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Research Report

Influence of *Pluchea indica* (L.) less extract supplementation on performance, physiological responses, meat quality, and microbial load in broiler chickens

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SUMMARY

This study evaluated the efficacy of Barnouf (*Pluchea indica* Less) extract (BE) as a natural antioxidant and immune-modulating supplement in broiler chickens. A total of 210 chicks were assigned to three treatments in a completely randomized design, with each treatment consisting of seven replicates of ten unsexed chicks. The control group received water without additives, whereas the other treatments received Barnouf extract (BE) at 3 or 6 mL/L. Supplementation with BE at both inclusion levels improved growth performance ($P \leq 0.05$). Birds receiving 6 mL/L exhibited greater body weight gain (BWG) from days 5 to 20 and days 21 to 33 compared with the control. Feed intake (FI) decreased ($P \leq 0.05$) in both supplemented groups, accompanied by improved feed conversion ratio (FCR); additionally, the 3 mL/L group demonstrated a higher performance index (PI) ($P \leq 0.05$) on day 33. Broilers receiving 3 or 6 mL/L of the extract showed increased water consumption (WC) and higher water-to-feed ratios between days 5 and 20 ($P \leq 0.05$), with the 6 mL/L group showing the greatest values, while still exhibiting reduced FI. Supplementation with BE increased abdominal fat percentage, live body weight, and relative weights of the gizzard and liver, whereas carcass percentage varied among treatments. Heart and spleen weights showed numerical reductions ($P \geq 0.05$) with increasing extract levels. Health-related biochemical indices were favorably altered by BE supplementation. Levels of aspartate aminotransferase (AST), urea, total lipids, and malondialdehyde (MDA) were reduced ($P \leq 0.05$), whereas concentrations of high-density lipoprotein (HDL), antioxidant enzymes—glutathione peroxidase (GPX), glutathione S-transferase (GST), and reduced glutathione (GSH)—and immunoglobulins G (IgG) and M (IgM) increased ($P \leq 0.05$). Meat quality also improved, as reflected by more stable pH values, lower microbial loads, and reduced storage-related water loss, with significant effects observed for color parameters ($P \leq 0.05$). Additionally, both supplementation levels increased *lactobacilli* count while reducing total bacterial count (TBC), *bacilli, Escherichia coli, coliforms*, and *Salmonella* ($P \leq 0.05$). In conclusion, dietary inclusion of BE at 3 or 6 mL/L enhanced growth performance, immune competence, antioxidant status, meat quality, and gut microbial balance in broiler chickens without adverse effects, supporting its potential as a safe natural feed additive.

Primary Audience: Nutritionists, poultry keepers, researchers, and feed mills.

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Description of problem

The recent population increase has led to increased demand for animal protein (Ojediran et al., 2024; Talib et al., 2024). As a result, the broiler sector has achieved significant progress in optimizing feed consumption and improving feed efficiency, increasing poultry weight at the time of marketing (Best et al., 2024; Kamal et al., 2025). Poultry products are considered an essential supply of animal protein and have proliferated across several industries owing to their economic viability, rapid production cycle, and significant nutritional advantages (Abu Qasim et al., 2024; Ejila et al., 2023). The poultry industry faces considerable obstacles stemming from traditional farming methods and increasing bird production, requiring enhancements in broiler growth, health, and well-being (Klein and Ciacciariello, 2025). The widespread use of conventional nutritional additives, including antibiotics, to enhance chicken growth and performance has resulted in the development of antibiotic-resistant microbes, presenting significant public health threats. Therefore, it is imperative to identify natural alternatives to antibiotics (Kamal et al., 2023a; Rashad et al., 2025).

El-Abbasy et al. (2025a, b) proposed that using herbal remedies or their extracts could improve broiler productivity. The Indonesian population extensively employs the leaves of the *Pluchea indica*, or marsh flea plant (*Pluchea indica* L.), a herbaceous species from the Asteraceae family, as a traditional medicinal herb (Ashrafi et al., 2022). Moreover, the leaves of *Pluchea indica* contain several beneficial compounds, such as alkaloids, tannins, flavonoids, essential oils, calcium, and vitamins A and C. Plant compounds augment their therapeutic effectiveness and nutritional value (Hanif and Manullang, 2022). The concentration of flavonoids is thought to enhance digestion, thus enhancing feed absorption and utilization, especially of proteins, which subsequently enhances poultry performance (Sudarman and Solikhah, 2011). The study examined the effects of varying doses of water extracts from marsh fleabane leaves on blood parameters, including white and red blood cell count, hemoglobin level, hematocrit, and glucose, in broiler chickens. The findings indicated that these compounds function as antibacterial agents without adversely affecting the blood components of the chickens (Lisnanti et al., 2023).

The use of *Pluchea indica* leaf meal or extracts in broiler diets improves hematology health, lowers cholesterol levels, and boosts the nutrient content of chicken and sheep outputs (Budiono et al., 2025; Sudarman and Solikhah, 2011). The foliage of the herbs is rich in minerals, including calcium, potassium, magnesium, and trace elements, thus enhancing the overall nutritional value of the diet. The unique sweet, astringent, and fragrant aroma of *Pluchea indica* reinforces its use as a feed additive to improve feed palatability (Wang et al., 2024). These outcomes correspond with the growing interest in phytogenic feed additives, acknowledged for their ability to enhance growth, nutrient digestion, immunological function, and intestinal wellness in birds. The research examined the utilization of BE as an antioxidant and anti-inflammatory in broiler chickens.

Materials and methods

The research was executed at the Poultry Research Farm, affiliated with the Department of Poultry in the Faculty of Agriculture at Zagazig University, Egypt. The experimental methods were executed in accordance with the protocols established by the Institutional Animal Care and Use Committee. The Ethics Committee at Zagazig University in Egypt authorized them.

Antimicrobial activity of Pluchea indica L. extract

The antibacterial activity of some plant extracts (BE) against Gram-positive and Gram-negative bacteria was assessed. The Gram-positive bacteria were *Staphylococcus aureus* and *Listeria monocytogenes,* and the Gram-negative bacteria were *Escherichia coli* and *Salmonella Typhimurium*. Three methods were used to confirm the results:

1- Agar well diffusion assay
2- Disc diffusion assay
3- Broth or agar dilution assay

In the first method, the disc was saturated with the extract and then placed on the surface of agar inoculated with the tested bacteria. The diameter of inhibition was measured. In this test, a single concentration and volume of the extract were used.

