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INTESTINAL PROFILE OF BROILERS FED *AVERRHOA BILIMBI* L.-ACIDIFIED TURMERIC UNDER HIGH STOCKING DENSITY

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Abstract

This study investigated the effects of acidified turmeric powder with Averrhoa bilimbi L. fruit filtrate on gut health in broiler chickens raised under high stocking density. A total of 285 Lohmann strain broiler chicks (14 days old, 370g±9.02g) were randomly assigned to four treatment groups: T0 (normal stocking density, 9 birds/m², basal diet), T1 (high stocking density, 16 birds/m², basal diet), T2 (16 birds/m², basal diet + 1% turmeric powder), and T3 (16 birds/m², basal diet + 1% acidified turmeric powder). At 37 days of age, ileal and cecal digesta samples were collected for bacterial analyze, and intestinal pH was measured. In the ileum, T3 showed reduced coliform counts ($p < 0.01$) compared to control, while lactic acid bacteria (LAB) was decreased in all treatment groups. Enterobacteriaceae counts did not differ significantly among treatments. Cecal bacterial populations were not significantly affected by the treatments. Intestinal pH values in the duodenum, jejunum, ileum, and cecum did not differ significantly among treatments. Acidified turmeric powder effectively recuce harmfull coliform while preserving the overall microbial balance reared high density. This findings highlight its potential as a alternative feed to promote gut health in intensive broiler production system.

Keyword: Broiler, *Averrhoa bilimbi L.*, **turmeric**, stocking density, gut health

INTRODUCTION

Broiler meat represents a significant proportion of global animal protein intake, with poultry contributing approximately 35–40% of total meat consumption worldwide (Lestingi et al., 2024). To meet this increasing demand, intensive production systems involving high stocking densities have become common. However, overcrowding can induce physiological stress, impair gut function, and increase the risk of enteric diseases, ultimately compromising growth (Dai et al., 2022). The ban on antibiotic growth promoters (AGPs) due to antimicrobial resistance concerns has further complicated broiler management, leading to reduced feed efficiency and growth rates (Zhu et al., 2021; Pratama et al., 2021). Consequently, there is an urgent need for natural alternatives to AGPs to maintain gut health and support optimal performance in broilers raised under intensive conditions.

Curcumin, the principal bioactive compound in turmeric (*Curcuma longa*), has attracted attention as a phytogenic feed additive (Akbar et al., 2016). It possesses antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory properties, which are beneficial for poultry health (Pan et al., 2022). Several studies have shown that dietary curcumin improves growth performance, gut morphology, and antioxidant capacity of broilers (Bondar et al., 2023; Hafez et al., 2022). However, its practical application is limited by poor water solubility and low bioavailability. Curcumin is relatively stable in acidic environments, which suggests that acidification may enhance its solubility and functional efficacy in the gastrointestinal tract (Mohanty et al., 2012).

A. bilimbi L., commonly known as bilimbi, is a tropical fruit known for its extremely low pH and high concentrations of organic acids such as citric, oxalic, and lactic acids (Pratama et al. 2021; Sugiharto et al., 2021). It also contains various bioactive compounds with antimicrobial activity, including flavonoids, vitamin c, and phenolic acids. Previous studies have shown that *A. bilimbi* L. can lower the intestinal pH, inhibit pathogenic bacteria, and support the growth of beneficial lactic acid bacteria (LAB) in broilers (Pratama et al., 2022). Therefore, acidifying curcumin with the *A. bilimbi* extract may increase its bioavailability and enhance its efficacy as a functional feed additive. Although both curcumin and A. bilimbi L. have been individually reported to improved gut health and microbial balance in broilers, limited information is available regarding their combined application, particularly under high stocking density conditions that predispose bird to physiological and intestinal stress. Regarding the synergistic potential between organic acid rich bilimbi filtrate and curcumin is essential to develop an effective natural alternative to antibiotic growth promoters in intensive broiler production system. This study aimed to evaluate the effects of acidified curcumin using *A. bilimbi* L. fruit filtrate on gut health and microbial population balance in broiler chickens raised under high stocking density.

MATERIAL AND METHODS

Preparation Acidified Turmeric

Ripe *A. bilimbi* L. fruits were collected from a garden near the campuss. *A. bilimbi* L. fruits were washed under running water, drained, blended without added water, and filtered through cheesecloth to obtain the fruit filtrate. Commercial turmeric powder was purchased from a local market in Semarang, Central Java, Indonesia. Acidified turmeric was prepared following Pratama et al. (2022) by mixing turmeric powder with *A. bilimbi* filtrate at a ratio of 1:3 (g:mL), incubating anaerobically at room temperature (±25°C) for 4 days, and subsequently sun-drying the mixture.

The pH was measured using a portable pH meter (OHAUS ST300), total acidity was determined by titration, antioxidant activity was evaluated using the DPPH assay (expressed as IC_{50}), and total phenolic content was analyzed using the Folin–Ciocalteu method.

