Effects of Spirulina Feed Additive on Duodenal Tissue Structure in Peking Duck (*Anas platyrhynchos*)

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Abstract. The use of synthetic feed additives in Peking ducks has adverse effects on their growth and productivity. *Spirulina* sp., a blue-green algae, has the potential to be used as a safe feed additive that can enhance the growth and productivity of ducks. This study aims to analyze the effects of Spirulina (*Spirulina* sp.) powder as a feed additive on the histomorphometry of the duodenum in Peking ducks (*Anas platyrhynchos*), focusing on the duodenal lumen diameter, villus height, villus width, mucosal tunic thickness, submucosal tunic thickness, and muscular tunic thickness. This study used a Completely Randomized Design (CRD) with 35 ducks divided into 5 treatments and 5 repetitions. The treatments included a control group and the addition of spirulina powder in feed at concentrations of 2.5%, 5%, 7.5%, and 10%. The data were analyzed by ANOVA with a significance level of 5%, followed by Duncan's test. The results of data analysis showed that spirulina powder had a significant effect ($P<0.05$) on villus height and duodenal mucosa tunica thickness. The conclusion of this study is that spirulina powder has the potential to be used as a feed additive to improve digestive performance in male Peking ducks, as evidenced by the increase in villus height and thick tunica mucosa in the small intestine. The novelty of this study lies in the utilization of spirulina powder as a feed additive to increase the size of the duodenal tissue structure in Peking ducks. The results of this study are expected to be a new reference on the potential of spirulina as a feed additive to improve poultry feed quality and digestive performance, especially in peking ducks.

Keywords: Microalgae; intestinal tract; antioxidant; flavonoid; cell differentiation

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INTRODUCTION

Duck is a kind of poultry that is reared mostly by Indonesian society. Raising ducks is a popular choice because ducks are a source of animal protein (Ramadhana et al., 2019). This type of poultry makes a significant contribution to meat and egg production (Tamzil et al., 2023). Peking duck is one type of duck that is classified as a broiler duck. This type of duck grows quickly in a relatively short time (Yulinda et al., 2022). The meat produced by Peking duck is known for its delicious taste and tender texture. These advantages make peking ducks in demand by consumers, thus encouraging breeders to increase their productivity. The increase is highly dependent on the performance of duck digestion and the use of feed additives (Muthmainnah & Jalali, 2022).

Feed additives are non-nutritive substances that are added to the feed (Nuningtyas, 2014). Feed additives are usually added in small amounts and are effective in producing optimal weight gain. One of the problems often faced by duck farmers is the use of antibiotics as feed additives. This type of feed additive has several disadvantages, including increasing bacterial resistance, leaving residues in poultry products that are harmful to health, and reducing the species diversity of gut microbiota (Ramirez et al., 2020). Previous research by Jammoul & Darra (2019) on 80 samples of chickens in Lebanese farms treated with antibiotics showed residues causing health problems such as toxicity, allergic reactions, and the development of resistance.

Spirulina flour might be used as an option for a natural feed additive for ducks (Widjastuti & Sudjana, 2019). The body weight growth can be enhanced, and the digestive system of Peking duck can be improved by supplementing spirulina to the diet of ducklings. Bellof & Alarkon (2013) reported that 5 % addition of Spirulina platensis had a positive effect both on final body weight and carcass quality of broiler. This microalgae species consists of some secondary metabolites such as alkaloids, saponins, and flavonoids (Suratno, 2012). These are well established to be involved in the control of the microflora in the small intestine. Using spirulina meal as an additive to feed may exert influences on the duodenal histomorphometry. The proportionate use of spirulina meal as a feed supplement in Peking duck farming by Indonesian farmers is still relatively low. More research on the utilization of Spirulina meal as a feed additive and its influence on duodenal small intestine histomorphometry in Peking ducks is required.

In this research, the addition of spirulina powder as a feed additive was novel to enhance the quality of poultry feed and influence the enlargement of the tissue structure in the duodenum. The findings in the present research will therefore be helpful in understanding and informing on spirulina powder, as a feed additive in the enlargement of the tissue structure of the duodenum in ducks and enhancing the digestive function and efficiency of the duodenum. This study is anticipated to provide important information for future research, particularly related to improving the immunity and production performance of Peking ducks.

METHODS

Experimental Design

The experimental design used in this study was a Completely Randomized Design (CRD) with 5 types of treatments, each repeated 5 times. The treatments were as follows: P0 (standard feed without spirulina flour 0%), P1 (standard feed + 2.5% spirulina flour), P2 (standard feed + 5% spirulina flour), P3 (standard feed + 7.5% spirulina flour), and P4 (standard feed + 10% spirulina flour).