In the second method, a well was made in the agar inoculated with the bacteria, and a volume and concentration of the extract were added. In this test, 250 μL, 500 μL, 750 μL, and 1000 μL were added, and the diameter of inhibition was measured. The minimum inhibitory concentration (MIC) was estimated by the microdilution broth assay described in European Committee on Antimicrobial Susceptibility Testing (Eucast 2003).

In the third method, the extract was added to the bacteria in the culture medium. Incubation followed by growth testing using the streaking method on the agar surface to estimate the minimum concentration or dilution that prevents microbial growth on the streaking agar surface. The lowest concentration that kills the bacteria was considered (MBC) (Ali et al., 2017).

Different concentrations and levels of the BE were used to determine the (MIC) and (MBC) and these tests were conducted 5 times to confirm the results obtained.

Barnouf solution formulation

The BE was acquired from a commercial entity named 2 M Group, situated in the 10th of Ramadan region in Sharqia, Egypt. To make aqueous Barnouf solutions, we soaked 333 g of *Pluchea indica* L. plants (leaves and thin trunks) in one liter of extraction solvent (water-ethanol). We preserved the solutions at ambient temperatures for no less than 24 h before filtration. We utilized filter sheets identified as Whatman No. 42 (Hawach Scientific Co., Ltd., Xi'an, China). The administration of BE in the drinking water commenced when the birds were 5 days old and persisted until they attained 33 days of age. During this period, we incorporated treatment into their water consumption.

Sample preparation

Two hundred mL of 70 % methanol was combined with ten g of Barnouf powder, agitated for three hours, and subsequently filtered utilizing Whatman No. 2 filter sheets. A Buchi water bath B-480 evaporator was utilized for methanol extraction from the extract at 45 °C under vacuum conditions. The extract was later lyophilized employing a freeze-dryer (Thermal Electric Corporation, Heto Power Dry LL 300 Freeze Dryer). The resultant extract was preserved at 20 °C until applied to additional tests (Ashour et al., 2020).

Assessing total phenolic compounds (TPCs)

The Folin-Ciocalteu test assessed the TPCs of the methanolic extract (1 g/mL) from each sample. Gallic acid equivalents (GAE), a common phenolic molecule, were utilized at different concentrations (10-1000 mg/mL) to establish the standard curve [$y = 0.001x + 0.0563$ ($R^2 = 0.9792$)], where y represents Ga absorbance and x indicate the level in mg/mL. 1 mL of every sample (standard GAE), 2 mL of 7.5 % Na_2CO_3, and 3 mL of diluted Folin-Ciocalteu were combined and incubated in the dark at 25 °C for thirty minutes. The absorbance of the mixture was detected at 760 nm using a spectrophotometer (JENWAY 6405 UV/Vis, U.K.) according to Ordonez et al. (2006).

Determination of total flavonoids

The extract must contain a total flavonoid (TF) concentration of 1000 mg/mL. Quercetin served as a standard phenolic substance at several concentrations (10-1000 mg/mL) to establish a typical curve [$y = 0.0012x + 0.008$ ($R^2 = 0.944$)], where x represents the quercetin level in mg/mL and y denotes absorbance. 1 ml of a 20 g/L $AlCl_3$ solution in ethanol was combined with 1 ml of quercetin extract. Ordonez et al. (2006) used a spectrophotometer to evaluate color absorption at 420 nm.

Assessing antioxidant activity

The ability of the BE to neutralize DPPH was employed to assess the antioxidant activity of the extract. One milliliter of each specimen was amalgamated with three milliliters of methanolic-DPPH solution. After a thirty-minute incubation, absorbance was assessed at 520 nm utilizing a spectrophotometer (DPPH test). Abdel-Hamid et al. (2020) discovered that the extract effectively scavenges around fifty percent of the ABTS and DPPH radicals (SC50). Table 2 delineates the chemical composition and antioxidant capability of BE, emphasizing its bioactive components and their prospective health advantages.

Diets, birds, and design

Two hundred ten 5-day-old Ross 308 chicks with similar initial weights were assigned to three treatments, each consisting of seven replicates of ten unsexed chicks, following a completely randomized design. The first group was the control and was not given any additives to the water, while the supplements BE were added to the drinking water of the second and third groups at a rate of 3.0 and 6.0 ml/L of water, respectively. The supplements were given every three days throughout the 33-day experiment period.

The chicks were reared at a temperature of 29 °C and a relative humidity of 70 % until the experiment concluded, 33 days later. The chicks were first kept in an open space at 33 °C for three days. Upon arrival, the chicks were exposed to a constant lighting routine of 22 h of light and 2 h of darkness. The baseline diets were formulated to meet the nutritional criteria established by the National Research Council (1994). Table 1 illustrates that the chicks were administered pellet-form meals from 5 to 33 days of age. The diet was implemented in two separate phases: the starter spanned from 5 to 21 days, and the finisher lasted from 22 to 33 days. Each chick was raised under uniform ecological, management, and hygienic conditions. The chicks were accommodated in standard cages of 110 × 110 × 50 cm, with unrestricted access to feed and water.

Data collection

The daily water consumption was recorded for each trial from 5 to 33 days of age, every two weeks during the experiment. The water-to-feed ratio was assessed during several periods of examination. The chicks were weighed separately at 5, 21, and 33 days of age to ascertain their live body weight (LBW). Furthermore, their daily body weight gain (DBWG) was computed for the intervals of 5-21 days, 22-33 days, and 5-33 days of age. The feed conversion ratio (FCR) and average daily feed intake (FI) were computed. We computed the performance index (PI) utilizing the following formula: PI = FBW kg divided by the FCR, multiplied by 100. (North, 1981).

Carcass characteristics

At the conclusion of the trial, we randomly chose 7 birds from each group to conduct carcass exams at 33 days of age. The birds were meticulously weighed and slaughtered. The carcass weight and the aggregate weight of all consumable components were recorded.

Table 1
Composition and calculated analysis of the basal diet.

