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The Use of Dietary Mint Leaves Powder (*Mentha piperita*) as a Phytobiotic on Intestinal Histomorphology of Broiler Chickens

Aris Karma Andhika[1], Irfan H. Djunaidi[2,*], Eko Widodo[2]
[1]Master Program of Animal Science, Faculty of Animal Science, University of Brawijaya, Malang, East Java, Indonesia-65145
[2]Department of Animal Nutrition and Feed Sciences, University of Brawijaya, Malang, East Java, Indonesia-65145
[*]Corresponding author: irjuna@gmail.com

***Abstract*—** *This study aimed to determine the effect of dietary mint leaves powder on the intestinal histomorphology of broiler chicken. The materials used in this study were 200 one-day-old male broiler chickens (Lohmann strain) that were kept for 35 days. The method used was an experiment using a completely randomized design with five treatments and five replications (each replication consisted of 8 birds). The treatments used were Control: basal diet without feed additive, AGP: basal diet + 0.01% zinc bacitracin, MLP0.5: basal diet + 0.5% mint leaves powder, MLP1.0: basal diet + 1% mint leaves powder, and MLP1.5: basal diet + 1.5% mint leaves powder. The variables observed in this study were villous height, basal villous width, apical villous width, villous number, villous surface area, and crypt depth. The results showed that the administration of peppermint leaves powder could increase apical villous width ($P<0.05$) and villous number ($P<0.01$). However, the treatments did not affect ($P>0.05$) on villous height, basal villous width, crypt depth, and villous surface area. It could be concluded that the use of mint leaves powder could beneficially increase the villous number of broiler chickens.*

***Keywords*—** *Mint leaves powder, villous number, villous surface area, broiler chickens.*

I. INTRODUCTION

Chickens come from the *Aves* class who are usually raised by the community. Broiler chicken is a superior type of chicken that has gone through a rigorous selection process and breeding program so that their body weight can reach 2.14 kg within 35 days [1]. The body weight gain of broiler chickens is influenced by several factors, including the health and conditions of the digestive tract. One of the causes of disease that often attacks broiler chickens comes from bacteria. Some pathogenic bacteria that often infect broiler chickens are *Salmonella* sp. and *Escherichia coli*.

In the last few decades, antibiotics are widely used as feed additives to prevent infection of pathogenic bacteria such as *Salmonella* sp. and *E. coli*. However, the use of antibiotics now has dangerous negative impacts, such as the emergence of resistant bacteria and the deposition of antibiotic residues in meat that can cause humans who consume it will experience resistance to the same bacteria. In 1999, European countries issued regulations that prohibit the use of antibiotics due to these adverse effects. Based on that, efforts should be made to find antibiotic replacement solutions using various herbal plants. The characteristics of herbal plants that can provide antibacterial effects are having active compounds such as phenols and flavonoids.

Mint (*Mentha piperita*) is a plant that often used in the manufacture of medicinal products. This plant is used worldwide to cure various diseases. In general, mint has the characteristics of square stems with a variety of flower colors such as white, pinkish and bluish. Mint originally comes from Europe and the Mediterranean. This plant includes in Labiatae class that has more than 3200 species. This plant is also characterized by its distinctive aroma that is pungent and minty. In a study by Ameri et al. [2], it was reported that mint contained bioactive compounds with antibacterial properties. For that reason, mint has potency as an antibiotic alternative.

Feed efficiency is highly dependent on the absorption of nutrients in the small intestine. Broiler chickens that have a healthy digestive tract can perform maximum bodyweight [3]. The small intestine has four cell layers, including mucosa, submucosa, tunica muscularis, and serosa. Villous are located on the outside of the mucosa, the longer the villous, the more efficient of nutrients absorption [4]. Therefore, this study aimed to determine the effect of dietary peppermint leaves powder as a phytobiotic on the intestinal histomorphology of broiler chickens.

II. MATERIALS AND METHODS

A. Preparation of Mint Leaves Powder

Mint was obtained from Bumiaji District, Batu City, Indonesia. Freshly harvested mint was washed, rinsed with water, and drained. Mint leaves were then picked from the stems and dried under the shade for seven days. After that, the dried mint leaves were ground using a blender and then filtered.

