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Effects of *Lactobacillus* spp. Isolated from Ensiled Swamp Forage on Gastrointestinal Tract Development and Nutrient Digestibility in Native Ducks

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ABSTRACT

The application of lactic acid bacteria (LAB), particularly *Lactobacillus,* as feed additives has demonstrated benefits for poultry, including enhanced gut function and better nutrient digestion. However, studies on LAB derived from swamp grass silage remain limited. The present study aimed to evaluate the effects of supplementing *Lactobacillus* spp. (Lacto) solution derived from ensiled swamp forage (*Hymenachne acutigluma*) at different concentrations through drinking water on the relative weight and length, as well as intestinal density and nutrient digestibility of native ducks. The relationship between intestinal tract density and nutrient digestibility was assessed using the Pearson correlation coefficient. The present study involved sixty 24-week-old native ducks, divided into five groups with four replicates each. The groups included a control group and groups that received Lacto solutions in their drinking water. The Lacto solutions were at concentrations of 1×10^6 CFU/mL (Lacto 1), 1×10^7 CFU/mL (Lacto 2), 1×10^8 CFU/mL (Lacto 3), and 1×10^9 CFU/mL (Lacto 4). The current results indicated that supplementation of the Lacto solution in drinking water increased the relative weights of the proventriculus, small intestine, duodenum, jejunum, ileum, and ceca. The relative weight and density of the intestine in the Lacto groups increased linearly with higher concentrations of Lacto solution. Compared to the control group, ducks receiving Lacto supplementation showed improved crude fiber digestibility (CFD) and a tendency to enhance organic matter digestibility (OMD). In 33-week-old ducks, a higher proventriculus density was associated with increased dry matter digestibility (DMD) and OMD. Additionally, the densities of the duodenum, jejunum, and ileum were positively correlated with DMD, OMD, and CFD. The present findings indicated that the administration of Lacto solution at increasing concentrations up to 10^9 CFU/mL via drinking water effectively improved the development of the small intestines of ducks, which was indicated by an increase in the intestinal relative weight and density, as well as enhancing the OMD and CFD in the diets.

Keywords: Drinking water, Lactic acid bacteria, *Lactobacillus*, Native duck, Nutrient digestibility, Swamp forage

INTRODUCTION

In recent years, the use of probiotics in poultry has risen as a substitute for antibiotics, which have been banned in several European countries and other nations, including Indonesia (Sihombing and Fajri, 2024). Probiotics are microbes that are beneficial to poultry when consumed, as they improve nutrient digestion and gut health (Ding et al., 2021). For example, several studies have shown that probiotic strains, particularly *Lactobacillus* spp., can modify gut development (Olnood et al., 2015), improve small intestine morphology (Wang et al., 2019), and increase nutrient digestibility and availability in broiler chickens, such as dry matter, fiber, protein, and minerals (Poberezhets et al., 2021). However, information on the effects of probiotics on the digestive tract density of poultry is still limited. In addition, most studies have used chickens as animal models to observe the effect of probiotics, while those on ducks are still rare.

As lactic acid bacteria (LAB), *Lactobacillus* strains have become popular and widely used as probiotics in poultry production (Halder et al., 2024). Several studies have utilized different sources to collect different LAB strains for probiotics, including digestive tract digesta and feces from healthy chickens (Ahmed et al., 2019; Chen et al., 2022) and traditional fermented foods (Poornachandra Rao et al., 2015; Suwannaphan, 2021). However, the use of LAB isolated from forage silage as a potential probiotic is still rare. There are several advantages to using forage silage as a source of LAB. Forage is relatively easy to obtain and abundant, particularly in tropical regions such as Indonesia, ensuring its sustainability (Perdinan et al., 2024). A previous study has shown that fermented swamp forage (*Hymenachne acutigluma*) produces *Lactobacillus* that have the potential to be used as probiotics, with properties such as resistance to low pH and the ability to inhibit the development of pathogenic bacteria after *in vitro* tests (Sandi et al., 2018). Previous studies suggested that administering *Lactobacillus* spp. (Lacto) solutions, isolated from ensiled swamp grass through