Items	Starter (1–21 days)	Finisher (22–33 days)
Ingredients (%)		
Soybean meal (44 %)	34.50	29.5
Yellow corn	54.10	58.7
Corn germ (62 %)	5.43	5.50
Dicalcium phosphate	2.00	1.75
Limestone	1.08	0.95
Premix[1]	0.30	0.30
Salt	0.30	0.30
DL-methionine	0.20	0.18
L-lysine	0.29	0.24
Soybean oil	1.80	2.58
Calculated analysis[2]		
CP %	23.12	21.10
ME (Kcal/kg)	2950	3110
Calcium %	0.99	0.89
Available phosphorus %	0.47	0.46
Methionine + cysteine %	1.00	0.90
Lysine%	1.40	1.22
Crude fiber%	3.68	3.43
Chemical analysis[3]		
CP%	21.68	19.86
Dry matter%	89.80	88.90
Ash %	6.80	6.40
Crude Fiber %	2.50	2.40
Starch %	38.35	40.15
Ether extract%	3.60	3.70

[1] Minerals and vitamins premix manufactured by Multi Vita Animal Nutrition® (Tenth of Ramadan City, Sharkia Governorate, Egypt) provides Vit A 12000 IU; Vit D3 2500 IU; Vit E 20 mg; Vit K3 2 mg; Vit B1 2 mg, Vit B2 5 mg, Vit B6 2 mg, Vit B12 0.05 ug, niacin 30 mg, biotin 0.05 ug, folic acid 1 mg, pantothenic acid 10 mg; manganese 60 mg; zinc 50 mg; iron 40 mg; copper 10 mg; iodine 0.6 mg; selenium 0.3 mg per 1 kg diet. DL-methionine (manufactured by Evonik Industries, Essen, Germany) contains 99 % methionine. Lysine = lysine hydrochloride (Evonik Industries) and includes 70 % Lysine. CP, crude protein; ME, Metabolizable energy.
[2] Calculated according to NRC (1994).
[3] Chemical analysis according to Near Infra-Red device (NIR).

Blood biochemical parameters

After an overnight fasting period, we slaughtered 7 birds from each group to obtain blood specimens. The blood was collected in non-heparinized tubes and centrifuged at 5000 revolutions/minute for 15 min at 4 °C. The serum was stored at −20 °C until it was ready for biochemical analysis. Serum concentrations of low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol (TC), triglycerides (TG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, creatinine, immunological parameters (IgG, IgM), total protein, and albumin were assessed utilizing commercial diagnostic kits from Bio Diagnostic Co. (Giza, Egypt). Globulin was computed utilizing the formula provided by Abdel-Hamid et al. (2020). One must comply with the manufacturer's instructions for commercial kits. The activities of malondialdehyde (MDA), glutathione peroxidase, glutathione-S-transferase, and reduced glutathione were evaluated as indicators of oxidative state, using the methodology outlined in a previous article (Abd El-Hack et al., 2017).

Meat quality

Natella et al. (2010) employed the Hunter Lab ColorFlex EZ (USA) to evaluate the color characteristics of broiler meat (L^*, a^*, and b^*). a^* represents redness, b^* indicates yellowness, and the L^* value (light index scale) spans from 0 (black) to 100 (white). Petri plates were inoculated with specimens consisting of a minced mixture of breast muscle tissue. The colorimeter sensor was placed directly on the petri dish. A HANNA pH meter (Woonsocket, USA) was utilized to assess the pH of samples at 1-, 5-, 10-, and 15-days post-storage.

The study quantified cooking yields by measuring the weight of patties pre- and post-cooking, assessing cooking loss through weight differences. Moisture retention was calculated as the moisture contained in 100 g of raw material, following Kumar and Sharma (2004). Water holding capacity (WHC) was determined using techniques of Warner (2014), involving the gradual addition of distilled water to stirring tubes. Five grams of the sample, comprising a minced amalgamation of breast muscles, were placed in a pre-weighed 50 ml centrifuge tube for each specimen. Following the thorough soaking of the mixture, specimens were centrifuged for 10 min at 4000 rpm. Decantation was utilized to remove the supernatant following centrifugation.

Microbial count in cecal samples

Five replicate cecal specimens were homogenized in a screw jar using a sterile saline peptone solution at a ratio of 1:10 (w/v). They conducted decimal serial dilutions up to 107. The diverse microorganisms were quantified in the medium. Total bacterial counts were conducted using Plate Count Agar (PCA) (Sheiha et al., 2020). We measured the aggregate quantity of coliforms on MacConkey agar medium. *Escherichia coli* was enumerated on Tryptone Bile Glucuronide Agar at 37 °C for 24 h, as reported by Paulsen et al. (2006). Additionally, Sieuwerts et al. (2008) described the enumeration of *Salmonella spp*. on S.S. agar, indicated by black colonies. Molds were quantified following Kurtzman et al. (2011). Lactic acid bacteria were isolated using MRS medium and MRS-cysteine agar, as detailed by Garcia et al. (2016). *Enterococcus spp*. enumeration was performed on Chromocult™ enterococci agar, with red colonies indicating their presence (Miranda et al., 2005).

Statistical analysis

SPSS version 27.0 was employed to examine the study results using a one-way analysis of variance and a general linear model. The outcomes are shown as M and SEM. The mean variations were evaluated with Duncan's test. Orthogonal contrasts were utilized to evaluate the linear and quadratic effects of dosage levels on the parameters. Graphs were produced on GraphPad Prism 8.0. The statistical methodology employed is as follows:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where μ represents the overall mean, Ti represents the treatment effect, and e_{ij} represents the random error. A ($P \leq 0.05$) was used to determine a statistically significant result. We show the mean and standard error of the results.

Results and discussion

Chemical characterization of Barnouf extract

The methanolic extract of Barnouf extract powder is shown in Table 2 along with its DPPH activity (SC50; mg mL^{-1}) and total phenolic (TP; mg GAE g^{-1} extract) and total flavonoid (TF; mg QE g^{-1} extract) contents. The total quantity of phenol in BE was 14.81 GAE g^{-1} extract, while the total amount of flavonoids was 4.06 mg QE g^{-1} extract. The extract's antioxidant activity at concentrations of 100, 250, 500, and 1000 was 13.00, 35.66, 61.66, and 76.33 mg mL^{-1}, respectively, as determined by its SC50 (mg mL^{-1}).

Barnouf extract exhibits strong free radical–scavenging activity, as demonstrated by its low SC_{50} values and high overall antioxidant capacity. Low SC_{50} values indicate that only a small concentration of the extract is required to neutralize 50 % of free radicals, reflecting stronger antioxidant potential. This activity is closely associated with the presence of polyphenolic compounds, such as flavonoids and phenolic acids, which can donate electrons or hydrogen atoms to stabilize reactive oxygen species (ROS). The leaves of *Pluchea indica* contain several beneficial compounds, such as alkaloids, tannins, flavonoids, essential oils, calcium, and vitamins A and C. Plant compounds augment their therapeutic effectiveness and nutritional value (Hanif and Manullang, 2022; Sudarman and Solikhah, 2012). The concentration of flavonoids is thought to enhance digestion, thus enhancing feed absorption and utilization, especially of proteins, which subsequently enhances poultry performance (Sudarman and Solikhah, 2011).

Table 2
Chemical composition of the antioxidant capacity Barnouf extract.

