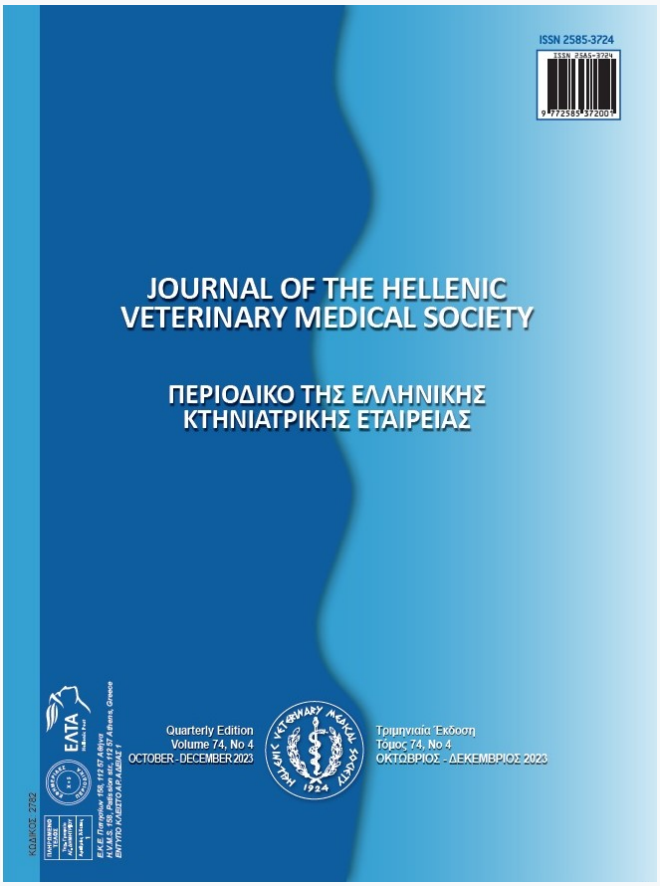
Journal of the Hellenic Veterinary Medical Society

Vol 75, No 1 (2024)



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doi: 10.12681/jhvms.34468

To cite this article:

Ullah, A., Anjum, A., Raziq, F., Khan, M., Gul, R., Rauf, M., Wadood, F., Ali, H., Abbas, G., Bughio, E., Younas, U., Nisa, Q., & Khan, K. (2024). Effect of ethanolic extract of Eucalyptus globulus leaves on growth enhancing, gut morphology, and intestinal absorption capacity in broiler chicken. *Journal of the Hellenic Veterinary Medical Society*, *75*(1), 7145–7154. https://doi.org/10.12681/jhvms.34468

J HELLENIC VET MED SOC 2024, 75 (1): 7145-7154
ΠΕΚΕ 2024, 75 (1): 7145-7154

Research article
Ερευνητικό άρθρο

Effect of ethanolic extract of *Eucalyptus globulus* leaves on growth enhancing, gut morphology, and intestinal absorption capacity in broiler chicken

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ABSTRACT: The goal of the current study was to assess the effects of an ethanolic extract of *Eucalyptus globulus* on the gut morphology and growth performance in broiler chicks. A total of 75 mixed sex one-day-old broiler chicks (Arbour Acres) with a body weight of 45.43 g were divided into five groups having 3 replicates with 15 birds each

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Date of initial submission: 10-05-2023
Date of acceptance: 06-07-2023

over the course of a 35-day feeding trial. These groups were as follows: 1 = PC (Positive control), 2 = NC (Negative control), 3 = Prob. (Probiotic), 4 = *E. globules* powder (EP), and 5 = *E. globules* extract (EE). The study was conducted under a completely randomized design. Growth performance was measured weekly. On the final day of the trial, birds were slaughtered, and samples of the duodenum, jejunum, and ileum, among other intestinal organs, were taken. In the meantime, the D-xylose test was conducted at 0 minutes, 30 minutes, 60 minutes, and 90 minutes. The results showed that the EE outperformed the other groups in terms of feed conversion ratio and body weight gain at 35 day of age, respectively. EE showed the most active and rapid intestinal absorption of D-xylose compared to other groups. The length of the gut villi's histomorphology revealed an increase in the villi's height, width, crypt, and depth. In conclusion, the ethanolic extract of *E. globulus* can replace low levels of antibiotics used in poultry feed as a growth stimulant, and it plays a part in the rapid absorption of nutrients in the gut by increasing villus height, width, and surface area.

