



# Evaluation of Body Weight and Intestinal Health in Broiler Chickens Supplemented with Encapsulated *Morinda citrifolia* L. Extract, Zinc, and Copper

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## ABSTRACT

The broiler poultry sector is strategically important, serving as a major contributor to animal protein supply and an essential component of national food security. The present study aimed to examine the effects of dietary supplementation of encapsulated noni fruit (*Morinda citrifolia* L.) extract (ENFE) enriched with zinc (Zn) and copper (Cu) on intestinal health and body weight in broiler chickens. A total of 200 eight-day-old broiler chickens, with an initial weight of  $233.69 \pm 7.28$  grams, were assigned to four treatments using a completely randomized design, with five replications per treatment. The treatment groups included a control group with a basal diet (T0), a basal diet with 0.06% ENFE, Zn at 40 ppm and Cu at 5 ppm (T1), a basal diet with 0.12% ENFE, Zn at 40 ppm and Cu at 5 ppm (T2), and a basal diet with 0.18% (T3) ENFE, Zn at 40 ppm and Cu at 5 ppm (T3). The body weight gain (BWG), counts of total lactic acid bacteria (LAB), coliform bacteria, pH value, villus height, crypt depth, relative length, and weight of each segment of the small intestine were assessed. The current results indicated that chickens in 0.12% (T2) and 0.18% (T3) significantly enhanced BWG, LAB populations, villus height, crypt depth, and length, and relative weight of the duodenum, jejunum, and ileum. Additionally, reduced duodenal and ileal pH, as well as coliform counts across all intestinal segments, were observed in 0.12% (T2) and 0.18% (T3). However, ENFE supplemented with Zn and Cu did not significantly affect jejunal pH. Administering ENFE at 0.12% supplemented with Zn and Cu represented the most optimal dose. Although the higher dose of 0.18% (T3) yielded statistically comparable results and was superior in some parameters, the 0.12% (T2) inclusion level is recommended as the most practical dose for enhancing BWG and intestinal health in broiler chickens.

**Keywords:** Body weight gain, Broiler chicken, Encapsulation, Intestinal morphology, *Morinda citrifolia*

## INTRODUCTION

Poultry farming, especially broiler chicken production, is crucial for providing animal protein and supports national food security. Nonetheless, successful production depends heavily on health, genetics, management practices, and on maintaining low mortality rates (Khan et al., 2023). Meanwhile, susceptibility to diseases, particularly gastrointestinal infections caused by pathogenic bacteria, can decrease productivity (Hashem et al., 2022).

Providing high-quality feed and incorporating herbal supplements, such as phytobiotics, are strategies to maintain gut health. Phytobiotics possess antimicrobial properties that may prevent infection and enhance the efficiency of the digestive system (Chodkowska et al.,

2022). The mechanism of action of antimicrobials in phytobiotics involves disrupting the cell membranes of harmful bacteria, thereby stopping the spread of disease.

Noni fruit (*Morinda citrifolia* L.) has the potential to serve as a source of phytochemicals due to its flavonoid compounds, which possess antibacterial and antioxidant properties (Sunder et al., 2016). Flavonoids in noni fruit can improve intestinal morphology, maintain microbiota balance, and support nutrient absorption efficiency, all of which positively affect the growth of broiler chickens (Song et al., 2022). To obtain beneficial active compounds, such as flavonoids, and to reduce antinutritional factors in noni fruit, extraction is an effective method (Widjastuti et al., 2025). The active compounds in noni fruit are unstable and easily degraded

due to the presence of short-chain unsaturated organic acids. Therefore, the addition of microminerals such as zinc (Zn) and copper (Cu) is necessary to protect these active compounds and maintain their utilization, especially in broiler chickens' intestine (Widjastuti et al., 2023). On the other hand, the addition of Zn and Cu minerals to noni extract supplementation can enhance intestinal morphology and maintain a balanced microbiota. Zinc is known to improve the histomorphometry characteristics of the small intestine, such as villus height, indicating an increased capacity for nutrient absorption (Dogra et al., 2023). Meanwhile, Cu can enhance intestinal morphology by increasing the small intestine's weight and length and by improving the beneficial microbiota composition, thereby supporting greater nutrient absorption (Nguyen et al., 2022). Bioactive compounds with natural antioxidant activity, such as flavonoids in noni fruit, are generally unstable under heat and oxidative conditions (Tanwiriah et al., 2024). To overcome this instability, the noni fruit extract is prepared using an encapsulation technique supplemented with Zn and Cu. Encapsulation technology is a method of coating active compounds to increase stability and bioavailability, and controlled secretion in target tissues (Temiz and Öztürk, 2018). In the present study, maltodextrin was used as a coating material due to its hygroscopic nature, which allows for a gradual release of active compounds, thereby enhancing their absorption and effectiveness in broiler intestinal tracts (Febrianta et al., 2022). The present study aimed to evaluate the effects of supplementing broiler chickens' diet with encapsulated *Morinda citrifolia* L. extract, Zn, and Cu on body weight and intestinal health.

