

# Histomorphometry of the Duodenum of Ducks (*Anas platyrhynchos*) after Administration of Nanochitosan in Feed

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**Abstract.** Poultry farming business has several problems such as lack of availability on raw materials, expensive feed prices, and egg production that is not optimal. To overcome the problem of high feed prices and duck productivity, we need to add feed additives such as nanochitosan. Poultry farming business has several problems such as lack of availability on raw materials, expensive feed prices, and egg production that is not optimal. To overcome the problem of high feed prices and duck productivity, we need to add feed additives such as nanochitosan. This study aimed to analyze the effect of nanochitosan feed on the digestive function of the intestine tenue (duodenum) as indicated by histomorphometric changes in the duodenum of Tegal ducks (*Anas platyrhynchos*). Duodenal histomorphometry related to digestive function was measured, including changes in lumen diameter, villi height, epithelial cell thickness, and muscular layer thickness. This study used a completely randomized design consisting 5 treatments with 5 replications. The research treatments included P0 as control (basic feed without nanochitosan particle additives) and P1, P2, P3, and P4 (basic feed with the addition of nanochitosan with the doses of 2.5; 5; 7.5; and 10 grams/kg feed respectively). The variables observed were lumen diameter, villi height, epithelial layer thickness, and duodenal muscle layer thickness. The research data were analyzed using the analysis of variance (ANOVA) with a 95% confidence level. The results showed that the addition of nanochitosan particles in the feed had a significant effect on the lumen diameter and thickness of the duodenal muscular layer ( $P < 0.05$ ) and had significant effect on the villi height and thickness of the duodenal epithelial layer in the intestine tenue ( $P > 0.05$ ). Tegal ducks. The conclusion of this study is that nanochitosan feed additives with a concentration of 2.5-10% can improve digestive function and improve duodenal histomorphometry in the intestine tenue of Tegal ducks as indicated by an increase in lumen diameter, villi height, and the thickness of epithelial cell thickening, and muscular layer. The novelty from this study is the production and the use of nanochitosan as feed additive to repair histomorphometry of duodenum in intestine tenue of Tegal ducks. From this research, people gets benefit by knowing the use nanochitosan as feed additive to improve digestion performance and productivity of Tegal ducks.

**Key words:** Tegal duck, duodenum, nanochitosan particles, lumen diameter, muscular layer

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## INTRODUCTION

Local poultry farming has very good prospects for development. One of the local poultry that contributes as a source of animal protein is duck. Local duck has superior characteristics, such as being able to adapt well in tropical environments, having good resistance to disease, and being able to produce eggs in very large numbers (Kasiyati *et al.*, 2019). This type of poultry has a longer egg production period than chickens, low mortality rate, and can be reared easily and efficiently. This animal has a fast growth rate, especially in the early period of growth (Alifah *et al.*, 2020). Therefore, duck farming has a relatively smaller risk, so it has the potential to be developed (Sunarno *et al.*, 2020).

The national poultry population in 2016-2018 has increased compared to the poultry population in 2015, which was 4.64% (Nuraeni *et al.*, 2019). Statistical data shows that the number of ducks in Central Java Province in 2018 was 4,587,436 heads (Pratiwi *et al.*, 2019). The Ministry Agriculture of Republic of Indonesia stated that in 2020, the population of laying

ducks has increased compared to 2019, from 57,229,088 heads/year to 58,243,335 heads/year. The production of laying ducks in small-scale farmers produces between 100-150 eggs per head per year (Rossida *et al.*, 2019). Local laying ducks in Indonesia generally have a production period about 15 months (Mas'adah *et al.*, 2018). The high population of ducks in Indonesia can be a solution to meet the needs of animal protein and could reduce the imports value of livestock products. Various efforts to increase the production of ducks continue to be made. The increase in duck production could not be separated from the improvement in the digestive performance of ducks. One of the external factors that affect digestion is feed. Good maintenance management on ducks could improve physiological processes related to duck digestive activities (Daryatmo and Hakim, 2017).