B. Birds and Experimental Design

The *in vivo* study was conducted in Karangploso District, Malang Regency, Indonesia, from 24 July until 27 August 2017. This study used 200 male broiler chickens (Lohmann strain) with an initial body weight of 46.10 g and a coefficient of variation of 9.69%. Broilers were reared for 35 days. This study used a completely randomized design with five treatments and five replications (each replication consisted of 8 broilers). The treatments used were Control: basal diet without feed additive, AGP: basal diet + 0.01% zinc bacitracin, MLP0.5: basal diet + 0.5% mint leaves powder, MLP1.0: basal diet + 1% mint leaves powder, and MLP1.5: basal diet + 1.5% mint leaves powder. The birds in each

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replicate were placed in a pen with a size of 1 m^2. The feed and drinking water were offered *ad libitum*. The basal diet was formulated to meet or exceed the nutrient specifications of Aviagen [5] with the following nutrient content (Table I).

TABLE I. Nutrient content of the basal diet

Nutrient content	Starter phase	Finisher phase
Metabolizable energy (Kcal/kg)	3,000	3,200
Crude protein (%)	23.00	19.50
Ether extract (%)	1.25	1.00
Lysine (%)	1.44	1.15
Methionine + Cysteine (%)	1.08	0.90
Methionine (%)	0.56	0.47
Calcium (%)	0.96	0.78
Phosphor (%)	0.48	0.39

C. Measurement of Intestinal Histomorphology

Intestinal morphology evaluation was carried out on 27 August to 27 September 2017 at the Laboratory of Anatomy Pathology, Faculty of Medicine, University of Brawijaya. The materials used were small intestine (ileum), aquadest, formalin (10%), paraffin, xylol solution, alcohol, hematoxylin, acidic alcohol (1%), ammonia lithium carbonate, and eosin. The apparatus used were surgical scissors, film pots, icebox, automatic tissue-tek processor, microtome, oven, test tubes, object glass, and cover glass.

One broiler chicken was taken from each replication and slaughtered. Ileum was collected from each bird by cutting 5 cm intestinal part from the ileocecal junction towards the ileum. The sample was put into a labeled film pot and placed into an icebox. After that, the sample was transported to the laboratory. The ileum sample was then fixed with 10% formalin for 7 hours. The sample was then inserted into a cassette and processed with an automatic tissue-tek processor for 90 minutes. The sample was then blocked with paraffin and cut to a thickness of 3-5 µm using a microtome. Deparaffinization was carried out in an oven with a temperature of 70-80 ^oC for 30 minutes. After that, the specimen was put into two tubes of xylol solution for 20 minutes of each, then put into four alcohol tubes for 3 minutes of each (hydration), and then washed with running water for 15 minutes. The staining process was carried out with hematoxylin for 10-15 minutes and then washed with running water for 15 minutes. Next, the specimen was put into a 1% acid alcohol solution as much as 2-5 dips, followed by 3-5 dips of lithium carbonate ammonia, and eosin for 10-15 minutes. After that, the graded alcohol was administered, starting from 70% alcohol for 3 minutes, 80% alcohol for 3 minutes, 96% alcohol for 3 minutes, and absolute alcohol for 3 minutes. The specimen was then cleared up twice using xylol for 15 minutes of each. After that, the mounting process was done with entellan and object glass, which was then covered with a cover glass. Next, the slide was left to dry at room temperature.

Measurement of intestinal morphology was done with a digital microscope using the OlyVia (Olympus) program with a micrometer (µm) size. Measurements were made by pulling angle 1 to angle 2 that has been determined. The villous height, basal villous width, apical villous width, and crypt depth were measured using a microscope equipped with a micrometer with 40x magnification. Measurement of villous surface area was conducted using the method of intestinal mucosa histology (light microscopy) using the formula of [(c + d) / 2] x a = e, where a: villous height, b: crypt depth, c: basal villous width, d: apical villous width, e: villous surface area, f: villous number [6].

III. Results and Discussion

Variables that can be used to evaluate intestinal histomorphology include villous height, basal villous width, apical villous width, villous number, and crypt depth [6]. These variables can be used to determine the villous surface area that can be used as an indicator of the nutrient absorption capacity in the ileum. Table II shows the effect of mint leaves powder administration on intestinal histomorphology of broiler chickens.

