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RESEARCH ARTICLE

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Impacts of Probiotic and Dietary Lamtoro Leaf Meal on the Growth Performance, Digestibility and Small Intestinal Morphometry of Kampung Chicken

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ABSTRACT

The present research aims to elucidate the interaction between the influence of Probiotic inclusion in drinking water and Lamtoro leaf meal (LLM) as an additional dietary fiber, which may act as a prebiotic. The research used 200 one-day-old chickens with a mean body weight of 25.8±1.36 g/head. Based on a completely randomized design, the chickens were divided into 5 treatment groups (P0-negative control; P1- P1-positive control; P2- 0.3% Probiotic; P3- 0.6% Probiotic; P4- 0.9% Probiotic) 4 replications with 5 chicks. Performances (body weight gain, feed intake, crude protein and fiber intakes), gross-morphometric indices of small intestine and histo-morphometric indices of ileum were measured. Along the experiment, there is no different response between the group fed basal diet (P0) and the group fed additional 3% LLM (P1). Along the 1st five weeks, the intakes of diet, crude protein and crude fiber were not significantly different among the five treatment groups. Along the 2nd five weeks, probiotic inclusion increased feed intake significantly. In line, probiotic inclusion significantly increased both the apparent digestibility of crude protein and crude fiber. Calculated FCR along the 1st and 2nd five weeks were significantly improved with increasing the level of probiotic inclusion. Moreover, probiotic inclusion also affected significantly to increase gross and histo-morphometric indices of the Ileum. In conclusion, the current research suggests that probiotic inclusion in drinking water and addition of 3% LLM as additional fiber resulted in a beneficial synergistic impact on significant improving the performance of Kampung chicken and absorption processes in small intestine.

Keywords: Kampung chicken, Probiotic, Lamtoro leaf meal, Performance, Small intestinal morphometry.

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INTRODUCTION

Kampung chicken is a general name given to all local or native chicken of Indonesia, or "without any specific exterior characteristic, and reared as egg, or meat producers (dual purposes). At least 30 clumps of Kampung chicken have been identified which having various morphological characteristics, and their ability as meat or egg producers. In general, the ability and efficiency as meat or egg producer is markedly lower than those of the exotic commercial breed of chicken (Muladno et al., 2013).

A lot of efforts have been conducted to improve productivity of Kampung chicken. In livestock production including poultry, using prebiotic and/or probiotic or in combination of both, and known as symbiotic, is an alternative solution to banning in using antibiotic as growth promotor. The main interest of current research is to reveal the responses of Kampung chicken treated probiotic inclusion in drinking water and fed dietary Lamtoro leaf meal.

According to FAO/WHO (2002) probiotics are defined as live microorganisms which when administered in

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adequate amount confer health benefits to the host. The commercial and potential probiotic microorganism species mainly belong to the species *Bifidobacterium,* and *Lactobacillus* (Fredua-Agyeman and Gaisford, 2019; Kwofie et al., 2020; Wendel, 2022). Species *Bifidobacterium,* and *Lactobacillus* are also the most extensively studied and widely used which mainly producing of the lactic acid. A study using several combinations of three probiotic species (*Lactobacillus casei, Lactobacillus acidophilus,* and *Bifidobacterium lactis*) through drinking water on the growth performance and nutrient absorption in broilers (Zhang et al., 2022; Gul & Alsayeqh, 2023). The results indicated that supplementation of single probiotics and their combinations improved the digestibility and absorption of nutrients by increasing the activities of digestive enzymes, improving the morphology of the digestive tract, and upregulating the expression of GLUT2 mRNA in the intestinal cell membrane to improve the production performance in broilers. These positive effects of combination probiotics were significantly better than those of the single probiotics, and in line with previous study results (Song et al., 2014; Ahmad et al., 2022; Chitura, 2024). Synergism effects among probiotic strains appeared to be necessary for adhesion to intestinal epithelial cells and abilities to form a barrier preventing colonization of pathogenic microorganism (Zhang et al., 2022).

