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Efficacy of *Acacia nilotica* bark extract as an alternative to antibiotics in broilers challenged with *Escherishia coli* from 15 to 28 days

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Abstract: The present experiment was conducted to compare the effect of *Acacia nilotica* bark extract and antibiotic on different biological parameters in broilers challenged with *E. coli*. Five hundred 2-week-old broiler chicks were divided into five treatments (Cont, NC, PC, AN-BE3, and AN-BE5) with ten replicates per treatment (10 chicks per replicate), and the experiment was extended over 2 weeks. All chicks, except that of Cont were challenged with *E. coli* (10^7 CFU/bird). Cont: basal diet only; NC: challenged with *E. coli*; PC: NC + Zinc Bacitracin 50 mg/kg diet; AN-BE3: NC + *A. nilotica* bark extract supplementation 0.3%; AN-BE5: NC + *A. nilotica* bark extract supplementation 0.5%. At the end of the experiment, one bird per replicate was selected randomly and slaughtered for sampling regarding blood biochemistry, intestinal morphology and cecal digesta. Weight gains in Cont, PC, and AN-BE5 were statistically similar but higher than that of NC. Similarly, feed conversion ratios in Cont, PC, and AN-BE5 were statistically similar but better than that of NC. The highest ($p < 0.05$) levels of blood glucose was observed in Cont (278.06 mg/dL) and the lowest in AN-BE5 (225.70 mg/dL). Concentrations of ALT and AST were significantly ($p < 0.05$) reduced in PC and *A. nilotica* bark extract supplemented groups than the other groups. Antioxidant status of birds in PC and *A. nilotica* bark extract supplemented groups was comparable and better than those of NC and Cont treatments. Villus heights in PC (1063.25 µm) and AN-BE5 (1061.63 µm) were similar but higher than those of Cont (1026.50 µm) and NC (1014.75 µm). In addition, counts of *Coliform* and *C. perfringens* were significantly reduced in PC and *A. nilotica* bark extract supplemented groups. *A. nilotica* bark extract could be an alternative to antibiotics in boilers challenged with *E. coli*.

Key words: *Acacia nilotica*, antioxidant capacity, blood chemistry, cecal microbiota, intestinal morphology

1. Introduction

Antibiotics are used to support the growth performance and to decrease the load of pathogenic bacteria in the gastrointestinal tract in poultry and other livestock animals. However, unrestricted use of antibiotics increases the cases of antibiotic resistance in pathogenic bacteria and also kills the beneficial bacteria [1]. This antibiotic resistance can transfer from one species to another and create many health issues in society [2]. To overcome these problems, the European Union banned the use of antibiotics at subtherapeutic levels in animal feeds. At present, the main issue is to find some suitable replacer of antibiotics which could support the growth performance in poultry under commercial farming systems and also protect them from attack of different diseases [3]. Work has been done and is still going on many alternatives, including probiotics, prebiotics, organic acids, and phytogenic feed additives, to check their efficacy against antibiotics. Every alternative has some merits and demerits regarding their use in poultry diets. Phytogenetic feed additives have shown better results regarding health and productivity in broilers [4]. Extracts of different plants contain many active compounds, such as alkaloids, volatile essential oils, resins, glycosides, phenolic oleosins, terpenes, and steroids, with potential antibacterial activities [5] and antioxidant effect [6]. It has been suggested that extracts

