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HISTOLOGICAL STRUCTURE OF BROILER DUODENUM AFTER FEEDING A COMBINATION OF ADDITIVES *SPIRULINA* FLOUR AND LIQUID NANOCHITOSAN

STRUKTUR HISTOLOGIS DUODENUM BROILER SETELAH PEMBERIAN KOMBINASI ADITIF TEPUNG *SPIRULINA* DAN NANOKITOSAN CAIR

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Abstract

Spirulina is a microalgae rich in protein, fat, carbohydrates, minerals, chlorophyll a, phycocyanin, beta-carotene, linoleic acid, and vitamins. Nanochitosan, a polymer derived from chitin, contains amine and carboxylate groups. Both additives enhance digestion, absorption, and duodenal structure in broilers. This study analyzed the effect of combining *spirulina* flour and liquid nanochitosan on broiler duodenal histology. A 3 × 2 factorial completely randomized design (CRD) with four replicates was used. The first factor was *spirulina* flour (0; 3; 6%), and the second was liquid nanochitosan (0 & 5%). Measured variables included duodenal weight, length, villus height, epithelial thickness, muscular layer thickness, and lumen diameter. Data were analyzed using ANOVA at a 5% significance level. Results showed *spirulina* alone affected duodenal lumen diameter ($P < 0.05$), while nanochitosan alone had no effect on duodenal length, villus height, epithelial thickness, muscular thickness, or lumen diameter ($P > 0.05$). The combination significantly increased duodenal weight ($P < 0.05$) but did not affect other variables. In conclusion, *spirulina* and nanochitosan improve duodenal weight but do not alter duodenal length, duodenal lumen diameter, villus height, or layer thicknesses.

Keywords: Epithelial thickness; Nanochitosan; Microalgae; Muscular layer; Villus height

Abstrak

Spirulina adalah mikroalga yang kaya akan protein, lemak, karbohidrat, mineral, klorofil a, fikosianin, beta-karoten, asam linoleat, serta vitamin. Nanokitosan adalah polimer yang berasal dari kitin, mengandung gugus amina, dan karboksilat. Kedua aditif pakan ini meningkatkan pencernaan, penyerapan, dan struktur duodenum pada ayam broiler. Penelitian ini bertujuan untuk menganalisis pengaruh kombinasi tepung spirulina dan nanochitosan cair terhadap histologi duodenum broiler. Rancangan yang digunakan adalah rancangan acak lengkap (RAL) factorial 3 × 2 dengan empat ulangan. Faktor pertama adalah tepung spirulina (0; 3; 6%), dan faktor kedua adalah nanochitosan cair (0 & 5%). Variabel yang diukur meliputi berat dan panjang duodenum, tinggi vili, ketebalan lapisan epitelium, ketebalan lapisan otot, dan diameter lumen duodenum. Hasil penelitian menunjukkan bahwa spirulina tanpa nanochitosan berpengaruh terhadap diameter lumen duodenum ($P < 0,05$), sedangkan nanochitosan cair tanpa tepung spirulina tidak berpengaruh pada panjang duodenum, tinggi villus, ketebalan epitel, ketebalan lapisan muskularis, dan diameter lumen duodenum ($P > 0,05$). Kombinasi keduanya secara signifikan meningkatkan bobot duodenum ($P < 0,05$), tetapi tidak memengaruhi variabel lainnya. Kesimpulan dari penelitian ini adalah, kombinasi spirulina dan nanochitosan cair meningkatkan bobot duodenum, tetapi tidak mengubah panjang duodenum, diameter lumen duodenum, tinggi villus, ketebalan epitel, dan lapisan otot pada duodenum.

***Kata Kunci**: Lapisan epitelium; Nanokitosan; Mikroalga; Lapisan muskularis; Tinggi vili*

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INTRODUCTION

Broilers are poultry that are widely cultivated in Indonesia. This poultry has a fast growth time, which is 4–5 weeks, and can be harvested with a body weight between 1.5–2 kg/head (Fahrunningsih & Septiningrum, 2021). The Direktorat Jenderal Peternakan dan Kesehatan Hewan (2022) stated that in 2019, broiler production in Indonesia was 3,495,090.53 tons, and in 2020 it decreased to 3,219,117.00 tons. Anggitasari et al. (2016) stated that one of the factors causing the decline in broiler production is that the need for nutrients during poultry growth is not met due to a decrease in feed quality. The decline in feed quality is a serious problem that requires a quick and systematic solution to the problem. Several researchers have conducted studies to address problems related to decreased feed quality, one of which is by incorporating feed additives into animal diets. Feed additives are substances included in feed in small quantities to enhance feed efficiency, support animal health, and improve overall productivity (Abd El Tawab et al., 2024).

