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Fulvic Acid from Oil Palm Empty Fruit Bunches as Feed Additive Improves Digestive Tract and Intestinal Morphology in Broilers

Aditif Pakan Asam Fulvat Tandan Kosong Kelapa Sawit untuk Memperbaiki Saluran Pencernaan dan Morfologi Usus pada Ayam Pedaging

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ABSTRACT

The use of Antibiotic Growth Promoters (AGPs) in the poultry industry led to farmers' dependence on antibiotics. Therefore, a safer alternative feed additive, such as fulvic acid, was needed. This study aimed to evaluate the effectiveness of fulvic acid extracted from oil palm empty fruit bunches (FA-OPEFB) as a feed additive on broiler chickens' digestive tract and intestinal morphology. A total of 150 broiler chickens were used in a Completely Randomized Design (CRD) consisting of three treatments and five replications: P0 (drinking water without FA-OPEFB), P1 (drinking water with 0.1% FA-OPEFB), and P2 (drinking water with 0.2% FA-OPEFB). Observed variables included the relative weight and length of the digestive tract and intestinal morphology. The results showed that the addition of FA-OPEFB in drinking water significantly affected the ileum percentage, villus height, crypt depth, and villus surface area. It was concluded that 0.2% FA-OPEFB in drinking water reduced the relative weight and length of the ileum and improved intestinal morphology in broiler chickens.

Key words: broiler chicken, digestive tract, fulvic acid, intestinal morphology, OPEFB

ABSTRAK

Penggunaan Antibiotic Growth Promoters (AGPs) dalam industri unggas menyebabkan ketergantungan peternak terhadap antibiotik. Oleh karena itu, diperlukan pakan tambahan alternatif yang lebih aman seperti asam fulvat. Penelitian ini bertujuan untuk mengevaluasi efektivitas asam fulvat yang diekstrak dari tandan kosong kelapa sawit (FA-OPEFB) sebagai pakan tambahan terhadap saluran pencernaan dan morfologi usus ayam broiler. Sebanyak 150 ekor ayam broiler dalam penelitian menggunakan Rancangan Acak Lengkap (RAL) yang terdiri dari tiga perlakuan dan lima ulangan: P0 (air minum tanpa FA-OPEFB), P1 (air minum dengan 0,1% FA-OPEFB), dan P2 (air minum dengan 0,2% FA-OPEFB). Variabel yang diamati meliputi berat dan panjang relatif saluran pencernaan dan morfologi usus. Hasil penelitian menunjukkan bahwa penambahan FA-OPEFB dalam air minum berpengaruh nyata terhadap persentase ileum, tinggi vilus, kedalaman kripta, dan luas permukaan vilus. Disimpulkan bahwa 0,2% FA-OPEFB dalam air minum mengurangi berat dan panjang relatif ileum dan memperbaiki morfologi usus pada ayam pedaging.

Kata kunci: asam fulvat, ayam broiler, morfologi usus, OPEFB, saluran pencernaan

INTRODUCTION

Indonesia has significant potential for broiler chicken production, making it an attractive opportunity for poultry farmers and entrepreneurs. Broiler chickens are among the most widely cultivated commercial poultry strains, both on a small and large scale (Siburian 2015). However, broilers are known to have weak immune systems and are highly susceptible to disease (Khairat & Qashlim, 2020). To address health and productivity issues in poultry, the use of antibiotics has become a common practice, leading to farmer dependence on antibiotic products, particularly Antibiotic Growth Promoters (AGPs). However, the use of AGPs has raised public health concerns due to their long-term adverse effects on consumers. According to Prasetyo *et al*. (2020), AGPs can lead to bacterial resistance and antibiotic residues in animal products. Similarly, Widhi & Saputra (2021) stated that AGPs leave residues in livestock products, which may contribute to antibiotic resistance in humans and reduce the effectiveness of antibiotics for therapeutic use. In response to these concerns, the Indonesian government issued Regulation No. 14/Permentan/PK.350/2017, Article 16, prohibiting the use of antibiotics as feed additives.