Research Variables

The independent variable consisted of spirulina flour in feed at concentrations of 2.5%, 5%, 7.5%, and 10%. The dependent variables included the diameter of the duodenal lumen, villi height, villi width, tunica mucosa thickness, tunica submucosa thickness, and tunica muscularis thickness.

Preparation of Test Animals

Male Pekin ducklings, 1 day old (DOD), were obtained from the distributor PT. Putra Perkasa Genetika in Secang District, Magelang Regency. Upon arrival, the ducklings were rested for 30 minutes and given a sugar solution of 30 g/liter of water. The ducklings were acclimatized for 7 days, during which they were provided with standard feed and water ad libitum. Subsequently, the test animals were weighed to determine their initial weight. The test animals consisted of 25 male ducklings with an average weight of approximately 300 g.

Feed Mixing with Spirulina Flour

The feed used in this study consisted of standard feed types B-11 and BR-2. The standard B-11 feed was used for ducks aged 0-21 days (starter phase), and the BR-2 feed was given to ducks aged 22-35 days (finisher phase). Spirulina flour was obtained from PT. Alga Bioteknologi Indonesia (ALBITEC), Gunung Pati District, Semarang City, Central Java. The feed preparation involved mixing the standard feed with spirulina flour according to the specified concentrations and homogenizing the mixture. The formulated feed was provided to the ducks for 39 days.

Table 1. Formulation of duck feed from spirulina flour

Feed ingredient composition	Treatment feed additive levels (%)				
	0	**2.5**	**5**	**7.5**	**10**
Standard feed (%)	100	97.5	95	92.5	90
Spirulina flour (%)	0	2.5	5	7.5	10
Total (%)	100	100	100	100	100

Experimental Animals

This research protocol was approved by the Ethics Committee of the Faculty of Medicine, Diponegoro University (Approval Letter No. 69/EC-H/KEPK/FK-UNDIP/VII/2023). The treatment of experimental animals was conducted over 39 days. For the first 7 days, they were given standard feed and water ad libitum, with an additional Vitastress supplement in the drinking water. The amount of feed provided was 75 g/duck/day during the starter phase and 150 g/duck/day during the finisher phase. At the ages of 7 and 14 days, the ducks were vaccinated with ND HB1 and ND La Sota vaccines through the eyes. Starting from 8 days old, the ducks were given feed with the spirulina flour additive. Feed was administered in the morning at 7:00 AM and in the afternoon at 4:00 PM. The ducks were weighed every five days at 7:00 AM using a hanging scale, and their body weights were recorded until termination.

Organ Isolation

Sampling of the small intestine was done by cutting the small intestine 10 cm from the stomach and taking 2 cm for the duodenum sample. The isolated organs were cleaned with a 0.9% physiological saline solution. They were then fixed in sample bottles containing 10% BNF solution to keep the tissue fresh and undamaged.

Preparation of Samples

The preparation of samples was done using the paraffin method and Hematoxylin-Eosin staining. The obtained tissue was fixed in 10% Buffer Neutral Formalin (BNF) solution. The duodenum samples were then dehydrated with graded alcohol. Dehydration was performed with 70%, 80%, 90%, 95%, and absolute alcohol for 30 minutes each. The duodenum samples were cleared in toluene for 1 hour. Then, infiltration was performed in liquid paraffin for 1 hour, followed by liquid paraffin II for 45 minutes, and liquid paraffin III for 45 minutes. The tissue embedded in paraffin blocks was sectioned at a thickness of 5 µm, and the sections were placed on glass slides (Suntoro, 1983). The next stage is Hematoxylin-Eosin staining, starting with deparaffinization by soaking the samples in xylene for 24 hours, followed by rehydration in graded alcohols (96%, 90%, 80%, 70%, 50%, 30%) for 1-2 minutes each. Samples were then immersed in Hematoxylin for 20 minutes and rinsed with distilled water. Next, the samples were immersed in graded alcohol for 3-5 minutes and then in Eosin for 5 minutes, followed by immersion in graded alcohol again for 3-5 minutes. The last stage after staining is de-alcoholization with xylene for 10 minutes, followed by closure with a cover glass. The histological structures in the prepared samples were observed using a microscope (Zainuddin et al., 2016).

Statistical Analysis

Data were analyzed to determine the distribution pattern and homogeneity using the *Shapiro-Wilk* normality test. The results of data analysis showed normal and homogeneous distribution, followed by Analysis of Variance (ANOVA) with a significance level of 5 percent. Duncan Multi Range Test at the 5% significance level using the SPSS (Statistical Product and Services) application for Windows version 25 was used to determine differences between treatment groups after ANOVA showed significant differences.