Lamtoro leaf meal used in this study is considered as an additional fiber source. Physiologically, dietary fiber can be defined as the consumable component of plants or analogous carbohydrates (polysaccharides, oligosaccharides, lignin, and associated plant substances) that are resistant to digestion and absorption in the small intestine with complete or partial fermentation in the large intestine (AACC 2001). Fiber is an element of plant - origin feed in the diet which is linked to enzymatic digestion which includes cellulose, non-cellulosic polysaccharides such as hemicellulose, pectic substances, gums, mucilages, and non-carbohydrate component lignin. In general terms, fiber can be classified as insoluble or soluble based on their solubility in water. Both fiber types have direct nutritional implications.

Gut microbes can readily ferment soluble fibers in the hind gut; however, they have also been shown to increase the viscosity of digesta (Cameron-Smith et al., 1994; Choct, 2015). Increased viscosity reduces passage rate, then causes increased satiety and ultimately reduces overall feed intake, and negatively changes intestinal microflora and reduction in nutrient absorption (Jha and Mishra, 2021). Nevertheless, there is a group of soluble fibers, integrated by oligosaccharides functioning as prebiotics, which positively modulate intestinal microbiota development and offer additional benefits, including fructose-oligosaccharides (FOS), galactic-oligosaccharide (GOS), trans-galactic-oligosaccharides (TOS), and mannan-oligosaccharide (MOS) (Tajeda and Kim, 2021). Additionally, there is a common reason for application of xylanases in poultry diet to reduce digesta viscosity (Choct et al. 1999; Yang et al., 2008; Zhang et al., 2014; Gonzalez-Ortiz et al., 2021). More studies of insoluble fiber inclusion in the broiler diet have impacts on the gut's structural morphology, the development of gastrointestinal organs, nutrient absorption, gut microbiota and growth performance (Slavin, 2013; Tajeda and Kim, 2021; Zhang et al., 2023; Rashid et al., 2023). Many reports use the LLM as a feedstuff in poultry ration, including chicken (broiler and layer). However, due to the presence of anti-nutritional substances, mainly mimosine, in the LLM resulted in a deleterious impact on their performances.

Against the background above, the aim of the research reported herein is to elucidate the impacts of including probiotics in drinking water and dietary LLM as an additional fiber on the growth performance, digestibility, and small intestinal morphometry of Kampung chicken.

MATERIALS & METHODS

All procedures of using animals in this research were in compliance with the guidelines for the care and use of animals in research and have been approved and certified by the Animal Ethics Committee of the Faculty of Medicine, Hasanuddin University, Indonesia (No: 783/UN4.64.5.31/PP36/2024). The experiment was conducted in the Animal House of the Animal Science Faculty – Hasanuddin University.

Design, Animals, Diets and Management

The experiment is a single-factor experiment that was arranged as a completely randomized design of 5 treatment groups with 4 replications. A total of 200 unsexing Kampung chickens (unsexed, with a body weight = 25.80±1.36g) supplied by the Hatchery House of Animal Science Faculty was used. Based on the design, the animals were randomly divided into 20 units containing 10 chickens per unit. There were 2 diets used during the experiment, basal and mixed diets (basal diet + 3% lamtoro leaf meal), and the nutrient composition of both diets is presented in Table 1. Probiotic supplementation as the treatment in this experiment was provided through drinking water, which were 0.3, 0.6 and 0.9%/L. Accordingly, the 5 treatment groups are:

P0 = basal diet (negative control)
P1 = mixed diet (positive control)
P2 = mixed diet & 0.3% probiotic/L
P3 = mixed diet & 0.6% probiotic/L
P4 = mixed diet & 0.9% probiotic/L

Table 1: Nutrient composition of a basal diet, lamtoro leave flour and mixed diet (basal + 3% lamtoro leaf flour) (%, dry matter basis)

Content	Basal Diet*	Lamtoro Leaf Flour (LLF)*	Mix Diet (Basal + 3% LLF)**
Proteins	24.22	23.83	24.21
Fat	16.52	17.79	16.55
Fiber	9.85	16.13	10.03
BETN	43.79	13.27	49.2
Ash	5.62	12.19	5.81

* = Alnalyzed in the Laboratory of Animal feed and nutrition, Animal Science Faculty, Hasanuddin University; ** = Calculation

