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Research Article
The Effect of Dietary Binahong [*Anredera cordifolia* (Ten.) Steenis] Leaf Meal Supplementation on Total Ileal Bacteria and Jejunal Histomorphology in Broiler Chickens

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Abstract

Objective: The aim of this study was to determine the effect of Binahong Leaf Meal (BLM) as a feed additive on the ileal bacteria and jejunal histomorphology of broiler chickens. **Methodology:** One hundred and ninety-two day old male broiler chickens were divided into six treatments groups of eight chickens each; each experiment was performed four times. The treatment groups were as follows: T0 (control negative), T1 (control positive; tetracycline 50 ppm), T2 (1% BLM), T3 (2% BLM), T4 (4% BLM) and T5 (8% BLM). **Results:** The addition of BLM significantly increased the amount of *E. coli* ileal bacteria, jejunum height and depth of jejunum crypts across groups T0-T5 ($p<0.01$). Addition of BLM significantly increased the amount of ileal lactic acid bacteria and width of jejunum villi across groups T0-T5 ($p<0.05$). **Conclusion:** The addition of 2% BLM increased the amount of *E. coli* ileal bacteria and ileal Lactic Acid Bacteria (LAB) and increased villi height and width and jejunum crypt depth in broiler chickens.

Key words: Binahong, feed additive, bacteria, histomorphology, broiler

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Antibiotics are used as Antibiotic Growth Promoters (AGP) to maintain health, accelerate growth and improve feed efficiency in the poultry feed industry. However, the use of antibiotics can lead to the emergence of bacterial resistance to antibiotics and the presence of residues in livestock products. Therefore, the Government of Indonesia issued a ban on the use of antibiotics as AGP as regulated by Regulation of Minister of Agriculture No. 14 of 2017 on the Classification of Animal Drugs to control the threat of antimicrobial resistance and to protect consumers from antibiotic residues contained in livestock products. Therefore, there is a need to develop an alternative to the use of antibiotics.

Phytobiotics are dietary supplements derived from active plant compounds added to livestock feed to improve production performance and health of livestock[1]. Phytobiotics can be obtained from leaves, flowers, stems, rhizomes, roots, or any parts of a plant and products may be dried or extracted[2]. The addition of phytobiotics as Natural Growth Promoters (NGPs) has been identified as an alternative to the use of antibiotics. Phytochemicals are divided into several categories, including phenolics and polyphenols, terpenoids and essential oils, alkaloids and lectins and polypeptides[1,3].

Phytobiotics can increase body weight and feed efficiency when used as a feed supplement[4]. They can also increase production performance by inhibiting the growth of pathogenic micro flora in the gut to maintain the balance in the digestive tract that indirectly stimulates the digestive organs and increases the absorption of nutrients in the feed[5,6].

Binahong [*Anredera cordifolia* (Ten.) Steenis] is a plant with medicinal compounds. The stems, leaves, flowers and roots contain several active chemicals, such as phenol, flavonoids, alkaloids, terpenoids, saponins and steroids, that play important roles as antimicrobials[7-11]. Phytobiotics work well as antimicrobials[3]. Flavonoids are the largest group of phenol compounds, which have properties to inhibit the growth of viruses, bacteria and fungi. Flavonoid compounds and derivatives act as antibacterials and can prevent viral infection. The leaf extracts, stems, flowers and tubers of binahong plants contain phenol compounds, flavonoids, saponins, terpenoids, steroids and alkaloids[7]. Leaves of the binahong have secondary metabolite content, including total phenol (85.30 mg kg^{-1}), total flavonoids (47.40 mg kg^{-1}), saponins (66.00 mg kg^{-1}) and alkaloids (2.60 mg kg^{-1})[12].

Binahong leaves may be used as feed additives to improve the gastrointestinal profile of broiler chickens. This study aimed to determine the effect of dietary Binahong Leaf Meal (BLM) on the bacterial count and histomorphology in broiler chickens.

