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Effect of dietary *atung* seed flour (*Parinarium glaberrimum*) on small intestine characteristics of broiler chickens

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Abstract. *Sangadji I, Hehanussa SCH, Kunda RM. 2024. Effect of dietary* atung *seed flour (Parinarium glaberrimum) on small intestine characteristics of broiler chickens. Nusantara Bioscience 16: 277-283.* This study was conducted to investigate the effect of feeding *atung* (*Parinarium glaberrimum* Hassk.) seed meal on intestinal microflora, pH, and micro-morphology in broiler chickens. A total of 168-day-old male New Lohmann broiler chickens were randomly assigned to six treatment groups with four replicates and seven birds in each replicate pen. The dietary treatments consisted of feeding the same corn-soybean meal basal diet with *P. glaberrimum* seed meal inclusions at levels of 0, 0.5, 1.0, 2.0, and 4.0%, respectively. Basal diet inclusion with 50 ppm of tetracycline was also used as a positive control treatment. The data was statistically analyzed using ANOVA in a completely randomized design, a robust methodology, and continued subsequently with Duncan's test for data with significant differences. Results showed that the number of lactic acid bacteria, duodenal and ileal villus height, villus width, and villus height to crypt depth ratio was increased ($p<0.05$) with the inclusion of 0.5-1.0% *P. glaberrimum* seed meal. Coliform numbers and intestinal pH were decreased ($p<0.01$) with 1.0% *P. glaberrimum* seed meal inclusion. With respect to the results of some response variables of intestinal pH and micro-morphology, additions of *P. glaberrimum* seed meal showed better results than the addition of 50 ppm tetracycline. It can be concluded that *P. glaberrimum* seed can be recommended as a green feed additive for replacing antibiotic growth promoters in the poultry diet based on the ability to improve gut microflora and micro-morphology.

Keywords: *Atung* seed, broiler chickens, intestinal microflora and morphology, phytobiotic

INTRODUCTION

The health of the digestive tract is important for supporting optimal animal production as it is related to the function of digestion and nutrient absorption and plays an essential role in the animal's defense system against disease infections. The intestinal microbiota has been known to play a crucial function in feed nutrient metabolism and animal intestinal health (Carrasco et al. 2019; Lee et al. 2019; Haberecht et al. 2020). Haberecht et al. (2020) suggested that a stable intestinal microflora has a significant effect on resisting pathogen infection. A healthy gut leads to efficiency in micronutrient digestibility and transportation. Colonization of non-pathogenic bacteria, such as *Lactobacillus* sp., in the gut of poultry is beneficial to inhibiting pathogen growth by producing lactic acid and short-chain fatty acid (Yadav and Jha 2019) and improving intestinal morphology and health (Li et al. 2018). Therefore, manipulation of animal's gut microbiota would be an important tool to maintain animal health and to enhance growth performance. Gut microflora can be influenced by diet (Ndotono et al. 2022), including additives such as antibiotics, prebiotics, probiotics, enzymes, synbiotics, and phytobiotics (Stanley et al. 2014; Yadav and Jha 2019).

Antibiotic Growth Promoters (AGPs) have been used in poultry production to improve growth performance for more than 60 years (Bajagai et al. 2020). AGP has a direct effect on the microflora in the digestive tract, which leads to reducing the nutrient competition between pathogenic bacteria and the host, reducing subclinical infections and toxic metabolites, and increasing feed efficiency due to the intestinal wall thinning (Bajagai et al. 2020; Tran et al. 2023).

Since AGP is no longer used as a feed additive in many countries due to the possible negative effects on the growing number of antibiotic-resistant bacteria (Tran et al. 2023), many studies have been conducted to find safer alternatives for AGP; among these are phytobiotics. Phytobiotics or phytogenic feed additives are a class of non-antibiotic growth promoters consisting of herbs, spices, or essential oils that are given to animals to improve growth performance and health (Wang et al. 2024). Contreras-López et al. (2024) reviewed the important properties of phytobiotics to improve growth performance in animals by improving gut microflora, nutrient digestibility, and morphology structure of the digestive tract. Some studies have shown that the inclusion of phytobiotics in poultry diets improved the balance of gut microflora (Abdelli et al. (2021) and intestinal morphology (Ndotono et al. 2022).

In this study, we supplemented diet of broiler chickens with a seed meal of *atung* (*Parinarium glaberrimum* Hassk.) to determine the probable positive effects on

the digestive tract profile, particularly the pH, microflora, and morphology of the small intestine. *P. glaberrimum* is a forest plant widespread in Maluku-Indonesia (Figure 1), whose seeds have historically been used to treat diarrhea and bleeding in pregnant women, as well as seafood preservatives (Hehanussa et al. 2022). In vitro studies have revealed that *P. glaberrimum* seed has antimicrobial properties (Pacana and Galarpe 2017b; Hehanussa et al. 2019) and antioxidant activities (Sarastani et al. 2002). To our knowledge, no studies have been published on the effect of *P. glaberrimum* seed meal supplementation on the pH, microflora, and morphology of the small intestine in broiler chickens.