## MATERIALS AND METHODS

### Ethical approval

All procedures involving animals were approved ethically by the Faculty of Animal and Agricultural Science, Diponegoro University, Semarang, Indonesia (Approval No. 61-10/A-30/KEP-FPP).

### Experimental design

The present study was conducted from August to September 2024 at the poultry farm of the Faculty of Animal and Agricultural Sciences, Diponegoro University, Indonesia. A total of 200 eight-day-old Ross 308 broiler chickens, with a mean weight of  $233.69 \pm 7.28$  g, were used for the present study. The stocking density was 10 chickens/m<sup>2</sup>, with each of the 20 pens ( $1 \times 1$  m<sup>2</sup>) housing 10 chickens. Temperature and humidity in the cages during the rearing period were between 28 and 32 °C, with humidity ranging from 46 to 59%. A completely

randomized design with four treatments was used. Each treatment was replicated five times, with ten broiler chickens per replicate. The treatment dose of noni fruit extract was determined according to the modified study by Krismiyanto et al. (2023), while the mineral doses for Zn and Cu were chosen based on the recommendations of the NRC (1994). The treatment groups included a control group receiving the basal diet (T0). The second group which received the basal diet supplementing with 0.06% noni fruit (*Morinda citrifolia* L.) extract (ENFE), Zn at 40 ppm and Cu at 5 ppm (T1), the third group was given 0.12% ENFE with Zn at 40 ppm and Cu at 5 ppm (T2), and the fourth group was administered 0.18% ENFE with Zn at 40 ppm and Cu at 5 ppm (T3). Following the method of Anjani et al. (2025), chickens were given adaptive feed until they reached 7 days old, comprising a mixture of commercial feed (B-11S, Charoen Pokhand, Indonesia) and experimental feed, with its composition gradually changed. The feed proportions on days 1-2 were 75% commercial feed and 25% experimental feed, on days 3-4, 50% commercial feed and 50% experimental feed, and on days 5-6, 25% commercial feed and 75% experimental feed. On day 7, all chickens were given the full treatment feed, and from day 8 to day 35, their diet was controlled as per the predetermined plan treatment. Vaccination was carried out on 3-day-old chickens using Newcastle Disease and Infectious Bronchitis vaccines (Medivac ND-IB, Medion, Bandung, Indonesia) administered via eye drops. Drinking water was given *ad libitum*. The composition of the diet ingredients is shown in Table 1.

**Table 1.** Composition of ingredients and nutritional content of diets for starter and finisher periods

Feed ingredients	Composition (%)	
	Starter (8-21 days)	Finisher (22-35 days)
Yellow corn	50.11	53.41
Rice bran	15.04	16.74
Soybean meal	24.00	19.00
Fish meal	10.00	10.00
Limestone	0.30	0.30
Premix	0.25	0.25
Lisin	0.10	0.10
Metionin	0.20	0.20
Total	100.00	100.00
<b>Nutrient content</b>		
Metabolic energy (kcal/g) <sup>2</sup>	2993.57	3018.24
Crude protein (%) <sup>1</sup>	21.22	19.33
Crude fat (%) <sup>1</sup>	4.45	4.59
Crude fiber (%) <sup>1</sup>	5.24	5.51
Calcium (%) <sup>1</sup>	1.04	1.07
Phosphorus (%) <sup>1</sup>	0.61	0.74

Note: <sup>1</sup>Result of Analysis of Animal Nutrition and Feed Science Laboratory, Faculty of Animal and Agricultural Sciences, University Diponegoro, Semarang (2024). <sup>2</sup>Based on the Bolton formula, Bolton (1967) as follows,  $40.81 (0.87 [\text{crude protein} + 2.25 \times \text{crude fat} + \text{nitrogen-free extract}] + 2.5)$ .