A commercial probiotic used is multi-strain microbiota containing of *Bacillus subtilis* (>1 x 10^8CFU/g), *Bifidobacterium longum* (>1 x 10^8 CFU/g), *Bifidobacterium bifidum* (> 1 x 10^8 CFU/g *and Lactobacillus bulgaricus* (>1 x10^8 CFU/g. Each treatment unit of 10 chicken were placed in a square cage (length x width x height = 1m x 1m x 80 cm) with 60w lighting. The experiment as rearing period was conducted along 70 days (10 weeks). Feed and water were provided *ad libitum*, and their intakes were monitored daily, the body weight was monitored weekly.

Digestibility Measurements

After 2 days of adjustment period at the last week of the experiment, the digestibility trial was conducted in the individual metabolism cage (30 x 50 x 80 cm, equipped with feeder and water container). The selected 3 chickens of each treatment (lightest, average, and heaviest) were used to determine nutrient digestibility. In the first day, feed was withdrawn for 24h, but drinking water provided as usual. The digestibility test was conducted for 3 days in the last week of the experiment. The total feed intakes were monitored for the 1st and 2nd days and withdrawn at 3rd d, while daily excreta during 3 days consecutively were collected, weighing, preserving by spraying with 0.2N HCl, storing, and preparation for chemical analyzing (McDonald, et al., 2010; Ginindza, et al, 2022). A proximate analysis of basal diet and Lamtoro leaf meal was done to analyze moisture, crude protein, crude fiber, ash and ether extract contents of the diet (AOAC, 2000), and the results are presented in Table 1. Apparent digestibility was calculated as follows:

Apparent digestibility (%) = [(nutrient intake - nutrient excreta)/nutrient intake] x 100

Gross- and Histo-morphometric indices of the small Intestine

Three chickens of each treatment group used in digestibility test were then used as representative chicken samples for examination of the small intestine indices. All chicken samples were slaughtered at day 70 (10 weeks old) after 12h fasting and weighing their body weights. The slaughtered chickens were cleaned and immersed into hot water (60-70ºC) for several minutes, which then plucked feathers, head and feed, eviscerated abdominal organs, and separating the small intestine from other organs. The small intestine was aseptically emptied by gently flushed twice with isotonic saline solution to remove luminal digesta. The length (cm) and weight (g) of whole and individual segment of duodenum (from gizzard outlet to the end of the pancreatic loop), Jejunum (from the pancreatic loop to Meckel's diverticulum), and ileum (from Meckel's diverticulum to the cecum junction) were measured. For histo-morphometric examination, sample of ileum segment (2-3 cm) was taken and then put into a sample bottle containing 10% buffered neutral formalin (BNF) for 24-48 hours. Histological slice of ileum segment samples was prepared with standard histological procedures by the Laboratory of Animal Pathology and Toxicology - Maros Veterinary Center. The histological slices of the ileum segment were observed and measured using a microscope – Zeiss Primo Star, interfaced with a camera of Optilab Prejector, vs 2.2 and connected to a computer to monitor a histological picture. Histo-morphometry of villus parameter were measured using an image processing system (software) of Axio vs 40v4.8.2.0 and digital images were captured for morphometric analysis. Villus height (VH) is from the tip to the base of lamina propria, villus width (WV) is average of apical part and basal part (at one third and two thirds) of VH, and crypt depth (CD) is from the base of the villus to the mucosa (de los et al., 2005). The surface area of the villus was estimated by considering a villus as a cylindrical structure. Villus surface area (VSA) was calculated using the formula (de los et al., 2015):

Villus surface area = 2 π × (average villus width/2) × villus length.

Statistical Analysis

Data were analyzed using a statistical package SYSTAT vs 13.2 (Wilkinson, 2009), based on one-way Analysis of Variance of a randomized completely design with GLM procedure of 5 treatments with 4 replications of 10 individual chicken per replication. For the parameters of the digestibility and intestinal morphometric indices, replication is of 3 sampled chicken. The significant differences between mean values are stated at a level of 5% maximum.