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drinking water at concentrations up to 1 × 10^9 CFU/mL, can influence the physical qualities of duck meat and eggs. These effects include increased cooking loss, enhanced water-holding capacity of meat, and a higher egg yolk index (Yosi et al., 2021). It is assumed that the improvement in egg and meat quality is closely related to improved digestive tract function and nutrient digestibility (Obianwuna et al., 2022). The Lacto supplementation would enhance digestive tract development, thereby improving nutrient digestibility. The present study aimed to assess how Lacto supplementation influences the density of the proventriculus, small intestine, and ceca, as well as the nutrient digestibility in the intestines and ceca of ducks. Additionally, the relationship between intestinal density and nutrient digestibility was examined.

MATERIALS AND METHODS

Ethical approval

The present experiment was conducted at the University of Sriwijaya, South Sumatra, Indonesia, following Regulation 18/2015 on Livestock, Animal Health and Welfare in Indonesia and ethical standards.

Experimental design

The experimental setup and methodology followed the description provided by Yosi et al. (2021). Sixty local female Pegagan laying ducks, aged 24 weeks and a mean weight of 1,341 ± 104.1 g, were obtained from a duck farm in Indralaya District, South Sumatra, Indonesia. After a 3-day acclimatization period, ducks were randomly assigned to 20 plots with daily air temperatures ranging from 24 to 35°C and relative humidity of 70 to 85%. Each plot measured 1 m x 1 m × 1 m. A round drinker and a round feeder were used for their setup, and they were maintained for 60 days. Treatments were divided into five groups, with four replicates assigned to each group. The control group did not receive the Lacto solution. The Lacto concentrations were 1×10^6 CFU/mL (Lacto 1), 1×10^7 (Lacto 2), 1×10^8 CFU/mL (Lacto 3), and 1×10^9 CFU/mL (Lacto 4). During the first 30 days of the experiment, the Lacto solutions were added to the drinking water at a dose of 10 mL per bird per day (Yosi et al., 2021). A diet based on corn, noodle meal, and concentrate was formulated to meet or exceed the nutritional requirements of laying ducks in accordance with Indonesian national standards. The diet was provided *ad libitum* throughout the experiment. Table 1 shows the nutrient composition and ingredients of one experimental diet, in accordance with a previous study of Yosi et al. (2021).

Table 1. Ingredients and chemical composition of the experimental diet for Pegagan laying ducks for 60 days

Ingredients (g/kg diet as fed basis)	**Composition (%)**
Corn meal	16
Dried noodle waste meal	40
Concentrate[a]	32
Bran	10
Premix[b]	1.0
Lysine	0.4
Methionine	0.6
Total	100
Calculated nutrient content[c]	
Metabolizable energy (Kcal/kg)	3007.2
Crude fiber (%)	4.60
Crude protein (%)	18.74
Calcium (%)	4.22
Available phosphorus (%)	0.46

[a]: A mixture of fish meal, soybean meal, meat and bone meal, coconut meal, peanut meal, wheat flakes, leaf meal, canola, vitamins, calcium, phosphate, and trace minerals. [b]: Provided per kilogram of diet, including Calcium (32.5%), Phosphorus (1%), Iron (6 g), Zinc (3.75 g), Manganese (4 g), Copper (0.3 g), Iodine (0.075 g), vitamin D3 (50,000,000 IU), and vitamin B12 (0.5 mg). [c]: Calculated according to the recommendation of the Indonesian National Standard.

The preparation of the *Lactobacillus* spp. solution

The preparation of the Lacto solution started with the preparation of swamp grass silage. The detailed procedure for the preparation of swamp grass silage was the same as previously described by Yosi et al. (2021). The freshly cut grass was chopped into pieces ranging from 2 to 5 centimeters in length. It was then allowed to wilt without direct sunlight at 27-30°C for at least 24 hours. Next, 500 g of the withered grass was mixed with 10 mL of molasses and 5 mL of water. The mixture was placed in a triple-layer plastic bag and stored at room temperature for 21 days under anaerobic conditions. After preparing the silage, the next step was to make the Lacto solutions and determine their concentrations according to Yosi et al. (2021). The *Lactobacillus* isolates were cultured in Man-Rogosa-Sharpe (MRS) broth (Oxoid CM0359B, England) at 37°C for 48 hours (Sandi et al., 2018). The isolates were then mixed with a peptone solution (Buffer Peptone Water, Oxoid, UK) in 90 mL until they matched the turbidity levels of McFarland reference solutions. The concentrations of the Lacto solutions in Lacto 1, Lacto 2, Lacto 3, and Lacto 4 corresponded to McFarland standards 1, 2, 3, and 4, respectively. These concentrations reflect the Lacto levels in the digestive systems of laying ducks, which range from 10^6 to 10^9 CFU/g (Rehman et al., 2007).