RESULTS AND DISCUSSION

The research data based on the normality test *(Shapiro-Wilk)* and homogeneity test *(Levene's Statistics)* were normally distributed and homogeneous. ANOVA results with 5% significance for the high variable villi and thickness of the tunica mucosa gave significantly different results between the control with treatment (P<0.05). ANOVA results on the variables villi width, lumen diameter, tunica thickness, submucosa, and thickness of the tunica muscularis showed no significant difference between the control with treatment groups. The results of research on the treatment of spirulina flour on Duodenal histomorphometry are shown in Table 2

Table 2. Average lumen diameter, height of villi, width of villi, thickness of tunica mucosa, thickness of tunica submucosa, and duodenal muscularis thickness in Peking ducks after adding spirulina flour to the feed for 39 days

Variable	Treatment				
	P0 (x̄ ± SD)	P1 (x̄ ± SD)	P2 (x̄ ± SD)	P3 (x̄ ± SD)	P4 (x̄ ± SD)
Diameter Lumen (μm)	2086.14 ± 9.35	2194.92 ± 13.42	2076.12 ± 16.64	2069.13 ± 9.01	2046.94 ± 5.22
Tall Villi (μm)	763.89^{a} ± 56.36	808.88^{ab} ± 67.11	841.33^{b} ± 33.01	859.27^{b} ± 41.44	970.05^{c} ± 56.95
Wide Villi (μm)	85.81 ± 2.20	86.32 ± 1.53	87.13 ± 1.39	87.37 ± 2.04	88.34 ± 2.23
Thick Tunica Mucosa (μm)	954.26^{a} ± 39.76	957.30^{a} ± 46.97	1027.62^{ab} ± 47.68	1040.66^{b} ± 92.57	1102.09^{b} ± 57.19
Thick Tunica Submucosa (μm)	32.4 ± 6.03	32.73 ± 1.51	33.01 ± 4.56	33.38 ± 5.69	34.52 ± 1.97
Thick Tunica Muskukaris (μm)	475.55 ± 33.39	482.43 ± 29.46	517.27 ± 50.82	510 ± 44.66	519.1 ± 21.17

Description: Data presented in the form of an average ± standard deviation (SD). Superscript with the same letter means different, not real, superscript with Different letters means significantly different things. P0 (control, feed standard without affix spirulina flour); P1, P2, P3, and P4 are feed treatments, respectively standard with affix spirulina flour 2.5%, 5%; 7.5%; and 10%.

The ANOVA results for villus height showed a significant difference between the control group (P0) and the treatment groups (P<0.05). Spirulina flour feed additives at levels between 2.5%-10% increased the villus height of the duodenum in Pekin ducks. This increase is suspected to be due to the presence of flavonoid compounds in spirulina that have been hydrolyzed into aglycones. Proteins involved in the proliferation of villus epithelial cells are activated by the presence of aglycones. Bioactive compounds in spirulina, such as flavonoids, play an important role in supporting the activity of protease enzymes in the digestive process, which produce amino acids. These amino acids resulting from the digestion process are subsequently absorbed by enterocytes in the duodenum, distributed to the liver through the bloodstream, and circulated to all target cells throughout the body. Within these target cells, amino acids are utilized as raw materials for intracellular protein synthesis (Sunarno et al., 2023a; Sunarno et al., 2023d). Amino acids absorbed by enterocytes in the intestinal villi are circulated to the liver and subsequently synthesized into proteins (Sunarno et al., 2023e). Increased protein levels in the plasma will affect the distribution and protein resynthesis in target cells (Sunarno et al., 2023b). Activated intracellular proteins affect the enhancement of the cytoskeleton and changes in the activity of catabolic metabolic enzymes.

Hidayah et al. (2023) stated that flavonoids bind to receptors connected to the catalytic domain of enzymes or G-proteins. This binding induces the activation of G-proteins on the inner surface of the cell membrane, followed by the activation of the adenylate cyclase enzyme. Marin-Aguilar et al. (2017) added that adenylate cyclase converts Adenosine Triphosphate (ATP) into cyclic adenosine monophosphate (cAMP), which acts as a second messenger in intracellular cascades. cAMP activates protein kinases, increasing catabolic activity, cellular respiration, and energy production required for cell proliferation and differentiation. Oh & Yoo (2019) stated that increased cell proliferation and migration can enhance villus height. The proliferation, migration, and differentiation of cells are closely associated with the availability of nutrients within target cells. Nutrients absorbed by intestinal cells are distributed to all target cells in the body and utilized by cells for energy production, supporting growth, differentiation, and increasing tissue biomass (Sunarno et al., 2023c). Proliferation is the process by which villus epithelial cells divide and increase to maintain the integrity and function of the intestinal epithelium. Kai et al. (2016) proposed that the intestinal epithelial layer is regularly replenished by stem cell differentiation and proliferation and that the formed mature cell types, enterocytes, goblet cells, and endocrine cells, move upward to the tip of the villi. The increased growth rate means that lots of new cells are pushed up to the apical villi, elongating the villi.