### Treatment preparation

Noni fruit was collected from Semarang City, Central Java, Indonesia. Noni fruit was dried in an oven (Binder ED56, Germany) at 50°C and ground into a fine powder. The extraction procedure was carried out according to the guidelines of Gouda et al. (2021). Noni fruit flour was dissolved in 96% ethanol at a 1:10 (w/v) ratio and stirred until homogeneous. The noni fruit and ethanol mixture was sonicated for 60 minutes at a controlled temperature of 37°C using a sonicator (Power Sonic 405, South Korea) set at 50 Hz. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was then evaporated in a rotary evaporator (RV 10, Germany) to obtain a thick extract. This extract was supplemented with 40 ppm Zn and 5 ppm Cu, which are considered microminerals (Tanwiriah et al., 2024).

The encapsulation technique was based on the method suggested by Agusetyaningsih et al. (2022). Maltodextrin as a coating material was dissolved in distilled water at a 1:3 (w/v) ratio. The noni fruit extract, supplemented with 40 ppm Zn and 5 ppm Cu, was then added to the maltodextrin solution at a 1:5 (v/v) ratio. The mixture was dried using a freeze dryer (Sp VirTis, USA) at -50°C. This process produced a crystal powder containing the encapsulated noni fruit extract, Zn, and Cu. The encapsulation efficiency was evaluated *in vitro* using an antimicrobial inhibition assay and antioxidant assays to verify whether the noni fruit extract, Zn, and Cu were successfully incorporated into the encapsulated matrix.

### Total intestinal bacteria and pH value

Total intestinal bacterial measurements involved collecting 2 cm of digesta from each small intestine segment, including the duodenum, jejunum, and ileum. The total counts of lactic acid bacteria (LAB) and coliform were determined using the total plate count method. Lactic acid bacteria were cultured on de Man Rogosa Sharpe agar (MRS), whereas coliform was cultured on MacConkey agar. Bacterial counts were determined after 48 hours of incubation at 44°C for LAB and 24 hours at 37°C for coliforms (Pratama et al., 2017). Results were expressed as log<sub>10</sub> colony-forming units (CFU) per gram. The pH was determined in three segments of the small intestine (duodenum, jejunum, and ileum) by using a pH meter (Eco Testr pH 1, Singapore).

### Villi height and crypt depth

The duodenum, jejunum, and ileum were cut into 3 cm segments and immersed in 10% buffered formalin for 24 hours. Tissues were processed, stained with

hematoxylin and eosin, and examined under a microscope (Binokuler Leica DM500<sup>®</sup>, Heerbrugg, Switzerland) using a 40x magnification objective lens connected to a screen and computer (Sohel et al., 2019).

### Length and weight

The small intestine was separated into the duodenum, jejunum, and ileum segments. The length of each segment was measured using a measuring tape, while the weight of each segment was measured using an analytical scale with an accuracy of 0.0001 g. Then, the relative length was calculated using the formula by Abdel-Kafy et al. (2022), and the relative weight was calculated using the formula by Rotiah et al. (2018).

$$\text{Relative length (\%)} = \frac{\text{length of the small intestine (g)}}{\text{weight life (g)}} \times 100\%$$

$$\text{Relative weight (\%)} = \frac{\text{weight of the small intestine (g)}}{\text{weight life (g)}} \times 100\%$$

### Body weight gain

Body weight gain (BWG) was determined by subtracting the initial body weight at 8 days of age and the final body weight, following the calculation method described by Jie et al. (2024).

$$\text{BWG} = \text{Final weight} - \text{initial weight}$$

### Statistical analysis

Data were analyzed using SPSS version 22.0. The data were evaluated for normality and homogeneity and were then analyzed using analysis of variance (ANOVA). When the one-way ANOVA revealed significant differences ( $p < 0.05$ ), the means were further compared using Duncan's Multiple Range Test (DMRT).

## RESULTS AND DISCUSSION

### Body weight

Encapsulation of ENFE supplemented with Zn and Cu had a significant effect on increasing the BWG in broiler chickens ( $p < 0.05$ ). The 0.06% (T1) level of ENFE did not differ from the control group (T0), whereas both 0.12% (T2) and 0.18% (T3) levels significantly increased BWG compared to control group (T0) and 0.06% (T1;  $P < 0.05$ ), with no significant difference between 0.12% (T2) and 0.18% (T3;  $P > 0.05$ ; Table 2).