Ingredient	Content
TPCs (mg GAE/g extract)	14.81
TFs (mg QE/g extract)	4.06
DPPH 100 %	13.00
DPPH 250 %	35.66
DPPH 500 %	61.66
DPPH 1000 %	76.33

TPCs, Total phenolic compounds; TFs, Total flavonoids; DPPH, Antioxidant activity.

Laboratory antimicrobial activity of Barnouf extract

The results shown in Table 3 reported that using different concentrations of Barnouf extract (250, 500, 750, and 1000 microliters) by diffusion in agar shows that Gram-positive and Gram-negative bacteria were not affected at the 250 and 500 microliter levels, while they were affected at the 750 and 1000 microliter concentrations. The inhibition diameters were (10, 13, 11, and 12 mm) for Gram-positive and Gram-negative bacteria, respectively.

The results obtained from the dilution method to calculate the minimum inhibitory concentration (MIC) indicate that both Gram-positive and Gram-negative bacteria were unaffected by extract concentrations of 1 and 2 ml, and Gram-negative bacteria were unaffected even at a concentration of 3 ml (Table 3). Conversely, Gram-positive bacteria were affected at concentrations of 3, 4, and 5 ml (25 %, 25 %, and 50 %), while Gram-negative bacteria were affected at 4 ml and 5 ml (50 % and 50 %, respectively). Therefore, concentrations of 3 and 6 ml of BE per liter of drinking water were chosen for the current experiment.

Growth performance

Table 4 displays the impact of incorporating BE in broiler drinking water on growth efficiency characteristics. On day 21, the birds that received BE at doses of 3 ml/L or 6 ml/L showed substantial differences ($P \leq 0.05$), with the highest growth performance found in the group receiving 6 ml/L. The BE groups had a lot more BWG from days 5–20 and 21–33 than the control. During days 21–33 and the entire growth period (days 5–33), the BE groups consumed significantly less ($P \leq 0.05$) than the control, which consumed the most. Birds that were administered BE had better FCR ($P \leq 0.05$) during days 21–33 and 5–33, and the FCRs

Table 3
Laboratory tests of different concentrations of Barnouf extract and their effect on some Gram-positive and Gram-negative bacteria.

Diffusion method in agar	Gram-positive bacteria (concentration in μL) and inhibition diameter in mm				
Concentration	250	500	750	1000	
Pluchea indica extract	Non	Non	10	13	
Gram-negative bacteria (concentration in mL) and inhibition diameter in mm					
Pluchea indica extract	Non	Non	11	12	
Minimum inhibitory concentration for Gram-positive bacteria ml					
Concentration	1	2	3	4	5
Pluchea indica extract	Non	Non	25 %	25 %	50 %
Calculating the minimum inhibitory concentration for Gram-negative bacteria					
Pluchea indica extract	Non	Non	Non	50 %	50 %

were better, especially in the 3 ml/L group. The PI on day 33 was also significantly ($P \leq 0.05$) higher in both BE groups than in the control, with a bigger effect on the 3 ml/L group. Overall, linear and quadratic trends in P values indicated that growth traits, feed efficiency, and PI all improved with increasing doses of BE compared to the control.

Table 5 shows how BE affects how much water birds drink. Broiler chickens given BE at 3 and 6 ml/L levels in their water consumption (WC) significantly ($P \leq 0.05$) more WC and had a higher water/feed intake ratio (WC/FI) than the control group from days 5 to 20. The 6 ml/L group had the highest WC (180.02 ± 0.96 ml/day) and WC/FI (2.77 ± 0.05), and both treatments were very different from the control ($P \leq 0.05$). Our findings showed that there were no differences in FI between treatments during this time. From 21-33 days, both treatment groups had a much higher WC/FI ratio, while the BE groups had a much lower FI than the control. From 5-33 days, the mean WC/FI ratio, on the other hand, showed no significant differences. In general, BE consistently raised the WC/FI ratio, especially at the higher dose of 6 ml/L.

The results indicated that the use of BE in chicken drinking water considerably enhanced growth efficiency, with BWG observed at the 3 ml/L dosage. Reduced levels of BE supplementation enhanced feeding effectiveness and productivity. These outcomes corroborate the recognized efficacy of grass-based nutrition supplements, which serve as natural growth promoters and beneficially affect digestion and immune regulation (Ashour et al., 2025a, b; Negm et al., 2025). Grass-based nutrients often contain bioactive chemicals such as polyphenols, flavonoids, and essential oils, which exhibit antioxidants, antimicrobial, and anti-inflammatory properties, enhancing nutrient absorption and reducing oxidative stress in poultry (Ibrahim et al., 2024; Lipinski et al., 2019). The improved feed conversion ratio, despite reduced feed intake, indicates higher nutrient utilization efficiency, a well-documented benefit of grass-based feed additives that mitigate metabolic stress and bacteria that impede digestion (Quintana-Ospina et al., 2023). The rise in PI underscores the enhanced integration of growth and wellness performance.

The improved FCR at 3 ml/L suggests optimal phytochemical concentrations, as higher dosages (6 ml/L) may promote growth but slightly reduce feed efficiency, a common pattern where moderate supplementation strikes a balance between growth and usage of feed (Ibrahim et al., 2024). The outcomes are consistent with Shalaby et al. (2024) and Reda et al. (2025), which suggest that herbal mixtures improve broiler growth, FCR, and immune responses while lowering TC levels and oxidative stress markers. Furthermore, Sudarman and Solikhah (2012) demonstrated that the incorporation of *Pluchea indica* leaf powder into the diet substantially affected ($P < 0.05$) the FCR, as broiler hens receiving diets with 2 % *P. indica* leaf exhibited an enhanced FCR. The supplementation of BE demonstrates significant benefits for broiler growth performance and feed efficiency.

The data indicate that broilers treated with BE at 3 and 6 ml/L demonstrated significantly increased WC and WC/FI relative to the control, especially at the 6 ml/L dosage, underscoring the extract's stimulatory effect on drinking behavior. The initial uniformity in FI suggests that the extract enhances hydration or modulates WC beyond simple fluid intake, subsequently leading to a decline in FI and an increased WC/FI ratio, consistent with phytochemical effects on thirst regulation or metabolic functions. Our results corroborate prior studies demonstrating that Moringa oleifera extract enhances waist circumference and optimizes FCR without increasing FI, likely due to bioactive chemicals affecting metabolism and digestive functions (Alabi et al., 2017; El-Abbasy et al., 2025a).