MATERIALS AND METHODS

Experimental design and diet

This experiment was conducted at the Department of Animal Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia. A total of 168 one-day-old male New Lohmann broiler chickens were reared in litter-floor pens of 85×100 cm^2 for 35 days. The chicks were randomly placed into six treatment groups, with four replicates and seven chicks in each replicate pen. The temperature was initially set at 32°C for four days before gradually decreasing in accordance with the New Lohmann MB 202 broiler maintenance standard. In the lighting program, the birds received continuous light for the first week, and then the lighting was set at 23 h light: 1 h dark until slaughtered. The temperature, humidity, light, and ventilation settings were consistent across all treatment groups.

Figure 1. *Atung* (*Parinarium glaberrimum* Hassk.) tree and seed (Hehanussa et al. 2022)

Table 1. Ingredients and nutrient composition of dietary treatments for broiler chickens (0-35 d of age)

Ingredients (%)	Dietary treatments[1]					
	NC	PC	T-0.5	T-1	T-2	T-4
Yellow corn	58.50	58.50	58.50	58.50	58.50	58.50
Soybean meal	24.25	24.25	24.25	24.25	24.25	24.25
Meat bone meal	6.75	6.75	6.75	6.75	6.75	6.75
Rice bran	2.00	2.00	2.00	2.00	2.00	3.00
Palm oil	3.00	3.00	3.00	3.00	3.00	2.00
DL-Methionine 99%	0.20	0.20	0.20	0.20	0.20	0.20
L-Lysine 78%	0.10	0.10	0.10	0.10	0.10	0.10
L-Threonine 78%	0.05	0.05	0.05	0.05	0.05	0.05
NaCl	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin-mineral premix[2]	0.20	0.20	0.20	0.20	0.20	0.20
Dicalcium phosphate	0.15	0.15	0.15	0.15	0.15	0.15
Calcium carbonate	0.50	0.50	0.50	0.50	0.50	0.50
Tetracycline (50 ppm)	-	+	-	-	-	-
P. glaberrimum seed meal	0.00	0.00	0.50	1.00	2.00	4.00
Filler (sand)	4.00	4.00	3.50	3.00	2.00	0.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient composition						
ME[3] (kcal/kg)	3017	3017	3035	3053	3088	3101
Crude protein (%)	21.01	21.01	21.04	21.07	21.13	21.35
Crude fat (%)	6.54	6.54	6.55	6.56	6.58	5.70
Crude fiber (%)	3.04	3.04	3.05	3.07	3.10	3.42
Calcium (%)	1.01	1.01	1.02	1.03	1.05	1.09
Available Phosphorus (%)	0.47	0.47	0.47	0.47	0.47	0.48
Methionine (%)	0.52	0.52	0.52	0.52	0.52	0.52
Lysine (%)	1.15	1.15	1.15	1.15	1.15	1.16
Threonine (%)	0.84	0.84	0.84	0.84	0.84	0.84

Note: [1]NC: Basal diet without additive (Negative Control), PC: Basal diet + 50 ppm of tetracycline (Positive Control), T-0.5: Basal diet + 0.5% *Parinarium glaberrimum* seed meal, T-1: Basal diet + 1.0% *P. glaberrimum* seed meal, T-2: Basal diet + 2.0% *P. glaberrimum* seed meal, T-4: Basal diet + 4.0% *P. glaberrimum* seed meal; [2]Vitamin-mineral premix composed of vitamin A 1.250.000 IU, vitamin D 250.000 IU, vitamin E 750 IU, vitamin K3 200 mg, vitamin C 5.000 mg, vitamin B1 250 mg, vitamin B2 400 mg, vitamin B6 100 mg, vitamin B12 1.2 mg, biotin 20 mg, folic acid 50 mg, nicotinic acid 3.000 mg, Ca-d pantothenate 400 mg, choline chloride 1.500 mg, copper 500 mg, iron 2.500 mg, iodine 20 mg, manganese 6.000mg, selenium 20 mg, zinc 7.000 mg, cobalt 20 mg, Lysine 16.000 mg, DL-methionine 5.000 mg; [3]ME: metabolizable energy

The first Newcastle Disease (ND) vaccine (Medivac ND La Sota, PT. Medion Farma, Bandung, Indonesia) was administered at 4 days of age via eye drop, and the second ND vaccine (Medivac ND La Sota, Bandung, Indonesia) was administered at 21 days of age via drinking water. Vaccination against infectious bursal disease (Medivac Gumboro A, PT. Medion Farma, Bandung, Indonesia) was carried out at the age of 18 days through drinking water. Birds in all groups were likely to consume feed and water on an ad libitum basis. All of the diets for the experiments were provided in mash form.