A. Villous Height

The use of dietary mint leaves powder is expected to increase the villous height in the ileum of broiler chickens. Villous is an extension of epithelial cells that function to absorb nutrients. The longer villous is expected to increase efficiency in the nutrient absorption process. Wider villous also could allow the digestive organs to reach feed better. Table II shows the results on the villous height starting from the highest to the lowest were MLP1.5 (483.91±113.94 µm), MLP0.5 (469.33±33.22 µm), MLP1.0 (450.67±118.34 µm), AGP (422.57±116.21 µm) and Control (374.69±47.25 µm). The inclusion of mint leaves powder tended to produce better villous height than AGP and control treatments. However, the results of statistical analysis showed that the use of mint leaves powder did not show any significant effect ($P>0.05$) on the villous height. This result was consistent with Cetingul et al. [7], who reported that the use of mint could not increase the villous height of broiler chickens. Observation of villous height was also carried out by Natsir et al. [8], who reported that the results had a highly significant effect. Measurement of villous height could be done in several parts of the small intestine, including ileum and duodenum. In a study by Kawalilak et al. [9], it was reported that the age had a significant effect on the villous height in the ileum and duodenum, in which the greater age could be followed by the higher villous height in the ileum and duodenum. The effectiveness of dietary mint leaves powder in the digestive tract of broiler chickens was influenced by several factors, one of them was in which intestinal part villous height was observed. According to Jamilah et al. [10], the highest villous height was found in the duodenum, followed by jejunum and ileum. The villous height in the ileum was the smallest than the other parts. In that study, it was also reported that the small intestine (duodenum, jejunum, and ileum) significantly affected the villous height of broiler chickens. The cause of the negligible effect of dietary mint leaves powder on the villous height was probably due to the diet viscosity factor. The feed ingredients used in this study were relatively similar, resulting in the same viscosity so that there was no significant

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effect in the villous height.

B. Basal Villous Width

Basal is the bottom part of the villous above the crypt. Basal villous helps the nutrients absorption process. As can be seen in Table II, the best result of basal villous width was MLP1.5 (217.24±53.43 µm), followed by MLP0.5 (210.25±4.56 µm), MLP1.0 (198.86±21.32 µm), Control (188.94±50.26 µm), and AGP (176.50±21.33 µm). The use of commercial antibiotic of zinc bacitracin did not provide better basal villous width than the use of dietary mint leaves powder. Based on the statistical analysis, the use of dietary mint leaves powder had no significant effect (P>0.05) on the basal villous width. Awad et al. [11] found that the use of prebiotics did not affect the villous width. In that study, feed containing probiotics contaminated with deoxynivalenol caused comparable villous width as control.

TABLE II. Effect of dietary mint leaves powder on intestinal histomorphology of broiler chickens

Treatment	Intestinal histomorphology					
	VH	BVW	AVW	VN	VSA	CD
Control	374.69±47.25	188.94±50.26	114.96±48.69^{b}	221.40±5.37^{A}	58.06±17.12	205.46±47.36
AGP	422.57±116.21	176.50±21.33	109.31±18.92^{a}	227.00±3.81^{A}	62.91±22.40	157.25±24.18
MLP0.5	469.33± 33.22	210.25±4.56	159.34±34.73^{b}	237.60±6.95^{B}	87.51±10.82	195.06±39.58
MLP1.0	450.67±118.34	198.86±21.32	160.32±20.03^{b}	246.20±6.30^{B}	81.82±25.98	217.05±12.45
MLP1.5	483.91±113.94	217.24±53.43	147.45±28.68^{b}	247.00±5.48^{B}	87.13±21.31	179.17±43.44

VH: villous height (µm), BVW: basal villous width (µm), AVW: apical villous width (µm), VN: villous number, VSA: villous surface area (mm^2), CD: crypt depth (µm)
^{ab} different superscript within the same column showed a significant difference (P<0.05)
^{AB} different superscript within the same column showed a highly significant difference (P<0.01)