The average height of duodenal villi in P0, P1, P2, P3, and P4 ranged from 763.89 to 970.05 μm. The overall mean villus height observed in this study, in control and treated groups, fell within the range of the normal villus. This finding is somewhat consistent with the results of Prakatur et al. (2019), which attained a villi height in ducks from 600 to 900 μm, outlined their postulation that P0, P1, P2 and P3 were statistically different from P4 by Duncan's test. P0 and P1 and P2 and P3 did not have statistically significant differences in the results. The elevated villous height in all the treatment groups is influenced by bioactive components present in spirulina, including flavonoids. It is reported that these bioactive compounds may have a significant influence in increasing the average villous height of the duodenum. Lestari et al. (2020) reported that flavonoids can inhibit bacterial growth by interacting with bacterial DNA, which would be detrimental to the permeability of bacterial cell walls. It does best when taken with flavonoids known to have antibacterial activity.

ANOVA for the thickness of tunica mucosa duodenum in Peking ducks, indicating significant differences between control (P0) and experimental ($P<0.05$). Addition of spirulina flour at the levels of 2.5%-10% in the diet can raise the thickness of the tunica mucosa duodenum intestinum tenue of Peking duck. This condition is thought to be related to flavonoid compounds contained in spirulina. Flavonoid compounds actively contribute to increasing the thickness of the tunica mucosa in the duodenum by protecting the duodenal mucosa. Setiawan et al. (2018) stated that flavonoid content can protect the duodenal mucosa by reducing inflammation. Flavonoids act as anti-inflammatory agents by inhibiting inflammatory signals on the NF-kB pathway through the suppression of pro-inflammatory cytokine production, such as TNF-α and stimulating anti-inflammatory cytokine production. Konan et al. (2012) stated that a well-protected mucosal wall can enhance nutrient absorption more optimally. Liu et al. (2019) added that the intestinal mucosa is a very dynamic layer because it is always directly exposed to the external environment through the transport and absorption of nutrients.

The average thickness of the duodenal tunica mucosa in this study ranged between 954-1102 μm. Pio et al. (2017) stated that the normal size of the tunica mucosa in poultry ranges between 900-1000 μm. Duncan's further test showed that the average thickness of the tunica mucosa differed significantly between the control and P3 and P4, but not significantly between P1 and P2. The average thickness of the tunica mucosa in P1 did not differ significantly from P2, but was significantly different from P3 and P4. The addition of spirulina flour feed additives caused an increase in the thickness of the duodenal tunica mucosa in Pekin ducks. The flavonoid content in spirulina, which acts as an antioxidant, is thought to affect the thickness of the tunica mucosa. Adawiah et al. (2015) stated that flavonoids act as antioxidants because they have hydroxyl groups that can donate hydrogen atoms to free radical compounds and stabilize reactive oxygen species (ROS). Blatama et al. (2023) also explained that flavonoid antioxidant activity can prevent oxidative stress and excessive cell damage in duodenal villi. The thickness of the tunica mucosa is also related to the depth of Lieberkühn's crypta and the height of the villi. Saleh (2012) showed that villi height affects the thickness of the tunica mucosa. The process of digestion and absorption of nutrients by enterocyte cells occurs at Lieberkühn's crypta and continues to increase to the apical part of the villi. Lieberkuhn's crypts are invaginations of the villi that extend to the basal layer of the mucosal lining. These crypts consist of stem cells that proliferate and differentiate to produce new cells to form the intestinal mucosa layer, such as enterocytes, goblet cells, and enteroendocrine cells (Sunarno et al., 2021). Zulfa et al. (2021) suggest that in the presence of high villi, which is combined with deep crypts, the tunica mucosa is thickened. This is apparent in the P3 and P4 treatments of the current investigation.

ANOVA results indicated that there was no significant difference in the mean duodenal villus width between the treatment and control groups ($P>0.05$). (Table 4.1) shows that spirulina meal as a feed additive does not have a significant influence, villous width is within the normal range. Feng et al. (2016) the normal data for the average width of duodenal villi of ducks from the study were between 84-101 μm. The relatively same width of duodenal villi among the treatments and controls showed that the supplementation of spirulina flour feed additives at a level of 2.5%-10% did not induce toxicity and had no damaging effect on the villi. The width of the normal villi may be attributed to the crude fiber level being within the normal range (3.78-5.87%). Umar et al. (2024) in this study reported that crude fiber at basal levels does not cause the villi to grow wide or otherwise block absorption of nutrients by the villi.