Hameed (2021) stated that several additives are added to poultry feed, including fermators, probiotics, prebiotics, antibiotics, enzymes, organic acids, emulsifiers, antioxidants, synthesizers, and hormones. Several researchers have conducted studies to address problems related to decreased feed quality, one of which is by incorporating feed additives into animal diets. Feed additives are substances included in feed in small quantities to improve feed efficiency, support animal health, and enhance overall productivity (Abdelli et al., 2021). Furthermore, several studies have reported that the effectiveness of feed additives may vary depending on their type, dosage, and animal species. Some feed additives have been shown to produce inconsistent or non-significant effects on growth performance or organ development under certain conditions, including the use of enzymes and organic acids such as sodium butyrate (El-Sabrout et al., 2024). Singh and Gaikwad (2020) stated that feed additives in the form of antibiotics made from synthetic chemicals have a variety of side effects, including disrupting the balance of microflora in the small intestine, hormone balance, antioxidant synthesis, disrupting metabolism, and growth in poultry. Additives to overcome farmers' problems have criteria, including being easy to obtain, natural, containing safe bioactive compounds, and affordable prices. These additives can also improve feed quality to support the formation of body cells, as a source of energy, increase growth, and broiler productivity (Lestingi et al., 2024). *Spirulina* and nanochitosan can be considered as feed additives.

Spirulina is a blue green microalgae that has the ability to live in various types of water. This microalgae has a spiral shape and contains protein, carbohydrates, fats, minerals, chlorophyll a, phycocyanin, beta carotene, linoleic acid, and vitamins. The microalgae's high nutrient content makes it suitable for use as a natural feed additive. The addition of *spirulina* in feed is not only to meet the nutrient needs of these birds, but also functions as an antimicrobial, antioxidant, and immunomodulator (Sugiharto, 2020a). Research conducted by Abouelezz (2017) showed that the use of *spirulina* as an animal feed additive is beneficial in spurring metabolism and improving broiler growth. The results of recent studies showed that the addition of *spirulina* in feed is beneficial for improving growth performance, feed conversion ratio, and intestinal morphology, including increased villi height in broilers (Lestingi et al., 2024).

Nanochitosan is a derivative of chitosan, which is found in the outer skin of crustaceans and is composed of amine, primary, and secondary carboxylic groups (Swiatkiewicz et al., 2015). The product is biodegradable and safe for consumption, making it suitable for use as an additive in animal feed. The provision of nanochitosan as a feed additive is beneficial in increasing nutrient absorption, poultry growth, and productivity (Abd El-Ghany, 2023), and reducing triglyceride formation in the liver (Allaw et al., 2016). Research conducted by Johnson and Nicola (2016) showed that the addition of nanochitosan in feed or drink gave a significant effect on the motility activity of the intestine tenue. The part of the intestine tenue that responds to motility activity is the duodenum.

The duodenum is the initial part of the small intestine tenue that first receives digestive products from the stomach. The process of digestion in the duodenum takes place through motility activity (Sherwood, 2014). Digestive motility is an activity in the form of coordinated contractions, including segmentation for mixing and peristaltic movements for propulsion (Patel & Thavamani, 2023). Mixing of digestive contents in the intestine involves contraction of circular and longitudinal muscles.

The result of the mixing is pushed towards the distal part of the small intestine giving rise to the gastroileal reflex and the transfer of digestive products from the lumen of the small intestine to the large intestine (Johnson & Nicola, 2016). The digestive process in the duodenum is equipped with enzymes secreted from the pancreas into the lumen so that it will respond directly to the absorptive surface of the duodenal mucosa (Johnson & Nicola, 2016). Segmental contractions that occur in the duodenum can cause luminal activity. This activity is indicated by changes in the histological structure of the duodenum, such as an increase in the surface area of the lumen (Kiarie et al., 2019), muscular layer thickness (Mazzoni et al., 2022), villi height (Lestingi et al., 2024), and epithelial layer thickness (Abdelli et al., 2021).

Changes in the histological structure of the duodenum can be known through histomorphometry and microanatomy of the duodenum as a whole. Pio et al. (2017) stated that histomorphometry is the process of measuring the volume, length, thickness, and width of cells or tissues. Dewi et al. (2022) stated that microanatomy is an activity of observing preparations with a microscope to see changes or damage to cells or organ tissues. Research on the combination of *spirulina* flour and liquid nanocitosan feed additives is still limited and has not provided information on the effect of both on the histological structure of the duodenum. Therefore, the purpose of this study was to analyze the effect of feeding a combination of *spirulina* meal additives and liquid nanochitosan and their interaction on the histological structure of the broiler duodenum.

MATERIALS AND METHODS

This study used a completely randomized design (CRD) with a 3 × 2 factorial arrangement consisting of *spirulina* flour (0; 3; and 6%) and liquid nanochitosan (0 & 5%). A total of 24 broilers with an average initial body weight of 452.29 ± 100.15 g were used in this study. Each treatment consisted of 4 replicates with 1 bird per replicate. The treatment groups included S0N0, S3N0, S6N0, S0N5, S3N5, and S6N5. The research variables consisted of duodenal weight and length, villi height, epithelial layer thickness, muscular layer thickness, and lumen diameter of the broiler duodenum.

