli* بواسطة 2% مسحوق أوراق الكونوكاريس كما ارتفع عدد بكتريا *Bifidobacterium spp* بنسبة اضافة 0.5% مسحوق الكونوكاريس. يُستنتج من هذه الدراسة: مسحوق أوراق الكونوكاريس ذو قيمة غذائية ويمكن استخدامه كإضافة علفية في علائق فروج اللحم.

الكلمات المفتاحية: بكتريا; طير; مضاد للأحياء المجهرية; معامل هضم العناصر الغذائية