Livestock business, especially on laying ducks, has several problems, including lack of availability on raw materials, expensive feed prices, and egg production that is not optimal. Various kinds of

problems have an impact on the laying duck farming business which has not been able to meet the needs of animal protein for the community. The development and utilization of local feed ingredients is an effort that can be done to overcome the problem of high feed prices. Feeding additives is one of the potential methods to increase duck productivity, especially on egg production (Marcelina *et al.*, 2020). Research conducted by Hamedi *et al.* (2011) showed that feed ingredients can affect digestive activity by changing the structure of the intestine tenue. Changes in the structure of the intestine tenue can cause changes in digestive performance and the ability to absorb nutrients from digestion (Zulfa *et al.*, 2020). Local feed ingredients that can be used as alternative feeds and abundantly available, especially in the province of Central Java, are nanochitosans.

Nanochitosan is widely used in the medical world, including in the manufacture and drugs processing, and as a carrier agent in gene therapy (Wang *et al.*, 2020). Chitosan nanoparticles are biodegradable, so they can be used to increase nutrient absorption and growth of poultry (Minqi *et al.*, 2011). The concentration of nanochitosan in feed as a feed supplement can be physically, chemically, and biologically affects the digestive activity of ducks, especially those related to the content of complex carbohydrates in the feed. Meanwhile, the maximum carbohydrate required by ducks at layer age is 15% (Kasiyati *et al.*, 2019). This is because carbohydrate supplements in feed have an important role in digestive activities, especially in the motility of the intestine as a digestive organ that mostly performs the function of digestion and absorption of feed nutrients (Johnson and Nicola, 2016).

The mixing activity of feed in the duodenum involves the addition of enzymes secreted from the pancreas into the lumen, so eventually there is a direct contact between all the feed ingredients with the enzymes and nutrients from digestion with the absorptive surface on the duodenal mucosal cells. The segmentation contraction that occurs helps to encourage the activity in the luminal section through stretching the surface area of the lumen (Yao *et al.*, 2006) and mucosal responses in the form of elevated villi (Sugito *et al.*, 2007). It also helps to increase movement activity in the muscular part of the duodenum (Kokoszynski *et al.*, 2018) and on the absorptive surface of the mucosal cells shown by a thickened epithelial layer (Ruttanavut *et al.*, 2009). The response of this luminal activity is part of the duodenal microanatomy work in the intestine tenue which supports the increased production of ducks by optimizing digestive activity (Daryatmo and Hakim, 2017).

Poultry has a low ability to utilize complex carbohydrates such as chitosan, but chitosan is still needed in certain concentrations to support optimal digestion and absorption of nutrients (Tossaporn, 2013). El Ashram *et al.* (2019) stated that duck fed with a feed additive in the form of nanochitosan with a concentration of 250-750 mg/100gr of feed was proven to have a significant effect on body performance and nutrient digestion in the intestines of ducks. Other research evidence showed that chitosan in low levels of feed can increase the activity of digestion and absorption of nutrients (Gopalakannan and Venkatesan, 2006). Giving chitosan as a feed additive to broilers, laying hens, and birds with concentrations below 1.4 g/kgbw per day is safe and has a hypolipidemic effect, which can reduce cholesterol, triacylglycerol, and free fatty acids in serum (El-Ashram *et al.*, 2019). The results of other studies showed that poultry receiving 0.025% and 0.05% chitosan supplements had a significant effect on reducing abdominal fat (Shraddha *et al.*, 2017). Giving 0.01% chitosan concentration can increase the growth rate in poultry (Swiatkiewicz *et al.*, 2015). The results of the study from Zhou *et al.* (2009) showed that chitosan supplementation in broiler feed can reduce triglyceride levels in blood serum.

Several other researchers have also proven the use of nanochitosan on digestive activity in poultry. Abdel-Wahhab *et al.* (2017) have proven that nanochitosan addition as much as 280 mg has a significant effect on changes in the biochemical profile of serum in rat test animals. Research by El-Naby *et al.* (2019) proved that nanochitosan supplementation in feed could significantly increase growth, total feed consumption, nutrient availability, and total protein serum in test animals. Nanochitosan also had a significant effect on metabolic performance, improving health status, carcass quality, intestinal microbial population, digestive enzyme activity, and fat digestion in quail (*Coturnix coturnix japonica*) test animals (El-Ashram *et al.*, 2020).