Sampling and measurements of the digestive tract

A total of two ducks of average weight (1,590 ± 81.6 g) in each group were randomly selected at the end of the experiment (33 weeks of age). The ducks were fasted for six hours before slaughter and given only drinking water. The ducks were euthanized by severing their throats and jugular veins with a sharp knife at the first vertebra (Nielsen et al., 2019). The contents of the gastrointestinal tract were removed after cutting it into individual segments. The digestive tract segments measured included the crop, esophagus, proventriculus, duodenum, jejunum, ileum, total small intestine, ceca, and colon. The length of the duodenum was measured from the gizzard outlet to the end of the pancreatic loop using a measuring tape. Jejunum length was measured from the end of the pancreatic loop to Meckel's diverticulum, while ileum length was measured from Meckel's diverticulum to the beginning of the cecal junction. The density of the digestive tract was assessed by dividing the empty weight of the digestive tract by its length (g/cm; Alshamy et al., 2018). The relative length and weight percentages of the digestive tract were calculated using the following formulas (Yosi et al., 2017).

Relative length of digestive tract (%) = Length of digestive tract segment (cm)/body weight (g) × 100

Relative weight of digestive tract (%) = Weight of digestive tract segment (g)/body weight (g) × 100

Measurement of nutrient digestibility

At the end of the experiment, at 33 weeks of age, one duck from each plot was placed in a metabolic cage. The excreta of each duck were collected over three days. Dry matter digestibility (DMD), organic matter digestibility (OMD), and crude fiber digestibility (CFD) were initially assessed using the AOAC (2016) procedure, using the following formulas (Yosi et al., 2016).

DMD (%) = Dry matter consumed - dry matter excreta /dry matter consumed × 100

OMD (%) = Organic matter consumed - organic matter excreted/organic matter consumed × 100

CFD (%) = Crude fiber consumed - crude fiber excreta /crude fiber consumed × 100.

Statistical analysis

SPSS statistical software package (IBM SPSS version 26) was used to analyze all experimental data. One-way analysis of variance (ANOVA) was applied to analyze the data. Duncan's multiple comparison test was used to determine differences between treatment groups. Orthogonal comparisons were performed using polynomial regression to determine the linear and quadratic effects of increasing concentrations of Lacto solution administered via drinking water. The Pearson correlation coefficient (*r*) between digestive tract density and nutrient digestibility was also calculated. The analyzed data were then presented in tables as means with pooled standard errors of the mean (SEM). To visualize the obtained correlations, heat maps were generated using the level plot++ function of the lattice package in R Studio (version 2023.06.0). Statistical difference was defined as $p < 0.05$.

RESULTS

Relative weight, length, and density of the digestive tract

Adding Lacto solution to drinking water in Lacto groups (Lacto 1 to 4) significantly affected the relative weights of the proventriculus, small intestine, duodenum, jejunum, ileum, and ceca ($p < 0.05$; Table 2). The relative weight of the small intestine was higher in the Lacto supplement groups (Lacto 1 to 4) compared to the control group ($p < 0.05$). Additionally, the relative weight of the small intestine in the Lacto groups showed a linear increase with higher Lacto solution concentrations ($p < 0.05$). The proventriculus and ceca weights were greater in Lacto 2 compared to the control group ($p < 0.05$), but then decreased in Lacto 4 ($p < 0.05$). The duodenum relative weight increased in Lacto 2, Lacto 3, and Lacto 4 Groups, respectively, compared to the control group ($p < 0.05$). The ileum relative weight increased in Lacto 3 and Lacto 4 Groups, respectively, compared to the control group ($p < 0.05$). The jejunum's relative weight was higher with Lacto supplementation than in the control group ($p < 0.05$), but no significant difference was found among the Lacto groups ($p > 0.05$).