The diets were formulated to meet the nutrient requirements of broilers according to the National Research Council US (1984) provision. The ingredients composition and nutrient content of the experimental diets are shown in Table 1. The Negative Control (NC) group was a corn-soybean meal basal diet without any additive supplementation, while the Positive Control (PC) group was a basal diet with 50 ppm of tetracycline supplementation. The T-0.5, T-1, T-2, and T-4 were the basal diet with 0.5%, 1.0%, 2.0%, and 4.0% *P. glaberrimum* seed meal supplementations, respectively. On day 35, one male chicken with a body weight close to the median of each replicate group was chosen and was slaughtered by cutting the trachea, esophagus, as well as jugular vein, and carotid artery on both sides. After the birds had completely died, small intestines were eviscerated immediately to obtain data on intestinal pH, microbial population, and histomorphology.

***Parinarium glaberrimum* seed meal preparation**

Parinarium glaberrimum fruits were obtained from Soya Village, Maluku Province, Indonesia. *P. glaberrimum* seeds were collected from the ripe fruit that had already fallen on the ground. The seeds were removed, air-dried, and ground to pass a 40-mesh screen for use in mixed diet treatments.

Bacterial enumeration

Samples content of the small intestine (ileum) were immediately removed after being slaughtered, collected into sterile tubes, tightly sealed, and stored in ice before the enumeration of total bacteria. The total bacteria enumeration was performed immediately after the sample was collected using the method described by Manafi et al. (2016). In each replication, one gram of ileum digesta was diluted with 9 mL of phosphate buffer saline (Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany). Initial dilution was used as a starting point for subsequent serial dilutions. Serial dilutions were made for *Lactobacillus* from 10^{-1} to 10^{-9} and 10^{-1} to 10^{-7} for coliforms. A 0.1 mL sample from each serial dilution was inoculated into the selective agar plate and spread with a sterile swab. *Lactobacillus* was grown anaerobically on MRS agar (Merck GmbH, Darmstadt, Germany) for 48 hours. Coliform was grown aerobically on Brilliance *Escherichia coli* coliform selective agar (Merck GmbH, Darmstadt, Germany) for 24 hours. After incubation, the colony of intestinal bacteria in every plate of each serial dilution was counted using a bacterial colony counter. The intestinal bacterial population was expressed in Log 10 CFU/g.

The pH of small intestine

The small intestine's acidity was measured immediately after the birds were slaughtered. The digestive tract was removed from the body, and the pH of each small intestinal segment was measured immediately using a digital pH meter (Hanna-HI 99121, Hanna Instruments, Woonsocket, USA) at three points in each section.

Intestinal micromorphology

Intestinal sample preparation and measurement of villus morphology were conducted following Popescu et al. (2021) and Alagbe et al. (2024). After the chicken was killed, the digestive tract was immediately removed from the body, followed by the separation of each part of the small intestine. Samples of the small intestine were collected from the endpoint gizzard to the ileocecal junction. Intestinal samples were taken approximately 6 cm from the middle of each section, flushed with phosphate-buffered saline (pH 7.4) gently, cut into 3 similar pieces, and fixed in 10% neutral buffered formalin solution (Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany) for about 48 hours. Samples of the intestines tissue were dehydrated with increasing concentrations (70, 80, 95, and 100%) of alcohol. The samples were then infiltrated with xylene and embedded into paraffin. The tissue samples were cut in 5 µm using a microtome (S35, CellPath Ltd, Newtown, Powys, UK), attached to slides, and stained with hematoxylin (Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany) and eosin (Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany). Histomorphology slide of the villi was performed using an optical microscope (Olympus CX23, Olympus Corporation, Tokyo, Japan) coupled with a digital camera software (optical viewer 3.3, PT. Miconos, Yogyakarta, Indonesia) to capture images of the villi. The morphology of the villi was then measured using a computer-integrated image program software (Image raster, Version 3.0, PT. Miconos, Yogyakarta, Indonesia). Villus height was measured from the villus tip to the villus crypt junction and measurement of crypt depth following the depth of the invagination between adjacent villi. Villus width was measured at its middle part (Kavoi et al. 2016).

Statistical analysis

Data were statistically analyzed using Oneway ANOVA in a completely randomized design with six treatments and four replications. Data from different statistical analysis results between treatments were tested further using Duncan's new multiple-range test. Data were expressed in means, and were statistically analyzed with SPSS for Windows tools (SPSS, version 25, IBM, New York, USA).

RESULTS AND DISCUSSION

Ileal microflora

Table 2 shows that dietary supplementation of *P. glaberrimum* seed meal has positive effects on the gut microfloral population, which increases lactic acid bacteria and reduces the coliform numbers in the gut. The reduction in coliform numbers in *P. glaberrimum* seed meal groups confirmed the antibacterial properties of *P. glaberrimum* seed against intestinal pathogens. Phytochemical compounds contained in *P. glaberrimum* seeds, such as phenols, tannins, flavonoids, alkaloids, and saponins (Pacana and Galarpe 2017a; Hehanussa et al. 2019) were reported to have antibacterial activity against pathogenic bacteria. In vitro studies have previously demonstrated that *P. glaberrimum* seed inhibited the growth of *E. coli*, *Salmonella* sp., and *Staphylococcus aureus* (Pacana and Galarpe 2017b; Hehanussa et al. 2019).