***Keywords*:** Antibiotic; Broiler; D-xylose absorption; Gut villi morphometry; Probiotic.

INTRODUCTION

The gizzard, duodenum, ileum, jejunum, and cecum make up the poultry intestinal system. The ability to absorb nutrients from food is strongly related to intestinal health. It is made up of a vast variety of microbiota (Saleem et al., 2018). The digestive system's gut is the area where food is processed for digestion, where various enzymes are used, where nutrients from food are absorbed in the intestine, and where immune system development takes place (Sommer and Bäckhed, 2013). Birds' guts are made up of numerous substances, tissues, and bacteria (Yegani and Korver, 2008). According to Liévin-Le and Servin (2006), the gut is the bodily component most exposed to infectious and hazardous particles. As a result of the gut's extensive exposure to infectious pathogens, changes in its physiology, chemistry, and integrity led to the emergence of illness, immunological suppression, and dyspepsia (McDevitt et al., 2006).

The rapid growth in human population has increased the demand for food. The chicken industry has grown quickly in response to the general population's protein deficiency. Flocks become crowded in order to provide fast broiler growth at a cheaper cost. The offered feed items are frequently found to be contaminated with dangerous microorganisms. Antibiotics are included in poultry feed to help fight bacterial outbreaks and prevalence (Manie et al., 1998). Antibiotics are used in the composition of chicken, cow, and pig feed at a low level to improve feeding efficiency and the growth conversion ratio. According to Sarmah et al. (2006), the usage of antibiotics as a growth promoter in feed has increased up to 80 times in the USA. Drug residue builds up in animal tissues as a result of inappropriate drug withdrawal procedures and irrational antibiotic use. As a result, there is a greater likelihood of mutagenicity, carcinogenicity, and immunopathology (Cortesi and Catellani, 1990). In poultry, antibiotic use is widespread, and bacteria have begun developing antibiotic resistance. The feed generated will be more challenging to treat because a significant portion of the bacterial micro flora is resistant to several antibiotics (Manie et al., 1998).

According to research, the development of resistance bacteria is mostly brought about by the overuse of antibiotics and antibiotic growth promoters. As a result, the use of antibiotic growth promoter was banned by the European Union (EU) in 2006. Scientists are looking for antibiotic alternatives due to the rising concern about antibiotic resistance (Mirzaei-Aghsaghali, 2012; Seidavi et al., 2022). The scientist has been looking for antibiotic alternatives for the past 20 years. Probiotics, prebiotics, organic acids, zinc, bacitracin, eucalyptol oil, an antimicrobial peptide, egg yolk, acidifiers, nutraceuticals, clay minerals, and zinc are among the ingredients on the list. The hunt for an antibiotic substitute is still ongoing (Thacker, 2013).According to Mohebodini et al. (2019), the gastrointestinal tract (GIT) microbiota changes, increased nutrient digestibility and absorption, improved immune response, histological and morphological modifications of the GIT, and antioxidant activity that aids in nutrient digestion are the plausible mechanisms of action of phytobiotic feed additive in broilers for growth promotion.

Plant oligosaccharides, which serve as a prebiotic, alter the intestinal function of the gut (Moure et al., 2006). According to Huyghebaert et al. (2011), the desired effects of natural growth promoters as an alternative to antibiotic growth promoters should include the treatment of subclinical infections, utilization of nutrients, increased gut absorption capacity, suppression of the anti-digestive factors, and immune modulation (Seidavi et al., 2022). The plant extract boosts the production of digestive enzymes and reduces the availability of nutrients for bacterial devel-

opment and multiplication (Pasteiner, 2006). According to Dorman and Deans (2000), plant extracts alter the lipid solubility of bacteria and destroy their membrane. According to Wang et al. (1998), plant extracts have been demonstrated to be effective supplements for animal production and can be utilized as a natural growth stimulant. The phytochemicals included in plant cells serve a variety of purposes, including enhancing digestibility through their antibacterial and antioxidant properties (Sethiya et al., 2009).

In contrast to the control group, it was discovered that the treated groups had higher levels of pancreatic, duodenal, and jejunal protease as well as pancreatic and duodenal amylase. The villi were shown to be positively affected, and the treated groups had reduced blood urea nitrogen levels. According to this study, the use of plant extract in the diet has a beneficial effect on intestinal absorption and feed efficiency (Yu et al., 2015). The D-xylose test is thought to be useful for determining how effectively the digestive tract functions. It efficiently absorbs from the small intestine and has been employed as a plasma technique to calculate the degree of bacterial and viral-caused malabsorption in birds (Doerfler et al., 2000). There have been few research done on the impact of *E. globulus* ethanolic extract on gut morphology and intestinal absorption. The purpose of the current investigation is to ascertain the impact of *E. globulus* extract on gut villus morphometry and gut absorption capacity in broilers. Increases in villus height and crypt depth ratio show that birds' guts are effectively absorbing nutrients (Ali et al., 2017).