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obtained from different spices and herbs enhance the production of endogenous digestive enzymes which ultimately improved the nutrients digestibility and growth performance in poultry birds, they also protect the birds from oxidative stress and pathogens [7]. *Acacia nilotica* belongs to the family Mimosaceae, which is a tree plant with yellow mimosa-like flowers. The bark of this plant has been used for the treatment of acute diarrhea [8]. *A. nilotica* is one of the important medicinal plants having numerous beneficial effects, such as enhanced growth performance and improved the bioactive compounds index in poultry meat [9]. It has been reported that extract of the bark of *A. nilotica* contains terpenoids, alkaloids, glycosides, and saponins, which exhibited antimicrobial activities against *Streprococcus viridans*, *S. aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Shigella sonne* [10]. Saponins facilitate the absorption of nutrients while tannins help to prevent the development of microorganisms [11]. In addition, during early life, chicks are more sensitive and their gut microbiota are relatively unstable and get disturbed by many factors including infection posed by pathogenic challenge. *Escherichia coli* is one of the most important pathogenic bacteria which could easily cause infection by attaching to epithelial surfaces [12]. Thus, the objective of this experiment was to compare the efficiency of *A. nilotica* bark extract as compared to antibiotics in broilers challenged with *E. coli.*

2. Materials and methods

2.1. Institutional review board statement

The protocol of this experiment was approved by committee of Ethical Handling of Experimental Birds, University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan, for the care and use of experimental birds with approval number: DAS/358, Dated 03/05/2021.

2.2. Experimental protocol

This experiment was conducted at the research and development farms of Sultan Feed Pvt. Ltd., Sargodha, Pakistan on 500 broiler chicks (Cobb-500). After being fed on a commercial starter diet (CP 21%; ME 3000 kcal/kg) for 2 weeks, chicks (mixed; male and female) were divided into 50 replicates (10 chicks per replicate) and 10 replicates were assigned to a specific group. Group 1 was control group without any challenge or supplementation; Group 2 was negative control (NC), challenged with *E. coli* only; Group 3 was positive control (PC) which was challenged with *E. coli* and supplemented with zinc bacitracin (50 mg/kg diet); Groups 4 and 5 were challenged with *E. coli* and supplemented with *A. nilotica* bark extract at the levels of 0.3% and 0.5%, respectively, and were designated as AN-BE3 and AN-BE5. *A. nilotica* bark extract was sprayed after pelleting on to the cooled pellets. Strain of *E. coli* used in this experiment was O157:H7. The birds were challenged with *E. coli* 10^7 CFU/bird by oral gavage [13]

Table 1. Ingredients and nutrient compositions of basal diet.

Ingredients	Percentage	Nutrients (dry matter bases)	Percentage
Corn	59.13	ME (kcal/kg)	3150
Canola meal	10	Crude protein	19.5
Soybean meal	20	Dig. lysine	1.05
Corn gluten 60%	1.89	Dig. methionine	0.42
Guar meal	3	Calcium	0.84
Veg. oil	3.84	Available phosphorus	0.42
Lysine sulfate	0.46		
DL-methionine	0.022		
L-theronine	0.07		
DCP	0.89		
Calcium carbonate	0.69		
Soda ash	0.12		
NaCl	0.2		
Vitamin and mineral premix*	0.5		
Total	100		

* Composition is per kg of premix; vita A 20,000,000 UI; vita D3 5,400,000 UI; vita E 48,000 mg; vita K3 4,030 mg; vita B1 4,000 mg; vitaB2 9,040 mg, vita B5 20,000 mg; vita B6 7,600 mg; vita B8 200 mg; vita B9 1600 mg; vita B12 20 mg; ferrous carbonate 60,060 mg; copper sulfate 10,000 mg; zinc oxide 123,750 mg; manganese oxide 132,000; cobalt carbonate 400 mg; selenium 360 mg.

at the age of 15 days. *E. coli* used in this experiment were collected from Institute of Microbiology, UVAS. Grower diet was formulated according to Cobb-500, Nutritional Requirement Manual Guide, 2018 (Table 1), and fed to the birds from 15 to 28 days. Ad libitum feeding and 24-h availability of fresh water were practiced throughout the feeding trial (15–28 days). In addition, all management and biosecurity practices were strictly practiced during the experiment.

2.3. Bark collection and extraction

Bark of health *A. nilotica* was collected from central cities of Punjab, Pakistan and thoroughly washed with tap water and finally with distilled water. It was oven dried at low temperature and ground to fine powder. Soxhlet extraction technique [14] was employed to get extract from bark power. Extract was concentrated by using rotary evaporator and placed in oven for 4 days at low temperature to remove moisture.