It was found that the density of the small intestine, particularly the duodenum and ileum segments, was significantly impacted by supplementing drinking water with Lacto solutions ($p < 0.05$; Figure 1). The overall small intestine density was higher in Lacto 4 than in the control and Lacto 1 Groups ($p < 0.05$). Duodenal density in Lacto 2 Group exceeded that of the control group ($p < 0.05$), though it was similar to Lacto 3 and Lacto 4 Groups. Furthermore, ileal density was higher in Lacto 3 than in the control group ($p < 0.05$).

Table 2. Relative weight and length of the digestive tract of 33-week-old Pegagan laying ducks after supplementation with *Lactobacillus* spp. solutions from ensiled *Hymenachne acutigluma* via drinking water

Variable		Control	Lacto 1	Lacto 2	Lacto 3	Lacto 4	SEM
Crop-esophagus (%)	W	0.52	0.37	0.53	0.48	0.36	0.053
	L	1.50	1.10	1.45	1.28	1.35	0.135
Proventriculus (%)	W	0.39^{ab}	0.34^{b}	0.43^{a}	0.38^{ab}	0.37^{b}	0.018
	L	0.30	0.41	0.37	0.32	0.33	0.036
Small intestine (%)	W	1.54^{c}	1.80^{bc}	2.11^{ab}	2.16^{ab}	2.32^{a}	0.139
	L	10.49	9.20	10.34	9.87	9.32	0.609
Duodenum (%)	W	0.28^{b}	0.32^{ab}	0.37^{a}	0.34^{a}	0.36^{a}	0.016
	L	1.69	1.64	1.80	1.66	1.58	0.133
Jejunum (%)	W	0.59^{b}	0.77^{a}	0.78^{a}	0.80^{a}	0.82^{a}	0.047
	L	4.36	3.65	3.99	3.75	3.64	0.329
Ileum (%)	W	0.68^{b}	0.71^{b}	0.96^{ab}	1.03^{a}	1.14^{a}	0.092
	L	4.43	3.91	4.55	4.46	4.10	0.290
Ceca (%)	W	0.31^{ab}	0.27^{b}	0.38^{a}	0.26^{b}	0.30^{ab}	0.024
	L	0.98	0.80	0.97	0.92	0.96	0.097
Colon (%)	W	0.16	0.12	0.12	0.11	0.14	0.020
	L	0.47	0.43	0.37	0.39	0.38	0.085

Lacto: *Lactobacillus* spp. isolated from ensiled *Hymenachne acutigluma*, SEM: Standard error of mean, W: Weight, L: Length, Control: Group without Lacto solution, Lacto 1, 2, 3, and 4: Groups supplemented with Lacto solution at concentrations of 1×10^6 CFU/mL, 10^7, 10^8, and 10^9, respectively. [a,b, and c] Means without common superscript letters in the same row differ significantly ($p < 0.05$).