Table 1. The nutritional composition of the feed mixture with *spirulina* meal

Spirulina levels (%)	Nutrition (%)				
	Ash	Water	Crude fat	Coarse fiber	Crude protein
0	9.46	5.78	5.68	3.68	19.94
3	9.74	5.77	5.67	4.08	21.31
6	9.96	5.58	5.77	4.59	22.82

Feed Manufacturing with the Addition of *Spirulina* Meal Feed Additives

12 kg of standard commercial feed was made into stock for 5 days. Every 2 kg of standard commercial feed was mixed with *spirulina* meal according to the treatment. The mixing of feed with *spirulina* flour was done by weighing the feed and *spirulina* flour according to each treatment, including S0 (2 kg of standard commercial feed without the addition of *spirulina* flour), S3 (1,940 g of standard commercial feed added 60 g of *spirulina* flour), and S6 (1,880 g of standard commercial feed added 120 g of *spirulina* flour). In the next stage, the weighed *spirulina* meal was added to the feed gradually and then stirred until homogeneous. The nutritional composition of the feed mixture with *spirulina* meal is shown in Table 1.

Treatment Given to Broilers

Broilers were placed in collective cages for a 2-week acclimation period. The experimental period lasted for 4 weeks (weeks 3–6). Each cage measured 100 × 51 × 72 cm^3 and was equipped with feed and water containers. The broilers were then transferred to battery cages measuring 40 × 34 × 35 cm^3 and received treatments during the experimental period. Feed and water were provided according to the treatment twice daily (07:00 and 15:00) on an *ad libitum* basis. Temperature and humidity were recorded daily, and cages were cleaned every morning and disinfected once a week.

Variables Measurement Observation

The variables observed and measured in this study were muscular layer thickness, villi height, lumen diameter, and epithelial layer thickness. Observations and measurements of the duodenal histology structure were made using a microscope and Optilab connected to a laptop. Each preparation was observed from 3 angles of view of each variable. Measurements of muscularis thickness, villi height, lumen diameter, and epithelial layer thickness were made using the following formulas and measurement techniques (Figure 1), $\hat{w} = \frac{w1+w2}{2}$, $\hat{y} = \frac{y1+y2}{2}$, $\hat{z} = \frac{z1+z2}{2}$, $\hat{x} = \frac{x1+x2}{2}$.

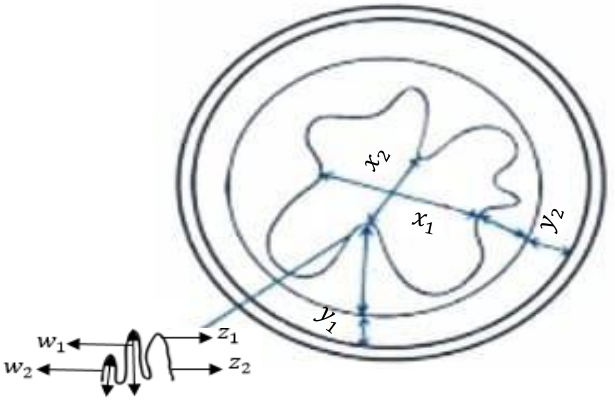

Figure 1. Duodenal histomorphometry technique. Note: w_1 = height of the tallest vili; w_2 = shortest vili height; y_1 = thickness of the muskularis layers; y_2 = thickness of the muskularis layers; x_1 = lumen diameter of the highest section; x_2 = lumen diameter of the shortest section; z_1 = thickness of the apical epithelial layer; z_2 = thickness of the basal epithelial layer

Data Analysis

Data from variable measurements were analyzed using Statistical Product of Service Solution (SPSS) Software for Windows version 26.0. The results of observations of the histological structure of the broiler duodenum were analyzed descriptively and qualitatively. The measurement data were tested for homogeneity using Levene's homogeneity test and tested for distribution patterns using the Shapiro-Wilk test. Data with normal and homogeneous distribution patterns are continued with a parametric difference test using the two-way Analysis of Variance (ANOVA) test with a significance level of 5%. Research data from significant parametric difference test results were further tested using the Least Significant Difference (BNT) test. The interaction test uses the Smallest Real Difference test (BNT) at 5% significance.

RESULTS

Observation of the histological structure of the broiler duodenum was carried out using an Optilab and a microscope connected to a laptop. The results of the observations in both treatment and control groups (Figure 2) showed a normal histological structure with intact villi, epithelial layer, muscular layer, and lumen at 40× magnification.

The lumen of the broiler duodenum in this study, based on histological observations presented in Figure 2, showed no changes or structural damage, and there were variations in thickness in each treatment. The results presented in Table 2 showed that *spirulina* supplementation had no significant effect on villi height (p= 0.143), epithelial thickness (p= 0.059), and muscularis thickness (p= 0.921), but significantly affected lumen diameter (p= 0.026). Nanochitosan (p= 0.755) and the interaction between *spirulina* and nanochitosan (p= 0.404) showed no significant effects on all measured variables.