The results of the ANOVA test that compared the diameter of the duodenal lumen of Peking ducks after the addition of spirulina flour additives (in Table 4.1) indicated no significant difference between the treatment and control. The diameter of the duodenal lumen was not affected by the inclusion of spirulina flour at any level. The cause of this might be the fiber concentration in the spirulina meal, which varied from 3.78% to 5.87%. Has et al. (2014) demonstrated that the inclusion of crude fiber at a level within the normal range of 8% content had the ability to maintain a stabilization of the digestive process inside the duodenal lumen without high energy. This normalizes the digestive process and accounts for the not much evidence of difference in lumen diameter between control and treated specimens reported in the current study.

The average duodenal lumen diameter of ducks in this experiment is between 2046.94 and 2194.92 μm, the duodenal lumen diameter of ducks is within the normal range. Zulfa et al. (2020) reported that the duodenal lumen of ducks had an average diameter of 2049-2800 μm. The normal diameter of the duodenal lumen determined in this study can be used as a basis for evaluating normality, the health of the duck, and for optimal activity of the digestive process. Ronnestad et al. (2014) reported that the lumen diameter of the duodenum is related to the maintenance of the mucosa layer, digestion, absorption, and duodenal motility, which occur efficiently, the amount of feed according to the capacity in the duodenum, and lumen pH conditions (neutral). Puspasari et al. (2020) reported that the optimal pH to keep the structure of the duodenum is 6–7. The fiber content in the diet with spirulina meal is also related to the normal duodenal lumen diameter.

ANOVA's results of the submucosal tunica of the duodenum there was no significant difference between the treatment and control groups (P>0.05). The mean tunica submucosa thickness was 32–34 μm, which falls within the range of normal. Prosekova et al. (2021) reported an average thickness of the tunica submucosa of the duodenum in poultry between 29-49 μm The similar average thickness between treatments and controls in this study suggests that spirulina flour at 2.5%-10% were not likely to have affected the thickness of the tunica submucosa duodenum in Peking ducks. What ducks eat is not that much, and it still falls under safe food for ducks. According to Suwignyo et al. (2021) that ducks can digest 8% fiber. The quite similar crude fiber content in feed for the treatment and the control can preserve the tunica submucosa structure of the duodenum, therefore, the thickness does not show a significant difference.

ANOVA analysis of muscularis tunica thickness found no significant difference between the control and treatments (P>0.05). The average thickness of the tunica muscularis varied between 475 and 519μm, this average is within the normal size of a rat. Zulfa et al. (2020) demonstrated that the normal thickness of the tunica muscularis in ducks is 358–513 μm. The normal size and insignificant difference of the tunica muscularis are influenced by the crude fiber content in the duck. Crude fiber of each treatment was between 3.78% and 5.87%, and the levels were appropriate for Peking ducks. Fiber at normal levels can increase peristaltic activity in the muscular tunica layer of the duodenum and optimize the absorption of nutrients in the duodenum. Sugiarto et al. (2024) stated that feeding with normal crude fiber causes protein to be optimally degraded, so that the rate of absorption of nutrients becomes more effective and efficient.. Zghair et al. (2019) stated that the muscular tunica consists of two layers of smooth muscle fibers, longitudinal and circular, which produce peristaltic movements. Fiber with a content that matches the duodenum's carrying capacity in the intestinum tenue will make the muscular tunica's work more optimal in performing peristaltic movements, thus enhancing the digestive and absorption functions in the duodenum. Fiber at normal levels will produce mucus secretion needed to protect the muscular membrane surface, supporting digestion and nutrient absorption by enterocyte cells in the duodenum.

Based on the histological structure of the duodenum in Figure 1, it is clear that the villi, tunica mucosa, muscular tunica, and duodenal lumen in Pekin ducks do not show changes in size and are within the normal range. The villi in the duodenum of Pekin ducks appear to protrude along the lumen surface. The structure of the tunica mucosa layer is clearly composed of the lamina propria, epithelium, and lacteal. The muscular tunica layer consists of circular and longitudinal muscles. Lisnahan et al. (2019) stated that the absorption process occurring in the duodenum is optimized by the presence of protruding villi along the lumen surface. Increased villi height can enhance the large surface area, making nutrient digestion more optimal.

Seyyedin's (2017) research showed that increased duodenal villi height is caused by epithelial cell growth in the villi, which functions to provide a larger villi surface area for the substrate to be digested and nutrients to be absorbed. This condition occurred in this study, showing an increase in villi height in the treatments compared to the control. The normal condition is related to the well-functioning proliferation process. According to Chen et al. (2024), epithelial cell growth in villi starts from proliferation (in poultry, this occurs every 3-5 days) followed by differentiation of stem cells located at the base of the crypts of Lieberkühn, eventually forming mature functional cells (maturation). Ramadhan et al. (2020) stated that mature epithelial cells function in synthesizing and secreting the enzyme enterokinase. A wider duodenal lumen diameter results in a better surface area for absorbing digested nutrients. Observations of the histological structure of the villi, tunica mucosa, muscular tunica, and duodenal lumen in Pekin ducks are presented in Figure 1.