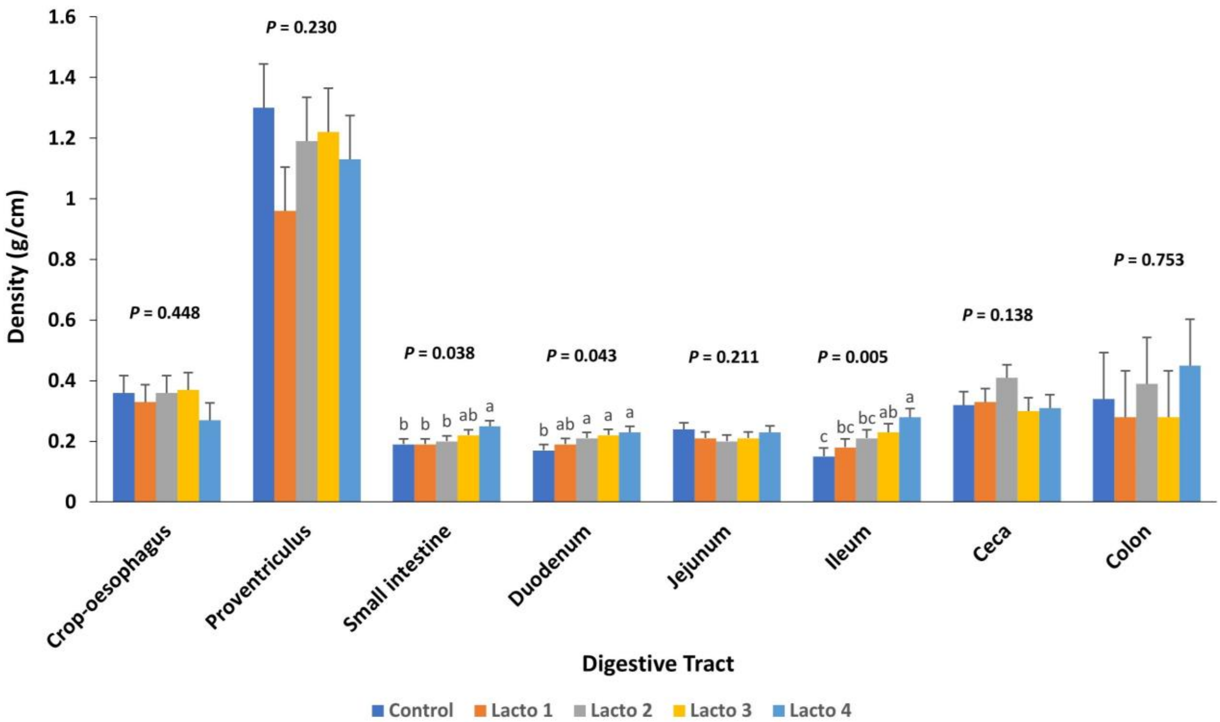

Figure 1. Differences in the digestive tract density of 33-week-old Pegagan laying ducks between the control group and groups supplemented with *Lactobacillus* spp. solutions from ensiled *Hymenachne acutigluma* via drinking water. Control: Group without Lacto solution, Lacto 1, 2, 3, and 4: Groups supplemented with Lacto solution at concentrations of 1×10^6 CFU/mL, 10^7, 10^8, and 10^9, respectively. [a,b, and c] Means without a common superscript letter on the bar graph of each gut segment are significantly different ($p < 0.05$).

Digestibility of feed dry matter, organic matter, and crude fiber

The addition of Lacto solution via drinking water in the Lacto groups significantly affected CFD ($p < 0.05$) and showed a trend toward influencing OMD compared to the control group, while it had no impact on DMD ($p > 0.05$; Figures 2 A-C). The CFD was higher in Lacto 2 Group than in the control and Lacto 1 ($p < 0.05$), but no significant differences were found among Lacto 2, Lacto 3, and Lacto 4 ($p > 0.05$). Additionally, there was a tendency for OMD to increase in Lacto 4 Group compared to the control group.