The increasing use of antibiotics to combat bacterial resistance has an impact on gut function (Apata, 2012). Additionally, there has been a drop in gut microbiota, and antibiotic residues have been found in the food supply for both humans and animals (Siddique et al., 2018). Antibiotics should therefore not be used to encourage growth instead using plant extract. The D-xylose test can be performed to determine how well the intestines can absorb nutrients. According to Siddique et al. (2018), D-xylose can be utilized to measure the gut malabsorption syndrome brought on by bacteria and viruses. The absorption of D-xylose varies depending to the health status of the individual birds (Shomali et al., 2012). Keeping this in mind, the study was designed to examine the effects of *E. globulus* on broiler chick growth promotion, gut morphology, and absorption capacity.

MATERIALS AND METHODS

Animal welfare

The care and use of birds and all experimental protocols were in accordance with the laws and regulations of Pakistan which were approved by Institutional Review Committee for Biomedical Research University of Veterinary & Animal Sciences (UVAS), Lahore-Pakistan.

Location and meteorological data

The effects of an ethanolic extract of E. globulus leaves on broiler chick growth, gut morphology, and intestinal absorption capacity were examined in an experiment. The research was carried out for 5 weeks in March and April 2019 at the Department of Poultry Production, University of Veterinary and Animal Sciences, Lahore (UVAS), Lahore, Pakistan. Lahore is located at 31° 1'0'' N, and 73° 50'60'' E with an altitude of 186 m (610 ft). This city experiences normally hot and humid tropical climate with temperature ranging from 5 °C in winter and +45 °C in summer.

Experimental site, management, birds, and husbandry

The poultry shed was thoroughly cleaned with a broom, and then washed with simple water. Bleach powder was then applied to the floor and left there for 24 hours. The floor was then cleaned with surf water. To get rid of any concealed pathogens, the roof, walls, floor, and utensils (feeders, drinkers, and partitions) were washed with hot water. Two inches of rice husk was placed on the ground. Partitions were used to form experimental groups. Five percent formalin spray was used to disinfect the shed, followed by formaldehyde fumigation. The shed was shut for a full day. All of the chicks received were kept at brooding temperatures with ventilation allowing fresh air to flow into the shed. Two groups of commercially available feed were made specifically for the experimental birds. In one group, feed was produced without antibiotics, while in the other, feed contained antibiotics. There were identical components and energy compositions in both meal groups. At various stages of growth, the chicks received starter, grower, and finisher feed. The on-site tap water that was available was utilized. Prior to any treatment, the pH of the water was measured. Water was available at all times. A total of 75 mixed sex one-day-old broiler chicks (Arbour Acres) with a body weight of 45.43 g were divided into five groups having 3 replicates with 15 birds each over the course of a 35-day feeding trial. These groups were as fol-

lows: 1 = PC (Positive control), 2 = NC (Negative control), 3 = Prob. (Probiotic), 4 = *E. globules* powder (EP), and 5 = *E. globules* extract (EE) (Table 2). The study was conducted under a completely randomized design.

Ethanolic extraction of Eucalyptus globulus leaves

Fresh leaves of the *E. globulus* tree were collected from Multan road, near Manga Mandi, Lahore-Pakistan. With the help of an ethanol solvent, plant extract was collected from dried leaves powder through Soxhlet apparatus (Kashyap et al., 2005). Manga Mandi is 212 meters (695.54 feet) above sea level and is situated at 31° 18'31'' N and 74° 3'5'' E. The typical climate in this city is hot and humid, with winter lows of 7.7 °C and summer highs of 43.7 °C.

Soxhlet apparatus method

Using an effective grinding apparatus with a sieve size of 2 mm, the dried *E. globulus* leaves that had been dried in the shade were ground into a powder. The Soxhlet apparatus was then used to mix 100 g of plant powder with 600 mL of pure ethanol in the bottom flask. In order to reduce the water vapours, the temperature was kept at or above 50 °C. The maximum amount of extracts was recovered in the bottom flask after 10 to 12 solvent cycles. The extract was obtained between 8 and 12 cycles later. On a rotating evaporator at a controlled temperature (40 to 50 °C), the plant's extract was concentrated under reduced pressure to more than 60 pascals. To eliminate the most moisture or ethanol, semisolid plant extracts were then transferred to big petri dishes and heated to 40 °C for a consecutive 4 days. The plant extract was then taken out of the petri dish, weighed to determine the yield percentage, and placed in plastic zip-top bags in the freezer for further investigation.