There was no significant difference between control group (T0) and 0.06% (T1) in BWG (Table 2), consistent with the gut microbiota response pattern at the 0.06% (T1) ENFE level (Table 3). In the segments of the duodenum, jejunum, and ileum, 0.06% (T1) demonstrated an increase

in LAB counts and a decrease in coliforms. However, the variations were not statistically significant enough to distinguish from the control group (T0;  $p > 0.05$ ). The present finding indicated that the 0.06% (T1) ENFE level might not have been high enough to induce significant changes in the microbiota and intestinal environment that could impact growth performance. Furthermore, the intestinal pH in 0.06% (T1) was close to the control group (T0) pH value, suggesting that the modulation of the luminal environment had not yet been optimized at this dose. Conversely, levels of 0.12% (T2) and 0.18% (T3) provided a significant increase in BWG compared to control group (T0) and 0.06 (T1;  $p < 0.05$ ). Although no significant difference was observed between 0.12% (T2) and 0.18% (T3;  $p > 0.05$ ), BWG increased gradually with increasing ENFE inclusion levels. The mentioned pattern suggested a dose-responsive physiological adaptation rather than a strict threshold effect, with 0.18% (T3) demonstrating the highest numerical values. The observed improvement could result from the antioxidant, antimicrobial, and metabolic activities of noni fruit extract

bioactive compounds, especially flavonoids, interacting with Zn and Cu.

Flavonoids present in noni fruit extract were bioactive compounds with antioxidant and antimicrobial properties that might contribute to improved growth performance by enhancing gut health and nutrient utilization. According to Kouvedaki *et al.* (2024), the presence of natural antioxidants promotes healthier digestive processes, strengthens the immune system, and increases the efficiency of nutrient absorption from the feed. The antioxidant and antimicrobial components in the flavonoids might have contributed to greater growth performance in broiler chickens. Although other factors, such as initial weight, can also affect weight gain.

Zinc stimulates increased appetite by regulating hormones (El-Hack *et al.*, 2017). Meanwhile, Cu contributes to the antioxidant mechanism by activating the superoxide dismutase (SOD) enzyme, which protects cells from oxidative damage, thereby strengthening the immune system, enhancing nutrient digestion, and promoting growth (Sharif *et al.*, 2021).

**Table 2.** Body weight gain in broiler chickens fed diets with encapsulated noni fruit extract supplemented with zinc and copper for four weeks

Treatments	T0	T1	T2	T3	P-value
Initial weight	227.38 ± 3.39	228.22 ± 3.23	235.12 ± 1.02	244.02 ± 4.35	0.436
Final weight	1902.67 ± 37.09 <sup>b</sup>	1929.98 ± 52.47 <sup>b</sup>	2011.82 ± 41.21 <sup>a</sup>	2030.50 ± 67.39 <sup>a</sup>	0.002
Body weight gain	1675.29 ± 36.57 <sup>b</sup>	1701.76 <sup>b</sup> ± 49.95	1776.70 ± 41.00 <sup>a</sup>	1786.48 ± 69.40 <sup>a</sup>	0.007

Note: <sup>a, b, and c</sup> Different superscript letters in the same row significantly differ ( $p < 0.05$ ). T0: Basal diet; T1: Basal diet + 0.06% ENFE supplemented with Zn, Cu; T2: Basal diet + 0.12% ENFE supplemented with Zn and Cu; T3: Basal diet + 0.18% ENFE supplemented with Zn and Cu.

**Table 3.** Total lactic acid bacteria, coliform, and pH value in intestinal segments of broiler chicken fed diets with encapsulated noni fruit extract supplemented with zinc and copper for four weeks

Treatments	T0	T1	T2	T3	P-value
<b>Duodenum</b>					
LAB (log cfu/g)	5.93 ± 0.45 <sup>b</sup>	6.09 ± 0.31 <sup>ab</sup>	6.50 ± 0.19 <sup>a</sup>	6.50 ± 0.21 <sup>a</sup>	0.021
Coliform (log cfu/g)	4.05 ± 0.40 <sup>b</sup>	3.91 ± 0.31 <sup>b</sup>	3.38 ± 0.27 <sup>a</sup>	3.37 ± 0.28 <sup>a</sup>	0.006
pH	6.21 ± 0.37 <sup>c</sup>	5.86 ± 0.04 <sup>b</sup>	5.59 ± 0.06 <sup>a</sup>	5.48 ± 0.05 <sup>a</sup>	< 0.001
<b>Jejunum</b>					
LAB (log cfu/g)	6.66 ± 0.40 <sup>b</sup>	6.83 ± 0.33 <sup>b</sup>	7.40 ± 0.35 <sup>a</sup>	7.47 ± 0.31 <sup>a</sup>	0.004
Coliform (log cfu/g)	4.83 ± 0.09 <sup>b</sup>	4.68 ± 0.13 <sup>b</sup>	4.05 ± 0.41 <sup>a</sup>	4.05 ± 0.47 <sup>a</sup>	0.002
pH	6.66 ± 0.30	6.43 ± 0.20	6.38 ± 0.17	6.38 ± 0.15	0.162
<b>Ileum</b>					
LAB (log cfu/g)	7.67 ± 0.22 <sup>b</sup>	7.96 ± 0.52 <sup>a</sup>	8.61 ± 0.42 <sup>a</sup>	9.05 ± 0.38 <sup>a</sup>	0.000
Coliform (log cfu/g)	4.88 ± 0.07 <sup>b</sup>	4.69 ± 0.24 <sup>b</sup>	4.54 ± 0.28 <sup>ab</sup>	4.33 ± 0.35 <sup>a</sup>	0.024
pH	6.92 ± 0.16 <sup>b</sup>	6.85 ± 0.04 <sup>ab</sup>	6.73 ± 0.17 <sup>a</sup>	6.70 ± 0.10 <sup>a</sup>	0.049