MATERIALS AND METHODS

This study was conducted at the Poultry Production Laboratory. This study used a single feed design and provided feed *ad libitum*. The research feed formulation used is shown in Table 1. The groups included 48 broiler chickens selected at random. Total bacteria *E. coli* and Lactic Acid Bacteria (LAB) and villi height, villi width and crypt depth were measured.

Animals and housing: One hundred and ninety-two DOC (New Lohmann MB 202 strain) male broiler chickens were divided into six treatment groups (eight chickens each) with four replications. The cage used had an open house design with a litter floor (rice husk) and a colony enclosure (1×1 m). There were 24 units equipped with feed and drinking water.

Experimental diets: The treatment groups were as follows: T0 (control negative), T1 (control positive, Tetracycline 50ppm), T2 (1% BLM), T3 (2% BLM), T4 (4% BLM) and T5 (8% BLM) (Table 1).

Data collection: The chickens were raised for 35 days and after 35 days they were slaughtered (two birds per replicate) and samples of small intestines and digesta were taken. The small intestines were kept in formalin for villi height, width and crypt depth measurements, while the digesta were immediately processed for *E. coli* and LAB counts.

Bacteria counts: *Escherichia coli* and ileal LAB were measured. Total bacteria were expressed as colony-forming units (CFU) per g of intestinal contents. Total Plate Count (TPC) is based on How to Test Microbe contamination, Indonesian National Standard, SNI 01-2897-1992[13,14].

Preparation of histology samples: Fresh intestine samples obtained for 2 cm segments for each segment of the small intestine jejunum were fixed in 10% buffered formalin and prepared for hematoxylin-eosin preparations. Each sample cut was hydrated through a series of concentrated alcohols (70, 80 and 90%) were left submerged in the alcohol concentration for 3 min each. Then, the sample was submerged in xylitol and dipped in paraffin. Using microtome samples of thin slices, hematoxylin-eosin staining was performed.

Table 1: Composition and calculation of nutrient content of the experimental feed

	Amount (kg)					
Feed ingredients	T0	T1	T2	T3	T4	T5
Yellow corn	54.50	54.50	54.50	54.50	54.50	53.50
Rice bran	3.00	3.00	3.00	3.00	3.00	2.00
White pollard	3.00	3.00	3.00	3.00	3.00	2.00
SBM	13.50	13.50	13.50	13.50	13.50	12.50
MBM	8.00	8.00	8.00	8.00	8.00	8.00
PMM	9.50	9.50	9.50	9.50	9.50	9.50
Palm oil	3.00	3.00	3.00	3.00	3.00	3.00
DL-Met	0.50	0.50	0.50	0.50	0.50	0.50
L-Lysin HCl	0.50	0.50	0.50	0.50	0.50	0.50
Calcium Phospate	0.50	0.50	0.50	0.50	0.50	0.50
NaCl	0.25	0.25	0.25	0.25	0.25	0.25
Binahong leaf meal	0.00	0.00	1.00	2.00	4.00	8.00
Tetrasiklin	0.00	++	0.00	0.00	0.00	0.00
Filler	4.00	4.00	3.00	2.00	0.00	0.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient content counts (%)						
Metabolizable energy (kcal kg^{-1})	3041.05	3041.05	3061.73	3082.40	3123.76	3091.87
Crude protein	21.24	21.24	21.39	21.54	21.83	21.63
Ether extract	7.60	7.60	7.65	7.70	7.81	7.83
Crude fibre	3.15	3.15	3.23	3.31	3.47	3.55
Calcium	1.48	1.48	1.50	1.51	1.53	1.58
P-available	0.53	0.53	0.53	0.54	0.55	0.56
Lysin	1.40	1.40	1.40	1.40	1.40	1.36
Methionin	0.66	0.66	0.66	0.66	0.66	0.65

SBM: Soybean meal, MBM: Meat bone meal and PBM: Poultry meat bone meal

Histological preparations in glass objects are readily observed and measured. Measurements of villi height and width and crypt depth was performed using an Olympus BX 51 microscope equipped with an Olympus projector at 40 times magnification.