2.4. Sampling and analysis

Initial and final weight of broilers was recorded to determine the weight gain during an experimental period of 2 weeks (15 to 28 days). Weighted amount of feed was offered to determine feed intake. From data of feed intake and weight gain, feed conversion ratio (FCR) was calculated.

At the end of the experiment, one bird per replicate was selected and slaughtered to get different samples. Blood samples were collected for analysis of blood biochemistry, antibody titer levels and antioxidant capacity. Jejunum samples were collected for morphometric analysis. Cecal contents were collected to determine the population of different bacteria. Serum was collected from blood samples and stored at –20 °C till further analysis. Serum was analyzed for glucose, total protein, albumin, globulin, triglyceride [15], alanine transaminase (ALT), and aspartate aminotransferase (AST) using the serum analysis kits. The antioxidant capacity of broilers was analyzed through levels of total antioxidant capacity (TAC) and thiobarbituric acid reactive substances (TBA) using ELISA kits (Cayman Chemical Company, MI, USA).

Small intestine was separated and divided into different parts. Jejunum samples were stored in 10% formalin solution after washing with normal saline. Samples were sectioned at 5 µm thickness by using a microtome and stained with hematoxylin-eosin followed by morphometric analysis.

Cecal contents were serially diluted [16] for determination of *Lactobacilli* counts on Rogosa agar (CM 0627) under anaerobic conditions for 48 h at 37 °C. The population of *Clostridium perfringens* was determined by using perfringens agar (CM 0543 OPSP) under anaerobic conditions at 37 °C for 24 h. *Coliforms* were determined on MacConkey agar (CM 0115) incubated aerobically for 24 h at 37 °C.

2.5. Statistical analysis

Data collected for each treatment were analyzed through one-way ANOVA using GLM procedure in SPSS 26. Statistical difference among the treatments was determined by Tukey's test and level of significance was set at $p < 0.05$.

3. Results

3.1. Growth performance

The results showed significant ($p < 0.05$) effect of dietary treatments on weight gain and FCR in broilers during this experiment; however, feed intake was not affected (Table 2). Weight gains in Cont, PC and AN-BE5 were statistically similar but higher ($p = 0.001$) than that of NC. Similarly, FCRs in Cont, PC, and AN-BE5 were statistically similar but better ($p = 0.001$) than that in NC. Highest mortality percentage was observed in the negative control followed by the control group while the lowest mortality was observed in PC.

3.2. Blood biochemistry

Data analysis showed that all parameters of blood biochemistry remained unaffected ($p > 0.05$) by treatments except glucose and triglyceride levels (Table

Table 2. Effect of *A. nilotica* bark extract on growth performance in broilers challenged with *E. coli*.

Parameters	Treatments					SEM	p-value
	Cont	NC	PC	AN-BE3	AN-BE5		
Feed intake (g)	797.62	777.98	801.56	790.25	797.77	4.19	0.053
Weight gain (g)	608.47 [a]	526.72 [b]	620.56 [a]	540.27 [b]	613.99 [a]	20.01	0.001
FCR	1.31 [b]	1.48 [a]	1.29 [b]	1.46 [a]	1.30 [b]	0.04	0.001
Mortality (%)	5.00	15	2.00	4.00	3.00	3.35	

Cont: basal diet only; NC: challenged with *E. coli*, 10^7 CFU/bird; PC: NC+Zinc Bacitracin, 50 mg/kg diet; AN-BE3: NC+*A. nilotica* bark extract 0.3%; AN-BE5: NC+*A. nilotica* bark extract 0.5%. SEM: Standard error of mean. Means with different superscript differ significantly ($p < 0.05$).

3). The highest levels of blood glucose (p = 0.001) were observed in Cont and the lowest in AN-BE5. Levels of triglyceride varied from 103.98 to 119.98 mg/dL in AN-BE5 and PC, respectively. Triglyceride levels of PC, NC, and Cont were statistically similar but higher (p = 0.002) than those of AN-BE3 and AN-BE5, while levels of total protein and globulin were not affected by dietary treatments (p > 0.05).