Note: <sup>a, b, and c</sup> Different superscript letters in the same row significantly differ ( $p < 0.05$ ). T0: Basal diet; T1: Basal diet + 0.06% ENFE supplemented with Zn, Cu; T2: Basal diet + 0.12% ENFE supplemented with Zn and Cu; T3: Basal diet + 0.18% ENFE supplemented with Zn and Cu; LAB: Lactic Acid Bacteria.

### Lactic acid bacteria, coliform, and potential hydrogen (pH)

Supplementing the broiler diet with ENFE, Zn, and Cu significantly affected LAB populations ( $p < 0.05$ ). A consistent increase in LAB population was observed in 0.12% (T2) and 0.18% (T3) compared to 0.06% (T1) and control group (T0; Table 3), although no significant difference was observed between 0.12% (T2) and 0.18% (T3;  $p > 0.05$ ). The mentioned response indicated that the inclusion levels of ENFE at 0.06% (T1) were correlated with a beneficial modulation of the intestinal microbiota population. In the ileum segment, an increase in LAB population was already evident at 0.06% (T1) ENFE, which was not significantly different from 0.12% (T2) and 0.18% (T3;  $P > 0.05$ ). Nevertheless, a gradual increase with higher ENFE levels was observed, with 0.18% (T3) exhibiting the highest numerical value among treatments. The higher LAB population observed at increased ENFE levels was likely related to the selective antimicrobial activity of flavonoids, as reported in previous studies. Flavonoids can inhibit bacterial growth, including *Escherichia coli*, due to their antibacterial properties (Pan et al., 2023). Additionally, Manner et al. (2018) found that flavonoids can inhibit quorum sensing in pathogenic bacteria by interfering with their communication, while flavonoids do not affect the communication among beneficial bacteria. Flavonoids form complex compounds with the extracellular proteins of pathogenic bacteria, causing cell membrane damage and inhibiting DNA, RNA, and protein synthesis, thereby inhibiting pathogen growth (Rodríguez et al., 2023).

Total coliforms in each segment of the small intestine of broiler chickens significantly decreased due to the ENFE, Zn, and Cu supplementation ( $p < 0.05$ , Table 3). As shown in Table 3, a similar statistical pattern was observed across all intestinal segments. In the duodenum and jejunum, the coliform levels in 0.18% (T3) were not significantly different from those in 0.12% (T2;  $P > 0.05$ ), although both were clearly lower than those in 0.06% (T1) and control group (T0). In the ileum, 0.18% (T3) demonstrated the lowest numerical values, followed by 0.12% (T2), whereas the highest coliform counts were recorded in the control group (T0). Although 0.18% (T3) was slightly lower than 0.12% (T2), these differences were not statistically significant ( $P > 0.05$ ), suggesting that a plateau in the inhibitory response might have been reached at the 0.12% (T2) inclusion level.

The reduction in coliforms was accompanied by increases in LAB counts and decreases in intestinal pH

observed in the 0.12% (T2) and 0.18% (T3). The mentioned pattern appears to be consistent with the activity of LAB, which have been reported to ferment carbohydrates into lactic acid and other organic acids, thereby lowering luminal pH and creating an environment that selectively favors beneficial bacteria while limiting the growth of coliforms. The enhanced LAB proliferation in 0.12% (T2) and 0.18% (T3) likely contributed to the formation of a more acidic microenvironment, thereby reinforcing competitive exclusion and limiting coliform proliferation. In broiler chickens, pathogenic bacteria are negatively correlated with LAB in the small intestine. The negative correlation between coliforms and LAB was explained by ecological competition between them in the intestine. Furthermore, flavanol compounds from noni fruit, such as quercetin, have been reported to interact with phospholipids in bacterial cell membranes, increasing membrane permeability and leading to leakage of intracellular components, which may impair bacterial survival in the intestinal environment (Ren et al., 2024).