RESULTS

The effects of Probiotic inclusion in drinking water on the performance of Kampung chicken fed dietary LLM are presented in Table 2. Probiotic inclusion in drinking water appear to gradually improve the performance of Kampung chicken with increasing the duration and levels of giving the probiotic. This response may be attributed with benefit alteration of functional and structural properties of digestive system. Addition of 3% LLM into the basal diet may also affect the performance along 10 weeks rearing. This response indicated by improving feed conversion ratio.

In the first 5 weeks, probiotic inclusion did not significantly affect feed intake, but resulted in a heavier body weight gain and a better feed conversion ratio, particularly indicated by highest level of probiotic inclusion (0.9%/L). In the second 5 weeks, the responses of Kampung chicken to probiotic inclusion in drinking water seem to strengthen the responses of the first 5 weeks. Feed intakes of the chicken of P2, P3, and P4 group were not significantly different, but body weight gain of P4 group was 6 and 12% significantly heavier than P2 and P3 ($P<0.05$) respectively. This result is also reflected significantly in feed conversion ratio ($P<0.05$). Taken together, 1 to 10 weeks rearing, the effects of probiotic inclusion significantly improved the performance of Kampung chicken, as indicated by heavier body weight gain, and better feed conversion ratio compared with control chicken (negative and positive). Mixing Lamtoro leaf meal into the basal diet did not affect the performance of the chicken of the positive control group compared to that of negative control group. Apparent digestibility of crude protein and crude fiber were measured in the last week of the experiment. The results indicated (Table 3) that apparent digestibility crude protein and crude fiber of the chicken treated with probiotic in drinking water were significantly higher compared to that of both the control chickens (negative and positive).

Table 2: Effect of probiotic inclusion in drinking water on the performance (body weight gain, intake of feed, crude protein, crude fiber, feed conversion rate, apparent digestibility of crude protein and crude fiber) of Kampung chicken fed Lamtoro leaf meal

Age	Parameter	P0	P1	P2	P3	P4
1 d	DOC Body weight (g)	26.00±2.71[a]	26.23±0.26[a]	25.63±0.75[a]	26.13±0.25[a]	26.25±1.26[a]
	Feed Intake (g/h/d)	29.10±3.38[a]	29.94±2.61[a]	29.44±2.22[a]	29.75±1.33[a]	30.35±1.55[a]
1 - 5 w	Body weight gain (g/h/d)	7.72±0.21[a]	7.64±0.32[a]	9.21±0.41[b]	9.64±0.43[b]	11.50±0.27[c]
	Crude Potein intake (g/h/d)	7.05±0.17[a]	7.27±0.19[a]	7.23±0.25[a]	7.26±0.28[a]	7.34±0.23[a]
	Crude Fiber intake (g/h/d)	2.87±0.08[a]	3.01±0.06[a]	2.97±0.06[a]	2.98±0.03[a]	3.05±0.05[a]
	Feed Conversion Ratio (%)	3.77±0.15[a]	3.92±0.06[a]	3.20±0.02[b]	3.09±0.03[c]	2.64±0.05[d]
	Feed Intake (g/h/d)	113.81±2.02[a]	14.37±2.19[a]	115.66±3.57[ab]	119.74±4.39[b]	123.92±5.64[b]
6 - 10 w	Body weight gain (g/h/d)	17.80±0.15[a]	17.88±0.11[a]	18.33±0.11[b]	19.37±0.10[c]	20.53±0.21[d]
	Crude Potein intake (g/h/d)	27.58±0.48[a]	27.69±0.32[a]	28.08±0.11[ab]	28.99±0.09[b]	30.03±0.15[c]
	Crude Fiber intake (g/h/d)	11.12±0.09[a]	11.27±0.26[ab]	11.61±0.31[ab]	12.01±0.04[b]	12.43±0.15[c]
	Feed Conversion Ratio (%)	6.39±0.08[a]	6.40±0.05[a]	6.31±0.01[b]	6.18±0.02[c]	6.04±0.04[d]
	Feed Intake (g/h/d)	71.38±0.86[a]	71.87±0.74[a]	72.87±0.17[b]	74.14±0.13[c]	74.73±0.27[c]
1 – 10 w	Body weight gain (g/h/d)	12.76±0.09[a]	12.94±0.07[a]	13.49±0.08[b]	14.50±0.08[c]	16.20±0.14[d]
	Crude Protein intake (g/h/d)	17.31±1.23[a]	17.40±1.24[a]	17.64±1.18[a]	18.09±1.57[ab]	18.75±1.42[b]
	Crude Fiber intake (g/h/d)	7.04±0.49[a]	7.21±0.38[a]	7.28±0.29[a]	7.48±0.35[ab]	7.77±0.33[b]
	Feed Conversion Rasio (%)	5.59±0.09[a]	5.56±0.06[a]	5.40±0.04[b]	5.11±0.04[c]	4.61±0.04[d]
	Apparent digestibility of crude protein (%)	62.78±0.50[a]	62.95±1.40[a]	65.24±0.29[b]	67.99±0.35[c]	70.83±0.93[d]
	Apparent digestibility of crude fiber (%)	54.73±1.47[a]	54.27±0.81[a]	56.49±1.27[b]	58.19±0.60[c]	60.78±0.29[d]