Statistical analysis: Data on total counts of *E. coli* and LAB and jejunal histomorphology (villi height, villi width and crypt depth) were subjected to a one-way ANOVA; $p<0.05$ was considered significant using orthogonal contras[15].

RESULTS AND DISCUSSION

Bacterial digestive tract: *Escherichia coli* and LAB were found in the digestive tract (Table 2).

***Escherichia coli*:** The BLM supplementation significantly increased ileal *E. coli* ($p<0.01$). The T1 group (Tetracycline 50 ppm) had lower *E. coli* (5.56 CFU g^{-1}) ($p<0.05$) compared to the T0 group (negative control) (6.56 CFU g^{-1}). Reducing tetracycline by as much as 50 ppm may inhibit the development of *E. coli* in the ileum. Tetracycline inhibits protein synthesis, thereby disrupting bacterial cell walls.

Table 2: Effect of addition of binahong leaf meal in feed to *Escherichia coli* and Lactic acid bacteria ileum bacteria (CFU g^{-1})

	TPC (CFU g^{-1})	
Treatments[1]	*Escherichia coli*	LAB
T0	6.56±0.52	8.53±0.14
T1	5.54±0.15	8.75±0.23
T2	5.77±0.09	8.57±0.44
T3	5.51±0.09	9.02±0.05
T4	5.40±0.23	8.60±0.08
T5	5.42±0.27	8.58±0.12
SEM	0.48	0.27
p-value[2]	0.005	0.048
	P-value for contrast[2]	
Contrast set	*Escherichia coli*	LAB
T0 Vs T1	0.025	0.045
T1 Vs T2.T3.T4.T5	0.838	0.883
T2 Vs T3.T4 .T5	0.002	0.515
T3 Vs T4.T5	0.352	<0.001
T3 Vs T1	0.682	0.156

[1]T0: Feed (negative control), T1: Feed + Tetracycline 50 ppm (positive control), T2, T3, and T5 are Feed plus 1, 2, 4, and 8% binahong leaf meal, [2]The value of P less than 0.05 shows a marked difference

Ileal *E. coli* did not differ in T1 group (Tetracycline 50 ppm) compared to T2, T3, T4 and T5 groups (1, 2, 4 and 8% BLM). Binahong leaves contain secondary metabolites,

including phenol (85.30 mg kg^{-1}), flavonoids (47.40 mg kg^{-1}), saponins (66.00 mg kg^{-1}) and alkaloids (2.60 mg kg^{-1})[12]. The secondary metabolites can match Tetracycline 50 ppm content to inhibit the development of *E. coli* bacteria by inhibiting the function of bacterial cytoplasm membranes, bacterial cell wall synthesis and nucleic acid synthesis[16]. Inhibition of cell wall synthesis can disrupt cell permeability, which can lead to inhibition of cell growth or cell death[17].

Ileal *E. coli* bacteria in chicken ileum were higher in the T2 group (1% BLM) (5.77 CFU g^{-1}) ($p<0.01$) compared to T3, T4 and T5 groups (2, 4 and 8%) (5.51, 5.40 and 5.42 CFU g^{-1}). Thus, addition of 1% BLM is not sufficient to inhibit ileal *E. coli*. Antibacterial compounds, bacteria type and concentration can influence lethal doses or damage to bacterial cells[18].

Ileal *E. coli* did not differ in T3 group (2% TLM) compared to groups T1, T4 and T5 (Tetracycline 50 ppm, 4% BLM and 8% BLM). The addition of 2% BLM can match Tetracycline 50 ppm and the addition of 4 and 8% BLM can produce relatively equal amounts of *E. coli* bacteria. The BLM used in this study has secondary metabolite content, including flavonoids, saponins, terpenoids, steroids and alkaloids, which have been proven to inhibit *E. coli* bacteria at 2%. At this level, BLM can balance micro flora in the digestive tract because, at higher levels (4% and 8%), LAB ileum is reduced.