The analysis of lumen diameter (Table 2) showed that *spirulina* supplementation significantly affected lumen diameter (p= 0.026), whereas nanochitosan (p= 0.755) and the interaction between *spirulina* and nanochitosan (p= 0.404) showed no significant effects. Broilers fed 3% and 6% *spirulina* without nanochitosan (S3N0 and S6N0) exhibited higher lumen diameter compared to the control (S0N0), with the highest value observed in the S6N0 treatment.

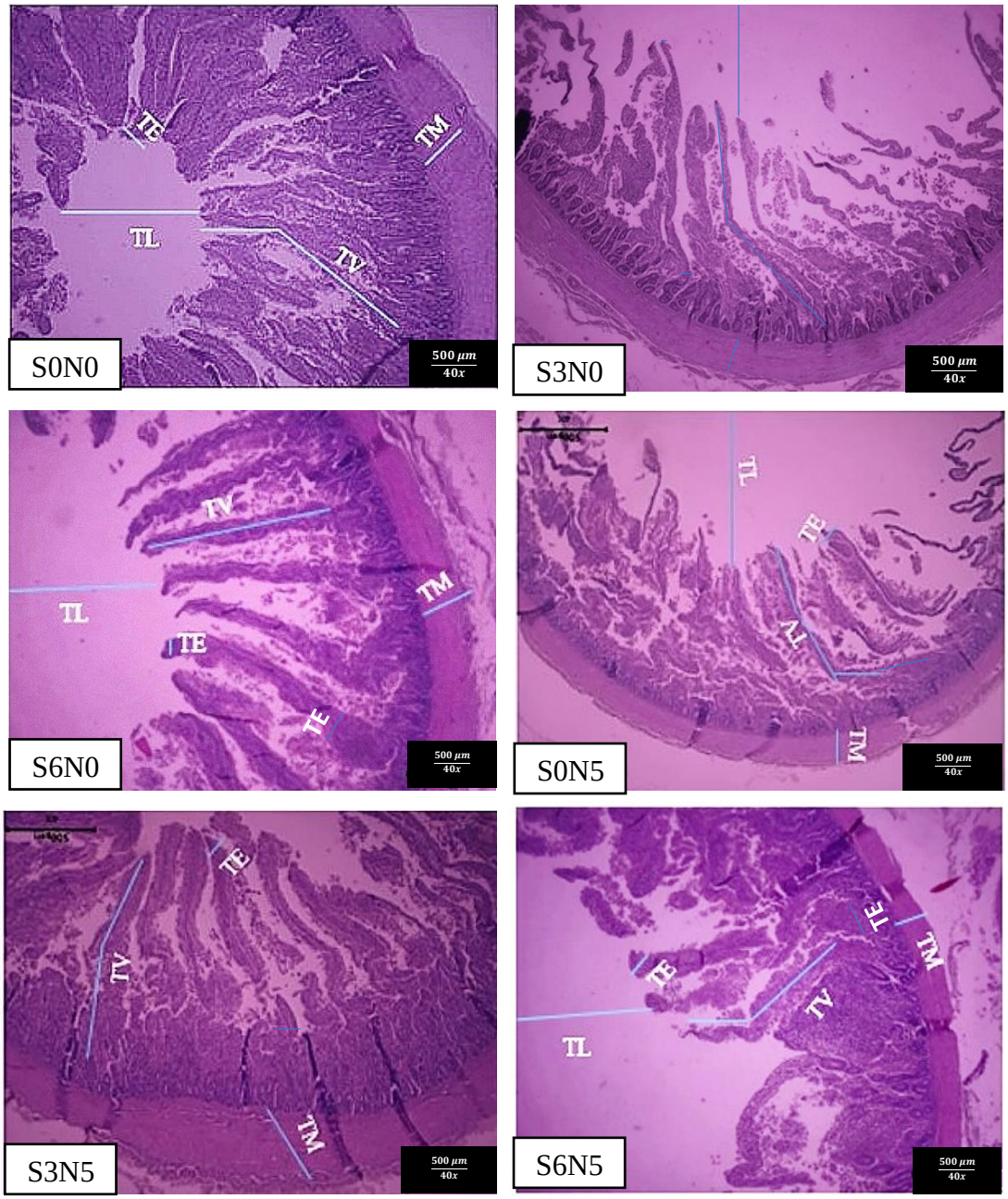

Figure 2. Histological structure of the broiler duodenum in the treatment and control. Note: Observation of histological structure of broiler duodenum with Hematoxylin-Eosin staining and 40× magnification. Villi height (TV), lumen thickness (TL), muscularis thickness (TM), epithelial thickness (TE) in treatments (S0N0), (S3N0), (S6N0), (S0N5), (S3N5), and (S6N5)

Table 2. Analysis of mean villi height, lumen diameter, muscularis layer thickness, and epithelial layer thickness