Duck farming with a high quality feed has an important role in supporting the development of the economic sector in Indonesia. In this regard, it is necessary to develop research through the use of local raw materials as feed additives for duck feed. The use of nanochitosan as a feed additive in duck farming is still limited, therefore it is very important to conduct this study to prove the effect of using nanochitosan as a feed additive on the histomorphometric structure of the duodenum in the intestine tenue by observing several variables including lumen diameter, villi height, epithelial layer thickness, and muscular layer thickness. This research was expected to support the optimization of digestive activity and nutrient absorption in increasing the productivity of Tegal

ducks in Indonesia. It was also expected to provide several benefits such as providing information about the potential of nanochitosan to repair the histology structure of duck intestine tenue (duodenum), and information to farmers about nanochitosan function to increase the productivity of laying ducks, as well as the use of nanochitosan as a feed supplement.

## METHODS

### Research plan

This study used a completely randomized design consisting of 5 treatments with 5 replications. The treatment was in the form of the addition of nanochitosan feed additive in the basic feed given to 25 Tegal ducks. Feed treatments with nanochitosan feed additives, including P0 as the control, group

(100% basic feed, without nanochitosan added), and P1, P2, P3, and P4 as the treatment groups with nanochitosan feed additive added to the basic feed with a concentration of 2.5; 5; 7.5; and 10 grams/kg feed respectively. The research variables consisted of nanochitosan as independent variables, and lumen diameter, villi height, and epithelial cell and muscular layer thickness, as dependent variables.

### Feed manufacturing with the addition of nanochitosan feed additives

Duck feed with the addition of nanochitosan feed additive was in the dry form and was given to the laying ducks in semi-wet conditions. The laboratory analysis results from the composition of nutrients content of feed are presented in Tables 1.

**Table 1.** Laboratory analysis of nutrient content of feed

Feed ingredients	Nanochitosan concentration (%)				
	0	2.5	5	7.5	10
Metabolic energy (ccal)	2,630.5	2,680.9	2,790.57	2,840.8	2,880.4
Crude protein (%)	17.22	17.56	18.30	19.56	20.08
Fat (%)	6.16	5.40	5.25	4.25	4.16
Calcium (%)	1.82	2.05	2.56	2.90	3.04
Crude fiber (%)	3.07	3.25	3.57	4.09	4.21

### Treatment giving to Tegal ducks

Tegal ducks were reared in cages measuring 160 x 80 x 50 cm. There were 5 rearing cages, each containing 5 ducks. Feed with the addition of nanochitosan was given to ducks with a frequency of twice a day, in the morning at 08.00 WIB and in the afternoon at 16.00 WIB. The amount of feed for each cage with 5 ducks was 400 grams for each feeding. Feed treatment was given according to the dose and drinking water was given ad libitum for 8 weeks, started from the ducks aged 21 weeks until 29 weeks. The day after the end of the treatment, the test animals were decapitated followed by surgery for sampling or isolation of the intestine tenue (duodenum).

### Intestinal organ isolation and fixation

The process of isolation and fixation began after the decapitation process according to the Kosher method, which were cutting blood vessels (jugular veins and carotid arteries), esophagus, and trachea. Isolated intestinal organ was taken and put into physiological saline solution (0.95% NaCl), then washed, followed by cutting the tip of 2 cm and for samples taken with a size of 2 cm afterward, then fixed using 10% BNF solution.

### Preparation of paraffin method

The preparation of histology analysis of the duodenum was using the paraffin method, which included the stages of fixation, dehydration, clearing, infiltration, embedding, section, and affixing based on method used by Zulfa *et al.* (2020).