Feed additives are substances incorporated into animal rations in small quantities with specific purposes. Their primary functions are to improve feed quality, enhance feed efficiency, and increase livestock productivity and product quality. One of the organic acids commonly used as a feed additive is fulvic acid. Fulvic acid is a naturally occurring organic acid formed during the decomposition of organic matter, particularly in the formation of humus, and is classified as a humic substance. It represents the fraction of soil organic matter that is soluble in both alkaline and acidic conditions. Fulvic acid contains various bioactive compounds such as carbohydrates and proteins, and it remains soluble across all pH levels (Herlambang *et al*. 2017). This compound is rich in a variety of reactive oxygen-containing functional groups, including hydroxyl, phenolic hydroxyl, methoxy, carboxyl, carbonyl, and quinone groups, which contribute to its diverse biological activity (Liu *et al*. 2023).

Fulvic acid is usually extracted from coal and resin that have been humified for thousands of years (Gong *et al*. 2020; Goenadi, 2001), which come from non-renewable sources. According to Lo *et al*. (2021), with ligno protein theory, fulvic acid can be synthesized from biomass that has lignin in it, one of which is oil palm empty fruit bunches (OPEFB). OPEFB is an abundant lignocellulosic waste from the palm oil industry, rich in lignin and commonly underutilized (Yunos *et al*. 2014). Extraction of fulvic acid from OPEFB has not been widely carried out, so this study emphasizes the novelty of using fulvic acid extracted from OPEFB.

Fulvic acid has been reported to support enzyme production, hormone structure formation, and the utilization of vitamins. It contains carboxyl and phenolic groups at concentrations of 6.2 me·g^{-1} and 3.2 me·g^{-1}, respectively (Oksana *et al*. 2024). In addition, fulvic acid possesses several bioactive properties, including antioxidant, anti-inflammatory, immunomodulatory, and antidiabetic effects. According to Mao (2019) supplementation with fulvic acid resulted in significantly higher body weight gain in broiler chickens. However, studies investigating the effects of fulvic acid as a feed additive on the digestive tract and intestinal morphology of broilers remain limited. Therefore, this study was conducted to evaluate the influence of fulvic acid supplementation on the gastrointestinal health and intestinal morphology of broiler chickens.

METHODS

Material

The animals used in this study were 150-day-old broiler chickens (DOC) of the Cobb500 strain, which were reared from day 1 to day 35. The chickens were housed in 15 bamboo and wire partition cages, each equipped with a brooder, incandescent lamp, feeder, drinker, thermohygrometer, digital scale, electric fan, measuring cup, and ration plastic bags. The feed ingredients used during the rearing period included corn, rice bran, soybean meal, meat bone meal (MBM), crude palm oil (CPO), calcium carbonate ($CaCO_3$), dicalcium phosphate (DCP), sodium chloride (NaCl), premix, DL-methionine, and L-lysine. The equipment used for intestinal morphology analysis included a microtome, glass slides, a microscope, and a computer. The materials used for sampling intestinal morphology included 2 cm segments of the intestine, 10% buffered neutral formalin (BNF), and paraffin. The experimental diets were formulated based on the nutrient requirements of Cobb500 broilers during the starter and grower phases (2022). The study used three treatment groups: P0 (control, drinking water without FA-OPEFB), P1 (drinking water with 0.1% FA-OPEFB), and P2 (drinking water with 0.2% FA-OPEFB).

Preparation of Fulvic Acid

The extraction of fulvic acid from oil palm empty fruit bunches (FA-OPEFB) used in this study was according to Dimawarnita *et al*. (2024) and has a purity of 24.71% (Figure 1).

Figure 1 Fulvic acid from oil palm empty fruit bunch (FA-OPEFB)

The FA-OPEFB solution was added to a 100 ml volumetric flask at an external temperature of 40–45°C, then purified by adding 12 ml of saturated sodium bicarbonate ($NaHCO_3$) solution in two stages with the aid of a hotplate stirrer. The purpose of this purification process was to remove residual hydrogen peroxide (H_2O_2) from the FA-OPEFB solution. Following purification, the FA-OPEFB solution was placed into a sample container containing potassium dichromate solution to test for the presence of hydrogen peroxide. A green coloration indicated the presence of residual peroxide, while a yellow color with no change indicated that the peroxide had been completely removed. The resulting pH values of the FA-OPEFB solutions ranged from 6.48 to 6.85.