D-xylose absorption capacity of broiler chicken

At the conclusion of the experimental trial, a D-xylose absorption test was conducted. Birds received three hours of thirst as a result. For sampling, five birds from each group were chosen at random. Each bird was weighed separately. The oral gauge was used to administer the D-xylose solution at a dose rate of 0.5 grammes per kg of bird weight. Prior to the D-xylose dose being consumed, blood samples were taken after 30, 60, and 90 minutes afterwards. Using sterile syringes, blood samples were taken from the ulnar vein in the wings at 0, 30, 60, and 90 minutes after D-xylose inoculation. The blood was drawn into a heparinized micro hematocrit capillary tube that is readily accessible on the market. To separate the plasma, the tubes were centrifuged for 15 minutes at 6000 rpm. 2 mL of the phloroglucinol reagent was added to each 20 mL of plasma and heated for 4 minutes at 100 °C. Each sample's absorbance was measured at 554 nm using a spectrophotometer after being cooled to room temperature (Regassa et al., 2016). By mixing 0.5 g of phloroglucinol with 100 mL of glacial acetic acid and 10 mL of concentration, the phloroglucinol colour reagent was created. HCl in a bottle with an amber color. D-xylose was dissolved in deionized water to create concentrations of 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, and 70 mg/2 mL to create the D-xylose standard solution. In these same standard concentrations, color reagents were also applied and processed. 2 mL of the phloroglucinol color reagent was added to Eppendorf along with the 20 L of each standard solution. After cooling, absorbance at 554 nm was measured after the solution had been heated for 4 minutes at 100 oC. D-xylose standards and sample optical densities (OD) values were used to create the standard curve. By comparing the optical density (OD) measurements to a standard absorbance value, it was possible to calculate the blood D-xylose concentration (Mansoori et al., 2007).

Data collection

By growing day-old broiler chickens, the *in-vivo* growth-enhancing effects of *Eucalyptus globulus* leaves extract in broiler chickens were determined. Body weight gain (BWG) and other growth performance metrics including feed conversion ratio (FCR) were calculated on a weekly basis.

At the conclusion of the trial, samples of the duodenum, jejunum, and ileum were taken. Through the use of image j software, the villus length, width, and crypt depth were measured. Five birds from each group were then randomly chosen and killed. The duodenum, jejunum, and ileum were the three sections that made up the chicken intestine (Figure 1). From each segment of the small intestine, a two-centimeter section was removed at the midline, flushed with saline, and split lengthwise. According to Awad et al. (2009), the intestinal segments were fixed in 10% neutral buffered formaldehyde before the tissues were prepared and stained with hematoxylin and eosin. Slides were analyzed using image j software after being inspected with a light microscope (LABOMED pixel pro, USA) equipped with a digital imaging system. Villus height, crypt depth, and villus height/crypt

Table 1. Experimental layout.

Sr. No.	Group	Birds per replicate
1	Positive control, mixed antibiotic feed/ Flavomycine @ 1 gm/liter) (PC)	05 05 05
2	Negative control, without antibiotic (NC)	05 05 05
3	Probiotic/Primalac @ 1 gm/800 liter) (Prob.)	05 05 05
4	*E. globulus* Extract (EE)	05 05 05
5	*E. globulus* leaves (EP) @ 0.60 gm/Kg feed	05 05 05