C. Apical Villous Width

Apical is the top of the villous. Apical villous could be stated as the head of the villous that helps absorb nutrients. As can be seen in Table II, the best result of apical villous width was MLP1.0 (160.32±20.03 µm), MLP0.5 (159.34±34.73 µm), MLP1.5 (147.45±28.68 µm), Control (114.96±48.69 µm), and AGP (109.31±18.92 µm). The use of commercial antibiotics of zinc bacitracin did not provide better apical villous width than the use of mint leaves powder. Based on statistical analysis, the use of mint leaves powder has a significant effect (P<0.05) on the apical villous width. This finding probably because mint leaves powder has flavonoid and phenol compounds that can inhibit pathogenic bacteria so that it can support the development of villous.

D. Villous Number

The villous number is a factor that dramatically affects the digestion and absorption of nutrients. The more villous number found in one small intestinal cross-section, the higher the nutrient absorption. Villous observations were also made by Rahim et al. [12], who observed the morphology of villous in the duodenum and ileum. The use of dietary mint leaves powder is expected to increase the villous number in the ileum. As can be seen in Table II, the results of villous number from the highest to the lowest were MLP1.5 (247.00±5.48), MLP1.0 (246.20±6.30), MLP0.5 (237.60±6.95), AGP (227.00±3.81), and Control (221.40±5.37). The higher the use of mint leaves powder has a positive effect on the villous number. The results of statistical analysis showed that the use of mint leaves powder had a highly significant effect (P<0.01) on the villous number of the ileum. In agreement with this finding, Natsir et al. [13] also showed that the use of a phytobiotic mixture of turmeric flour (*Curcuma domestica*) and Ginger (*Zingiber officinale*) had a highly significant effect on the villous number. The use of mint leaves powder gave better results on the villous number compared to AGP and control treatment. Increasing the administration level of mint leaves powder could increase the number of active compounds entering the digestive tract so that it could increase the villous number. In contrary to this current finding, Emma et al. [14] reported that the use of lime could not increase the villous number of broiler chickens. Fitasari [15] also reported that the use of the papain enzyme could not increase the villous number of villous.

E. Villous Surface Area

The small intestine is a digestive organ that plays a vital role in the digestion process and nutrients absorption. The nutrient absorption capacity can be seen from the villous surface area. The higher the villous surface area, the higher the digestion and absorption of nutrients. The results of villous surface area from the highest to the lowest were MLP0.5 (87.51±10.82 µm^2), MLP1.5 (87.13±21.31 µm^2), MLP1.0 (81.82±25.98 µm^2), AGP (62.91±22.40 µm^2), and Control (58.06±17.12 µm^2). Based on the results of statistical analysis, the use of dietary mint leaves powder had no significant effect (P>0.05) on the villous surface area.

F. Crypt Depth

Crypt depth is beneficial to support the process of nutrients absorption. Crypt is the root of villous. The higher the crypt, the higher the nutrient absorption. Based on Table II, the best crypt depths was MLP1.0 (217.05±12.45 µm), followed by Control (205.46±47.36 µm), MLP0.5 (195.06±39.58 µm), MLP1.5 (179.17±43.44 µm), and AGP (157.25±24.18 µm). Based on the results of statistical analysis, the use of mint leaves powder had no significant effect (P>0.05) on the crypt depth. A different result was revealed by Cetingul et al. [7], who reported that the use of mint leaves powder at the level of 0, 1, 2, 3, 4, and 5% could increase the crypt depth. In that study, the superior levels of mint leave powder administration were 1 and 5%. Observations of villous height, crypt depth, and the villous number of broiler chickens were also carried out by Natsir et al. [16] with the addition of garlic and *Phyllanthus niruri* L. in the form of powder or encapsulated.

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In another study, Burkholder et al. [17] reported that the treatment had a significant effect on crypt depth. In a study conducted by Houshmand et al. [18], the small intestinal part also affected crypt depth. In that study, crypt depth in the duodenum was better than the jejunum. In other studies, the use of feed additives does not affect crypt depth, but the use of prebiotics could influence crypt depth.

IV. CONCLUSION

It could be concluded that the use of mint leaves powder provides benefit to improve villous number in the ileum of broiler chickens.

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