Notes: P0 (Basal Diet (Negative Control), P1 (Mixed Diet (Positive Control), P2 (Mixed Diet & 0.3% Probiotic/L), P3 (Mixed Diet & 0.6% Probiotic/L), P4 (Mixed Diet & 0.9% Probiotic/L); [a,b,c,d,] Mean values within the same row followed with different superscript letters are significantly different (P<0.05).

Table 3: Effect of Probiotic (%/L) inclusion on the gross morphometric indices - the length/weight ratio of individual segment of small intestine in Kampung chicken fed Lamtoro leaf meal

Segments	Treatments/Groups				
	P0	P1	P2	P3	P4
Duedenum (cm/g)	3.85±0.02[a]	3.99±0.25[a]	4.31±0.06[b]	4.36±0.12[b]	4.65±0.22[c]
Jejenum (cm/g)	5.19±0.13[a]	5.22±0.16[a]	5.58±0.13[b]	5.63±0.07[b]	5.91±0.16[c]
Ileum (cm/g)	3.58±0.21[a]	3.53±0.16[a]	3.88±0.24[b]	3.88±0.28[b]	3.96±0.23[b]

Notes: P0 (Basal Diet (Negative Control), P1 (Mixed Diet (Positive Control), P2 (Mixed Diet & 0.3% Prebiotic/L), P3 (Mixed Diet & 0.6% Probiotic/L), P4 (Mixed Diet & 0.9% Probiotic/L); [a,b,c,d,] Mean values within the same row followed with different superscript letters are significantly different (P<0.05).

Moreover, the gross morphometric of individual segments of the small intestines in the chicken group treated with probiotics responded positively but not significantly, except the ratio of the ileum was significantly higher compared to that of both control groups (negative and positive). However, histo-morphometric indices were significantly affected by probiotic inclusion in drinking water. Villus high, villus wide, villus crypt depth and villus surface area increased significantly (P<0.05), while there was no difference between positive and negative control.