Turmeric powder had a pH of 5.90, total acidity of 2.34%, antioxidant activity of 70.9 ppm, and total phenols of 3.47%. After acidification, the pH decreased to 3.70, total acidity increased to 6.41%, antioxidant activity reached 81.2 ppm, and total phenols were 3.35%.

In Vivo Experiment

The in-vivo experiment was approved by the Animal Ethics Committee, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro (No. 57-01/A-2/KEP-FPP). A total 285 broiler *Lohmann* MB 202, each 14 days old and weighing an average of 370±9.02 g (mean±standard deviation), were used in a completely randomized design. Before the experimental treatments began, the chicks were reared under standard management conditions and fed a commercial pre-starter diet containing 23% crude protein, 5% crude fat, 5% crude fiber, and 7% ash (according to label of feed) from day 1 to day 14. On the day 14, the chicks were randomly divided into four treatment groups, each with five replicates.

According to Mocz et al. (2022), the stocking density of broiler chickens ranges from 31 to 41 kg/m² or 13-18 birds/m². The treatment groups were as follows T0: chicks kept at a normal stocking density of 9 birds/m² and fed a basal diet; T1: chicks kept at a higher stocking density of 16 birds/m² and fed a basal diet; T2: chicks kept at 16 birds/m² and fed a basal diet supplemented with 1% turmeric powder; and T3:, chicks kept at 16 birds/m² and fed a basal diet supplemented with 1% acidified turmeric powder. The chicks were housed in open-sided cages with rice husk litter, and each pen was equipped with feeders and drinkers. The basal diet for the experiment was formulated in accordance with the Indonesian National Broiler Feed Standards. All groups received the basal diet with the turmeric powder or acidified turmeric powder treatments were applied on top of the feed from day 15 until harvest at day 37, coinciding with the stocking density challenge. Vaccination with Newcastle disease vaccine (NDV) was administered on days 4 and 18 of age, while vaccination against infectious bursal disease was provided via drinking water on day 12 of age. The ingredient composition and chemical composition of the finisher diets are presented in Table 1.

At 37 days of age, the birds were slaughtered. Digesta samples from the ileum and cecum were collected to quantify selected bacterial populations. Additionally, the pH values of the duodenum, jejunum, ileum, and cecum were measured using a digital pH meter (OHAUS ST300). The enumeration of lactic acid bacteria (LAB) was conducted using De Man, Rogosa, and Sharpe (MRS) agar (Merck KGaA, Darmstadt, Germany), with the samples incubated under anaerobic conditions at 38°C for 48 hours. The population of coliform, lactose-negative Enterobacteriaceae (LNE), and total Enterobacteriac were assessed using MacConkey agar (Merck KGaA, Darmstadt, Germany) following aerobic incubation at 38°C for 24 hours.

Table 1. Ingredients and Chemical Composition of Finisher Diets

Item	(%)
Corn	57,95
Soybean meal	34,80
Palm oil	3.50
DL-methionine, 990 gm	0.19
Bentonite	0.75
Premix[a]	0.34
Monocalcium phosphate (MCP)	1
Chlorine chloride	0.07
Salt	0.40
Limestone	1
Chemicals content	
Crude protein	20.0
Crude fiber	5.48
ME (kcal/kg)[b]	3,029
Ca	1.02
P (available)	0.52

[a]Premix composition on each kg of diet: 1.10 gm Zn, 1.0 gm Mn, 0.85 gm Fe, 75 mg Cu, 4 mg Se, 6 mg Co, 19 mg I, 1.23 gm K, 1.23 gm Mg, 1,250,000 IU vit A, 1.35 gm pantothenic acid, 250,000 IU vit D_3, 1.88 gm vit E, 250 mg vit K_3, 250 mg vit B_1, 750 mg vit B_2, 500 mg vit B_6, 2.5 gm vit B_{12}, 5.0 gm niacin, 125 gm folic acid, and 2.5 mg biotin.

[b]Metabolizable energy (ME) was calculated using the formula: 40.81(0.87 (crude protein + 2.25 crude fat + nitrogen-free extract) + 2.5))

Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) with SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA). When significant differences among treatments were identified ($p < 0.05$), further comparisons were conducted using Duncan's multiple range test to determine specific differences between treatment means.

RESULT AND DISCUSSION

Intestinal Bacteria Profile

In the ileum, T3 showed a reduction in coliform counts ($p < 0.01$) compared to the control group (T0). Coliform and Enterobacteriaceae counts tended to decrease in treatment compared than control. Total Enterobacteriaceae counts showed no significant differences ($p > 0.05$). In the cecum, LAB, coliform and specific bacteria counts were not significantly different ($p > 0.05$). The intestinal bacterial populations of broilers are presented in Table 2.