3.3. Liver enzymes concentration

Effect of treatments on liver health was evaluated by analyzing the levels of Alanine transaminase (ALT) and Aspartate aminotransferase (AST) in blood (Table 4). Significantly (p = 0.001), the highest concentration of ALT was observed in the NC followed by Cont, while the concentrations of AST were statistically similar in NC and Cont but higher than those of the other groups.

3.4. Antioxidant capacity

Concentrations of total antioxidant capacity (TAC) and thiobarbituric acid reactive substance (TARS) were determined in blood serum of broilers to determine their antioxidant capacity (Figure 1). The TAC concentration was significantly higher (p = 0.001) in PC than those of NC and Cont but similar to those of AN-BE3 and AN-BE5. The TARS concentration was not affected by treatments (p > 0.05).

3.5. Intestinal morphology

The results of jejunum morphology are represented in Table 5. Significant (p = 0.001) effect of dietary treatments was observed on villus height while crypt depth and VH:CD were not affected (p > 0.05). Villus heights in PC and AN-BE5 were similar (p > 0.05) but higher (p = 0.001) than those of Cont and NC.

3.6. Cecal bacterial counts

From cecal contents, populations of *Lactobacili*, *Coliform*, and *C. perfringens* were enumerated and are presented in Figure 2. *Lactobacili* counts were not changed (p > 0.05) by treatment. Populations of *Coliform* and *C. perfringens* were significantly (p = 0.001) reduced in PC and *A. nilotica* bark extract supplemented groups. Counts of *Coliform* in NC were reduced in PC and AN-BE5. Similarly, count of *C. perfringens* in NC was 5.94 logCFU/g, which was decreased in PC and in AN-BE5.

4. Discussion

The results suggest that *A. nilotica* bark extract contains some active compounds which protect the broilers under pathological conditions as done by antibiotics. As reported earlier, bark extract of *A. nilotica* contains terpenoids, alkaloids, glycosides, tannins, and saponins that have antimicrobial activities against *E. coli*, and other pathogenic

Table 3. Effect of *A. nilotica* bark extract on blood biochemistry in broilers challenged with *E. coli*.

Parameters	Treatments					SEM	p-value
	Cont	NC	PC	AN-BE3	AN-BE5		
Glucose (mg/dL)	278.06 [a]	232.39 [b]	236.17 [b]	227.77 [b]	225.79 [b]	9.68	0.001
Total protein (g/dL)	2.79	2.57	2.58	2.66	2.76	0.04	0.060
Albumin (g/dL)	1.44	1.42	1.36	1.44	1.36	0.02	0.644
Globulin (g/dL)	1.34	1.15	1.22	1.22	1.40	0.05	0.221
Triglyceride (mg/dL)	116.75 [ab]	115.18 [abc]	119.98 [a]	105.18 [bc]	103.98 [bc]	3.22	0.002

Cont: basal diet only; NC: challenged with *E. coli*, 10^7 CFU/bird; PC: NC+Zinc Bacitracin, 50 mg/kg diet; AN-BE3: NC+*A. nilotica* bark extract 0.3%; AN-BE5: NC+*A. nilotica* bark extract 0.5%. SEM: Standard error of mean. Means with different superscript differ significantly (p < 0.05).

Table 4. Effect of *A. nilotica* bark extract on liver enzymes concentration in broilers challenged with *E. coli*.