Feed Types and Nutritional Composition

The feed used in this study was in crumble form and was produced by PT. Jendela Fauna, Bandung. The feed was divided into two types: starter feed (0–14 days old) and grower-finisher feed (15–35 days old). Nutrient requirements were based on the Broiler Performance and Nutrition Supplement for Cobb500 (2022). The composition and nutrient content of the broiler feed used in this study are presented in Table 1.

Table 1 Composition and nutrient content of broiler feed for Cobb500 Strain

Feed ingredients	Starter (1-14 days)	Grower (15-35 days)
	Percentage (%)	
Yellow corn	59.00	61.00
Rice bran	3.00	3.50
Soybean meal	26.50	25.50
MBM	8.00	5.00
CPO	2.00	3.00
$CaCO_3$	0.20	0.70
DCP	0.00	0.00
NaCl	0.20	0.20
Premix[1]	0.50	0.50
L-lysine	0.30	0.30
DL-Methionine	0.30	0.30
Nutrient Content		
Dry matter (%)[2]	90.39	90.35
Crude protein (%)	22.04	20.28
Metabolic energy (kkal kg^{-1})	2931.75	2957.25
Ca (%)	0.94	0.83
P (%)	0.60	0.45
Lysine (%)	1.32	1.21
Methionine (%)	0.58	0.55
Methionine + cysteine (%)	0.85	0.81
Na (%)	0.18	0.16
Cl (%)	0.21	0.20
Linoleic acid (%)	1.47	1.47

[1]Composition of premix kg-1 (PT Medion): Vitamin A 1.200.000 IU, Vitamin D3 200.000, Vitamin E 800 IU, Vitamin K3 200 mg, Vitamin B2 500 mg, Vitamin B6 50 mg, Vitamin B12 1.200, Vitamin C 2.500, Calcium-D-pantothenate 600 mg, Niacin 400 mg, Colin chloride 1.000 mg, Methionine 3.000 mg, Lysine 3.000, Manganese 1.200, Iron 2.000 mg, Iodine 20 mg, Zinc 10.000 mg, Cobalt 20 mg, Copper 400 mg, Antioxidant 1.000 mg, Adjuvants 1 kg.
[2]Calculation results using the trial-and-error method

Broiler Management and Sampling Procedures

A total of 150 Day-Old Chickens (DOC) were divided into 15 cages, with each cage consisting of 10 chicks. Initial weighing and administration of anti-stress vitamins were carried out upon the arrival of the DOC. Chicken body weight and feed consumption were measured weekly. The measurement of water needs and water residue was conducted daily. Litter replacement was done periodically and adjusted according to the condition of the litter. Feed was given based on the chicken's growth phase, namely the starter and grower-finisher phases. Starter feed was provided for broiler chickens aged 1–14 days, while grower feed was given from 15–35 days of age. Feed was given every 2 hours during the first week, every 3 hours during the second week, and every 6 hours from the third to the fifth week. Routine activities during the maintenance period included cleaning the cages, drinkers, feeders, and the surrounding environment. In addition, records of the environmental conditions such as temperature and humidity were also kept. Slaughtering was conducted when the broiler chickens reached 35 days of age. A total of 15 male chickens (one from each replicate) were slaughtered. Prior to slaughter, the chickens were fasted for 12 hours. Each chicken was weighed and marked according to its treatment group. The slaughter weight of each chicken was obtained from this weighing. The selected chickens had body weights that were close to the average weight of their respective replicates. The chickens were then taken to the Poultry Nutrition Laboratory (NTU) for dissection and measurement of the digestive tract as part of the research variable analysis.

Measurement of Digestive Tract and Intestinal Histomorphology

The measurement of research variables was carried out on one chicken from each replicate across all treatments. The measurements included the digestive tract and intestinal morphology. Digestive tract measurements involved weighing and measuring the length of the small intestine. The intestines were separated into the duodenum, jejunum, ileum, cecum, and colon. Each segment was weighed using an analytical balance and its length was measured using a measuring tape. Morphological analysis of the intestine was conducted by collecting intestinal samples from 15 chickens, consisting of 3 treatments with 5 replicates each. Fresh intestinal samples were taken from a 2 cm section of the ileum for each treatment: control (P0), supplementation with 0.1% FA-OPEFB (P1), and supplementation with 0.2% FA-OPEFB (P2), each with five replicates. The collected intestinal segments were rinsed with distilled water (aquadest) and placed in bottles containing 10% Neutral Buffered Formalin (NBF) for fixation. Histological preparations were then made. The histological slides