Improvement of the *Lactobacillus* and reduction of the coliform bacteria numbers in the intestine could be attributed to the presence of phenolic compounds in *P. glaberrimum* seed. Polyphenols are absorbed in small amounts in the intestine (that was approximately 5-10%). Unabsorbed polyphenols, on the other hand, have a significant impact on the intestinal environment by suppressing or stimulating the growth of certain intestinal microbes. Phenolics that were unabsorbed and their metabolites may play an essential role in maintaining the intestinal environment by modulating microbiota growth and population.

A decrease in pathogenic bacteria in the intestine confirmed by dietary supplementation with *P. glaberrimum* seed leads to a decrease of toxic metabolites, which might result in a favorable condition of the intestine for beneficial bacteria growth. The results of this study were supported by Gheisar and Kim (2017), who suggested that a reduction in the number of pathogenic (e.g., *E. coli*) may lead to positive effects in the digestive tract by increasing the population of beneficial bacteria (e.g., *Lactobacillus* sp.). On the other hand, *Lactobacillus* produces Short-Chain Fatty Acids (SCFA), among these acetate, propionic, and butyrate, as well as lactic acid, that also have anti-microbial activities. Organic acids produced by *Lactobacillus* might inhibit the growth of pathogenic bacteria in the intestine (Yadav and Jha 2019). The result of the current study was similar to other researchers (Aleman and Yadav 2024), which reported that phytobiotic inclusions reduced the population of pathogenic bacteria in the gut and increased the population of beneficial bacteria, such as *Lactobacillus*. Wang et al. (2018) stated that increased *Lactobacillus* spp. and decreased coliform numbers lead to a favorable intestinal environment which may improve gastrointestinal function, nutrient digestibility, and growth performance.

The pH of small intestine

Statistical analysis (Table 3) showed that feeding broiler chickens with a diet supplemented with *P. glaberrimum* seed meal reduced intestinal pH, particularly in the treatments of 1-2% supplementations, 1-4% supplementation, and 0.5-1% supplementation which were significantly decreased ($p<0.01$) in the duodenum, jejunum, and ileum compared to that of in the negative control. A lower pH environment of the intestine is necessary for maintaining gut health because it inhibits the growth of harmful bacteria (Lee et al. 2019; Haberecht et al. 2020), and it can reduce metabolic requirements and increase the availability of nutrients for the host.

The decreased pH in the intestine of broiler chickens fed a diet with *P. glaberrimum* seed meal inclusions was probably linked to an elevated *Lactobacillus* population in the gut. *Lactobacillus* sp. produced SCFA that had the possibility to lower pH in the intestine of broiler chickens (Haberecht et al. 2020). Dono et al. (2014) stated that lower intestinal pH is associated with beneficial microbial colonization and may also be related to better energy and nutrient utilization efficiency. As a result, feeding *P. glaberrimum* seed meal might have the potency to improve energy utilization efficiency and nutrient utilization by lowering intestinal pH and suppressing the growth of pathogenic microbiota.

The findings were consistent with other studies that found supplementing broiler chicken diet with phytobiotics lowered the intestinal pH. Ferdous et al. (2019) showed that feeding phytobiotic additives decreased the intestinal pH of broiler chickens when compared to that of the negative control, positive control, or probiotic treatments. Administration of phytobiotic *Artemisia annua* L. has been shown to lower the ileal and cecal pH of broiler chickens (Lee et al. 2014). Similarly, Anugom and Ofongo (2019) discovered a decrease in ileal and cecal pH in 28-day-old broiler chickens fed a diet supplemented with *Ocimum gratissimum* L. leaf extract.

Intestinal micromorphology

Results in Table 4 showed that *P. glaberrimum* seed meal supplementations improved duodenal and ileal villus height but had no effect on jejunal villus height. When 0.5 and 4% *P. glaberrimum* seed meal were added to the diet, villus height in the duodenal section increased ($p<0.05$) when compared to that of the negative control group. The diet of 1-4% *P. glaberrimum* seed meal increased villus height in the ileum ($p<0.05$) when compared to that of the negative control group. The inclusion of 0.5-2% and 0.5-4% *P. glaberrimum* seed meal increased ($p<0.05$) both the duodenal and ileal villus width, respectively, when compared to those of the negative control group. Likewise, the inclusion of 0.5, 1.0, and 4.0% *P. glaberrimum* seed meal increased ($p<0.05$) villus width of the jejunal wall when compared to that of the negative control group.