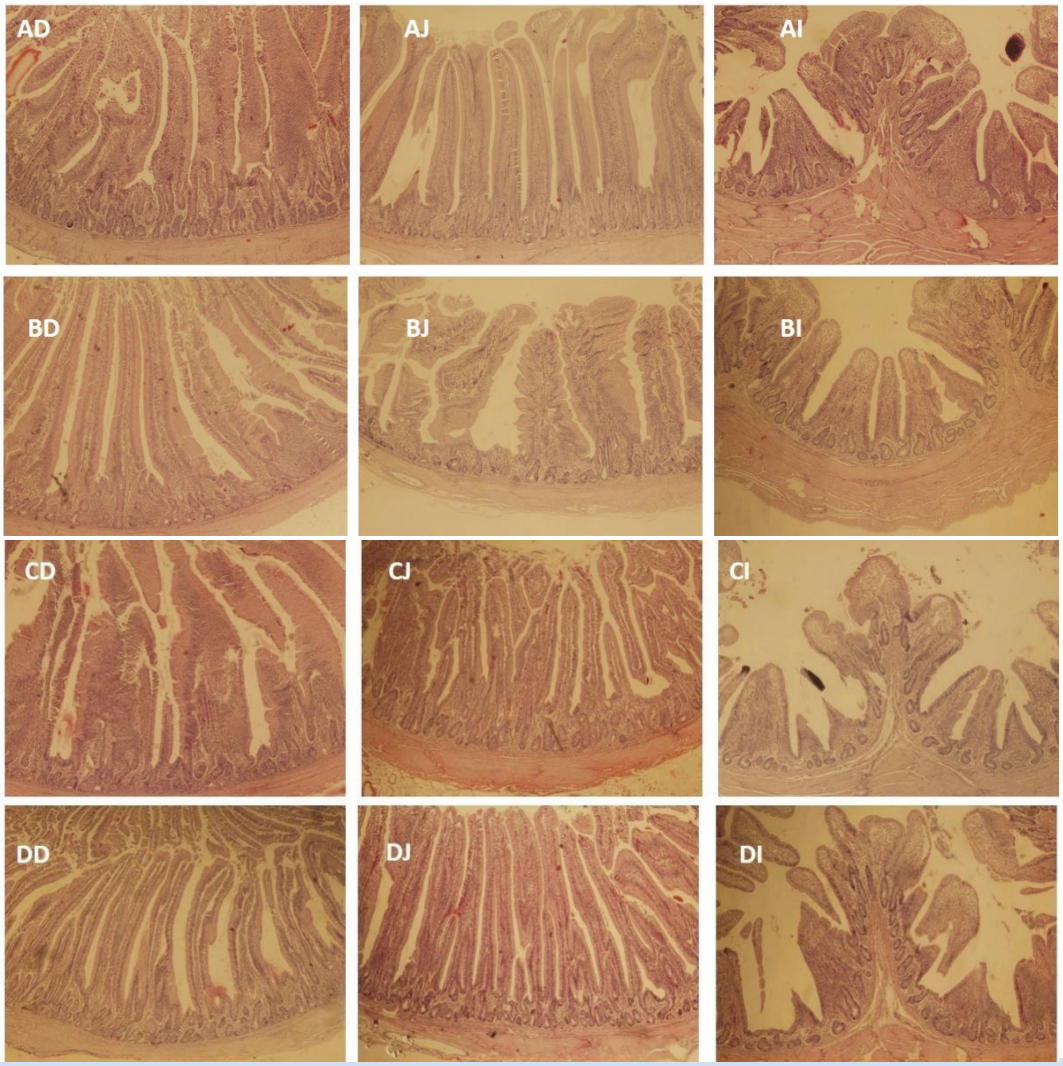

Figure 1. Villus parameter of Negative control group (AD = duodenum, AJ = Jejunum, AI= Ileum); B) Villus parameter of antibiotic (Positive control) group (BD = duodenum, BJ = Jejunum, BI = Ileum); C) Villus parameter of Probiotic group (CD = duodenum, CJ = Jejunum, CI = Ileum); D) Villus parameter of Ethanolic extract of *Eucalyptus globules* group (DD = duodenum, DJ = Jejunum, DI = Ileum)

Table 2. Villi morphometry of different experimental groups.

Groups	VH (µm)	VW (µm)	CD (µm)	VH/CD	VSA (µm²)
NC	864.9 ±108.9[a]	93.4±11.6[a]	175.6±19.1[abc]	5.0±1.1[abc]	492.9±76.9[a]
PC	990.9±153.2[b]	97.8±10.2[a]	184.2±28.7[c]	5.5±1.2[c]	608.0±108.1[b]
Prob.	1048.4±10.9[b]	118.3±15.5[b]	169.3±19.9[ab]	6.3±1.3[ab]	776.9±208.0[c]
EE	1165.9±78.3[c]	122.9±13.5[b]	180.7±15.3[bc]	6.5±0.8[bc]	905.5±131.0[d]
EP	1074.7±136.3[b]	94.1±20.1[a]	166.6±20.7[a]	6.3±1.2[a]	600.3±165.8[b]
Sig.	0	0	0.002	0	0

[a,b,c]Different superscript in different rows of same column show statistically significant difference at P≤0.0
NC = Negative control; PC = Positive control; Prob. = Probiotic; EE = *E. globulus* extract; EP = Ether powder
Sig = Significance.

depth ratio were the morphometric variables. Villus height was calculated using the approach of De et al. (2007) from the villus end point to the villus crypt junction, and crypt depth was taken into account from its base root up to the region of shift between crypt and villus (Table 2).

Statistical analysis

Using SPSS, enumeration data were reported as mean Standard Deviation (SD) and compared using one-way Analysis of Variance (ANOVA) and the Tukey technique at a significance threshold of P<0.05.

RESULTS

Body weight gain

The body weight of the broiler was determined on a weekly basis. Body weight gain at 35 days of age increased considerably in group EE followed by Prob. and EP, PC, and NC (Table 3 and Figure 2).

Table 3. Average weekly weight (Mean ± STD) of different experimental groups in broiler chicks (35 days).

Groups	Day-0	Day 7	Day 14	Day 21	Day 28	Day 35	P-value
NC	41.5± 1.2[b]	164.5± 9.7[a]	469.5± 33.3[c]	828.0±16.9[c]	1214.5± 9.5[a]	1654.5±113.[1]a	0.018
PC	41.3± 1.1[b]	164.5± 3.2[a]	407.0± 14.1[a]	763.0± 19.9[ab]	1215.0± 42.9[ab]	1647.5±74.[4]a	0.999
Prob.	41.3± 1.1[b]	165.0± 7.7[a]	434.0± 13.9[b]	790.0±73.9[b]	1287.5± 21.8c	1734.0±38.[6]b	0.000
EE	41.3± 1.1[b]	164.5± 7.4[a]	429.5± 16.3[b]	770.0± 13.3[ab]	1285.5± 33.4[c]	1794.0±67.[3]c	0.000
EP	40.0± 1.2[a]	165.0± 16.3[a]	400.0± 19.8[a]	750.0±20.9[a]	1256.0± 24.5[b]	1665.0±17.[0]a	0.000