Treatment	Variabel (μm)			
	Villi height	Lumen diameter	Muscularis layer thickness	Epithelial layer thickness
Spirulina (S)				
S0	1097.66 ± 147.65	1771.76[a] ± 323.41	163.16 ± 57.82	52.48 ± 9.07
S3	1000.87 ± 92.68	1766.23[a] ± 262.55	165.60 ± 52.58	61.72 ± 13.84
S6	1115.50 ± 101.82	1871.01[b] ± 176.71	154.62 ± 51.02	69.84 ± 13.76
Nanochitosan (N)				
N0	1104.31 ±111.17	1886.04 ± 309.14	168.07 ± 60.27	57.40 ± 8.50
N5	1038.37 ± 116.92	1719.96 ± 199.31	154.18 ± 47.35	65.29 ± 15.95
(S) × (N)				

Treatment	Variabel (µm)			
	Villi height	Lumen diameter	Muscularis layer thickness	Epithelial layer thickness
S0N0	1148.18 ± 115.94	1635.54 ± 534.36	203.02 ± 86.30	52.34 ± 9.38
S3N0	993.53 ± 100.19	2023.05 ± 266.13	137.43 ± 55.97	53.76 ± 5.31
S6N0	1171.22 ± 117.40	1999.54 ±126.92	163.76 ± 38.54	66.10 ± 10.81
S0N5	1047.13 ± 179.36	1907.99 ± 112.47	123.30 ± 29.35	52.61 ± 8.77
S3N5	1008.21 ± 85.17	1509.41 ± 258.97	193.77 ± 49.19	69.68 ± 22.38
S6N5	1059.78 ± 86.23	1742.48 ± 226.50	145.49 ± 63.51	73.58 ± 16.72

Note: Data presented are mean ± standard deviation. Different superscripts in the lumen diameter column (S) indicate significant differences between treatments and there is no interaction effect between *spirulina* flour and liquid nanocitosan ($P < 0.05$). S0N0 (standard commercial feed, without liquid nanochitosan), S3N0 (standard commercial feed with 3% *spirulina* meal, tap water), S6N0 (standard commercial feed with 6% *spirulina* meal, tap water), S0N5 (standard commercial feed, 5% liquid nanochitosan), S3N5 (standard commercial feed with 3% *spirulina* meal, 5% liquid nanochitosan), and S6N5 (standard commercial feed with 6% *spirulina* meal, 5% liquid nanochitosan)

DISCUSSION

The results suggest that the levels of *spirulina* flour and nanochitosan added in feed and drinking water do not exceed the limit so as not to cause toxic properties (Figure 2). Recent studies have shown that villus development is influenced by the activity of intestinal stem cells located at the base of the crypts of Lieberkühn (Gehart & Clevers, 2019). This activity occurs during the growth and renewal phase, characterized by continuous cell proliferation and differentiation into functionally mature epithelial cells with specific morphology and functions (Beumer & Clevers, 2020). Epithelial cells that have passed the differentiation phase will migrate from the basal to the apical part of the villi and turn into more specific, mature epithelial cells. Bogucka et al. (2016) explained that epithelial cells that have undergone differentiation will migrate to the apical part of the duodenal villi to be involved in the process of digestion and absorption of nutrients. Stem cells at the base of Lieberkühn's crypt will activate mitosis and differentiate to replace duodenal villous epithelial cells that have undergone apoptosis. Elhassan et al. (2022) stated that the normal muscular layer is composed of elongated smooth muscle coated with connective tissue on the outside and circular on the inside. Reston et al. (2023) suggested that the smooth muscles that make up the muscular layer of the broiler duodenum are formed in the grower phase.

Recent studies have shown that the muscular layer of the duodenum is considered normal when the circular and longitudinal smooth muscle layers are clearly distinguishable (Bonis et al., 2021). The circular smooth muscle is located internally and is thicker, while the longitudinal smooth muscle is located externally with a thinner layer. The muscularis layer functions in coordinated contraction and relaxation to mix and propel intestinal contents. The addition of *spirulina* flour, liquid nanochitosan, or a combination of the two additives is not toxic and does not interfere with the nutrient absorption process.

Compared to turmeric supplementation in poultry, which has been reported to improve intestinal morphology, such as villi height and epithelial structure due to the antioxidant and anti-inflammatory activity of curcumin, the present study showed a different response pattern. In this study, only lumen diameter was significantly affected, while villi height, epithelial thickness, and muscularis layer thickness were not significantly changed. This suggests that *spirulina* and nanochitosan may exert a more limited or different modulatory effect on intestinal morphology compared to curcumin-based phytobiotics. This finding is in line with the statistical results of the present study, which showed that most intestinal parameters were not significantly affected by the treatments except for lumen diameter.

An increase in lumen diameter is associated with an increase in absorption surface area (Apriliyani et al., 2016). Research conducted by Sugiharto (2020b) proved that the utilization of *spirulina* at doses below 10% did not have a negative impact on digestive organs. *Spirulina* has a beneficial effect in improving the morphological and anatomical structure of the broiler's small

intestine. Sunarno et al. (2021) added that the provision of nanochitosan at levels of 2.5–10 g/kg feed can increase the diameter of the lumen.