Increased villus height in treated birds might correspond to the increased digestive and nutrient absorption surface area, intestinal enzyme expression, and transport nutrient system (Yadav and Jha 2019). Nurhayati et al. (2021) reported that a larger villus correlated with a larger surface area for nutrient absorption. Improvement in villus height due to the *P. glaberrimum* seed meal inclusion in the present study might have beneficial properties to improve micro-nutrients uptake for better growth performance.

Table 2. The effects of *Parinarium glaberrimum* seed supplementation on gut microflora in ileal digesta of 35-day-old broiler chickens

Bacteria (Log10 CFU/g)	Dietary treatments[1]						SEM[2]	*p*
	NC	PC	T-0.5	T-1	T-2	T-4		
Lactobacillus	6.92^{a}	7.95^{abc}	8.46^{c}	7.28^{ab}	7.74^{abc}	8.05^{bc}	0.158	0.044
Coliform	3.83^{b}	2.70^{a}	2.84^{a}	2.66^{a}	3.19^{a}	3.22^{a}	0.112	0.007

Note: ^{a,b,c}Mean in the same row without common letter are different at p<0.05; [1]NC: Basal diet without additive (Negative Control), PC: Basal diet + 50 ppm of tetracycline (Positive Control), T-0.5: Basal diet + 0.5% *P. glaberrimum* seed meal, T-1: Basal diet + 1.0% *P. glaberrimum* seed meal, T-2: Basal diet + 2.0% *P. glaberrimum* seed meal, T-4: Basal diet + 4.0% *P. glaberrimum* seed meal; SEM[2]: Standard Error of Mean

Table 3. The effect of *Parinarium glaberrimum* seed supplementation on the intestinal pH of 35-day-old broiler chickens

Intestinal segments	Dietary treatments[1]						SEM[2]	*p*
	NC	PC	T-0.5	T-1	T-2	T-4		
Duodenum	5.70^{bc}	5.49^{ab}	5.90^{c}	5.2^{a}	5.3^{a}	5.4^{ab}	0.676	0.002
Jejunum	5.72^{c}	5.97^{c}	5.43^{bc}	4.7^{a}	5^{ab}	5.4^{ab}	0.117	0.003
Ileum	6.09^{bc}	6.57^{c}	5.18^{a}	5.3^{a}	5.5^{ab}	5.8^{ab}	0.128	0.002

Note: ^{a,b,c}Mean in the same row without common letter are different at p<0.05; [1]NC: Basal diet without additive (Negative Control), PC: Basal diet + 50 ppm of tetracycline (Positive Control), T-0.5: Basal diet + 0.5% *P. glaberrimum* seed meal, T-1: Basal diet + 1.0% *P. glaberrimum* seed meal, T-2: Basal diet + 2.0% *P. glaberrimum* seed meal, T-4: Basal diet + 4.0% *P. glaberrimum* seed meal; SEM[2]: Standard Error of Mean

Table 4. The effect of antibiotic and *Parinarium glaberrimum* seed supplementation on the intestinal morphology of 35-day-old broiler chickens

Attribute	Dietary treatments[1]						SEM[2]	*p*
	NC	PC	T-0.5	T-1	T-2	T-4		
Villus height (µm):								
Duodenum	1762^{a}	1946^{ab}	2147^{b}	1989^{ab}	1914^{ab}	2098^{b}	38.458	0.033
Jejunum	1398	1437	1459	1487	1655	1523	32.647	0.281
Ileum	634^{a}	712^{ab}	698^{ab}	713^{b}	799^{bc}	861^{c}	21.255	0.013
Villus width (µm):								
Duodenum	170^{a}	179^{ab}	218^{c}	212^{bc}	211^{bc}	171^{a}	6.116	0.023
Jejunum	139^{a}	213^{b}	208^{b}	175^{ab}	219^{b}	198^{b}	8.158	0.022
Ileum	133^{a}	184^{b}	180^{b}	193^{b}	203^{b}	203^{b}	6.786	0.011
Crypt depth (µm):								
Duodenum	297	305	233	250	256	258	10.242	0.282
Jejunum	236	186	212	205	214	206	6.049	0.293
Ileum	155	137	122	123	130	130	4.715	0.389
VH/CD ratio:								
Duodenum	6.10^{a}	6.61^{ab}	9.37^{d}	8.05^{bcd}	7.61^{abc}	8.51^{cd}	0.292	0.002
Jejunum	6.06^{a}	7.88^{b}	7.03^{b}	7.31^{b}	7.73^{b}	7.20^{b}	0.170	0.013
Ileum	4.29^{a}	5.24^{b}	5.75^{bc}	5.86^{bc}	6.24^{bc}	6.53^{c}	0.190	<0.01