[abc]Different superscript in different rows of same column show statistically significant difference at P≤0.05
NC = Negative control; PC = Positive control; Prob. = Probiotic; EE = *E. globulus* extract; EP = Ether powder

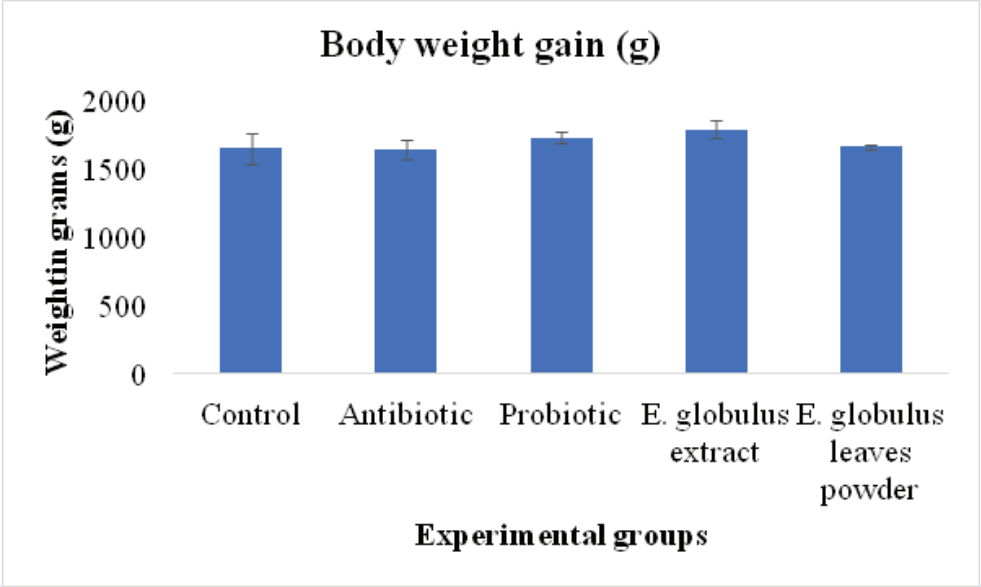

Figure 2. Weight gain at 35 day of age (Mean ± STD) of different experimental group

Feed conversion ratio

Weekly calculations were made for the feed conversion ratio. At 35 days of age, the FCR considerably improved in group EE, followed by Prob. and PC, NC, and EP (Table 4 and figure 3).

Absorption of D-xylose

According to the findings, group EE absorbed D-xylose at a rate that was higher than that of the other treatment groups in the first half hour, an hour, and a half and hour (Table 5).

Villus morphometry

Significantly, the EE group had the highest duodenal villus height, followed by Prob., PC, and CN (Table 6). When compared to all other groups, the group fed with EE had a considerably wider duodenal villus. Similar to this, it was discovered that the EE group's villi surface area was the greatest, followed by Prob. PC and CN (Table 6).

Similar to this, EE had the largest ileum villus height, followed by Prob. NC and PC (Table 6). The

Table 4. Weekly FCR of different experimental groups of broiler chicks.

Groups	Age				
	Day 7	Day 14	Day 21	Day 28	Day 35
NC	1.04	1.07	1.33	1.56	1.89
PC	0.97	1.10	1.39	1.56	1.80
Prob.	0.91	1.10	1.32	1.43	1.69
EE	0.96	1.13	1.34	1.46	1.64
EP	0.91	1.24	1.43	1.52	1.93

NC = Negative control; PC = Positive control; Prob. = Probiotic; EE = *E. globulus* extract; EP = Ether powder