### Staining preparation with Hematoxylin-Eosin (HE)

Hematoxylin-eosin is a histological dye used to mark different tissue structures, for clarity. Hematoxylin marked the cell nucleus with a blue-black color that showed the nucleus clearly, and eosin colored the cell cytoplasm which most of them were connective tissue fibers in various colors and color intensities, such as pink, orange, and red (Zulfa *et al.*, 2020). The next process was mounting, the process of closing the preparation with a cover glass. Before mounting, the histological preparation were put in xylol for 24 hours. The stained preparation were covered with Canada balsam and labeled for further observation using a microscope.

### Variable measurement observation

Histomorphometric observations of the duodenum were carried out descriptively and quantitatively with the micrometer contained in the Olympus photomicrograph. Calculation of lumen diameter,

villi height, epithelial layer thickness, and muscular layer thickness were referred to Zulfa *et al.* (2020).

### Data analysis

The research data were analyzed to determine the distribution pattern and its homogeneity. If the results of the data analysis show a normal and homogeneous distribution pattern, then the analysis of variance (ANOVA) was continued at the 95% confidence level. If there were differences between groups, then the data were proceed with Duncan's test at a significance level of 5% to determine the different groups.

**Table 2.** The mean value of lumen diameter, villi height, epithelial layer thickness, and muscular layer thickness of intestine tenue (duodenum) after the administration of nanochitosan for 8 weeks

Variable ( $\mu\text{m}$ )	Concentration of nanochitosan particles (g/kg feed)				
	P0	P1	P2	P3	P4
Lumen diameter	2,425.2a $\pm$ 225.6	2,793.7a $\pm$ 210.4	3,242.9b $\pm$ 287.8	3,811.9c $\pm$ 256.3	3,516.1bc $\pm$ 202.1
Villi height	1,126.3a $\pm$ 110.6	1,129.6a $\pm$ 109.6	1,460.3b $\pm$ 116.2	1,542.1b $\pm$ 119.3	1,446.9b $\pm$ 117.6
Epithelial layer thickness	37.4a $\pm$ 3.5	37.6a $\pm$ 3.2	45.2b $\pm$ 3.5	47.9b $\pm$ 3.4	44.1b $\pm$ 3.0
Muscular layer thickness	317.6a $\pm$ 32.2	388.8b $\pm$ 38.5	438.2b $\pm$ 43.6	453.4b $\pm$ 44.2	448.5b $\pm$ 44.2

Note: The data displayed are the average  $\pm$  standard deviation. Different superscripts in the same column showed significant differences between treatments (P 0.05).

The results showed that the lumen diameter of the duodenum in the intestine tenue in Tegal ducks treated with nanochitosan was higher than the control (P<0.05). This means that nanochitosan is able to increase the lumen diameter of the duodenum of the test animals. The average diameter of the duodenal lumen in all treatments (P0, P1, P2, P3, and P4) are still in the normal category according to (Zulfa *et al.*, (2020). The result obtained show that nanochitosan has the ability to improve the function of digestion, absorption, and maintain cellular integrity of the cell components that make up the duodenum in the intestine tenue of Tegal ducks. Nanochitosan given in an appropriate levels can improve digestive function and increase nutrient absorbtion activity in the intestine tenue (duodenum) (Younus *et al.*, 2020).

Another histomorphometric variable, the height of the duodenal villi, showed a significant difference between the treatment and the control (P<0.05). The data in Table 2 show that nanochitosan can increase the height of the duodenal villi in Tegal ducks. Based on Duncan's test at 95% confidence level, it showed that the mean height of the duodenal villi between P2, P3, and P4 treatments were not significantly different, but significantly different from the control and P1.

The thickness of the epithelial lining of the duodenal villi in the intestines of Tegal ducks administered with feed additive also showed a significant difference compared to the control

## RESULT AND DISCUSSION

Analysis of variance at 95% confidence level on duodenal histomorphometry data, which included lumen diameter, villi height, epithelial layer thickness, and muscular layer thickness showed that the nanochitosan feed additive in the feed had a significant effect on several histomorphometric variables of the intestine tenue (duodenum). The mean values of several duodenal histomorphometric variables are presented in Table 2.

(P<0.05). The data in Table 3 show that the experimental animals in treatment P2, P3, and P4 had a thicker epithelial layer and were significantly different than the control and P1.