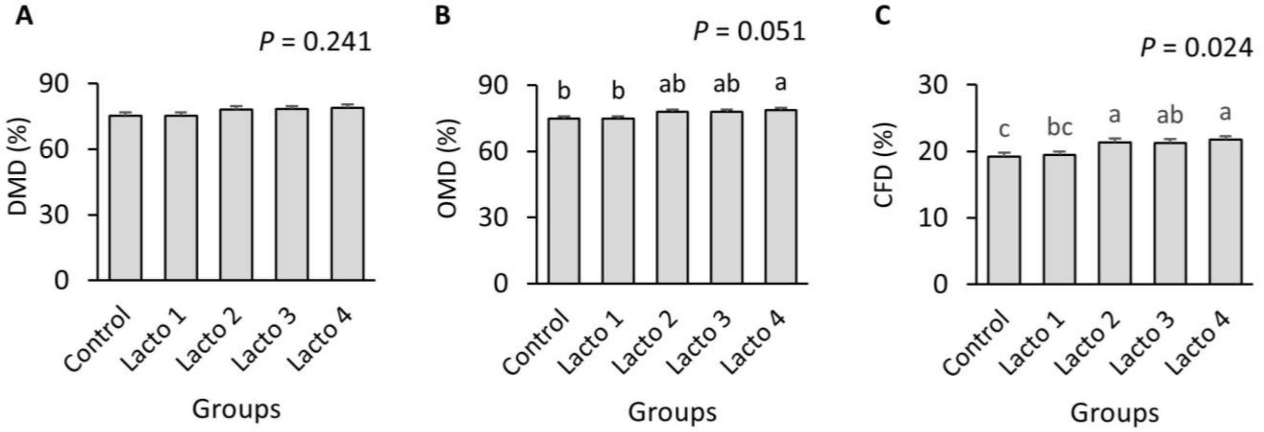

Figure 2. Differences in the dietary digestibility of dry matter, organic matter, and crude fiber in 33-week-old Pegagan laying ducks between the control group and groups supplemented with *Lactobacillus* spp. solutions via drinking water. **A**: Dry matter digestibility (DMD), **B**: Organic matter digestibility (OMD), **C**: Crude fiber digestibility (CFD), Control: Group without Lacto solution, Lacto 1, 2, 3, and 4: Groups supplemented with Lacto solution at concentrations of 1×10^6 CFU/mL, 10^7, 10^8, and 10^9, respectively. [a,b, and c] Means without common superscript letters in the bar graph are significantly different ($p < 0.05$).

Correlation of digestive tract density with nutrient digestibility

According to the Pearson correlation coefficient, the DMD was positively correlated with the density of the proventriculus, duodenum, and total small intestine in 33-week-old Pegagan laying ducks ($p < 0.05$, r = 0.31-0.53; Figure 3a). A positive relationship was found between the CFD and the density of the duodenum, jejunum, and ileum ($p < 0.05$, r = 0.41-0.47; Figure 3b). Additionally, the density of the proventriculus, duodenum, ileum, and total small intestine showed a positive correlation with the OMD ($p < 0.05$, r = 0.32-0.63; Figure 3c).