Lactic acid bacteria: Addition of BLM increased ileal LAB ($p<005$). Group T1 (Tetracycline 50 ppm) increased LAB by 8.75 CFU g^{-1}, which was higher than that of T0 group (negative control) (8.53 CFU g^{-1}) ($p<0.05$). Therefore, addition of Tetracycline 50 ppm is more effective in inhibiting the development of *E. coli* bacteria than LAB. The mechanism of antibacterial action on the permeability of bacterial cell wall occurs through cell wall damage by inhibiting or altering its formation[17].

The LAB did not differ in T1 group (Tetracycline 50 ppm) (8.7 CFU g^{-1}) compared to groups T2, T3, T4 and T5 (1, 2, 4 and 8% BLM) (8.57 9.02, 8.60 and 8.58 CFU g^{-1}). These data show that BLM has secondary metabolite content, including flavonoids, saponins, terpenoids, steroids and alkaloids, which have active antibacterial activity. The antibacterial mechanism of BLM is believed to be due to different responses between gram-negative and gram-positive bacteria to secondary metabolites. Gram-negative bacteria (*E. coli*) have thicker cell walls than gram-positive bacteria[17]. Gram-positive bacteria become dehydrated after contact with phenolics and their pores shrink, which decreases cell wall permeability and membrane function. In gram-negative bacteria (*E. coli*), phenolics extract lipids from the cell wall and expands pores, which causes cell seepage and increased membrane function due to uncontrolled absorption; this damages the cell wall and eventually the bacterial cells will die.

Ileal LAB was higher in group T3 (2% BLM) (9.02 CFU g^{-1}) ($p<0.01$) compared to groups T2, T4 and T5 (1, 4 and 8% BLM) (8.57, 8.60 and 8.58 CFU g^{-1}). These data indicate that 2% BLM is optimal to increase ileal LAB. Inhibition is caused by the concentration of the tested compound, pH, temperature and microbial properties, including the type and age of bacteria[19].

Jejunal histomorphology: Villi height, villi width and crypt depth are shown in Table 3.

Table 3: Effect of adding binahong leaf meal in feed to villi height, villi width and crypt depth of jejunum (μm)

	Jejunum Histomorphology (μm)		
Treatments[1]	Villi height	Villi width	Crypt depth
T0	1083.42±40.97	149.79±18.63	182.61±14.82
T1	1228.72±81.17	160.39±17.05	208.16±29.36
T2	1184.45±31.75	158.29±23.45	224.85±53.28
T3	1344.61±134.20	202.87±53.55	278.04±29.11
T4	892.74±47.40	141.41±31.34	218.10±10.68
T5	702.91±80.45	124.66±8.22	137.86±10.96
SEM	231.07	35.59	50.40
p-value[2]	<0.001	0.029	<0.001
	p-value for contrast[2]		
Contrast set	Villi height	Villi width	Crypt depth
1. T0 Vs T1	0.029	0.614	0.188
2. T1 Vs T2,T3,T4,T5	0.009	0.828	0.711
3. T2 Vs T3,T4 ,T5	<0.001	0.907	0.651
4. T3 Vs T4,T5	0.002	0.001	0.005
5. T3 Vs T1	0.200	0.054	0.015

[1]T0: Feed (negative control), T1: Feed + Tetracycline 50 ppm (positive control), T2, T3, and T5 are Feed plus 1, 2, 4, and 8% binahong leaf meal, [2]The value of P less than 0.05 shows a marked difference

Villi height: The BLM addition at 1, 2, 4 and 8% increased the height of the jejunum (p<001). The T1 group (tetracycline 50 ppm) had higher jejunum villi (1228.72 μm) (p<0.05) compared with group T0 (negative control) (1083.42 CFU g^{-1}). Addition of Tetracycline 50 ppm in feed is effective in inhibiting the development of ileal *E. coli* (Table 2). Inhibition of pathogenic bacteria in the gastrointestinal tract may affect the gastrointestinal micro flora toward more favorable conditions so that more intestinal villi are protected from damage caused by colonization and pathogenic bacterial infection of the intestinal wall. Colonization and pathogen infection in the intestinal wall may protect intestinal villi from damage[20,21].