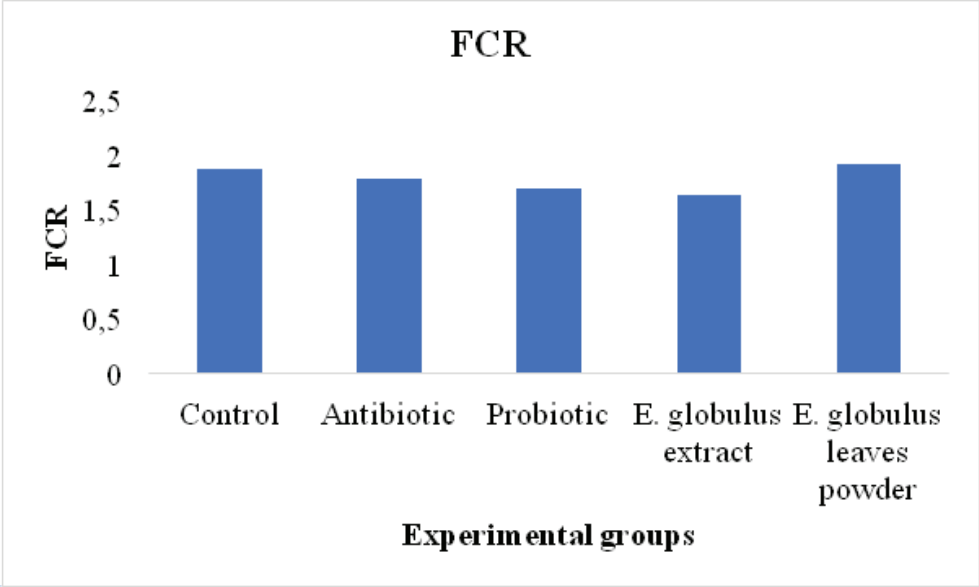

Figure 3. FCR of different experimental groups at 35 day

Table 5. D-xylose absorption test at 0 min, 30 min, 60 min, 90 min broiler chicks at 35 days.

Groups	Time Duration			
	0 hr	Half hr	One hr	One and half hr
NC	0	6.95±2.49^{a}	5.65±1.47^{a}	2.75±1.12^{a}
PC	0	43.40±10.86^{b}	39.22±8.26^{bc}	9.25±2.50^{bc}
Prob.	0	55.70±5.41^{c}	35.12±7.26^{bc}	6.52±0.62^{b}
EP	0	62.82±6.86^{c}	44.22±11.47^{cd}	9.90±3.08^{bc}
EE	0	75.80±5.55^{d}	54.27±6.80^{d}	10.47±2.72^{c}
Sig.	0	0.01	0.01	0.02

^{a,b,c} Different superscript in different rows of same column show statistically significant difference at P≤0.05
NC = Negative control; PC = Positive control; Prob. = Probiotic; EP = *E. globulus* powder; EE = *E. globulus* extract; EP.

Table 6. Villus morphometry of duodenum, ileum, and jejunum in broiler chicks at 35 day of age.

Group	VH (µm)	VW (µm)	CD (µm)	VH/CD	SA (µm²)
Villus parameter of duodenum					
CN	358.8±13.1	47.5±3.9	45.0±8.5	7.91[a]	35.87[a]
PC	332.5±83.8	67.5±31.9	59.8±8.2	6.57[b]	83.19[b]
Prob.	589.5±6.5	51.3±13.6	62.5±14.5	7.80[a]	95.02[b]
EE	658.2±50.3	78.0±42.0	44.3±37.6	9.43[c]	161.2[c]
Villus parameter of ileum					
NC	275.0±13.4	43.7±10.3	50.75±2.8	3.73[a]	27.5[b]
PC	156.3±10.3	48.3±15.2	39.25±12.6	3.98[a]	23.7[a]
Prob.	224.5±15.8	81.2±7.2	38.25±10.2	5.87[b]	57.2[bc]
EE	526.7±59.8	75.7±12.4	40.66±7.9	4.19[c]	125.1[c]
Villus parameter of jejunum					
CN	440.5±28.9	65.2±14.5	80.0±9.0	8.80[c]	44.1[b]
PC	526.0±9.6	39.7±15.0	61.0±22.0	6.34[b]	98.6[c]
Prob.	334.5±127.3	73.8±7.2	41.3±6.2	8.75[bc]	77.6[bc]
EE	306.5±104.9	51.1±4.9	36.8±27.3	4.96[a]	37.6[a]

[a,b,c] Different superscript in different rows of same column show statistically significant difference at P≤0.05
VH = Villus Height; VW = Villus Width; CD = Crypt Depth; VH/CD = Villus height/Crypt Depth; SA = Surface Area; NC = Negative control; PC = Positive control; Prob. = Probiotic; EE = *E. globulus* extract.

ileum villus width considerably increased in the group fed Prob., followed by EE, PC, and NC (Table 6). PC had the highest jejunum villus heights followed by CN, Prob. and EE (Table 6). It was discovered that the jejunum villus width was considerably highest in group Prob., followed by CN, EE, and PC (Table 6).