The insignificant difference between the three variables and the control is thought to be due to the cross-linking of *spirulina* content and nanochitosan constituent compounds with other molecules in the digestive process, but the levels added have not been able to support an increase in villus height, epithelial layer thickness, and muscularis layer thickness (Table 2). *Spirulina* bioactive compounds, including phycocyanin, xanthophyll, and beta-carotene have the ability to bind with amine groups and hydroxyl groups of nanochitosan constituents. The crosslinking that occurs between *spirulina* bioactive compounds and nanocitosan constituent groups results in increased activation of protease enzymes to break down proteins into amino acids which are then circulated for the maintenance and regeneration of duodenal cells. Sunarno et al. (2023) stated that *spirulina* bioactive compounds such as phycocyanin, xanthophyll, and beta-carotene, act as protease enzyme activators through the formation of cross-links with amine groups and hydroxyl groups of nanochitosan.

Recent studies have shown that protease enzymes play a role in hydrolyzing proteins into amino acids (Hall, 2021). These enzymes are secreted by the pancreas as inactive zymogens and are activated in the small intestine, particularly the duodenum, to support protein digestion. Protease enzymes bind to proteins on the active site of the enzyme. The enzyme hydrolyzes proteins into amino acids, which are then absorbed through the duodenal epithelial cells via active transport and facilitated diffusion.

Recent studies have shown that proteins are essential nutrients that play a key role in growth and in the maintenance of body and organ structures (Nelson & Cox, 2021). Satimah et al. (2019) added that protein has an important function in the formation and regeneration of cells and the formation of body tissues. Rokhati (2018) suggested that nanochitosan is able to bind to fat and cholesterol in digestion causing the viscosity of chyme to increase and put pressure on the surface of the villi. The average value of villi height, epithelial layer thickness, and duodenal muscularis layer thickness in this study is considered normal, because it has a relatively similar size with the results of research by Bondar et al. (2022), Zhang et al. (2020), Elnaga and Selim (2018), respectively, which ranged from 943.81–1148.20 µm; 40–80 µm; and 136–213 µm.

The difference is thought to be due to the bonding between the bioactive compounds of *spirulina* and liquid nanochitosan with other molecules in the digestive process. Phycocyanin, xanthophyll, and beta-carotene compounds can bind with amine groups and hydroxyl groups of nanochitosan to increase protease enzyme activity. The hydroxyl group of nanochitosan is able to bind with fat and cholesterol, resulting in increased chyme viscosity that presses on the villi surface, causing the lumen diameter to widen but not accompanied by an increase in villi height, epithelial layer thickness, and muscularis layer thickness. Ismoyowati and Sumarmono (2019) stated that phycocyanin, xanthophyll, and beta-carotene form cross-links with the constituent groups of nanochitosan and protease to increase the catalytic activity of proteins into amino acids. Amine groups and hydroxyl groups will form cross-links and increase the stability and activity of the protease in protein degradation into amino acids. The degraded amino acids will be absorbed by the villi and distributed to small intestinal cells, liver, lymphatic system, tissues, and other organs. Mirzaie et al. (2020) stated that *spirulina* contains bioactive compounds, such as phycocyanins, carotenoids, and beta-carotene, which play a role in improving the development and morphology of the broiler small intestine.

Bhalamurugan et al. (2018) suggested that bioactive compounds in *spirulina*, such as lycopene and phycobiliproteins, act as antioxidant agents to neutralize excess free radicals so as to prevent oxidative damage to broiler tissues. The results of research conducted by Elhady and El-ghalid (2018) showed that giving *spirulina* with a concentration of 3–6% had a positive impact on improving cell growth. Tahir et al. (2023) added that the addition of nanochitosan as an additive can stimulate the expansion of surface area, but does not cause an increase in the depth of Lieberkühn's crypts in the duodenum. Research conducted by Sunarno et al. (2021) proved that the addition of liquid nanochitosan additives plays a role in maintaining the structure of the cell membranes that make up the duodenum.

CONCLUSION

Based on the statistical analysis, the combination of *spirulina* meal and liquid nanochitosan significantly affected lumen diameter ($P < 0.05$), whereas no significant differences ($P > 0.05$) were observed in villi height, epithelial layer thickness, and muscularis layer thickness of broilers. Further research using different inclusion levels, longer feeding periods, and additional intestinal morphology parameters is recommended to clarify the effects of spirulina meal and liquid nanochitosan on broiler intestinal health.

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REFERENCES

Abdelli, N., Solà-Oriol, D., & Pérez, J. F. (2021). Phytogenic feed additives in poultry: Achievements, prospects and challenges. *Animals, 11*(12), 3471.

Abouelezz, F. M. K. (2017). Evaluation of Spirulina algae (*Spirulina platensis*) as a feed supplement for Japanese quail: Nutritional effect on growth performance egg production, egg quality, blood metabolites, sperm egg penetration, and fertility. *Egyptian Poultry Science Journal*, *37*(3), 707-719.