were analyzed using the Hematoxylin-Eosin (HE) staining method. The intestinal samples were immersed in 10% Neutral Buffered Formalin for fixation over 72 hours (3×24 hours). Following fixation, the tissues were processed for embedding in paraffin to facilitate the preparation of thin sections. The paraffin blocks containing jejunal tissue were sliced into thin sections (approximately 3–5 micrometers) using a microtome for sectioning. The prepared histological slides were placed on glass slides, then observed and measured under a microscope at 100x magnification. The histopathological examination of the jejunum included measurements of villus height, crypt depth, villus surface area, and the villus height to crypt depth ratio (VH:CD). The percentage calculation of the variables was performed using the following formula:

$$\text{Percentage of digestive tract weight (\%)} = \frac{\text{Weight of digestive tract (g)}}{\text{Slaughter weight (g)}} \times 100\%$$

$$\text{Relative intestinal lenght (cm 100g}^{-1}\text{)} = \frac{\text{Intestinal lenght (cm)}}{\text{Slaughter weight (g)}} \times 100$$

$$\text{Villus surface area } (\mu m) = \frac{[\text{Basal width } (\mu m) + \text{Apical width } (\mu m)}{\text{Basal width } (\mu m)} \times \text{Villus height } (\mu m)$$

$$\text{VH:CD ratio} = \frac{\text{Villus height } (\mu m)}{\text{Crypt depth } (\mu m)}$$

Experimental Design and Data Analysis

The research design used in this study was a completely randomized design (CRD) with three treatments and five replications. Each replication consisted of one broiler chicken.

- P0: Drinking water without the addition of FA-OPEFB (control)
- P1: Drinking water + 0.1% FA-OPEFB/drinking water requirement/per bird
- P2: Drinking water + 0.2% FA-OPEFB /drinking water requirement/per bird

The data obtained were analyzed using Analysis of Variance (ANOVA). The values presented were means and standard deviations. The ANOVA was used to determine the effect of treatments on the observed variables. If the ANOVA results showed a significant effect, Duncan's Multiple Range Test (DMRT) was performed using SPSS 25 to identify treatment interactions and assign superscripts for mean comparisons.

RESULT AND DISCUSSION

Percentage of Digestive Tract Weight and Relative Length in Broiler Chickens

The calculation of digestive tract weight and relative length in broiler chickens was an important parameter in evaluating the physiological efficiency and morphological adaptation of the digestive system in response to nutritional treatments. Therefore, measurements were carried out to understand the response to the administration of drinking water supplemented with FA-OPEFB on the weight and relative length of the digestive tract. The effect of FA-OPEFB addition through drinking water on the percentage of digestive tract weight and relative length in 35-day-old broiler chickens including the duodenum, jejunum, ileum, cecum, and colon is presented in Table 2.

The addition of drinking water supplemented with FA-OPEFB did not show a significant effect on the percentage of weight and relative length of the duodenum, jejunum, cecum, and colon. In this study, the average duodenum weight ranged from 0.61% to 0.71%, and the relative length ranged from 0.98 cm 100 g^{-1} to 1.26 cm 100 g^{-1}. These results were consistent with the findings of Isroli *et al*. (2019), who reported that the relative weight of the duodenum ranged between 0.54% and 0.63%. The duodenum played a role in active digestion with the assistance of enzymes from the pancreas and bile from the liver, which broke down starch, fats, and proteins (Ananda *et al*. 2023). The phenolic and polyphenolic compounds contained in FA-OPEFB acted as antioxidants that modulated the gut microbiota. This potentially allowed FA-OPEFB to enhance the integrity of the intestinal mucosa and epithelial barrier function. Moreover, FA-OPEFB increased the activity of digestive enzymes such as amylase and lipase, which contributed to the efficiency of nutrient digestion by stimulating enterocyte (intestinal epithelial) cells through interaction with G protein-coupled receptors (GPRs), leading to increased expression and secretion of digestive enzymes (Liu *et al*. 2024).