Differences in histomorphometric variables between treatment and control were also seen in the thickness of the duodenal muscular layer. Tegal ducks fed with nanochitosan as feed additive had a thicker muscular layer than the control.

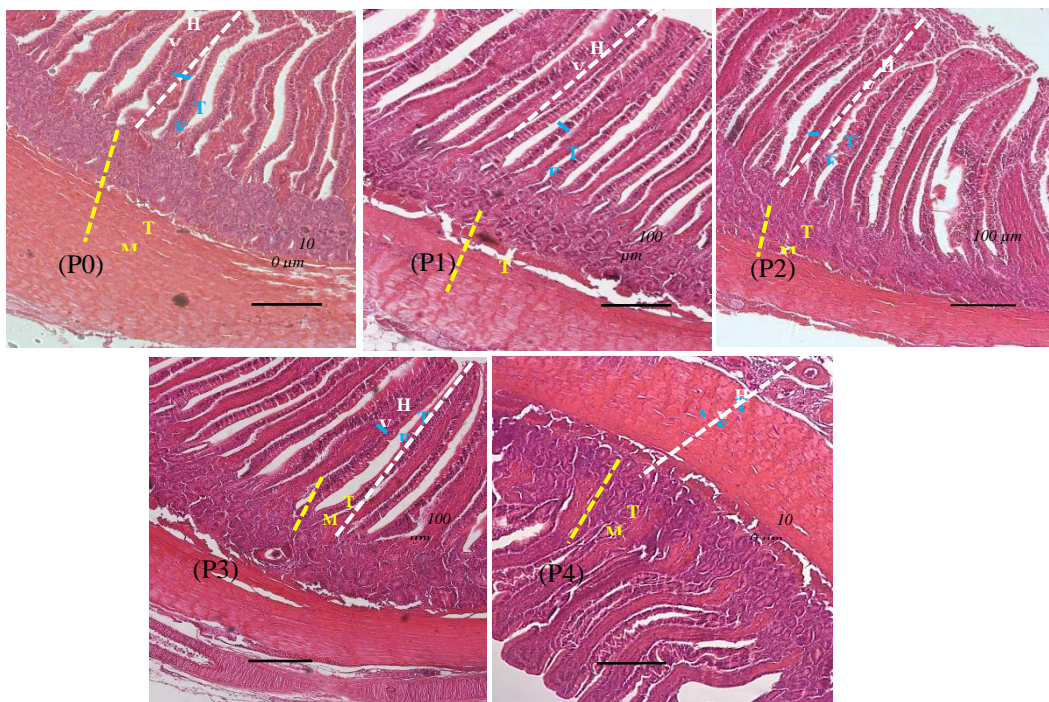
Based on the results of ANOVA at the 95% confidence level, there was a difference between the treatment and the control (P<0.05), which means that nanochitosan has the influence in increasing the thickness of the muscular layer in the duodenum of the test animals. The results of Duncan's test at the 95% confidence level showed that the P1, P2, P3, and P4 treatments were not much different from each other, but significantly different from the control. The results showed that nanochitosan given as a feed additive could increase the villi height, and increase the thickness of the epithelial cells and muscular layer, of the intestine tenue (duodenum) in Tegal duck. This means that nanochitosan has the ability to improve the function of digestion, absorption, and maintain the cellular integrity of the cell components that make up the duodenum. These results are in accordance with the results of Tahir *et al.* (2019) which stated that nanochitosan as a feed additive can

increase the height, surface area, and ratio of crypt depth to villus height in the duodenum.

The results obtained in this study showed the improvement in the availability of nutrients from the digestive process which was marked by an improvement in the histomorphometric variable of the intestinum tenue (duodenum). This shows that when the quality of the feed is improved by giving nanochitosan as the feed additive, there will be an increase in growth performance indicated by the histomorphometric variable of the intestine tenue (duodenum) as evidenced by this study. Although digestive enzymes were not examined in this study, the benefits of nanochitosan have been shown to increase digestive activity in the duodenum through evidences of improvement in duodenal histomorphometric variables. Digestive enzymes in

the duodenum function to hydrolyze feed nutrients, such as carbohydrates, fats, and proteins into their derivative products so that eventually those nutrients can be easily absorbed by the intestinal villi and available for body cells that need them (Zhang *et al.*, 2019).