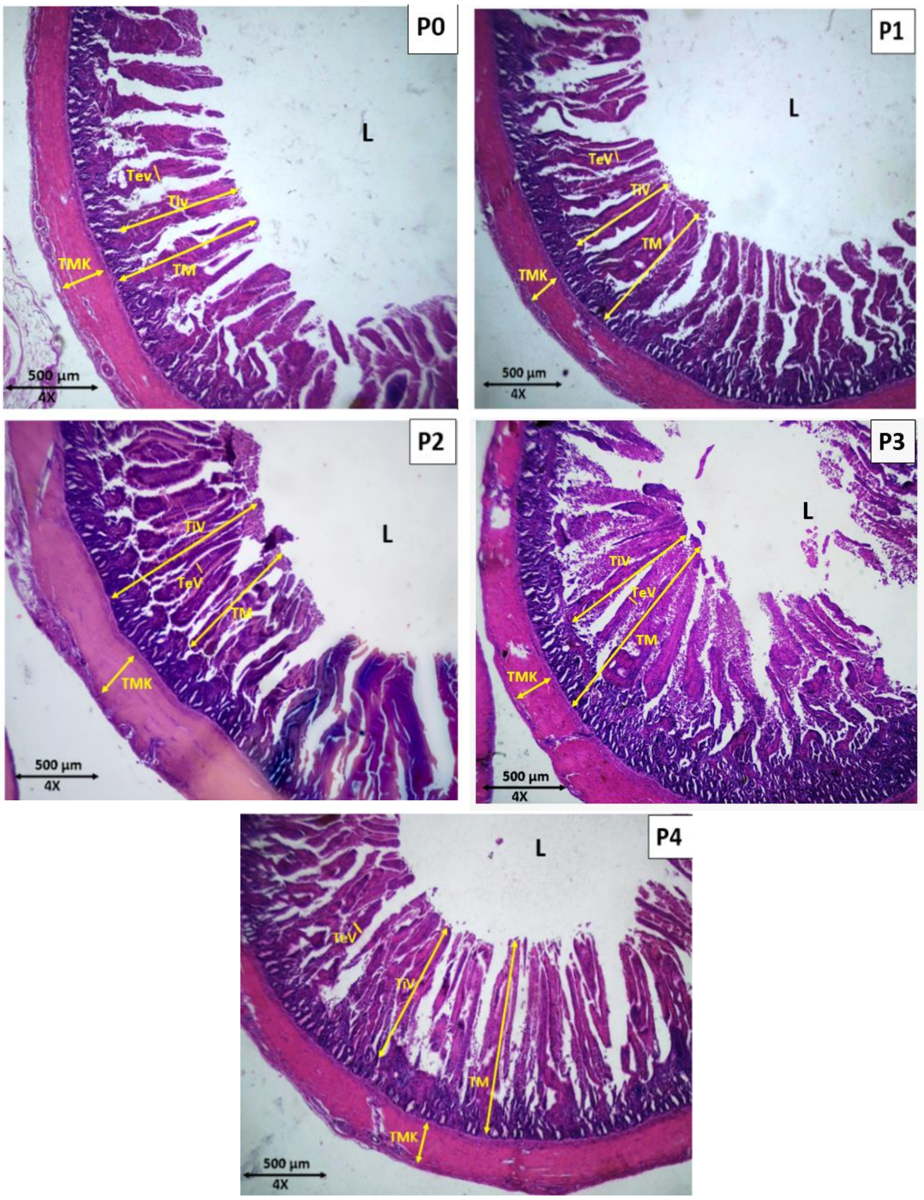

Figure 1. Villous height, villous thickness, tunica mucosa thickness, muscularis thickness, and duodenal lumen of male Peking ducks (coloring Hematoxylin -Eosin, magnification 40x). P0= Feed with spirulina flour (control) 0%, P1= feed with spirulina flour 2.5%, P2= feed with spirulina flour 5%, P3= feed with spirulina flour 7.5%, P4= feed with spirulina flour 10%. TM= mucosal thickness, TeV= villous thickness, TMK= muscularis thickness, TiV= high villi , L=lumen.