The results of the study showed that the average weight of the jejunum ranged from 0.91% to 1.07%, and the relative length ranged from 3.89 cm 100 g^{-1} to 4.15 cm 100 g^{-1}. These findings were consistent with the study by Isroli *et al*. (2019) which reported that the relative weight of the jejunum ranged from 1.07% to 1.23%. The jejunum is the middle part of the small intestine, located between the duodenum and the ileum. Its functions in the digestive system include digesting food with the help of intestinal enzymes and absorbing nutrients such as sugars, amino acids, and fatty acids (Sari *et al*. 2025). Fulvic acid contains phenolic and polyphenolic compounds with antioxidant properties, which were able to protect the intestinal mucosal integrity from oxidative stress, strengthen the tight junctions between epithelial cells, and support a healthy microbial balance. Moreover, fulvic acid, which has a low molecular weight, was easily absorbed and could stimulate the increased availability of essential minerals such as zinc, magnesium, and iron, which are required for nutrient transport and metabolism processes in the jejunum.

The results of the study showed that the average weight of the cecum ranged from 0.31% to 0.38%, and the relative length ranged from 1.15 cm 100 g^{-1} to 1.43 cm 100 g^{-1}. This was in line with the findings of Isroli *et al*. (2019), who reported that the relative cecal weight

Table 2 Percentage of digestive tract weight and relative length in 35 day old Cobb Strain broiler chicken

Variable		Treatment			*P Value*
		P0	P1	P2	
Slaughter weight	(g)	1980.00±98.87	1797.00±91.56	1873.00±89.76	
Duodenum	(%)	0.61±0.10	0.71±0.09	0.63±0.20	0.524
	(cm 100 g^{-1})	1.26±0.54	0.98±0.58	1.15±0.42	0.692
Jejunum	(%)	1.07±0.19	0.91±0.22	0.99±0.11	0.441
	(cm 100 g^{-1})	4.01±0.43	4.15±0.38	3.89±0.31	0.568
Ileum	(%)	1.05±0.11^{b}	0.88±0.11^{a}	0.89±0.10^{a}	0.047
	(cm 100 g^{-1})	4.47±0.38^{b}	3.97±0.17^{a}	4.19±0.21^{ab}	0.042
Cecum	(%)	0.31±0.06	0.38±0.06	0.32±0.05	0.204
	(cm 100 g^{-1})	1.43±0.55	1.15±0.44	1.19±0.52	0.647
Colon	(%)	0.09±0.02	0.13±0.04	0.10±0.01	0.074
	(cm 100 g^{-1})	0.39±0.05	0.49±0.09	0.44±0.06	0.127

P0 = drinking water without fulvic acid (control); P1 = drinking water + 0.1% fulvic acid; P2 = drinking water + 0.2% fulvic acid. Different superscripts in the same row indicate significant differences (p< 0.05).

ranged from 0.48% to 0.58%. The cecum functioned in the absorption of water, sodium, chloride, and fiber with the help of microorganisms (Shivus 2014). Additionally, the cecum contained lymphoid tissue (cecal tonsils), which was part of the gut-associated lymphoid tissue (GALT) and played an important role in immune defense against antigens entering through the digestive tract and in neutralizing pathogenic microorganisms. Active compounds in fulvic acid, such as polyphenols and phenolics, acted as antioxidants and anti-inflammatory agents that protected the cecal mucosa from oxidative stress-induced damage and stabilized epithelial cell membranes, thereby supporting optimal cecal development (Feng *et al*. 2022). Polyphenolic compounds acted as antioxidants capable of neutralizing free radicals and reducing oxidative stress. They neutralized reactive oxygen species (ROS), such as hydroxyl (OH^{-}) and peroxide (H_2O_2), by donating hydrogen atoms from phenolic groups, thereby interrupting the chain reactions of free radicals in intestinal tissues (Rossi *et al*. 2025).

In this study, the average weight of the colon ranged from 0.09% to 0.13%, and the relative length ranged from 0.39 cm 100 g^{-1} to 0.49 cm 100 g^{-1}. This was in line with the findings of Agustina *et al*. (2022), who reported that the relative weight of the colon ranged from 0.12% to 0.15%. The colon functioned to transport the remaining food from the small intestine to the cloaca and served as a site for water reabsorption. Histologically, the colon wall consisted of the tunica mucosa, tunica submucosa, tunica muscularis, and tunica serosa (Hidayati *et al*. 2019). The phenolic groups in fulvic acid acted as natural antioxidants capable of neutralizing free radicals in the colonic mucosa. With this antioxidant activity, oxidative stress in the colon could be reduced, thereby maintaining the integrity and function of the intestinal epithelium.