Table 2. Bacteria Intestine of Broiler

Item	T0	T1	T2	T3	*p-v*alue	SEM
Ileum						
Coliform	6.34	5.26	5.60	5.26	0.05	1.66
LAB	10.77[a]	9.86[b]	7.90[c]	7.88[d]	<0.01	0.35
LNE	5.95	5.26	5.79	5.26	0.37	0.17
Enterobacteriaceae	6.47	5.26	5.80	5.26	0.10	0.20
Caecum						
Coliform	5.78	5.75	5.26	6.50	0.44	0.26
LAB	10.48	10.85	10.28	10.72	0.60	1.51
LNE	5.26	6.62	5.26	6.30	0.10	0.25
Enterobacteriaceae	5.82	6.66	5.26	7.09	0.14	0.31

SEM, standard error of the mean; LAB, lactic acid bacteria; LNE, lactose-negative Enterobacteriaceae.

Intestinal pH Profil

In the duodenum, jejunum, ileum, and cecum, the pH values did not differ significantly ($p > 0.05$) among treatments. The intestinal pH of broilers is presented in Table 3.

Table 3. pH Intestine of Broiler

Item	T0	T1	T2	T3	*p v*alue	SEM
Duodenum	5.97	5.89	6.21	5.99	0.11	0.09
Jejenum	5.87	5.12	5.26	5.49	0.96	5.11
Ileum	5.27	5.42	5.33	5.23	0.92	0.96
Caecum	7.16	6.95	7.14	6.79	0.55	0.10

SEM, standard error of the mean; LAB, lactic acid bacteria; LNE, lactose-negative Enterobacteriaceae.

The administration of acidified turmeric powder influenced the gut microbiota profiles in broiler chickens. In the ileum, showed a reduction in coliform counts, indicating a potential antimicrobial effect of acidified turmeric against pathogenic bacteria such coliform (Dai et al., 2022). This reduction can be attributed to the increased acidity and enhanced phytogenic compound bioavailability in acidified turmeric, consistent with turmeric's documented antimicrobial properties (Hettiarachchi et al., 2021; Sohn et al., 2021). However, LAB counts in the ileum were also lower in tretatment compared to control, suggesting that while acidified turmeric effectively suppresses coliform populations, it does not selectively promote LAB proliferation in the ileum under the tested conditions. The population of LAB in the ileum may be smaller compared to the control group due to the challenging environment of the gastrointestinal tract. Despite their reduced presence, these LAB are still capable of offering substantial health benefits through their metabolic processes (Quinto et al., 2014). Enterobacteriaceae counts were observed no significant differences across treatments, indicating that the acidified turmeric did not induce overgrowth or significant suppression of these bacterial populations. The complex pharmacological characteristics of curcumin, which are affected by its poor bioavailability, are highlighted by the bioactive breakdown and microbial metabolism products of curcumin, along with their regulatory impacts on gut microbiota (Kumar et al., 2017; Shen et al., 2017).

In the caecum, coliform, LAB, and Enterobacteriaceae counts did not differ significantly among the treatments. This may reflect the complex and stable microbial ecosystem of the caecum, which can buffer against dietary interventions, especially over the relatively short experimental period (Savino, et al., 2016). Overall, the administration of acidified turmeric powder under high stocking density conditions resulted in a balanced modulation of gut microbiota, reducing potentially pathogenic coliforms without significantly disrupting the overall microbial equilibrium in the ileum and caecum.

Measurements of gastrointestinal pH revealed no significant differences among treatments in the duodenum, jejunum, ileum, or caecum. Although acidified turmeric powder had a low pH (3.70), this did not translate into a significant reduction in gut pH in vivo. This finding contrasts with some reports indicating dietary organic acids can lower intestinal pH in broilers (Saleem et al., 2020; Paul et al., 2007), which may be due to physiological buffering mechanisms in the gut, including pancreatic and biliary secretions, that stabilize luminal pH despite acidified feed intake (Ma et al., 2021). Moreover, high stocking density may cause physiological stress and digestive organ dysfunction, reducing nutrient absorption(Li et al., 2024). Under these conditions, lactic acid bacteria (LAB) did not grow well, possibly due to the sensitivity of LAB to curcumin (Kai et al., 2020). Although curcumin has antimicrobial properties, it may also suppress beneficial microbes like LAB, limiting their function in gut health. These results highlight the complexity of dietary interventions. While phytobiotics and acidified feed can offer benefits, their effects on gut microbiota require further investigation.

CONCLUTION

Averrhoa bilimbi L. acidified turmeric was also able to modulate intestinal microbiota in broiler under high stocking density reducing coliform population in the ileal segmen without distrupting overall microbial balance. However, it did not significantly affect intestinal PH.

These findings indicated that acidified turmeric has potential as a natural alternative antibiotic growth promoters, particularly through its antimicrobial and phytogenic effect under stress conditions. Further studies are needed to optimize its application and evaluate long-term impact on broiler performance

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CONFLICT OF INTEREST

No potential conflicts of interest relevant to this article are reported.

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