Herbal extracts dissolved in water have demonstrated an ability to increase water use preference and alter consumption behaviors without significantly impacting FI, indicating an influence on behavioral hydration cues or palatability, as supported by studies on herbal emulsions enhancing drinking preference in broilers (Mariadi et al., 2025). The substances present in these extracts, including phenolics and antioxidants, may influence gut function, mucosal integrity, and systemic hydration levels. These factors may elucidate the increase in water consumption and the alteration in WC/FI ratios (Sosnówka-Czajka and Skomorucha, 2013). The subsequent reduction in FI may be linked to satiety signaling or modifications in energy metabolism induced by the extract's components, potentially improving nutritional efficacy as demonstrated in herbal supplements (Ndelekwute et al., 2014; 2018).

The administration of BE, especially at the increased dosage of 6 ml/L, demonstrates a dose-dependent enhancement in LBW and belly fat percentage, hence facilitating development and fat storage. Bioactive substances in BE may enable improved feed absorption or metabolic control, contributing to this rise in belly fat. Fouad and El-Senousey (2014) indicated that the specific herbal extracts and nutrients that affect lipid metabolism led to similar increases in belly fat. Moreover, the addition of specific plant extracts or emulsifiers has been associated with enhanced fat accumulation by modifying lipogenic enzymes and lipid transport pathways in broilers (Ding et al., 2022). Other supplements, such as L-carnitine, usually help people lose belly fat by speeding up the breakdown of fatty acids. This suggests that the results depend on the bioactive composition of the extract (Ganna et al., 2022).

Carcass traits

Table 6 illustrates how BE affects the traits of carcasses. BE added to the diet had a big effect ($P \leq 0.05$) on some carcass traits of broiler chickens when compared to the control. There were no significant differences ($P \geq 0.05$) in live body weight between the treatments. Carcass yield significantly increased with BE 6 ml/L at 74.11 % compared to 71.74 % in Control ($P \leq 0.05$). Heart and spleen percentages diminished

Table 4
Effect of dietary Barnouf extract on broiler growth performance.

Traits	Age (days)	Treatments			SEM	*P* value		
		Control	BE 3 ml/L	BE 6 ml/L		T	L	Q
BW (g/ bird)	5	108.85	109.36	109.28	0.40	0.865	0.677	0.740
	21	823.80^{b}	852.19^{b}	894.82^{a}	8.65	0.001	0.000	0.582
	33	2011.45	2049.61	1992.08	14.63	0.276	0.588	0.134
BWG (g/ bird/ day)	5-20	47.66^{b}	49.52^{b}	52.37^{a}	0.56	0.000	0.000	0.557
	21-33	91.35^{a}	92.103^{a}	84.40^{b}	1.32	0.023	0.022	0.951
	5-33	67.94	69.29	67.24	0.51	0.271	0.577	0.132
FI (g/ day)	5-20	64.56	64.49	64.95	0.23	0.707	0.512	0.618
	21-33	143.91^{a}	128.971^{b}	128.18^{b}	2.75	0.022	0.014	0.173
	5-33	104.23^{a}	96.73^{b}	96.56^{b}	1.42	0.033	0.021	0.181
FCR (g feed/ g gain)	5-20	1.35^{a}	1.30^{b}	1.24^{c}	0.01	0.000	0.000	0.967
	21-33	1.57^{a}	1.40^{b}	1.52^{ab}	0.02	0.024	0.396	0.009
	5-33	1.53^{a}	1.39^{b}	1.43^{b}	0.01	0.001	0.006	0.004
PI	33	131.16^{c}	147.03^{a}	138.93^{b}	1.98	0.001	0.043	0.001

SEM, Standard deviation of means; ^{a,b,c} Means within a row followed by different superscripts are significantly different ($P \leq 0.05$). BW, body weight; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio; PI, performance index.

Table 5
Effect of dietary Barnouf extract on broiler water consumption.

Traits	Age (days)	Treatments			SEM	*P* value		
		Control	BE 3 ml/L	BE 6 ml/L		T	L	Q
WC (bird/ml/d)	5-20	172.45^{c}	177.59^{b}	180.02^{a}	0.72	0.000	0.000	0.002
FI (g/ day)	5-20	64.56	64.49	64.95	0.23	0.707	0.512	0.618
WC/FI ratio	5-20	2.67^{b}	2.75^{a}	2.77^{a}	0.01	0.005	0.002	0.248
WC (bird/ml/d)	21-33	292.65^{b}	274.37^{b}	292.75^{a}	5.30	0.277	0.994	0.114
FI (g/ day)	21-33	143.91^{a}	128.97^{b}	128.18^{b}	2.30	0.021	0.013	0.166
WC/FI ratio	21-33	2.03^{c}	2.13^{b}	2.28^{a}	0.05	0.000	0.000	0.898
WC (bird/ml/d)	5-33	232.55	225.98	236.38	2.59	0.267	0.546	0.133
FI (g/ day)	5-33	104.23^{a}	96.73^{b}	96.56^{b}	1.54	0.031	0.020	0.174
WC/FI ratio	5-33	2.23^{c}	2.34^{b}	2.45^{a}	0.03	0.000	0.000	0.686

SEM, Standard deviation of means; ^{a,b,c} Means within a row followed by different superscripts are significantly different ($P \leq 0.05$). WC, water consumption; FI, feed intake; WC/FI, water consumption/feed intake ratio.

with rising BE levels; however, these alterations were not consistently statistically significant. With BE additions, the percentages of gizzard and liver went up a lot, with the highest value being in the 6 ml/L group.

The decrease in the relative weights of the heart and spleen with increasing BE dosages, although not statistically significant ($P \geq 0.05$), may suggest a redistribution of growth resources or an anti-inflammatory effect, considering the roles of these organs in cardiovascular function and immune response. Specific herbal supplements are acknowledged for their capacity to affect the growth and functionality of immune organs, evidenced by changes in organ size (Ogwuegbu et al., 2022). The notable rise in liver and gizzard % with BE supplementation corresponds with improved metabolic activity and digestive efficacy. The liver is vital for metabolic processes and detoxification, whereas the gizzard is important for mechanical digestion. The augmented relative weights of these organs have been recorded with botanical extracts that elevate the secretions of digestion and enzyme activity, thereby improving FCR (El-Abbasy et al., 2025a; Eltanahy et al., 2025).

Blood indicators

Table 7 illustrates the effect of BE on hematological parameters. The results indicate that the addition of BE at 3 and 6 ml/L to drinking water substantially ($P \leq 0.05$) affected several hematological parameters relative to the control group. Significant reductions ($P \leq 0.05$) were observed in AST, urea, TG, TC, and LDL levels in the treated groups, while HDL levels exhibited a substantial increase. At the maximum dosage, ALT levels increased a little, although it constituted a significant alteration ($P \leq 0.05$). The A/G ratio and globulin changed a lot, but albumin only went up slightly at the lowest addition rate. There were no major changes in creatinine or total protein.