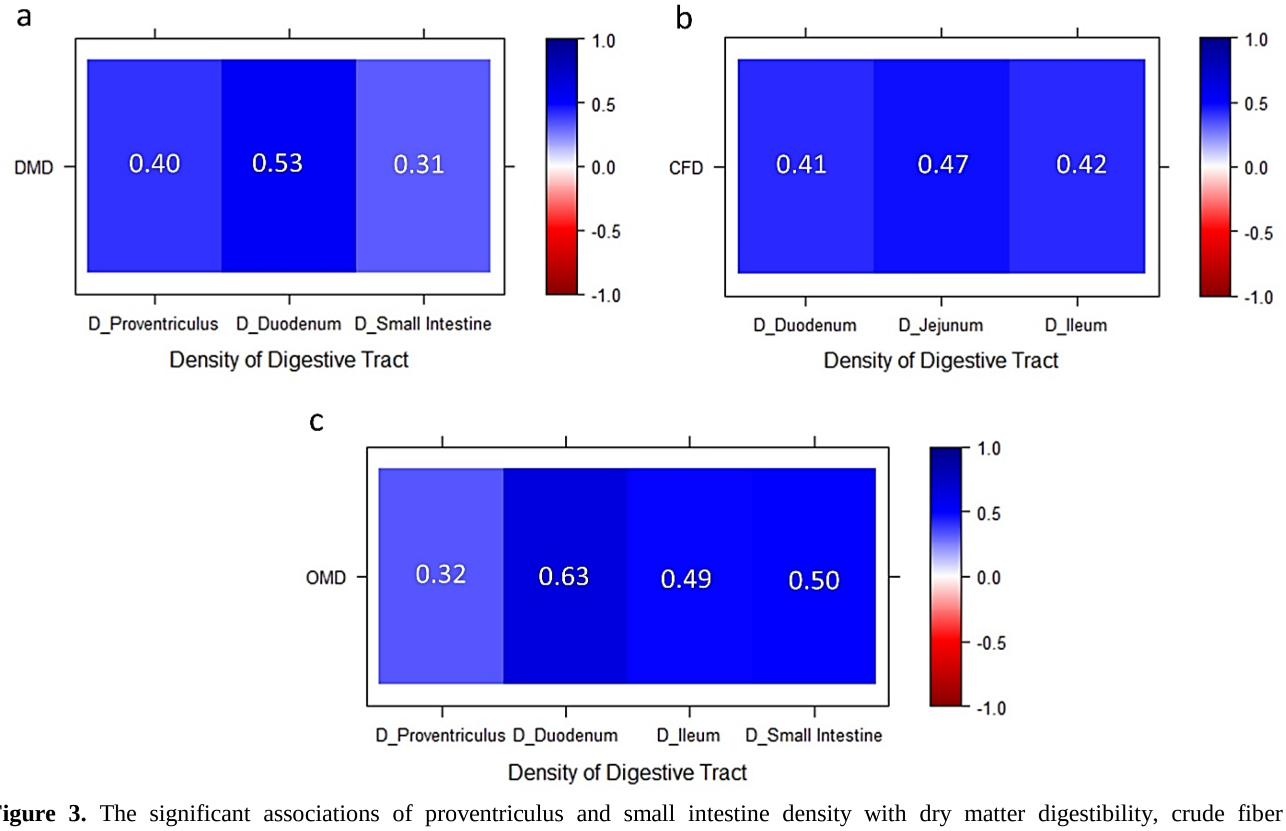

Figure 3. The significant associations of proventriculus and small intestine density with dry matter digestibility, crude fiber digestibility, and organic matter digestibility in 33-week-old Pegagan laying ducks. **a**: Dry matter digestibility (DMD), **b**: Crude fiber digestibility (CFD), **c**: Organic matter digestibility (OMD).

DISCUSSION

The development of the digestive tract of poultry is very important to improve the digestibility of nutrients, which can be supported by providing probiotics as a feed additive (Halder et al., 2024). In the current study, adding Lacto solutions derived from ensiled swamp forage and administered via drinking water appeared to influence the relative weight of the gastrointestinal tract and alter the density of the small intestine, particularly in the duodenum and ileum, in 33-week-old ducks. It was confirmed that the administration of Lacto solution through drinking water was effective in increasing CFD, with less effect on OMD.

In the present study, adding Lacto solutions to drinking water increased the relative weights of the proventriculus and small intestine. These findings aligned with those of Pedroso et al. (2003), who found that probiotics containing *Lactobacillus* spp. (*L. johnsonii* and *L. reuteri*) in drinking water significantly improved broiler intestinal weight at three weeks. The same results for digestive tract improvement were found when *Lactobacillus* was administered in the chicken diet. Olnood et al. (2015) discovered that the addition of four strains of *Lactobacillus*, including *L. salivarius*, *L. crispatus*, *L. johnsonii*, and an unidentified *Lactobacillus* sp., in the diet improved the relative jejunal and ileal weights of 3 and 6-week-old chickens compared to the control, which indicated that both probiotic routes (Diet and drinking water) showed similar responses to changes in small intestinal weight. Overall, the effect of probiotics on the weights of organs in animals remains uncertain, and the underlying mechanism is unknown. It has been suggested that probiotics in the diet or drinking water may modify the intestinal surface by increasing or decreasing its length or the height of the villi. Changing the size of the villi will result in modifications in the surface area for digestion and absorption of feed (Olnood et al., 2015). Additionally, Awad et al. (2009) suggested that an increase in the small intestine relative to body weight when using probiotics may indicate histological changes. The increased villus height, resulting from a larger absorptive surface area, improved nutrient transport mechanisms, and greater expression of brush border enzymes, is believed to coincide with enhanced digestive and absorptive functions of the intestine (Ravindran and Abdollah, 2021). The increased villus height is proposed to be an indicator of stimulated intestinal villus activity (Wang et al., 2025). Furthermore, the higher density of the small intestine, duodenum, and ileum in the present study reinforced the notion that the intestinal villi were more developed after supplementing Lacto solutions. It is assumed that higher density indicates more villi per unit area (Garic et al., 2025). Based on the current results, it is conceivable that the villus development and function might be activated after supplementation of Lacto solutions in drinking water. Nevertheless, histomorphological examination of intestinal villi is essential to validate improved intestinal density and development.