Note: ^{a,b,c}Mean in the same row without common letter are different at p<0.05; [1]NC: Basal diet without additive (Negative Control), PC: Basal diet + 50 ppm of tetracycline (Positive Control), T-0.5: Basal diet + 0.5% *P. glaberrimum* seed meal, T-1: Basal diet + 1.0% *P. glaberrimum* seed meal, T-2: Basal diet + 2.0% *P. glaberrimum* seed meal, T-4: Basal diet + 4.0% *P. glaberrimum* seed meal; SEM[2]: Standard Error of Mean

The result of the histo-morphology examination showed that *P. glaberrimum* seed meal inclusions had no effect on the villus crypt depth of the small intestine. The crypts are villus factories, and deeper crypts have faster tissue turnover, allowing for villus renewal as needed in response to normal shedding or inflammation caused by pathogens or their toxins. Deeper crypts indicate a greater need for new tissue regeneration, resulting in higher energy and protein requirements for intestinal maintenance (Mfoundou et al. 2022). However, no response in crypt depth with the dietary *P. glaberrimum* seed meal inclusion groups might indicate that chickens in all treatments were repairing or renewing villi at the same rate.

From the results obtained, there was a significant difference in the negative control treatment with the administration of *P. glaberrimum* seeds. It can be seen that the administration of 0.5-4% *P. glaberrimum* seed meal increased the ratio of villus height to crypt depth of the duodenum (p<0.05), jejunum (p<0.05), and ileum (p<0.01). Improvement of the villus height to crypt depth ratio in the duodenum, jejunum, and ileum could be attributed to the increased villus height with no alteration in crypt depth at the same time. Ali et al. (2018) stated that the increase in villus height to crypt depth ratio is associated with

improved host growth performance. The findings in the current experiment suggested that *P. glaberrimum* seed meal may promote a healthier intestinal environment with improved nutrient absorption capacity, allowing for improved growth performance in broiler chickens.

In general, *P. glaberrimum* seed meal inclusion improved the villus structure in the intestinal wall of broiler chicken, which is linked to better intestinal microflora balance in the digestive tract (Table 1). An increased number of *Lactobacillus* in groups with *P. glaberrimum* seed meal inclusion may also promote the improvement of intestinal structure. Li et al. (2018) reported that *Lactobacillus* inclusion improved intestinal morphology and gut health. Nurhayati et al. (2021) also stated that colonization of beneficial bacteria, such as *Lactobacillus*, in the digestive tract might improve intestinal villus structure. *Lactobacillus* sp. produced SCFA, which has been known as an essential energy source in stimulating intestinal epithelial cell proliferation, particularly butyric acid (Ali et al. 2018; Yadav and Jha 2019; Nurhayati et al. 2021). Improvement of the morphology structure of the small intestine in the current study, on the other hand, could be linked to the presence of polyphenols, particularly flavonoids, in the *P. glaberrimum* seed. Wang et al. (2020) stated that the health-promoting properties of flavonoids for intestinal health were related to the ability to modulate barrier permeability, protect the mucus layer, regulate the intestinal immune system, fight against oxidative stress, and positively shape the intestinal microbiome.

It has been reported in some research that diet inclusion with several green feed additives, or plant origins feed additives, improved the intestinal morphology of poultry, which was consistently found in the current experiment. For instance, 1.0-2.0% garlic meal inclusion or inclusion of 1.2% *Moringa oleifera* Lam. leaf meal (Khan et al. 2017) increased villus height in all small intestine segments of broiler chickens. Furthermore, dietary inclusion of *M. oleifera* leaf meal also increased villus height to crypt depth ratio on the wall of ileum. Boka et al. (2014) showed that 2.0 and 3.0% dietary inclusion of black cumin increased villus height, villus height to crypt depth ratio, and crypt depth. Kiczorowska et al. (2016) reported that incorporating a diet with 3-4% *Boswellia serrata* Roxb. ex Colebr. resin increased villus height to crypt depth ratio in the wall of duodenum.

In conclusion, based on the ability to improve gut microflora and morphology, *P. glaberrimum* seed meal can be recommended as a green feed additive to replace AGP in the poultry diet.

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REFERENCES

Abdelli N, Solà-Oriol D, Pérez JF. 2021. Phytogenic feed additives in poultry: Achievements, prospective and challenges. Animals 11: 3471. DOI: 10.3390/ani11123471.

Alagbe EO, Sung JY, Lindsey K, Pasternak JA, Adeola O. 2024. PSI-21 Optimizing sample size for accurate measurements and prediction equations for intestinal morphology in broiler chickens. J Anim Sci 102 (2): 281-282. DOI: 10.1093/jas/skae102.320.

Aleman RS, Yadav A. 2024. Systematic review of probiotics and their potential for developing functional nondairy foods. Appl Microbiol 4: 47-69. DOI: 10.3390/applmicrobiol4010004.

Ali N, Alkassar S, Alkassar A. 2018. The effect of using organic acid as an alternative to antibiotics drugs on productive and physiological performance of broilers ross – 308. Adv Anim Vet Sci 6 (9): 359-365 DOI: 10.17582/journal.aavs/2018/6.9.359.365.