DISCUSSION

Two factors are involved in this research, namely the probiotics inclusion in drinking water and 3% lamtoro leaf meal which is mixed into basal diet of one selected commercial brand. These two independent factors are eventually expected to contribute through their interaction, which in turn to result in improving the performance of the Kampung chicken. Lamtoro or *Leucaena leucocephala* is a versatile tree legume, planted widely in many tropical areas and in Indonesia named petai cina. Lamtoro have been used as feedstuff for livestock including chicken as its high basic nutrient content, particularly crude protein-amino acid, crude fiber, vitamin and mineral. However, Lamtoro (leaf or seed) contain at least two deleterious agents, mimosine (Ross and Springhall, 1963; D'Mello and Acomovic 1989) and tannin (D'Mello and Acomovic 1989; Sethi and Kulkarni 1995). Deleterious effects in the performance of chicks were indicated when the LLM inclusion in the diet at the concentration as low as 50 g/kg and further depressed performance were resulted from higher concentration LLM in diet (D'Mello and Acomovic 1989; Zanu et al., 2012), and a similar result was indicated in laying hen (Bhatnagar and Kataria, 1999; Abou-Elezz, et al., 2012). In contrast, it was also reported that up to 20% inclusion of LLM did not affect the broiler performance (Mandey et al., 2015). The results of the present study showed that 3% inclusion of LLM into the basal diet (P1, positive control) Kampung chicken did not affect all parameter measured along the experiment compared to those of negative control (P0), which means that there is no negative effects of LLM at 3% level. Another previous study (Tirajoh et al., 2021) on KUB (a crossbred of Indonesian Kampung chicken and exotic commercial breed) reported that there was no negative effect of inclusion of LLM up to 7%. There is a potential manner to reduce mimosine content of LLM (Putra et al., 2021) in broiler diet, which is by fermentation of LLM before mixed with the other stuff, and fermented Lamtoro leaf flour can replace the use of the commercial ration of broiler up to 20%. Almost similar results were already reported using fermented mulberry leaf powder in broiler diet to reduce fiber content and anti-nutritional agent of tannin (Ding et al., 2021)

Responses of Kampung chicken fed 3% LLM and probiotic inclusion in drinking water were examined in the 1st five weeks (week 1 to 5) and the 2nd five weeks (week 6 to 10). During the 1st five weeks, there was no effect of probiotic inclusion on feed, crude protein and crude fiber intakes, while body weight gain was heavier than that of the controls (P0 and P1), which resulted in increasing feed utilization efficiency, or improvement of feed conversion ratio. During the 2nd five weeks, probiotic inclusion in drinking water appeared to strengthen the effects on increasing feed intake, body weight gains and feed conversion ratio. It appears that probiotic inclusion in drinking water to Kampung chicken fed 3% LLM interacted

Table 4: Effect of Probiotic inclusion on the histo-morphometric indices of ileum segment of the small intestine in Kampung chicken fed Lamtoro leaf meal

	Probiotic Inclusion				
	P0	P1	P2	P3	P4
Villus High (µm)	564.25±69.16[a]	563.72±49.08[a]	597.48±45.59[b]	670.70±31.37[c]	883.42±66.07[d]
Crypt Depth (µm)	65.26±2.06[a]	64.37±2.11[a]	61.64±2.60[ab]	60.05±1.44[b]	60.55±2.40[b]
VH/CD	8.65±0.48[a]	8.78±0.62[a]	9.69±0.83[b]	11.17±1.31[b]	14.59±1.19[c]
Villus Surface Area (mm[2)]	0.156±0.014[a]	0.154±0.008[a]	0.196±0024[b]	0.261±0.007[c]	0.297±0.012[d]

P0 (Basal Diet (Negative Control), P1 (Mixed Diet (Positive Control), P2 (Mixed Diet & 0.3% Prebiotic/L), P3 (Mixed Diet & 0.6% Probiotic/L), P4 (Mixed Diet & 0.9% Probiotic/L); [a,b,c,d,] Mean values within the same row followed with different supescript letters are significantly different (P<0.05).

and complemented each other in determining the performance than only the LLM or probiotic.

In relation with this performance, apparent digestibility of crude protein and crude fiber (Table 3) measured at the 2[nd] 5 weeks increased significantly, and the increase was due to increased level of probiotic inclusion. In the current study, a commercial probiotic used contains *Bacillus subtilis, Bifidobacterium longum. Bifidobacterium bifidum* and *Lactobacillus bulgaricus*. These four genera-species of bacteria are commonly used as probiotics, and found as communities of beneficial microbiota in the caecum of chicken. Several previous studies have shown that increasing the digestion and absorption of nutrients is a major mechanism responsible for the enhanced growth performance of broilers in response to probiotic (Gao et al., 2017; Haque et al. 2021; Zhang et al., 2022; Ali et al., 2022; Mohammed et al., 2022).