The increased OMD in the Lacto supplementation groups indicated that more nutrients, such as carbohydrates, protein, fat, minerals, and vitamins from the feed, can be absorbed in the intestinal tract of ducks compared to the control group. This suggestion is supported by the results of Chichlowski et al. (2007), which indicated that broiler chickens receiving a multi-probiotic with *Lactobacillus* showed enhanced passive nutrient absorption, particularly for glucose and proline. Additionally, the Lacto supplementation probiotics were found to improve lipid digestibility and the levels of several minerals, such as calcium, phosphorus, and nitrogen, in broiler chickens (Apata, 2008; Wu et al., 2019). The production of extracellular enzymes could play a role in enhancing digestion and nutrient uptake (Jha et al., 2020). A study by Jin et al. (2000) showed that supplementation with *L. acidophilus* and a mixture of *L. fermentum*, *L. acidophilus*, *L. brevis*, and *L. crispatus* was able to increase amylase levels in the small intestine and reduce intestinal glucuronidase activity in broiler chickens after 40 days of feeding. It is suggested that *Lactobacillus* probiotics selectively influence the utilization of major nutrients (Jha et al., 2020). In addition, CFD improved by the administration of Lacto solutions through drinking water. *Lactobacillus* may contribute to an increase in the number of beneficial bacteria in the digestive tract, which can assist in digesting crude fiber and increase the absorption of fermentation-derived metabolites, such as short-chain fatty acids, that serve to improve gut function and health (Silva et al., 2020).

The DMD and OMD were found to be positively linked to the density of the proventriculus. It appeared that an increase in the density of the proventriculus correlated with a rise in the DMD or OMD in the diet. The density of the proventriculus indicated the number of glands or cells per unit area (Langlois, 2003), which means that increasing the density of the proventriculus could increase the number of columnar epithelial cells and glands in the proventriculus that function to secrete mucus and digestive enzymes, especially pepsinogen, to digest protein (Zhu, 2015). Similarly, the density of the small intestine, which includes the duodenum, jejunum, and ileum, exhibited a positive correlation with DMD, OMD, and CFD, indicating that higher small intestine density leads to increased digestibility of these components. A higher density in the intestine indicates more villi, increasing the surface area for absorption (Kai, 2021), which boosts the small intestine's capacity to digest and absorb nutrients, improving overall digestive efficiency. Likewise, the density of the small intestine, including the duodenum, jejunum, and ileum, was positively correlated with DMD, OMD, and CFD, indicating that higher small intestine density is associated with greater digestibility of these components. High intestinal density indicates more villi, thereby increasing the absorption surface area (Kai, 2021). This results in more nutrients being digested and absorbed by the small intestine, enhancing digestion efficiency.

CONCLUSION

Supplying *Lactobacillus* spp. solutions derived from swamp grass silage at concentrations of up to 1×10^9 CFU/mL via drinking water may enhance the development of the proventriculus, small intestine, and ceca, indicated by increased relative weight and density, along with improved digestibility of nutrients and crude fiber. The correlation findings highlighted the importance of proventriculus and small intestine density in improving feed nutrient digestibility, which is essential for the growth performance of ducks. For future studies, a more comprehensive understanding requires histomorphological examination of the small intestine and ceca in Pegagan laying ducks.

DECLARATIONS

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Authors' contributions

Fitra Yosi conceptualized the study, collected and analyzed the samples, processed and interpreted the data, and wrote the initial manuscript. Sofia Sandi and Nuni Gofar participated in the design of the study and interpreted the data. Eli Sahara and Meisji Liana Sari participated in data analysis and interpretation. Aryantini Safitri and Farin Farandhita performed animal experiments, collected and analyzed samples. All authors reviewed, revised, and approved the final edition of the manuscript.

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Competing interests

The authors declared no conflict of interest.

Ethical considerations

The present study has not been published elsewhere. The authors have checked the plagiarism index and confirmed that the article is original based on their scientific results.

Availability of data and materials

The data to support the present study are available upon reasonable request from the corresponding author.

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