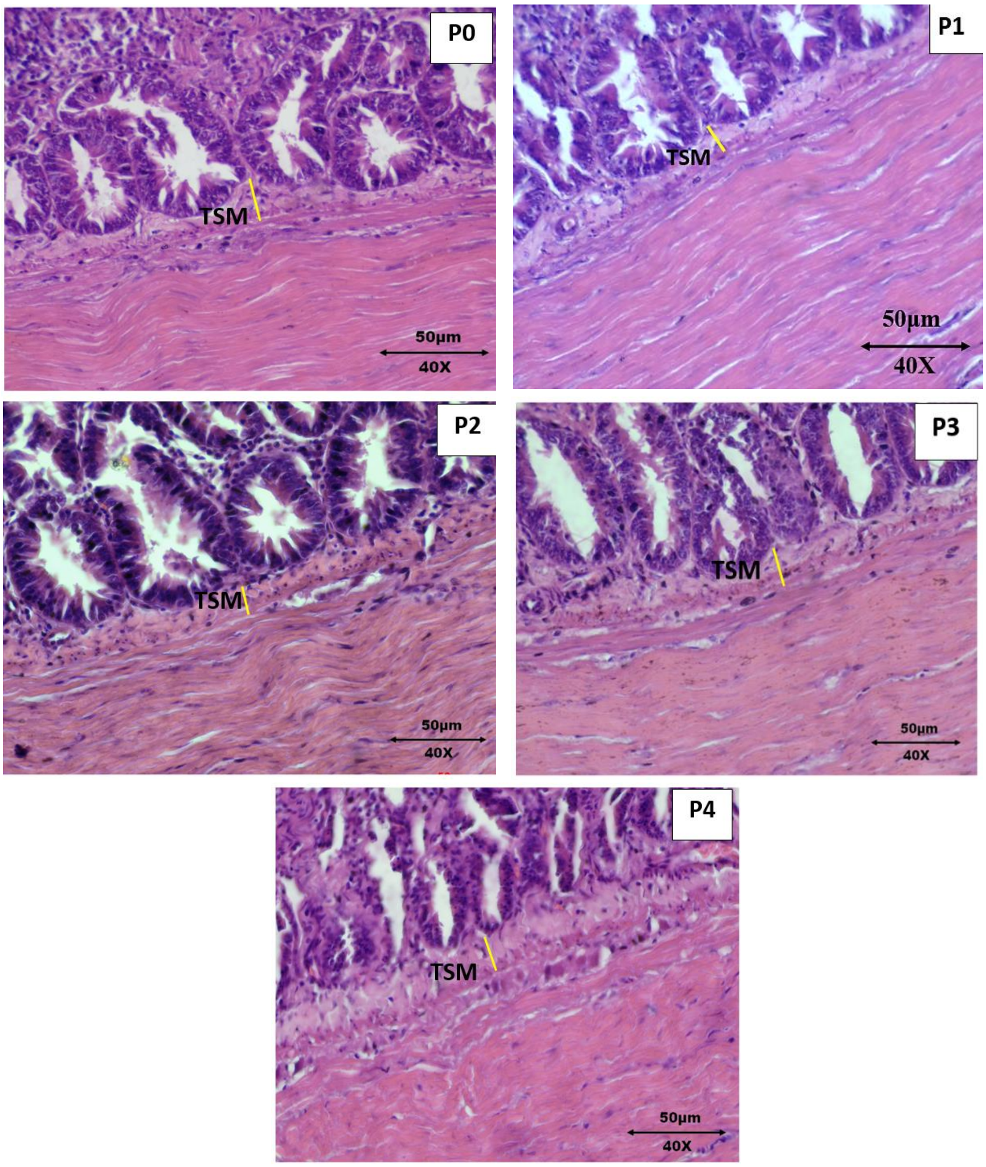

Figure 2. Peking duck duodenal submucosal tunica (Coloring Hematoxylin-Eosin, magnification 400x). P0= Feed with spirulina flour (control) 0%, P1= feed with spirulina flour 2.5%, P2= feed with spirulina flour 5%, P3= feed with spirulina flour 7.5%, P4= feed with spirulina flour 10%. TSM= Submucosal thickness .

Histological observations tunica duodenal subumoxa is seen in Figure 2. in normal conditions and no damage to cells occurs. The submucosa consists of loose connective tissue, blood vessels, lymphatic vessels, nervous tissue, and Brunner's glands. According to Al-Taee (2017), the loose connective tissue in the submucosa provides strength and flexibility during contractions of the small intestine during peristalsis. Small blood vessels, arterioles, and venules function to supply blood and nutrients to the mucosa and muscular tunica. Optimal absorption and distribution of nutrients play a crucial role in maintaining and repairing tissue structure in body organs (Kwardoyo et al., 2023). Lymphatic vessels transport absorbed fats in the form of chylomicrons and immune cells from the mucosa. Nervous tissue, specifically the submucosal plexus, controls mucosal secretion and small intestine motility. Brunner's glands function to lubricate the small intestine so as to avoid irritation. Putra et al. (2021) stated that submucosal thickness is influenced by its components, especially the number of Brunner's glands. Submucosa consists of dense irregular connective tissue, blood vessels, lymphatics, and nerves.