Anugom YO, Ofongo RTS. 2019. Impact of aqueous *Ocimum gratissimum* (Lyn) leaf extract on growth performance, gut pH and bacterial counts in broiler chickens. Intl J Poult Sci 18 (7): 309-316. DOI: 10.3923/ijps.2019.309.316.

Bajagai YS, Alsemgeest J, Moore RJ, Van TT, Stanley D. 2020. Phytogenic products, used as alternatives to antibiotic growth promoters, modify the intestinal microbiota derived from a range of production systems: An in vitro model. Appl Microbiol Biotechnol 104: 10631-10640. DOI: 10.1007/s00253-020-10998-x.

Boka J, Mahdavi AH, SamieAH, Jahanian R. 2014. Effect of different levels of black cumin (*Nigella sativa* L.) on performance, intestinal *Escherichia coli* colonization dan jejunal morphology in laying hens. J Physiol Anim Nutr 98: 373-383. DOI: 10.1111/jpn.12109.

Carrasco JMD, Casanova NA, Miyakawa MEF. 2019. Microbiota, gut health and chicken productivity: what is the connection? Microorganisms 7: 374. DOI: 10.3390/microorganisms7100374.

Contreras-López G, Carrillo-Lopez LM, Vargas-Bello-Pérez E, García-Galicia IA. 2024. Microencapsulation of feed additives with potential in livestock and poultry production: A systematic review. Chil J Agric Anim Sci 40 (1): 229-249. DOI: 10.29393/chjaas40-21mfgi40021.

Dono ND, Sparks NH, Olukosi OA. 2014. Association between digesta pH, body weight, and nutrient utilization in chickens of different body weights and at different ages. J Poult Sci 51: 180-184. DOI: 10.2141/jpsa.0120151.

Ferdous FM, Arefin SM, Rahman MM, Ripon MMR, Rashid MH, Sultana RM, Hossain MT, Ahammad MU, Rafiq K. 2019. Beneficial effects of probiotic and phytobiotic as growth promoter alternative to antibiotic for safe broiler production. J Adv Vet Anim Res 6 (3): 409-415. DOI: 10.5455/javar.2019.f361.

Gheisar MM, Kim IH. 2017. Phytobiotics in poultry and swine nutrition. Italian J Anim Sci 10 (1): 1350120. DOI: 10.1080/1828051X.2017.1350120.

Haberecht S, Bajagai YS, Moore RJ, Moore RJ, Van TT, Stanley D. 2020. Poultry feeds carry diverse microbial communities that influence chicken intestinal microbiota colonisation and maturation. AMB Express 10: 143. DOI: 10.1186/s13568-020-01077-5.

Hehanussa SC, Zuprizal Z, Hanim C, Dono ND. 2022. Performance and haematological profile of broiler chickens fed diet containing *atung* (*Parinarium glaberrimum* Hassk.) seed powder. Buletin Peternakan 46 (2): 104. DOI: 10.21059/buletinpeternak.v46i2.73251.

Hehanussa SCH, Zuprizal, Dono ND, Hanim C. 2019. Potential of *atung* seeds (*Parinarium glaberrimum* Hassk.) as a phytobiotic candidate in poultry ration based on nutrient composition, phytochemical and antibacterial properties. IOP Conf Ser: Earth Environ Sci 387: 012072. DOI: 10.1088/1755-1315/387/1/012072.

Kavoi BM, Gakuya DW, Mbugua PN, Kiama SG. 2016. Effects of dietary *Moringa oleifera* leaf meal supplementation on chicken intestinal structure and growth performance. J Morphol Sci 33: 186-192. DOI: 10.4322/jms.095915.

Khan I, Zaneb H, Masood S, Yousaf MS, Rehman HF, Rehman H. 2017. Effect of *Moringa oleifera* leaf meal supplementation on growth performance and intestinal morphology in broiler chickens. J Anim Physiol Anim Nutr 101: 114-121. DOI: 10.1111/jpn.12634.

Kiczorowska B, Al-Yasiry ARM, Samolinska W, Marek A, Pyzik E. 2016. The effect of dietary supplementation of the broiler chicken diet with *Boswellia serrata* resin on growth performance, digestibility, and gastrointestinal characteristics, morphology, and microbiota. Livest Sci 191: 117-124. DOI: 10.1016/j.livsci.2016.07.019.

Lee H, Lee S, Kim IS, Oh S, Kim J, Sung NJ. 2014. Effect of dietary supplementation of *gaeddongssuk* (*Artemisia annua* L.) on the meat quality in the chicken (Woorimatdag). J Agric Life Sci 48: 235-250. DOI: 10.14397/jals.2014.48.3.235.