Abd El-Ghany, W. A. (2023). Chitosan and its derivatives as promising feed additives in poultry nutrition: A review. *Journal of Advanced Veterinary Research*, *13*(2), 1-10.

Abd El Tawab, A. M., Liu, Q., Xu, G., & Han, X. (2024). Feed additives strategies to control methanogenesis in ruminants: Review. *Archiva Zootechnica*, *27*(2). doi: 10.2478/azibna-2024-0017.

Allaw, A., Taha, A., & Aldeenabdulwahid, A. S. (2016). The effect of adding different levels of chitosan to the diet in the some physiological characters on broiler chickens. *Journal for Agricultural Sciences*, *16*(3), 111-120.

Anggitasari, S., Sjofjan, O., & Djunaidi, I. H. (2016). Pengaruh beberapa jenis pakan komersial terhadap kinerja produksi kuantitatif dan kualitatif ayam pedaging. *Buletin Peternakan*, *40*(3), 187-196.

Apriliyani, N. I., Djaelani, M. A., & Tana, S. (2016). Profil histologi duodenum berbagai itik lokal di Kabupaten Semarang. *Bioma*, *2*(1), 144-150.

Beumer, J., & Clevers, H. (2020). Cell fate specification and differentiation in the adult mammalian intestine. *Nature Reviews Molecular Cell Biology*, *11*(1), 39-53.

Bhalamurugan, G. L., Valerie, O., & Mark, L. (2018). Valuable bioproducts obtained from microalgal biomass and their commercial applications: A review. *Environmental Engineering Research*, *23*, 229-241.

Bondar, A., Slencu, B. G., Popovici, I., & Solcan, C. (2022). Effect of turmeric (*Curcuma longa*) on duodenal structure in broiler chickens. *Brazilian Journal of Poultry Science*, *25*, 1-8.

Bogucka, J., Dankowiakowska, A., Elminowska-Wenda, G., Sobolewska, A., Szczerba, A., & Bednarczy, M. (2016). Effect of prebiotics and synbiotics delivered in ovo on broiler small intestine histomorphology during the first days after hatching. *Folia Biologica*, *64*, 131-143.

Bonis, V., Rossell, C., & Gehart, H. (2021). The intestinal epithelium fluid fate and rigid structure from crypt bottom to villus tip. *Frontiers in Cell and Developmental Biology*, *9*, 661931.

Dewi, P., Widiatningrum, T., Mubarok, I., Tirtasari, N. L., Walandipa, G., Wahidah, T. H., … Mailani. (2022). *Potensi senyawa aktif bahan alam*. Malang: Unisma Press.

Direktorat Jenderal Peternakan dan Kesehatan Hewan. (2022). *Livestock and animal health statistics 2022*. Jakarta: Kementerian Pertanian, Republic of Indonesia.

Elhady, A. A., & El-Ghalid, O. A. H. (2018). Spirulina platensis algae (SPA): A novel poultry feed additive effect of SPA supplementation in broiler chicken diets on productive performance, lipid profile, and calcium phosphorus metabolism. Proceedings of the VI Mediterranean Poultry Summit (18-20 June 2018). Torino, Italy.

Elhassan, M. M. O., Ali, A. M., Eissa, L., & Taha, A. A. (2022). Histologi usus halus broiler. *Jurnal Ilmu Kedokteran Hewan Universitas Bahri*, *1*(2), 5561.

Elnaga, M. K. A., & Selim, S. (2018). Influence of early feeding with different diet composition on performance and intestinal morphology of layer-type chicks during the brooding period. *Journal of Animal and Feed Sciences, 27*, 268-275.

El-Sabrout, K., Landolfi, S., & Ciani, F. (2024). Feed additives and enrichment materials to reduce chicken stress, maximize productivity, and improve welfare. *Poultry Science*, *103*(1), 103456.

Fahrunningsih, R., & Septiningrum, R. (2021). Analisis usaha peternakan ayam broiler: Studi kasus peternak mandiri di Kelurahan Maklalut. *Journal of Tropical Animal Research (JTAR)*, *2*(1), 1-7.

Gehart, H., & Clevers, H. (2019). Stem cells and intestinal homeostasis. *Nature Reviews Gastroenterology & Hepatology*, *16*(1), 19-34.

Hameed, H. M. (2021). Feed additives in poultry. *Assiut Veterinary Medical Journal*, *67*(168), 1-14.

Hall, J. E. (2021). *Guyton and Hall textbook of medical physiology (14th ed)*. Philadelphia: Elsevier.

Ismoyowati., & Sumarmono, J. (2019). Duck production and food security. The 1st Animal Science and Food Technology Conference (AnSTC) 2019. *IOP Conf. Series: Earth and Environmental Science, 371*, 1-11.

Singh, J., & Gaikwad, D. S. (2020). Phytogenic feed additives in animal nutrition. In M. Naeem, M. Ansari, S. Gill, & R. Gandhi (Eds.), *Natural bioactive products in sustainable agriculture* (pp. 273-289). Singapore: Springer.