The addition of drinking water supplemented with FA-OPEFB showed a significant effect (p<0.05) in reducing the percentage of weight and relative length of the ileum. Based on Table 2, the results of the study showed that the addition of FA-OPEFB at levels of 0.1% and 0.2% significantly reduced these parameters compared to the control group (P0), although no significant difference was observed between the 0.1% (P1) and 0.2% (P2) treatments. In this study, the average weight of the ileum ranged from 0.88% to 1.05%, and the relative length ranged from 4.15 cm 100 g^{-1} to 4.89 cm 100 g^{-1}. This was consistent with the findings of Isroli *et al*. (2019) who reported that the relative weight of the ileum ranged from 0.86% to 1.03%. The ileum functioned in the continued absorption of various digested nutrients, such as amino acids, free fatty acids, monoglycerides, and glucose that had previously been broken down by pancreatic enzymes. In addition, the ileum absorbed essential minerals such as calcium, phosphorus, and iron, as well as water, which were necessary for maintaining electrolyte balance and fecal consistency (Shivus 2014). Water absorption in the ileum occurred passively, driven by solute uptake. The absorption of nutrients such as amino acids and glucose required active transport that depended on ions like sodium and calcium. Fulvic acid contained various oxygen-rich reactive functional groups, such as carboxyl, phenol, and carbonyl, which could bind to metal ions such as sodium and calcium, thus helping maintain mineral solubility in the digestive tract. Phenolic compounds are characterized by one or more hydroxyl groups attached to aromatic rings (benzene), while polyphenols contain more than one phenolic group in a single molecule and have stronger antioxidant properties. Moreover, the phenolic content in fulvic acid acted as electron or hydrogen atom donors that neutralized free radicals, thereby preventing the chain reactions of lipid peroxidation that could damage intestinal cell membranes. This was supported by Tang *et al*. (2023) who stated that fulvic acid could influence the structure of the intestinal mucosa. The intestinal mucosa is the innermost layer of the intestinal wall, consisting of the epithelial layer, muscularis mucosa, villi, and microvilli, which play a primary role in the absorption of nutrients.

Intestinal Morphology

Fulvic acid played an important role in supporting digestive tract health through its effects on the histological structure of the intestine, particularly on morphological parameters such as villus height, crypt depth, and absorptive surface area. This compound worked by enhancing mucosal integrity, stimulating epithelial cell regeneration, and supporting the population of beneficial microbes that contributed to optimal intestinal conditions. The effect of FA-OPEFB addition in drinking water on the intestinal morphology of 35-day-old broiler chickens including villus height, crypt depth, villus surface area, and villus height to crypt depth ratio is presented in Table 3.

The addition of drinking water supplemented with FA-OPEFB had a significant effect ($p<0.05$) on villus height, crypt depth, and villus surface area. Based on Table 3, the results of the study showed that the addition of 0.1% FA-OPEFB (P1) resulted in significantly lower villus height ($p<0.05$) compared to the control group (P0) and the 0.2% FA-OPEFB group (P2). However, the 0.2% FA-OPEFB treatment (P2) did not show a significant difference compared to the control (P0). This indicated that the 0.1% FA-OPEFB supplementation (P1) was insufficient to optimally stimulate intestinal mucosal development, or it may have reflected an initial adaptation response of the gut to the bioactive compounds such as phenolic and polyphenolic substances. These compounds acted as antioxidants and anti-inflammatory agents, which could influence intestinal mucosal homeostasis. In contrast, the 0.2% FA-OPEFB supplementation (P2) appeared to enhance villus height to a level approaching that of the control group (P0), suggesting a positive activation effect on villus elongation and nutrient absorption. Fulvic acid played a role in increasing the expression of tight junction proteins such as occludin and claudin, which are crucial for maintaining intestinal epithelial integrity and preventing the translocation of pathogenic bacteria. The expression of these proteins was essential for maintaining mucosal structure and enterocyte differentiation, thereby supporting the elongation of the villi. According to Aristimunha *et al*. (2020) the villus height in broiler chickens ranged from 872.47 to 972.79 µm. The villi are lined with a single layer of columnar epithelial cells equipped with microvilli and interspersed with goblet cells. The growth in villus length is associated with the small intestine's potential to absorb nutrients. The longer the intestinal villi, the more effective the nutrient absorption through the intestinal epithelial cells (Lenhardt & Mozes 2003).