The pH values of the duodenum and ileum segments decreased significantly following the inclusion of ENFE supplemented with Zn and Cu in the broiler diet ( $p < 0.05$ ). In the duodenum, a significant reduction ( $p < 0.05$ ) was observed in, while 0.18% (T3) did not differ statistically from 0.12% (T2;  $P > 0.05$ ) but still indicated a slightly lower numerical value than the other treatment groups (Table 3). The current findings indicated that the primary acidification response occurred at a 0.12% (T2) inclusion level, while the decrease at 0.18% (T3) was not statistically significant ( $P > 0.05$ ). A non-significant reduction in ileal pH was observed at the 0.06% (T1) ENFE supplementation level compared to the control group (T0;  $P > 0.05$ ; Table 3). Meanwhile, the jejunum did not exhibit a significant response to dietary supplementation with ENFE, Zn, and Cu ( $p > 0.05$ ). The observed decrease in small intestine pH occurred concurrently with higher LAB counts (Table 3), indicating a close association between microbial dynamics and luminal activity. Consistent with the present findings, Dityana et al. (2024) reported that an increase in LAB population led to higher production of bacterial metabolites, such as organic acids, which contributed to a decrease in small intestinal pH.

Mechanistically, organic acids, such as short-chain fatty acids (SCFA), release positive hydrogen ions ( $H^+$ ) that lower pH and make the digestive tract more acidic (Chukwudi et al., 2024).

### Intestinal segment morphology

The villi height in broiler chickens was significantly affected by the addition of ENFE supplemented with Zn and Cu ( $p < 0.05$ ). Villus height in the duodenum and ileum increased significantly in 0.12% (T2) and 0.18% (T3). Groups 0.12% (T2) and 0.18% (T3) demonstrated significantly higher villus height compared to 0.06% (T1) and control group (T0;  $P < 0.05$ ), with no significant difference between 0.12% (T2) and 0.18% (T3;  $P > 0.05$ ; Table 4). In the jejunum segment, 0.06% (T1) exhibited an increase in villus height, followed by 0.12% (T2), with the highest increase found in 0.18% (T3). Although no significant differences were detected between 0.12% (T2) and 0.18% (T3) across all intestinal segments ( $P > 0.05$ ), the numerically highest villus height consistently appeared in 0.18% (T3). The present results indicated a dose-dependent increase, yet the effect at 0.18% (T3) was not biologically greater than lower doses. Despite statistical

equivalence between 0.12% (T2) and 0.18% (T3), the consistent improvement in villus morphology across the small intestine indicated that ENFE, supplemented with Zn and Cu, enhanced mucosal development. The antimicrobial properties of bioactive compounds in noni fruit extract, when combined with Zn and Cu, have been reported to suppress pathogenic bacteria, thereby reducing toxin production and mucosal irritation and creating ideal conditions for epithelial cell turnover and villus development. The present findings are in line with those of Prihambodo et al. (2021), who reported that flavonoids exhibited anti-inflammatory and antioxidant activities, neutralizing free radicals, minimizing cellular damage, and enhancing epithelial regeneration, thereby supporting villus development. Small intestinal villi are closely related to nutrient absorption capacity, where longer villi increase absorption efficiency (Abdellatif et al., 2022).

**Table 4.** Villus height, crypt depth, relative length, and weight of intestinal segments in broiler chicken fed diets with encapsulated noni fruit extract supplemented with zinc and copper for four weeks