A 3% Lamtoro leaf meal is mixed to basal diet in this research is mainly to be an additional fiber source paired with probiotic inclusion in drinking water. In general, fiber is classified into soluble or insoluble based on their solubility in water. Both fiber types have direct nutritional implications in broiler diets. Insoluble fiber in broiler diets modulates intestinal morphology, digestive organ development, nutrient absorption, growth performance, and intestinal microbiota. Soluble fiber is thought to increase intestinal viscosity and is associated with negative changes in intestinal microflora and reduction in nutrient absorption. Nevertheless, there is a group of soluble fibers, integrated by oligosaccharides, that function as prebiotics positively modulating intestinal microbiota (Sklan et al., 2003; Hetland et al., 2004; Sadeghi et al., 2015; Jha and Mishra, 2021; Röhe and Zentek, 2021; Morgan, 2023).

Small intestine is an organ in the gastrointestinal tract where most of enzymatic digestion and absorption of ingested nutrient of food takes place. In addition to the activities of digestive enzymes as functional responses, probiotic inclusion in drinking water and dietary Lamtoro leaf meal may result in modification of morphology (gross- and histo-morphology) of the small intestine. There are three segments of small intestine, duodenum, jejunum and ileum, which microscopically look similar, but may have important specific function of individual segment of the small intestine. The results of the current study, gross morphometric indices are presented as the length/weight ratio of each segment of the small intestine (Table 4). There is a dearth of reports linking the effects of nutrients supplementation and especially that of probiotic inclusion in drinking water and 3% Lamtoro leaf meal in diet as prebiotic on gross morphometric indices of the whole or individual segment of the small intestine. The length/weight ratios of duodenum, jejunum and ileum increased significantly, which may result from interaction between probiotic in drinking water and Lamtoro leaf meal as prebiotic, as there is no difference between negative control and positive control of additional Lamtoro leaf meal, in addition the response increased with increasing level of probiotic inclusion. Histo-morphometric of ileum segment also indicated a consistent response to increase markedly as resulted from increasing probiotic inclusion with feed dietary containing 3% Lamtoro leaf meal.

Alteration in gross morphometric indices of each small intestine segment and alteration in histo-morphometric indices (Table 4) maybe complement each other. Alteration in the length/weight ratio of individual segments indicate the opportunity for longer or shorter time of digestion and absorption processes, while alteration in villi height (VH) or crypt depth (CD) or villus surface area (VSA) or VH/CD ratio are an indication of mucosal thickness alteration which is associated with enzymatic processes and absorption surface area **(**Pelicano et al., 2005; Izadi et al., 2013; Al-Baadani et al., 2016; He et al., 2019; Alshamiri et al., 2021; Marchewka et al., 2021 Zhang et al., 2022; Naghibi et al., 2023). A comparison study between Lohmann dual purpose (LD) and Ross 308 Broiler line (Alshamy et al., 2018) indicated that with the same body weight (but different aged achieved), LD had a significant heavier gizzard, shorter intestine, longer jejunum villi and thicker ileal tunica muscularis than those found in Ross 308. A longer in jejunum villi was consistently followed with a thicker of intestinal mucosa of the individual segment, not length of the segment. The scanning electron micrograph indicated that there was no visible difference in villi density (VD) of individual segment resulted from different levels of chicory fructan or inulin (Yusrizal and Chen, 2003). These findings are corroborated with further results (Pelicano et al., 2007; Oliveira et al., 2008; Izadi et al., 2013; Alshamy et al., 2018), which indicated that no difference in VD in the duodenum and jejunum was seen with the use of probiotics and prebiotics, although higher VD were seen in the ileum. Briefly, the alteration in villus indices initiate alteration in the thickness of the mucosal intestine which resulted in increasing villus surface area – means absorptive capacity and efficiency rather than increasing in the intestinal length.

Conclusion

Taken together, it can be concluded that probiotic inclusion in drinking water increased the performance - body weight gain, feed efficiency - of Kampung chicken fed containing 3% Lamtoro leaf meal. Lamtoro leaf meal treat as prebiotic in the diet to synergistically interact with probiotic in drinking water, which resulted in improving function and structure of the small intestinal morphometric.

Conflict of Interest

All authors declare no conflict of interest.

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Author Contribution

SYEF: Acquisition of data. DPR and SP: Conception and design of study. SYEF, DPR, and SP: analysis and/or interpretation of data and drafting the manuscript.

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