Lee S, La T, Lee H, Choi I, Song CS, Park S, Lee J, Lee S. 2019. Characterization of microbial communities in the chicken oviduct and the origin of chicken embryo gut microbiota. Sci Rep 9: 6838. DOI: 10.1038/s41598-019-43280-w.

Li W, Wang W, Liu D, Guo Y. 2018. Effects of *Lactobacillus acidophilus* on the growth performance and intestinal health of broilers challenged with *Clostridium perfringens*. J Anim Sci Biotechnol 9: 25. DOI: 10.1186/s40104-018-0243-3.

Manafi M, Hedayati M, Khalaji S. 2016. Effectiveness of phytogenic feed additive as alternative to bacitracin methylene disalicylate on hematological parameters, intestinal histomorphology and microbial population and production performance of Japanese quails. Asian-Australas J Anim Sci 29 (9): 1300-1308. DOI: 10.5713/ajas.16.0108.

Mfoundou JDL, Guo Y, Yan Z, Wang X. 2022. Morpho-histology and morphometry of chicken testes and seminiferous tubules among yellow-feathered broilers of different ages. Vet Sci 9: 485. DOI: 10.3390/vetsci9090485.

National Research Council (US). Subcommittee on Poultry Nutrition. 1984. Nutrient Requirements of Poultry: Eighth Revised Edition, 1984. The National Academy of Sciences Press, Washington DC. DOI: 10.17226/19397.

Ndotono EW, Khamis FM, Bargul JL, Tanga CM. 2022. Gut microbiota shift in layer pullets fed on black soldier fly larvae-based feeds towards enhancing healthy gut microbial community. Sci Rep 12: 16714. DOI: 10.1038/s41598-022-20736-0.

Nurhayati, Hartutik, Sjofjan O, Widodo E. 2021. The effect of using mannan oligosaccharides extracted from fermented palm kernel cake and cassava by-product mixture in ration on broiler gut profile. Livest Res Rural Dev 33: 39.

Pacana JM, Galarpe Van RKR. 2017a. Phytochemical screening and toxicity of *Atuna racemosa* Rafin. chrysobalanaceae shell and seed extracts. Intl J Adv Appl Sci 4 (11): 110-115. DOI: 10.21833/ijaas.2017.011.017.

Pacana JM, Galarpe Van RKR. 2017b. Antibacterial property *of Atuna racemosa* Rafin. chrysobalanaceae shell and kernel extracts (aqueous, methanol, ethyl acetate, and decoction). Intl J Biosci 11 (1): 443-448. DOI: 10.12692/ijb/11.1.443-448.

Popescu RG, Voicu SN, Grădișteanu Pîrcălăbioru G, Gharbia SA, Hermenean AO, Georgescu SE, Panaite TD, Turcu RP, Dinischiotu A. 2021. Impact of dietary supplementation of flaxseed meal on intestinal morphology, specific enzymatic activity, and cecal microbiome in broiler chickens. Appl Sci 11 (15): 6714. DOI: 10.3390/app11156714.

Sarastani D, Soekarto ST, Muchtadi TR, Fardiaz D, Apriyantono A. 2002. Aktivitas antioksidan ekstrak dan fraksi ekstrak biji Atung (*Parinarium glaberrimum* Hassk.) [Antioxidant Activities of *Parinarium glaberrimum* Hassk. Extracts and their Fractions]. Jurnal Teknologi dan Industri Pangan 13: 149-149. [Indonesian]

Stanley D, Hughes RJ, Moore RJ. 2014. Microbiota of the chicken gastrointestinal tract: Influence on health, productivity and disease. Appl Microbiol Biotechnol 98: 4301 - 4310. DOI: 10.1007/s00253-014-5646-2.

Tran C, Horyanto D, Stanley D, Cock IE, Chen X, Feng, Y. 2023. Antimicrobial properties of *Bacillus* probiotics as animal growth promoters. Antibiotics 12 (2): 407. DOI: 10.3390/antibiotics12020407.

Wang J, Deng L, Chen M, Che Y, Li L, Zhu L, Chen G, Feng T. 2024. Phytogenic feed additives as natural antibiotic alternatives in animal health and production: A review of the literature of the last decade. Anim Nutr 17: 244-264. DOI: 10.1016/j.aninu.2024.01.012.

Wang J, Ji H, Wang S, Liu H, Zhang W, Zhang D, Wang Y. 2018. Probiotic *Lactobacillus plantarum* promotes intestinal barrier function by strengthening the epithelium and modulating gut microbiota. Front Microbiol 9: 1953. DOI: 10.3389/fmicb.2018.01953.

Wang M, Zhao H, Wen X, Ho CT, Li S. 2020. Citrus flavonoids and the intestinal barrier: Interactions and effects. Compr Rev Food Sci Food Saf 20: 225-251. DOI: 10.1111/1541-4337.12652.

Yadav S, Jha R. 2019. Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance, and health of poultry. J Anim Sci Biotechnol 10: 2. DOI: 10.1186/s40104-018-0310-9.