Johnson, W. M., & Nicola, M. M. (2016). Understanding the physics of functional fiber in the gastrointestinal tract: An evidence-based approach to resolving eduring misconceptions about insoluble and soluble fiber. *Journal of the Academy of Nutrition and Dietetics, 1*, 1-14.

Kiarie, E., Bhandari, S., Scott, M., & Krause, D. O. (2019). Role of feed additives in modulating gut health and nutrient utilization in poultry. *Frontiers in Veterinary Science, 6*, 82.

Lestingi, A., Alagawany, M., Di Cerbo, A., Crescenzo, G., & Zizzadoro, C. (2024). Spirulina (*Arthrospira platensis*) used as functional feed supplement or alternative protein source: A review of the effects of different dietary inclusion levels on production performance, health status, and meat quality of broiler chickens. *Life*, *14*(12), 1537. doi: 10.3390/life14121537.

Mazzoni, M., Zampiga, M., Clavenzani, P., Lattanzio, G., Tagliavia, C., & Sirri, F. (2022). Effect of chronic heat stress on gastrointestinal histology and expression of feed intake-regulatory hormones in broiler chickens. *Animal*, *16*(8), Article 100600. doi: 10.1016/j.animal.2022.100600.

Mirzaie, S., Sharifi, S. D., & Khattab, F. Z. (2020). The effect of a chlorella by product dietary supplement on immune response, antioxidant status, and intestinal mucosal morphology of broiler chickens. *Journal of Applied Phycology*, *32*, 1771-1777.

Nelson, D. L., & Cox, M. M. (2021). *Lehninger principles of Biochemistry (8th ed)*. New York: W.H. Freeman.

Patel, R. M., & Thavamani, A. (2023). *Physiology, digestive motility*. Florida: *StatPearls Publishing*.

Pio, P. O., Ardana, I. B. K., & Suastika, P. (2017). Efektivitas berbagai dosis ayam organik dan anorganik sebagai acidifer terhadap histomorfometri duodenum ayam pedaging. *Indonesia Medicus Veterinus*, *6*(1), 44-51.

Reston, U., Suatha, I. K., Heryani, L. G. S. S., & Setiasih, N. I. E. (2023). Gambaran struktur dan morfometri usus halus itik Bali pada umur berbeda. *Buletin Veteriner Udayana*, *15*(1), 95-105.

Rokhati, N., Susanto, H., Haryani, K., & Pramudono, B. (2018). Enhanced enzymatic hydrolysis of chitosan by surfactant addition. *Periodica Polytechnica Chemical Engineering*, *62*(3), 286-291.

Satimah, S., Yunianto, V. D., & Wahyono, F. (2019). Bobot relatif dan panjang usus halus broiler yang diberi ransum menggunakan cangkang telur mikropartikel dengan suplementasi probiotik *Lactobacillus* sp. *Jurnal Sains Peternakan Indonesia*, *14*(4), 396-403.

Sherwood, I. (2014). *Fisiologi manusia*. Jakarta: EGC.

Sugiharto. (2020a). *Chlorella vulgaris* dan *Spirulina platensis*: Kandungan nutrisi dan senyawa bioaktifnya untuk meningkatkan produktivitas unggas. *Indonesian Bulletin of Animal and Veterinary Sciences*, *30*(3), 123-138.

Sugiharto. (2020b). Nutraceutical aspects of microalgae spirulina and chlorella on broiler chickens. *Livestock Research for Rural Development*, *32*, 6.

Sunarno., Solikhin., & Budiraharjo, K. (2021). Histomorfometri duodenum bebek (*Anas platyrhynchus)* setelah pemberian nanokitosan pada pakan. *Jurnal Pendidikan Biologi & Biologi*, *13*(3), 27-274.

Sunarno., Handyka, M. A. N., Mulyani, S., & Suparmi. (2023). Spirulina flour-based feed additive improves the physical and chemical eggs quality of Tegal-laying duck. *Biosaintifika*, *15*(3), 326-340.

Swiatkiewicz, S., Arczewska, M. A., & Jozefiak, D. (2015). Chitosan and its oligosaccharide derivatives (chito-oligosaccharides) as feed supplements in poultry and swine nutrition. *Journal of Animal Physiology and Animal Nutrition*, *99*(1), 1-12.

Tahir, I., Justitia, M., Karna, W., Mudasir, Roswanira, A. W., & Widi, K. (2023). Optimization of thiamine chitosan nanoemulsion production using sonication treatment. *Brazilian Journal of Poultry Science*, *15*(3), 181-189.

Zhang, Z., Zhouyang, Z., Huang, J., Xiaoting, S., Muhammad, H., Shakeel, A., … Li, X. (2020). Molecular characterization of a potential receptor of *Eimeria acervulina* microneme protein 3 from chicken duodenal epithelial cells. *Parasite*, *27*(18), 1-10.