Based on Table 3, the results of the study showed that the addition of 0.1% FA-OPEFB (P1) resulted in a significantly lower crypt depth ($p<0.05$) compared to the control group (P0) and the 0.2% FA-OPEFB group (P2). However, the 0.2% FA-OPEFB treatment (P2) produced a higher crypt depth value. According to Dani *et al*. (2024) the crypt depth in broiler chickens ranged from 218.94 to 244.67 µm. A greater crypt depth is associated with enhanced digestive and nutrient absorption capacity in the intestines, thereby positively influencing livestock growth (Kusuma *et al*. 2020). Polyphenols and antioxidants contained in fulvic acid could help prevent oxidative stress, thereby reducing epithelial cell damage and contributing to the stabilization of crypt depth increases. According to Ebrahimi *et al*. (2017), crypt depth is influenced by several factors, including feed composition, genetics, age, and gut health of the chicken. The villus surface area is influenced by various factors, such as feed quality, intestinal infections, and genetic background (Ebrahimi *et al*. 2017). Phenolic compounds and macro minerals in fulvic acid may stimulate the expression of genes associated with the proliferation and differentiation of intestinal absorptive cells, thus increasing the villus surface area.

Based on Table 3, the results of the study showed that the addition of 0.1% FA-OPEFB (P1) resulted in a villus surface area that was not significantly different from the control group (P0). However, the administration of 0.2% FA-OPEFB (P2) resulted in a higher villus surface area. The epithelial cells on the villi contained goblet cells that secreted mucus, which assisted the absorptive cells in the process of nutrient absorption. A broader villus surface area indicated greater efficiency in nutrient absorption, as the intestinal villi were equipped with microvilli that functioned as absorptive surfaces (Ramadhan *et al*. 2022).

The addition of drinking water supplemented with FA-OPEFB did not show a significant effect on the villus height to crypt depth ratio. According to Saragih *et al*. (2017) the VK ratio in broiler chickens ranged from 3.49 to 8.06. A high VK ratio indicated tall intestinal villi with shallow crypts, which was favorable for nutrient absorption in the intestines.

Table 3 Intestinal morphology of Cobb strain broiler chickens at 35 days of age

Variable		Treatment			*P-Value*
		P0	P1	P2	
Villus height	(µm)	805.20±33.34^{b}	662.25±23.38^{a}	799.62±54.63^{b}	0.000
Crypt depth	(µm)	227.35±15.48^{b}	179.72±9.63^{a}	250.43±38.33^{b}	0.002
Villus surface area	(mm^2)	1277.31±93.24^{a}	117.84±44.41^{a}	1485.56±100.85^{b}	0.000
Villus height to crypt depth ratio (VH:CD)		3.55±0.34	3.69±0.29	3.24±0.49	0.216

P0 = drinking water without fulvic acid (control); P1 = drinking water + 0.1% fulvic acid; P2 = drinking water + 0.2% fulvic acid. Different superscripts in the same row indicate significant differences ($p < 0.05$).

Based on the study by Budiartawan *et al*. (2018), an optimal crypt depth depended on villus height, villus surface area, and crypt depth. Increases in villus height, surface area, and VK ratio enhanced nutrient absorption capacity, which in turn could promote broiler growth (Ibrahim 2008). A low villus-to-crypt depth ratio described an intestine with fewer absorptive cells and a larger number of secretory cells (which secrete mucin). Changes in the quantity or composition of mucin on the intestinal mucosal surface could reduce nutrient absorption and increase the energy requirement needed to maintain intestinal function (Prakatur *et al*. 2019). A high villus to crypt (V:C) ratio indicated a lower rate of tissue turnover, resulting in reduced nutritional demand to compensate for villus atrophy or inflammation-related damage caused by pathogens (Jahanian *et al*. 2021).

CONCLUSIONS

The addition of FA-OPEFB in drinking water at a level of 0.1% reduced the percentage of ileum weight and relative length, and had a positive effect by decreasing crypt depth as well as the villus height to crypt depth ratio (VH:CD).

DECLARATIONS

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Ethical statement
This research was conducted in accordance with ethical standards and approved by the Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, IPB University.

Statement of conflict of interest
The authors have declared no conflict of interest.

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