Substantial decreases in AST, urea, TG, TC, and LDL, along with increased HDL, indicate improved liver function and a beneficial cholesterol profile. This corresponds with research demonstrating that plant-derived compounds—such as flavonoids, polyphenols, and terpenoids—exhibit hepatoprotective effects and enhance lipid metabolism in chickens (Chen et al., 2022). Moreover, botanical extracts like bamboo leaf, curcumin, and licorice have been shown to reduce serum TG, TC, and LDL by influencing genes related to lipid synthesis and degradation, such as fatty acid synthase, acetyl-CoA carboxylase, and lipid receptors (Ashayerizadeh et al., 2025; Gowthaman et al., 2024). The slight rise in ALT at the highest dose of BE, while significant, may suggest minimal liver enzyme induction rather than damage, which is a common response to some bioactive substances that boost metabolic activity. Variations in the A/G ratio and globulin concentrations signify modifications in protein catabolism and immune system functionality. Globulins primarily consist of immunoglobulins and acute phase proteins, which are influenced by nutritional status, metabolic processes, and metabolic circumstances (Kadi et al., 2025).

The formulation, including 4 % *Pluchea indica* flour in the concentrate, is the most efficacious for lowering blood total cholesterol levels (Budiono et al., 2025). The absence of notable alterations in creatinine and total protein indicates that renal function and overall protein levels were stable, hence affirming the extract's safety at the studied concentrations. The findings demonstrate that BE substantially influences lipid metabolism and hepatic biochemical pathways, likely through the modulation of lipid-associated gene expression and antioxidant mechanisms, as evidenced by similar phytogenic treatments (Ding et al., 2022). The decrease in TC, TG, and LDL, along with an elevation in HDL, signifies reduced cardiovascular risks and improved metabolic health in broilers, potentially resulting in enhanced growth efficiency (Ahmadpour and Zarrin, 2024).

Antioxidants and immunity indicators

Table 8 shows that adding 3 or 6 ml/L of BE to the drinking water of broiler chickens made their antioxidant and immune systems much better ($P \leq 0.05$) than the control. Specifically ($P \leq 0.001$), the levels of GPX, GST, and GSH went up, while the levels of MDA went down when the concentration of BE went up. This result shows that the antioxidant capacity is higher. The groups that got BE also had higher levels of immunoglobulins (IgG and IgM), which means that their immune systems worked better.

The outcomes demonstrate that the addition of 3 or 6 ml/L of BE to broiler chicken drinking water significantly improved antioxidant and

Table 6
Effect of dietary Barnouf extract on broiler carcass traits.

Traits	Treatments			SEM	*P* value		
	Control	BE 3 ml/L	BE 6 ml/L		T	L	Q
LBW	2005.57	2074.43	1997.71	34.08	0.103	0.101	0.558
Carcass %	71.74^{b}	72.12^{ab}	74.11^{a}	0.98	0.029	0.187	0.018
Heart %	0.70	0.63	0.55	0.03	0.121	0.043	0.981
Gizzard %	1.19^{a}	0.98^{b}	1.07^{ab}	0.05	0.000	0.000	0.288
Liver %	3.17^{a}	2.44^{c}	2.79^{b}	0.08	0.000	0.014	0.000
Spleen %	0.10	0.08	0.10	0.01	0.724	1.000	0.428
Abdominal fat %	0.39^{b}	0.43^{b}	0.67^{a}	0.02	0.001	0.000	0.074

SEM, Standard deviation of means; ^{a,b,c} Means within a row followed by different superscripts are significantly different ($P \leq 0.05$). LBW, live body weight.

Table 7
Effect of dietary Barnouf extract on broiler blood parameters.

Traits	Treatments			SEM	*P* value		
	Control	BE 3 ml/L	BE 6 ml/L		T	L	Q
ALT (U/L)	10.76^{b}	10.67^{b}	11.24^{a}	0.09	0.019	0.023	0.067
AST (U/L)	142.50^{a}	137.01^{b}	137.47^{b}	0.84	0.006	0.007	0.050
Urea (mg/dl)	41.67^{a}	37.15^{b}	36.96^{b}	0.51	0.000	0.000	0.000
Creatinine (mg/dl)	0.66	0.65	0.65	0.01	0.972	0822	0.948
TP (g/dl)	6.45^{a}	6.39^{ab}	6.38^{b}	0.01	0.059	0.027	0.367
Albumin (g/dl)	3.07^{b}	3.10^{a}	3.06^{b}	0.01	0.055	0.930	0.017
Globulin (g/dl)	3.38^{a}	3.29^{b}	3.31^{b}	0.00	0.010	0.025	0.023
A/G ratio	0.90^{b}	0.94^{a}	0.92^{ab}	0.08	0.006	0.083	0.005
TC (mg/dl)	59.02^{a}	52.59^{b}	53.17^{b}	3.66	0.000	0.000	0.004
TG (mg/dl)	76.87^{a}	55.77^{b}	52.69^{b}	2.47	0.000	0.000	0.000
HDL (mg/dl)	27.12^{a}	31.862^{b}	32.57^{b}	0.59	0.000	0.000	0.001
LDL (mg/dl)	15.34^{a}	11.23^{b}	11.46^{b}	0.51	0.000	0.000	0.005

SEM, Standard deviation of means; ^{a,b,c} Means within a row followed by different superscripts are significantly different ($P \leq 0.05$). ALT, alanine transaminase; AST, aspartate aminotransferase; TP, total protein; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

immunological parameters, supporting existing knowledge regarding herbal and phytochemical supplements in diets for poultry. Increased levels of GPX, GST, and GSH, along with decreased MDA, indicate enhanced antioxidant defenses and lower oxidative stress. In broilers, heightened activity of these antioxidant enzymes enhances the ability to reduce reactive oxygen species and protect cellular components from oxidative damage (Albarrak, 2021). Research on natural extracts abundant in polyphenols and antioxidants, including Rhazya stricta extract, has shown comparable enhancements in enzyme activity and decreases in lipid peroxidation indicators like MDA, indicating enhanced oxidative resilience and better health status (Albeshri et al., 2021; Sugiharto and Ayasan, 2023). The noted elevation in IgG and IgM levels in the BE-treated groups signifies enhanced humoral immunity.