Parameters (U/L)	Treatments					SEM	p-value
	Cont	NC	PC	AN-BE3	AN-BE5		
Alanine transaminase	8.99[b]	9.94[a]	6.48[c]	6.50[c]	6.49[c]	0.74	0.001
Aspartate aminotransferase	202.20[a]	213.92[a]	182.89[b]	183.19[b]	182.06[b]	6.48	0.001

Cont: basal diet only; NC: challenged with E. coli, 10^7 CFU/bird; PC: NC+Zinc Bacitracin, 50 mg/kg diet; AN-BE3: NC+*A. nilotica* bark extract 0.3%; AN-BE5: NC+*A. nilotica* bark extract 0.5%. SEM: Standard error of mean. Means with different superscript differ significantly (p < 0.05).

bacteria [10]. In addition, saponins facilitate the absorption of nutrients and medicine while tannins help to prevent the development of microorganisms [11]. All these points suggest that bark extract of *A. nilotica* could support the growth performance in boilers as supported by antibiotics. In addition, Abudabos et al. [17] also stated that extract and phytogenic feed additives obtained from different herbs and spices could support the growth performance in boilers under pathologically challenged or unchallenged conditions [9,18]. Festus et al. [19] stated that extract of *A. nilotica*, *Moringa oleifera*, and wild mushroom could be used to replace antibiotic growth promoters with no negative effect on growth performance in broilers. Similarly, Marimuthu and D'Souza [20] stated that choline chloride could be replaced with herbal mixture of *Curcuma longa* and *A. nilotica* in broilers. A possible reason for improved growth performance might be the enhanced secretion of digestive enzymes which increase the digestion, and availability of the nutrients to the birds [21].

Parameters of blood biochemistry were not affected by dietary treatments except blood glucose and triglyceride levels which were reduced in antibiotic and *A. nilotica* bark extract supplemented groups. In contrast, triglyceride concentration was not affected by using other plant extracts in comparison with antibiotics in a previous study [22]. Toghyani et al. [23] stated that blood protein and albumin levels were slightly increased by supplementation of thyme powder in boilers diet. In addition, it has been proven that *A. nilotica* extract controls blood sugar level in hyperglycemic rats and also maintains normal blood lipid profile [24]. The inconsistency that exists in blood biochemical response in broilers fed different feed additives [7,23,24] might be due to the presence of different types of active compounds in plants extracts, ways of feeding, duration and its concentration, genetic makeup, or experimental design.

The results suggest that liver health was improved by *A. nilotica* bark extract supplementation as done by

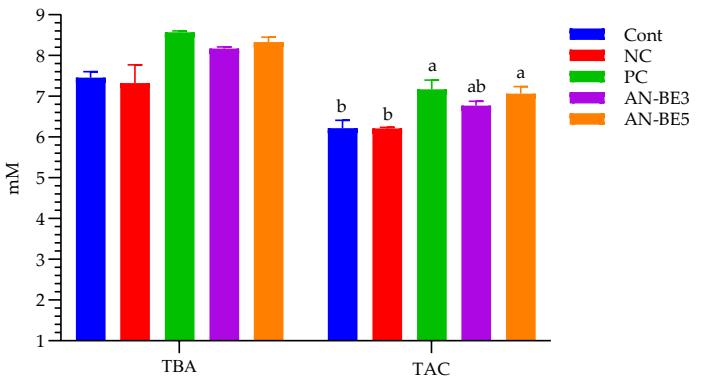

Figure 1. Effect of *A. nilotica* bark extract on antioxidant capacity in broilers challenged with *E. coli*. TBA, thiobarbituric acid reactive substances; TAC, total antioxidant capacity. Cont: basal diet only; NC: challenged with *E. coli*, 10^7 CFU/bird; PC: NC+Zinc Bacitracin, 50 mg/kg diet; AN-BE3: NC+*A. nilotica* bark extract 0.3%; AN-BE5: NC+*A. nilotica* bark extract 0.5%. Bars with different superscript differ significantly ($p < 0.05$).

Table 5. Effect of *A. nilotica* bark extract on intestinal morphology in broilers challenged with *E. coli*.