Variables	Treatments	T0	T1	T2	T3	P-value
<b>Duodenum</b>						
Villus height ( $\mu\text{m}$ )		1297.01 $\pm$ 100.76 <sup>b</sup>	1391.46 <sup>b</sup> $\pm$ 109.75 <sup>b</sup>	1536.21 $\pm$ 93.98 <sup>a</sup>	1557.28 $\pm$ 51.21 <sup>a</sup>	< 0.001
Crypt depth ( $\mu\text{m}$ )		242.74 $\pm$ 8.63 <sup>b</sup>	248.73 <sup>b</sup> $\pm$ 3.35 <sup>b</sup>	276.37 $\pm$ 21.55 <sup>a</sup>	280.48 $\pm$ 12.42 <sup>a</sup>	< 0.001
Length (%)		1.12 $\pm$ 0.36 <sup>b</sup>	1.15 <sup>a</sup> $\pm$ 0.41 <sup>a</sup>	1.18 $\pm$ 0.58 <sup>a</sup>	1.26 $\pm$ 0.62 <sup>a</sup>	0.004
Weight (%)		0.34 $\pm$ 0.10 <sup>b</sup>	0.50 <sup>a</sup> $\pm$ 0.06 <sup>a</sup>	0.52 $\pm$ 0.02 <sup>a</sup>	0.52 $\pm$ 0.04 <sup>a</sup>	< 0.001
<b>Jejunum</b>						
Villus height ( $\mu\text{m}$ )		992.39 $\pm$ 103.47 <sup>c</sup>	1157.18 $\pm$ 43.09 <sup>b</sup>	1241.88 $\pm$ 54.64 <sup>ab</sup>	1294.21 $\pm$ 78.60 <sup>a</sup>	< 0.001
Crypt depth ( $\mu\text{m}$ )		201.92 $\pm$ 11.31 <sup>b</sup>	225.12 $\pm$ 13.21 <sup>a</sup>	241.52 $\pm$ 5.12 <sup>a</sup>	246.07 $\pm$ 24.13 <sup>a</sup>	< 0.001
Length (%)		3.62 $\pm$ 0.16 <sup>c</sup>	3.92 $\pm$ 0.12 <sup>b</sup>	3.92 $\pm$ 0.18 <sup>b</sup>	4.15 $\pm$ 0.07 <sup>a</sup>	< 0.001
Weight (%)		0.92 $\pm$ 0.06 <sup>b</sup>	0.99 $\pm$ 0.07 <sup>b</sup>	1.19 $\pm$ 0.15 <sup>a</sup>	1.22 $\pm$ 0.09 <sup>a</sup>	< 0.001
<b>Ileum</b>						
Villus height ( $\mu\text{m}$ )		471.33 $\pm$ 79.94 <sup>b</sup>	496.58 $\pm$ 75.83 <sup>b</sup>	826.71 $\pm$ 63.77 <sup>a</sup>	830.59 $\pm$ 121.64 <sup>a</sup>	< 0.001
Crypt depth ( $\mu\text{m}$ )		117.37 $\pm$ 16.40 <sup>b</sup>	127.27 $\pm$ 7.47 <sup>b</sup>	170.94 $\pm$ 17.91 <sup>a</sup>	156.34 $\pm$ 19.52 <sup>a</sup>	< 0.001
Length (%)		3.61 $\pm$ 3.32 <sup>b</sup>	4.10 $\pm$ 3.62 <sup>a</sup>	4.19 $\pm$ 1.89 <sup>a</sup>	4.16 $\pm$ 1.72 <sup>a</sup>	0.006
Weight (%)		0.77 $\pm$ 0.03 <sup>c</sup>	0.95 $\pm$ 0.06 <sup>b</sup>	1.06 $\pm$ 0.06 <sup>a</sup>	1.13 $\pm$ 0.04 <sup>a</sup>	< 0.001

Note: <sup>a, b, and c</sup> Different superscript letters in the same row significantly differ ( $p < 0.05$ ). T0: Basal diet; T1: Basal diet + 0.06% ENFE supplemented with Zn, Cu; T2: Basal diet + 0.12% ENFE supplemented with Zn and Cu; T3: Basal diet + 0.18% ENFE supplemented with Zn and Cu.

The crypt depth of every segment of the small intestine was significantly affected by the addition of ENFE supplemented with Zn and Cu ( $p < 0.05$ ). Crypt depth was significantly greater in the duodenum and ileum of 0.12% (T2;  $p < 0.05$ ). In the jejunum, however, an increase in 0.06% (T1) was already observed compared with the control group (T0). The duodenal crypt depth in 0.18% (T3) did not differ from 0.12 (T2) but was significantly greater than in 0.06% (T1) and control group

(T0;  $p < 0.05$ , Table 4). In the jejunum, the crypt depth in 0.18% (T3), 0.12% (T2), and 0.06% (T1) was significantly greater than in the control group (T0;  $P < 0.05$ ). Meanwhile, the ileal crypt depth in 0.18% (T3) was similar to that in 0.12% (T2), and both were significantly higher than in 0.06% (T1) and control group (T0;  $p < 0.05$ ; Table 4). Although the jejunum demonstrated an additional significant response at 0.18% (T3), other critical morphological parameters, such as villus height and crypt

depth in the duodenum and ileum, stabilized at the 0.12% (T2) ENFE dosage.