DISCUSSION

It was hypothesized that *E. globulus* leaves might promote growth in broiler chicks. The potential of *Eucalyptus globules*' bioactive substances to stimulate the release of digestive and pancreatic enzymes may be responsible for the increase in performance in the current study (Hashemipour et al., 2013). In a similar vein, Mashayekhi et al. (2018) observed that the addition of *E. globulus* extract had a substantial impact on bird weight increase. With the addition of *E. globules* leaves to the broiler birds' feed, the carcass and breast weights increased. The beginning weight of the birds, the sex of the birds, and the ventilation available can all have an impact on the birds' weight and FCR. Regarding the leaves of *E. globules*, similar findings were published by Karthivashan et al. (2016). According to Barbour et al. (2011), *E. globulus* leaf powder improved feed intake and growth performance. In a related study, birds fed diets supplemented with 0.1% of *Eucalyptus globules* leaves had the highest growth rates, live weights, and live weight gains. This suggests that *Eucalyptus globules* leaves can be used as a natural feed additive in broiler diets to achieve the best performance and the highest income per chicken. *Eucalyptus globulus* leaves have also been observed to boost the growth and production of laying Japanese quails (Hassan et al., 2011). According to Chen et al. (2017), broiler production efficiency, growth rate, live body weight increase, and feed conversion ratio can all be improved with the addition of 0.1% *Eucalyptus globules* leaves to the diet. Mashayekhi et al. (2018) found that adding 0.5% *Eucalyptus leaf* powder to the food considerably increased the carcass weight and relative breast weight, and was an effective substitute for the antibiotic (Virginiamycin). The nutrients and antinutritive elements found in plants may have a favorable or negative impact on certain production indices. According to Farhadi et al. (2017), broilers fed a diet containing powdered *Eucalyptus globulus* leaf demonstrated decreases in feed intake but had little effect on FCR. It can have a strong correlation with the bird's FCR and increase in body weight (Mansoori et al., 2015).

A study suggested using the D-xylose absorption test to assess how well nutrients are absorbed through the intestines. It is a reasonably easy and cost-effective strategy that has been used frequently on both humans and animals (Mansoori et al., 2009). D-xylose, a pentose sugar that is poorly metabolizable and well absorbed in chicken intestine, can be utilized to accurately assess the malabsorption syndrome in poultry by monitoring changes in plasma concentration over time (Mansoori et al., 2009; Yu et al., 2015). Extract

from *E. globulus* leaves is essential for improving gut morphology (Giannenas et al., 2018). The thymol and carvacrol in essential oils may be to blame for this (Lee et al., 2003). Therefore, the D-xylose absorption test is employed to determine how the leaves of *E. globulus* affect intestinal absorption. Similar to this, offering *E. globulus* leaves to broiler chicks has been shown to boost digestibility (Mohebodini et al., 2021). Increased intestinal absorption may be the cause of this variance. In this experiment, more absorption led to greater growth performance (Awad et al., 2009). The 1, 8-cineole's phenolic concentration may be the cause of this.

E. globulus leaves are essential for the ileal digestibility of broiler chickens. The thymol and carvacrol in essential oils may be to blame for this (Lee et al., 2003). A higher production of digestive enzymes caused by an increase in duodenal height and crypt depth lowers the risk of mortality at high weight (Mustafa, 2019). The essential oil from *E. globulus* leaves may be the cause of this variation (Luis et al., 2016; Dhakad et al., 2018). Additionally, according to Wahlstrom (2013), an increase in the duodenum's height and depth causes a high release of digesting enzymes, which lowers the risk of mortality at high weight. Intestinal weight and length increased when *E. globulus* leaves were introduced to the quail diet (Hassan et al., 2011).According to Lee et al. (2003) and Dhakad et al. (2018), the essential oil (thymol and carvacrol) from *E. globulus* leaves may be the cause of this variation. According to research, an imbalance between oxidative and antioxidant activity affects how well the gastrointestinal tissues operate (Luis et al., 2016). The 1, 8-cineole, a phenolic compound, is primarily responsible for the *E. globulus* leaves' significant antioxidant activity (Olayinka et al., 2012). Additionally, Luis et al. (2016) confirmed the antioxidant activity of *E. globulus* leaves, which contain the phenol 1, 8-cineole. The intestinal mucosa and structure improved as a result of the plant extract's protection of the mucosa from free oxidative radicals and maintenance of the oxidation reduction cycle (Mustafa, 2019). This variance may result from the essential oil's ability to remove free radicals (Luis et al., 2016; Jin et al., 2020). The plant extract also works as immunostimulants, disrupts bacterial cell membranes, reduces bacteria's virulence by producing hydrophobicity, and promotes the growth of villus and crypt cells in the gut, which increases the production of digestive enzymes (Jamroz et al., 2002; Vidanarachchi et al., 2006). In this trial, higher absorption levels were associated with better development outcomes. Regarding *E. globulus* leaves meal, similar results were reported by (Nkukwana et al., 2015; Khan et al., 2017).

CONCLUSIONS

According to the results of the current study, the ethanolic extract of *E. globulus* can replace antibiotics used in poultry feed as a growth stimulant, and it could play a part in the rapid absorption of nutrients in the gut by increasing villus height, width, and surface area. The addition of *E. globulus* ethanolic extract in broiler diet may improve nutritional absorption and boost growth of the birds.

ACKNOWLEDGEMENTS

This work is funded by the Higher Education Commission (HEC) of Pakistan approved project of "Replacement of low level of antibiotic added in poultry feed" with the project code number NRPU PROJECT 2101. The author also thanks the professors from the microbiology department at the University of Veterinary & Animal Sciences (UVAS), Lahore, Pakistan, for their assistance in making this study possible.

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