The addition of spirulina flour as a feed additive at levels of 2.5%-10% is not toxic and does not interfere with the structure of the duodenum in Peking ducks. The activity of bioactive compounds in spirulina, such as flavonoids, tannins, saponins, and alkaloids, acts as anti-inflammatory, antibacterial, and plays a role in cell mitosis. Sherwood et al. (2014) explained that new cells are formed due to high mitotic activity, which migrate to the apical crypta of the villi to replace aging cells. Shaw et al. (2012) noted that cell proliferation can increase villi height, crypt depth, mucosal thickness, and surface area. Antioxidant compounds in spirulina also affect activity in the duodenum. Flavonoids are a group of phenolic compounds found in spirulina. Feeding spirulina flour with a balanced amount of flavonoids does not have a negative effect on the digestive organs and can have a positive impact on the duodenum. According to Setiawan et al. (2018), the free radicals in cells due to the oxidative reaction can be blocked by flavonoids as an antioxidant. The inhibitory effect of flavonoid compounds prevents the duodenal free radicals that may injure the mucosa, reducing the excessive apoptosis and inflammation. Moustafa et al. (2021) reported that flavonoids have the ability to scavenge free radicals in the small intestine through the intervention of the generation of reactive oxygen species (ROS). They have an antioxidative effect through the donation of hydrogen atoms to scavenge opposite free radicals in substance. Flavonoids are also antibacterial by inhibiting the permeability of bacterial walls. Flavonoids have been said to disrupt the permeability of bacterial cell walls, bacterial microsomes, and lysosomes (Kusmayadi & Rahayu, 2020). The hydroxyl group of flavonoid compounds has induced alterations on organic content and nutrient transportation, leading to a toxic effect on pathogenic bacteria. Substances like the tannins which exist in spirulina, can help to harden the mucosal lining of the small intestines. This is also justified by Buyse et al. (2022) mentioned that tannins can function to inactivate cell adhesins of pathogenic bacteria and exert disruptive activities on the transit of proteins across the inner layer of the bacterial cells. Kusmayadi & Rahayu (2020)) further explained that tannins acted on the polypeptide region of the bacterial cell wall, thus leading to the incomplete formation of the bacterial cell wall membrane, and the lysis of the bacterial cells ensued through osmotic pressure. Saponin could destroy the cytoplasmic membrane of bacterial cells, so that the permeability of the cytomembrane of bacteria was weakened. Alkaloids disrupt the synthesis of peptidoglycan in bacterial cells, preventing the full development of bacterial cell walls, and leading to the cells' death.

Based on the result of this research, it can be taken as a new reference for duck (especially in Indonesia) and generally poultry industry about how to improve the quality of feed given to poultry by adding spirulina. It is also correlated with larger tissue structure of the duodenum, enhanced digestibility, productivity, and product quality of Peking ducks. The development of natural supplements (eg spirulina powder) as an animal feed supplement could also be an alternative to costly commercial supplements and can have health benefits for the ducks and consumers. Larger duodenal tissue structure size could enhance digestion and absorption of nutrients; thus promoting the improvement of productivity and product quality of ducks. It is expected that the result of this study can be the base in conducting further research to find the optimum dose of spirulina as a natural feed additive or isolating natural bioactive molecules from spirulina to be utilized in product development and feed quality, leading to an improvement in the poultry productivity and quality in Indonesia.

CONCLUSIONS

Spirulina flour is a prospective feed additive enhancing duodenal performance in male pekin ducks, as observed by villi height and mucosal layer thickness in the small intestine. Further research is needed on the bioavailability and distribution of bioactive compounds in spirulina flour such as flavonoids, tannins, alkaloids and saponins in the blood and target tissues in the duodenum Intestinum tenue in Peking ducks.

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INFORMED CONSENT STATEMENT

Informed consent was obtained from all subjects involved in the study referring to Ethical Clearance (EC) Number 69/EC-H/KEPK/FK-

UNDIP/VII/2023. Prior to participation, each subject was given a detailed explanation of the purpose, procedures, potential risks, and benefits of this study. All participants voluntarily agreed to participate and signed a written informed consent form.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest related to the publication of this paper.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI)-based tools were used in the preparation, analysis, or writing of this manuscript. All aspects of the study, including data collection, interpretation, and preparation of the manuscript, were carried out entirely by the authors without the assistance of AI-based technology.

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