The observed increase in crypt depth might be due to the biological activity of flavonoids, which have been reported to exert antimicrobial effects that suppress pathogenic bacteria, promote LAB populations, and enhance SCFA production. The SCFAs are known to interact with G protein-coupled receptors (GPCRs), potentially stimulating glucagon-like peptide-1 (GLP-1) secretion, thereby supporting epithelial cell growth at the base of the crypt (Zhang et al., 2019; Paredes-López et al., 2024). Crypt depth is often used as a marker of epithelial cell proliferation and is crucial for villus renewal. Therefore, deeper crypts along with taller villi indicate enhanced mucosal turnover and improved intestinal function, potentially boosting digestive efficiency and nutrient absorption in broiler chickens (El-Sabry and Yalcin, 2022).

The relative length and weight of each segment of the small intestine were significantly affected by the addition of ENFE supplemented with Zn and Cu ( $p < 0.05$ ). A significant increase in the relative length of the duodenum, jejunum, and ileum was observed in 0.06% (T1;  $P < 0.05$ ). Similarly, the duodenum and ileum weights increased in T1, while the jejunal weight increased by 0.12% (T2). The duodenum's weight and length were significantly higher in 0.06% (T1), 0.12% (T2), and 0.18% (T3) than in the control group (T0;  $P < 0.05$ ). The jejunum length in 0.06% (T1), 0.12% (T2), and 0.18% (T3) was significantly higher than in the control group (T0;  $P < 0.05$ ). The ileal length and weight in 0.06% (T1), 0.12% (T2), and 0.18% (T3) were significantly higher than in the control group (T0;  $P < 0.05$ ). The ileum had the longest length and the highest weight in 0.12% (T2) and 0.18% (T3; Table 4).

The increase in the length and relative weight of the small intestine, particularly in 0.12% (T2) and 0.18% (T3), generally correlated with an increase in villi height. Villi height probably enhanced the absorption surface area, resulting in more nutrient uptake, as observed in the increased intestinal weight and length. Ravindran and Abdollahi (2021) highlighted that absorbed nutrients, especially proteins, are essential for cell formation and the regeneration of epithelial cells in the intestine and different body tissues. Improved intestinal microbial balance, as reflected by an increase in LAB and a decrease in coliforms, was likely related to increases in the length and weight of each intestinal segment. Flavonoids might influence the gut ecosystem by promoting the growth of LAB, possibly suppressing some harmful microbes, and helping sustain a healthier balance of gut microbiota

(Rodsatian et al., 2023). Increased LAB populations improve mucosal health by lowering pathogenic pressure and inflammation. This promotes epithelial proliferation, leading to enhanced villus development and an expanded absorptive surface (Luo et al., 2024). Thus, the enhanced absorptive function supports the growth and development of intestinal tissues, as reflected in the increased relative lengths and weights of the duodenum, jejunum, and ileum observed during the present study.

While the highest numerical values were occasionally observed in 0.18% (T3), 0.12% (T2) consistently demonstrated substantial, statistically significant improvements in intestinal structure, microbial balance, and organ metrics, similar to those observed in 0.18% (T3) across most parameters. Accordingly, the 0.12% (T2) supplementation level can be considered the practical and effective optimal dose for promoting intestinal development in broiler chickens.

## CONCLUSION

A positive response was observed with the inclusion of encapsulated noni fruit extract (ENFE) combined with Zn and Cu at 0.12%. This indicated an optimal inclusion level that substantially improved BWG, gut microbiota balance, and intestinal morphology in broiler chickens. Additionally, the higher ENFE level at 0.18% (T3) yielded statistically comparable results. Therefore, 0.12% (T2) is recommended as the most optimal level for commercial broiler production. Future studies should evaluate the economic feasibility of ENFE supplementation under commercial production conditions to confirm its cost-effectiveness.

## DECLARATIONS

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### Authors' contributions

The study was conceptualized by Linda Nur Baetavianti, who also carried out data collection, data analysis, and manuscript drafting. Vitus Dwi Yuniato supervised the research process. Under the supervision of Mulyono, a manuscript was drafted and reviewed. The initial text was prepared by Lilik Krismiyanto, who also originated the idea and design of the study. All authors have read and approved the statistical analysis as well as the final edition of the manuscript.

### Ethical considerations

All authors have reviewed the study for potential ethical concerns, including duplicate publication, redundancy, data fabrication or falsification, misconduct, or plagiarism. The authors originally wrote the article without seeking any help from available AI software.

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### Availability of data

All data generated during the study are included in this article. Any additional information is available upon reasonable request from the authors.

### Competing interests

The authors have declared that no competing interest exists.

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