Parameters	Treatments					SEM	p-value
	Cont	NC	PC	AN-BE3	AN-BE5		
Villus height (µm)	1,026.50 ^{bc}	1,014.75 ^{c}	1,063.25 ^{a}	1,037.96 ^{b}	1,061.63 ^{a}	9.56	0.001
Crypt depth (µm)	155.00	154.75	160.75	156.49	158.94	1.16	0.642
VH:CD	6.62	6.56	6.62	6.66	6.69	0.02	0.981

Cont: basal diet only; NC: challenged with E. coli, 10^7 CFU/bird; PC: NC+Zinc Bacitracin, 50 mg/kg diet; AN-BE3: NC+*A. nilotica* bark extract 0.3%; AN-BE5: NC+*A. nilotica* bark extract 0.5%. SEM: Standard error of mean. Means with different superscript differ significantly ($p < 0.05$).

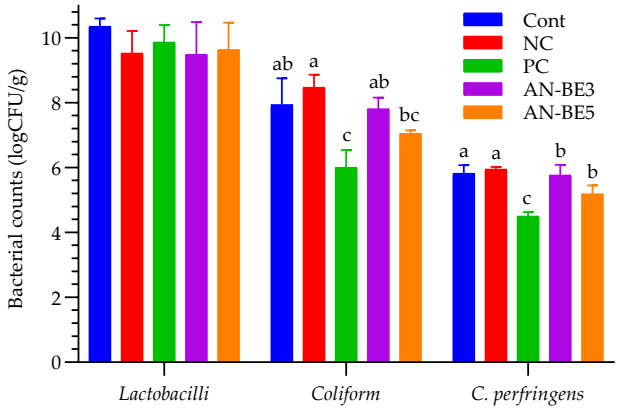

Figure 2. Effect of *A. nilotica* bark extract on cecal bacterial counts in broilers challenged with *E. coli*. Cont: basal diet only; NC: challenged with *E. coli*, 10^7 CFU/bird; PC: NC+Zinc Bacitracin, 50 mg/kg diet; AN-BE3: NC+*A. nilotica* bark extract 0.3%; AN-BE5: NC+*A. nilotica* bark extract 0.5%. Bars with different lowercase letters differ significantly ($p < 0.05$).

antibiotic because serum ALT and AST were reduced in both antibiotic and *A. nilotica* bark extract supplemented groups. Serum ALT and AST are indicators of liver health [25], and their concentrations increase under pathological conditions [26]. *A. nilotica* extract also minimizes the complication of liver and kidney [27]. Other phytogenic feed additives also reduced the serum AST and ALT levels in broilers [28].

In the present experiment, total antioxidant capacity was significantly increased by *A. nilotica* bark extract supplementation in broilers challenged with *E. coli*, which suggested the protective role of *A. nilotica* extract against the reactive species and reduced the oxidative stress in broilers. The results are supported by Amos et al. [29], who suggested that *A. nilotica* could be efficiently used against free radical-mediated diseases including diabetes, inflammation, and cancer. Phenolic compounds present in the extract have hydroxyl groups which have a role in scavenging the free radicals, which might be the reason of improved antioxidant capacity in present study [30].

The results suggest that *A. nilotica* bark extract supplementation significantly enhanced the villus height than control and it was similar to that of antibiotic treated group, while crypt depth and VH:CD were not affected by treatments. Improved gut health might be the reason of improved feed efficiency and better growth rate in the present study. Intestinal microbiota also affects the health and production in broilers. In addition, *A. nilotica* contains different active compounds (terpenoids, alkaloids, glycosides, tannins and saponins) [10], which prevent the development and proliferation of pathogenic bacteria in broilers [11].

From the present study, it could be stated that *A. nilotica* bark extract supports the growth performance in broilers under pathological conditions by improving the antioxidant status and gut health. *A. nilotica* bark extract could be a potential alternative to antibiotic growth promoters in boilers challenged with *E. coli*.

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Conflict of interest
The authors declare no conflicts of interest for this article.

Institutional review board statement
The protocol of this experiment was approved by committee of Ethical Handling of Experimental Birds, University of Veterinary and Animal Sciences, Lahore, Pakistan, for the care and use of experimental birds with approval number: DAS/358, Dated 03/05/2021

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