Immunoglobulins are critical components of the adaptive immune response, with IgM typically indicating the initial response and IgG promoting long-term immunity (Hasted et al., 2021). Phytochemicals influence immune function through antioxidant characteristics and directly interact with immune cell signaling, leading to improved pathogen defense and immunological readiness (Williams et al., 2020). Studies on dietary antioxidants from fruits and herbal extracts have shown a positive effect on both innate and adaptive immunity in poultry, as indicated by enhanced antibody production and alterations in cytokine profiles, thus supporting the observed immunostimulatory effects (El-Abbasy et al., 2025b; Phillips et al., 2023). The dose-dependent increase in antioxidant enzymes and immunoglobulins, coupled with a reduction in oxidative damage, indicates that BE has a protective effect against oxidative stress and may improve immune function in broilers. This study aligns with the established notion that oxidative stress diminishes immune function and that enhancing antioxidant capacity can elevate poultry health and productivity (Azzam et al., 2023; Bortoluzzi et al., 2021). These enhancements can result in improved growth, health, and well-being in broiler production systems.

Table 8
Effect of dietary Barnouf extract on broiler antioxidant and immunity indicators.

Traits	Treatments			SEM	*P* value		
	Control	BE 3 ml/L	BE 6 ml/L		T	L	Q
GPX (U/mL)	164.85^{c}	168.28^{b}	171.28^{a}	0.70	0.000	0.000	0.808
GST (U/mL)	4.69^{b}	5.77^{a}	5.83^{a}	0.12	0.000	0.000	0.000
GSH (U/mL)	72.79^{b}	78.89^{a}	79.63^{a}	0.73	0.000	0.000	0.000
MDA (nmol/L)	3.98^{a}	3.27^{b}	3.11^{b}	0.07	0.000	0.000	0.101
IgG (ng/ml)	214.93^{b}	234.43^{a}	228.81^{a}	2.67	0.003	0.013	0.010
IgM (ng/ml)	17.90^{b}	18.73^{a}	19.08^{a}	0.15	0.001	0.000	0.325

SEM, Standard deviation of means; ^{a,b,c} Means within a row followed by different superscripts are significantly different ($P \leq 0.05$). GPX, Glutathione peroxidase; GST, Glutathione-S-transferase; GSH, Glutathione reduced; MDA, malondialdehyde.

Meat quality

Table 9 illustrates that the dietary BE had a big effect on several meat quality traits in contrast to the control. The pH levels at 0 and 10 days, as well as after 15 days of storage, were substantially ($P \leq 0.05$) lower in the 3 cm/L BE. The 6 cm/L group had higher pH values on storage for 15 days. The BE groups had lower APC at 5 and 10 days, especially at 6 ml/L, which showed better control of microbes. The L^* (lightness) and b^* (yellowness) values for meat color were not considerably different ($P \leq 0.05$), but the a^* (redness) value was somewhat lower in the BE groups. There were no substantial ($P \leq 0.05$) changes in the WHC or purge loss between groups. Cooking loss decreased significantly ($P \leq 0.05$) in groups with BE, with the 3 ml/L group showing the least loss.

The significant reduction in pH at 0 and 10 days, and after 15 days in the 3 ml/L BE group, contrasted with the increased pH in the 6 cm/L group at 15 days, suggests that lower doses of BE improve acidification after death and may more efficiently preserve meat freshness. The pH of meat is a vital factor influencing shelf life and microbial growth; lower pH levels generally inhibit the activity of spoilage bacteria (Shaltout, 2024). Furthermore, research demonstrates that natural antioxidants reduce meat pH and microbial load during storage, exemplified by bamboo leaf extract on broiler meat and olive leaf extract on minced beef (Horbańczuk et al., 2019; Shen et al., 2019; Yuan et al., 2025). The increased pH and improved microbial regulation in the 6 cm/L group may indicate that *Pluchea indica's* unique bioactive profile affects microbial populations variably at different levels.

The decreased APC in BE groups, especially at 6 ml/L, corroborates their antimicrobial efficacy, consistent with findings indicating plant extracts and natural antioxidants enhance meat microbiological safety by inhibiting spoilage or pathogenic bacteria (Horbańczuk et al., 2019; Salem et al., 2018). This microbial inhibition enhances shelf life and quality. The stable L^* (lightness) and b^* (yellowness) values, along with a minor decrease in a^* (redness) in *P. indica*-treated meat, indicate a minimal impact on visual attractiveness but a noticeable effect on redness, an important indicator of meat oxidation and myoglobin status. Plant-derived antioxidants frequently impede pigment oxidation, preserving color stability; yet minor fluctuations in redness may arise from phenolic interactions (Shen et al., 2019; Zhu et al., 2024). No significant differences in water-holding capacity and purge loss align with findings suggesting that while antioxidant supplementation can reduce protein

Table 9
Effect of dietary Barnouf extract on meat quality.

Traits		Treatments			SEM	*P* value		
		Control	BE 3 ml/L	BE 6 ml/L		T	L	Q
PH	0 day	6.50[a]	6.31[b]	6.33[b]	0.02	0.000	0.000	0.000
	5 days	6.54	6.48	6.50	0.02	0.646	0.564	0.648
	10 days	6.42[c]	6.45[b]	6.51[a]	0.01	0.000	0.000	0.094
	15 days	7.32[a]	6.55[b]	7.41[a]	0.07	0.001	0.412	0.001
APC	1 day	4.79[c]	4.89[b]	5.28[a]	0.05	0.000	0.000	0.001
	5 days	6.40[a]	5.58[b]	5.51[b]	0.10	0.000	0.000	0.006
	10 days	6.77[b]	6.82[b]	6.57[a]	0.03	0.009	0.014	0.038
*L**		38.27	42.00	40.12	0.76	0.137	0.310	0.084
*a**		13.60[a]	11.08[ab]	10.30[b]	0.60	0.060	0.025	0.462
*b**		21.23[a]	21.10[a]	18.54[b]	0.42	0.008	0.005	0.113
WHC		62.16	58.56	61.97	1.57	0.600	0.963	0.319
Purge		4.38	3.57	3.81	0.33	0.614	0.503	0.474
Cooking		33.46[a]	28.27[c]	31.42[b]	0.58	0.000	0.029	0.000

SEM, Standard deviation of means; [a,b,c] Means within a row followed by different superscripts are significantly different ($P \leq 0.05$). APC, Aerobic plate counts; WHC, water-holding capacity; *L**, lightness; *a**, redness; *b**, yellowness.

oxidation and drip loss, its impact on water-holding capacity may require specific conditions or polyphenol profiles to be meaningful (Mazur-Kuśnirek et al., 2019; Sugiharto, 2022). The significant reduction in cooking loss in the 3 cm/L group suggests that BE improves the meat's ability to retain moisture during cooking, hence enhancing juiciness and tenderness—crucial factors for consumer quality.