Duodenal histomorphometry in several treatments are presented in Figure 1. The duodenum histomorphometry observations of the ducks showed that there were differences between the treatment and the control, and there were no signs of damage to the structure of the duodenum. This histomorphometric evidence shows that nanochitosan has an effect on the widening of the lumen diameter of the duodenum, the height of the villi, and the thickening of the epithelial and muscular layer of the intestinum tenue (duodenum) in Tegal ducks.



**Figure 1.** Histomorphometry of the intestinum tenue (duodenum) in Tegal ducks after 8 weeks of nanochitosan treatment

Note: histomorphology of villous height, epithelial thickness, and muscular thickness P0, P1, P2, P3, and P4. The height of the villi (HV), the thickness of the epithelium (TE), and the thickness of the muscular layer (TM).

Based on the histomorphometric results as shown in Figure 1, it shows that the duodenum is histologically in normal condition. The nanochitosan given can improve the histological profile of the duodenum. Histologically, the duodenum consists of four layers, which include the mucosa, submucosa, muscularis, and serosa. The improvement in duodenal morphometry means an increase in the size of the studied variables involved in the process of nutrient absorption resulting from the digestion process that takes place in the intestine tenue (duodenum).

Histological observations showed that the mucosal layer of the duodenum consists of villi that appear high or elongated and Lieberkuhn's crypts that are covered by simple columnar cylindrical epithelial cells that are tall, and have a striated border and large oval-shaped nucleus at the bottom of the cell near the basal membrane. The intestinal villi of the duodenum are composed of absorptive columnar epithelial cells (enterocytes) that have cilia (brush border) and mucus-secreting goblet cells. The epithelial cells of the duodenal villi will continue into the open sections

of the Lieberkuhn's crypts at the base of the villi, these crypts appear as invaginations from simple branching tubular glands. The elastic tissue surrounding the crypts and smooth muscle extends to the villi. Intestinal villi and crypts of Lieberkuhn's did not appear to be degenerated and inflamed.

The provision of nanochitosan feed additives will affect the mitotic activity of intestinal epithelial cells in the duodenal villi. The differentiated cells will then migrate to the apex of the villi to support the function of digestion and absorption, and some of the epithelial cells will undergo apoptosis or be extruded into the duodenal lumen (Onderci *et al.*, 2006). Cells that are actively mitotic in the crypts will constantly replenish the epithelial cells of the duodenal villi. The results of the research by Incharoen and Yamauchi (2010) support the results of this study, which stated that the activity of epithelial cells in the duodenal villi is influenced by digestive activity and nutrient absorption. Nanochitosan in the threshold range had an effect on increasing lumen diameter, villi size, epithelial cell layer thickness, duodenal muscular layer compared to control and remained in normal size. Increasing some of these histomorphometric variables will improve digestive function and affect the quality of growth, immunity, and productivity of Tegal ducks (Sumiati, 2003)

Although this research is still limited in the study and analysis of the function of nanochitosan on the histomorphometric aspects of the intestine tenue (duodenum) in Tegal ducks, the histological studies carried out in this study are expected to open opportunities for further research in the future about the mechanistic effects of nanochitosan in improving digestive performance of Tegal ducks or research on hematological status, microbiological, and immunological responses in effort to improve the quality and productivity of Tegal ducks. The novelty of this study is about the production and the used of nanochitosan as feed additive to repair histomorphometry of duodenum in intestine tenue of Tegal ducks. The benefits of this research are the use nanochitosan as feed additive to improve digestion performance and productivity of Tegal ducks.

## CONCLUSION

The addition of nanochitosan feed additives in the feed can increase the lumen diameter, increases the villi height, and increase the thickness of epithelial cells and the muscular layers of the duodenum intestine tenue in Tegal ducks.

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