There was no difference in jejunum velocity in group T1 (1228.72 μm) compared to groups T2, T3, T4 and T5 (1, 2, 4 and 8% BLM) (1184.45, 1344.61, 892.74 and 702.91 μm). The BLM has secondary metabolite content that can inhibit the development of *E. coli* bacteria (Phase 1 Study). The reduction in *E. coli* due to the addition of BLM and Tetracycline in feed encourages the development of bacterial micro flora in the digestive tract to become more favorable and increase the amount of LAB in the digestive tract so that it will eventually increase the height of the villi. Increasing the height and width of the villi in the digestive tract can increase the production of short chain fatty acids, which play a role in stimulating the proliferation or multiplication of epithelial cells of the small intestine wall[22]. The T2 group (2% BLM) (1344.61 μm) increased the height of villi compared to groups T1, T4 and T5 (1, 4 and 8% BLM) (p<0.01) (1184.45, 892,74 and 702.91 μm). These data show that addition of 2% BLM is optimal to increase the height of villi.

Villi width: The effects of BLM in feed on villi width is shown in Table 3. Addition of 1, 2, 4 and 8 % BLM increased villi width (p<0.05). The villi width of T1 group (Tetracycline 50 ppm) was 149.79 μm; this value was not significantly different from the T0 group (negative control) (160.39 μm) or the other groups (T2: 158.29; T3: 202.87; T4: 141.41; T5: 124.66 μm). These results suggest that a reduction of *E. coli* bacteria, as a result of Tetracycline 50 ppm treatment, did not affect the amount of LAB in the jejunum.

The T3 group (2% BLM) had wider (202.87 μm) villi (p<0.01) compared to the other groups (T2: 158.29 μm; T4: 141.41 μm; T5: 124.66 μm). Therefore, 2% BLM addition does not impact on the balance of ileum micro flora, while 4 and 8% BLM decrease the number of *E. coli* bacteria and LAB in the ileum

Crypt depth: The BLM addition to feed significantly increased crypt depth (p<0.01). There was no difference between the T1 group (50 ppm) (208.16 μm) compared to the T0 group (negative control) (182.61 μm) or other groups (T2: 224.85; T3: 278.04; T4: 218.10; T5: 137.86 μm). These results show that addition of tetracycline 50 ppm did not produce better crypt depth, as there was no effect on the number of LAB on the jejunum.

The T2 group (2% BLM) had a deeper crypt depth (278.04 μm) (p<0.01) compared to groups T1 (208.18 μm), T2 (224.85), T4 (218.10) and T5 (137.86 μm). Addition of 2% BLM improved the histomorphology of the gut, as reflected by villi height, villi width and crypt depth. These results are likely due to the secondary metabolites of BLM, including saponins, total phenol, flavonoids, steroids and alkaloids, which can maintain the balance of gastrointestinal micro flora by inhibiting bacterial development pathogens such as *E. coli* (Table 2). Reduction in infections due to pathogenic bacteria in the gastrointestinal tract may be followed by depletion of the gastric mucosa in the gastrointestinal tract, as goblet cells do not produce excessive mucus and can ultimately disturb the development and extension of the intestinal villi. The goblet cells in the crypt and intestinal villi produce mucus cells that act as layers during pathogenic bacterial infections[23].

CONCLUSION

Addition of 2% BLM to feed can inhibit the development of *E. coli* bacteria, as well as increase the number of LAB and increase villi height, villi width and crypt depth.

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SIGNIFICANCE STATEMENT

This study found that a BLM supplement can be used as an alternative to antibiotics in broiler chicken feed. This study will help researchers better understand the use of BLM as an antibiotic against *E. coli* and LAB to improve the histomorphology of the intestine by increasing villi height, villi width and crypt depth jejunum in broiler chickens.

STATEMENT ON CONFLICTS OF INTEREST

All authors have declared no competing interests.

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