Microbial analysis

Figure 1 demonstrates that the dietary inclusion of BE significantly ($P < 0.05$) modified the cecal microbiota of broilers during the feeding period. The results demonstrated an increase in the number of lactobacilli in both BE groups, combined with a significant decrease in the count of bacteria, including TBC, *bacilli, E. coli, coliforms*, and *Salmonella*, compared to the control. The outcomes indicate that BE, at concentrations of 3 or 6 ml/L, regulates gut microbiota by suppressing pathogenic populations, hence improving intestinal wellness in broilers.

The results demonstrate that BE treatment at 3 or 6 ml/L significantly modifies the cecal microbiota of broilers, enhancing beneficial microbes such as *lactobacilli* while suppressing pathogenic populations like TBC, *bacilli, E. coli, coliforms*, and *Salmonella*. This effect is consistent with current literature on phytogenic and herbal extracts in poultry feeds, suggesting that these compounds can selectively promote beneficial microbial growth while suppressing pathogens, thereby enhancing intestinal health and overall efficiency (Meng et al., 2023; Sapsuha et al., 2021).

The decrease in TBC, *E. coli, coliforms*, and *Salmonella* is a vital indicator of improved gut health. *Escherichia coli* and *Salmonella* are prominent pathogens in chicken, associated with enteric diseases and food safety risks (Orimaye et al., 2024). The inhibition of these bacteria by BE corresponds with research on other herbal extracts, including garlic and grape seed, which have exhibited comparable antimicrobial effectiveness in broilers (Aswathi et al., 2023; Meng et al., 2023). The ability of herbal extracts to inhibit pathogenic bacteria is important for reducing antibiotic use and combating antimicrobial resistance in poultry production (Cheng et al., 2024; Rehman et al., 2025).

Conclusions and applications

1. The results indicated that administering Barnouf extract to broilers drinking water at doses of 3 or 6 ml/L enhanced their growth performance.
2. The 3 ml/L group had the most favorable outcomes regarding BWG and FCR. The birds that gave BE had lower FI and had better PI and health metrics, as shown by lower levels of AST, urea, lipids, and MDA, as well as higher levels of HDL and antioxidant enzymes.
3. Additionally, the extract helped stabilize meat pH, decrease microbial counts, and improve meat quality, with no adverse impacts detected.
4. Overall, Barnouf extract suggests that it is a beneficial natural feed additive for broiler production.

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Disclosures

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

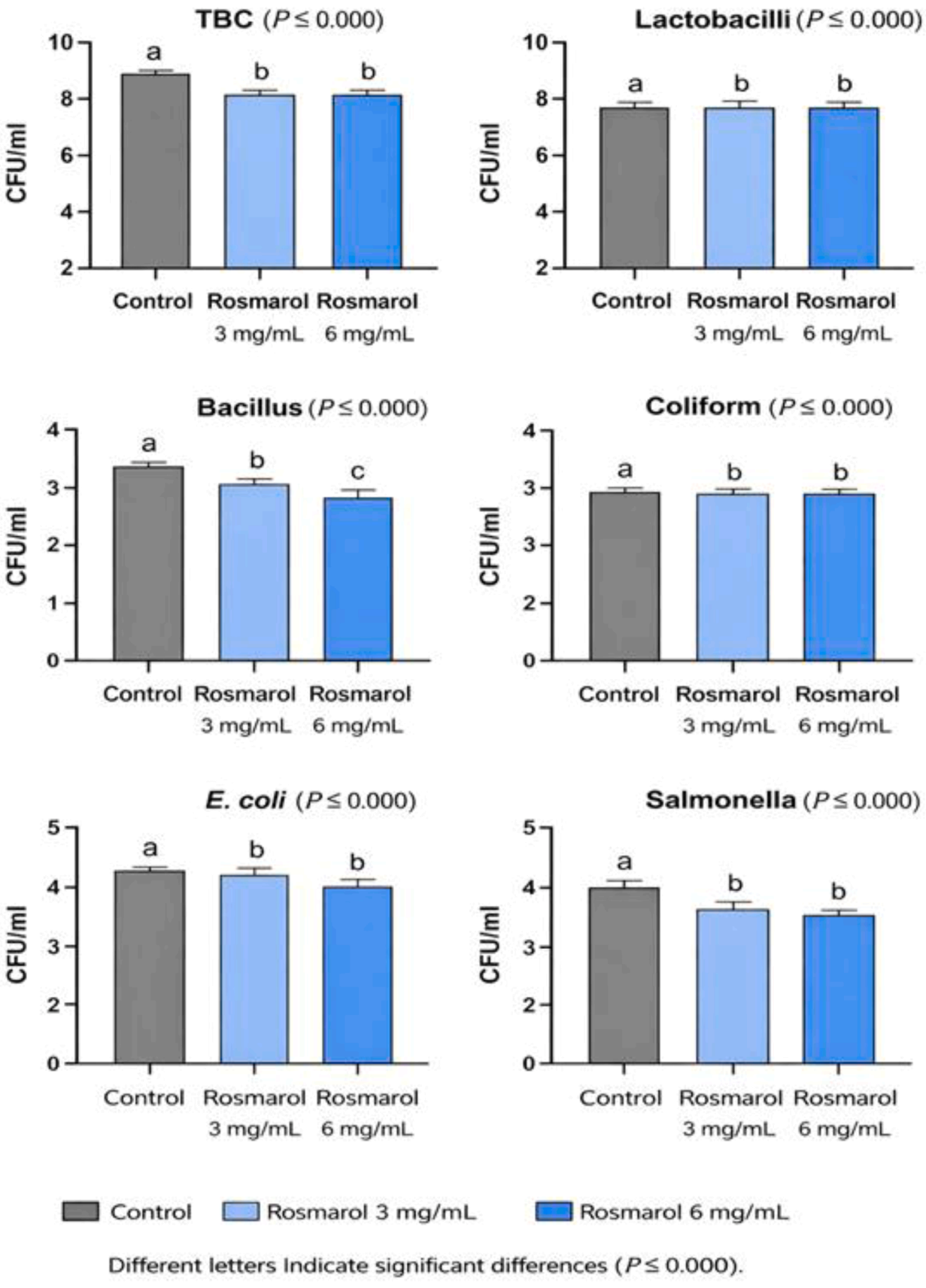

Figure 1. Effect of Barnouf extract on cecal microbiota (TBC, *lactobacilli, bacillus, E. coli*, coliform, and *Salmonella*) represented by (Log CFU/ml) in broiler during feeding.

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