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Visceral Organ Weight of Pengging Duck after the Addition of Nanochitosan as Feed Additive

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Abstract. The productivity of pengging ducks is determined by optimizing the function of the body's organs. The feed consumed greatly determines the growth and development of the internal organs in the pengging duck. Nanochitosan is a polysaccharide that has potential as a feed additive which functions to increase feed digestibility, nutrient absorption, promote growth to help optimize the function of visceral organs. This study aims to analyze the effect of nanochitosan as a feed additive on the function of the visceral organs of pengging ducks in terms of the weight of the visceral organs. The visceral organs studied included the ventriculus, intestines, liver, pancreas, spleen, and heart. This study was designed in a completely randomized design (CRD) consisting of 5 treatments (0; 2.5; 5; 7.5; 10 g nanochitosan/kg feed) with 5 replications. Nanochitosan feed additive treatment was given for 8 week. Variables that were measured in this study included weight of ventriculus, intestinal, liver, pancreas, spleen, and heart of pengging ducks. The research data showed that nanochitosan as a feed additive had no significant effect on visceral weight of pengging ducks (P>0.05). The conclusion of this study is that nanochitosan as a feed additive does not cause changes in the function of the visceral organs based on the indication of the weight of the visceral organs measured, namely the ventricles, intestines, liver, pancreas, spleen, and heart. The novelty of this research is the use of nanochitosan as a feed additive to improve the performance of penging ducks by maintaining the size of the visceral organs. It is hoped that the results of this research on nanochitosan feed additive can be used by local laying duck farms in Indonesia because it is safe and as an effort to improve the performance and productivity of ducks.

Keywords: feed additives; nanochitosan; pengging duck; standard feed; visceral organs

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INTRODUCTION

Pengging duck is a type of poultry that has the potential as a source of animal protein, crosses between mojosari ducks and magelang ducks (Marcelina *et al*., 2020). The Directorate General of Livestock and Animal Health stated that the national duck population in 2020 increased from 2019, which was 1.68% with a population of 48.59 million head. The problem faced by duck breeders in general is the high cost of feed. Expenditures for feed costs can reach 70% of production costs. Duck breeders try to provide feed ingredients that are cheap, of good quality, do not compete with human needs, and can increase the productivity of ducks, especially laying ducks (Khattab *et al*., 2021).

Duck productivity is influenced by the optimization of organ functions. Growth, development, and function of the digestive organs and internal organs are affected by the feed consumed by livestock (Sahara *et al*., 2019). Animal feed with poor quality, contaminated with mycotoxins can cause abnormalities in several internal organs and lymphoid ducks which is characterized by an increase in the relative weight of the organs due to swelling. This condition can further trigger a decrease in the weight of the visceral organs and result in animal death (Lakkawar *et al*., 2017).

Adding additives to feed can be done to improve feed quality and livestock performance. Feed additives are additional feed ingredients given to livestock to increase livestock productivity and health (Zufan *et al*., 2023). The addition of antibiotic growth promoter (AGP) as a feed additive has been banned because it can cause bacterial resistance effects in livestock and humans who consume these livestock products. An alternative effort that can be done by farmers is to use feed additives made from natural ingredients, quality, cheap, and do not compete with human needs. One of them is nanochitosan which has been approved for use in feed by the FDA (The U.S. Food and Drug Administration) (Wang *et al*., 2020).

Nanochitosan is a chitin-derived poly saccharide derived from crustacean waste, has biodegradable properties so it can be used to increase nutrient absorption and growth of poultry, is non-toxic, antibacterial, antioxidant, immunostimulatory, anti-inflammatory, and can have a hypocholesterolemic effect (Minqi *et al*., 2011). El-Ashram *et al*. (2020) stated that nanochitosan with a concentration of 250-750 mg/100g in duck feed has been shown to have a significant effect on the digestion of nutrients in the intestines, and body performance. Research Sunarno *et al*. (2021) reported that the addition of nanochitosan in feed with a concentration of 2.5-10% can improve digestive function and increase the ability to absorb nutrients in feed. The digestive process in the digestive tract produces derivative products from carbohydrates, proteins, and fats which are then absorbed by intestinal cells and transported throughout the body as raw materials for metabolism. Simple molecules that enter the blood vessels are transported with the help of the heart to various organs in the body that need them, such as the ventriculus, intestines, liver, pancreas, spleen, and the heart itself. Metabolic processes that occur in various organs produce energy that is used for physical activity, controlling body temperature, strengthening the immune system, tissue formation, reproduction and productivity (Falasifah *et al*., 2018). Various effects of feed and energy use are associated with changes in visceral organ weight (Lestari *et al*., 2020).

The use of nanochitosan as a feed additive has not provided much information regarding its effect on the weight of duck visceral organs (ventriculus, intestine, liver, pancreas, heart and spleen). Based on the above background, it is necessary to conduct research to determine the effect of adding nanochitosan in the feed on the weight of the visceral organs of pengging ducks which include the ventriculus, intestines, liver, pancreas, heart and spleen.

This research is an attempt to obtain important data related to the optimization of visceral organ function in layer phase pengging ducks which can be used to analyze the effect of nanochitosan feed additive levels. These feed additives have been known to improve digestive and reproductive performance, and do not cause disturbances to the visceral organs. The data from this study are very useful as a reference in administering nanochitosan as a feed additive to local ducks reared by breeders in Indonesia and as important information for further research on nanochitosan administration in the grower phase.

METHODS

Research Plan

This study used a completely randomized design (CRD) which consisted of 5 treatments with 5 replications. Treatment with nanochitosan addition in feed was given to 25 female pengging ducks according to the treatment group. Treatment of addition of nanochitosan in standard feed each with levels of 0; 2.5; 5; 7.5; and 10 g/kg feed. The research variables consisted of nanochitosan concentration as the independent variable, and ventriculus weight, intestinal weight, liver weight, pancreas weight, spleen weight, heart weight as the dependent variable.

Maintenance and Acclimation of Pengging Duck

Pengging ducks were placed into 5 plots of cages to be acclimated for one week. Each cage plot measuring 100 × 150 × 70 cm^3 used a litter system with rice husk bedding, equipped with a place to feed and drink. Each plot contained 5 female pengging ducks aged 5 months and entering the production period. Duck feed is given 2 times a day, morning and evening. The amount of feed for each cage with 5 ducks is 400 g for each feeding. Treatment of feed and drinking water was given ad libitum for 8 weeks.

Feed Mixing with Nanochitosan

A standard feed of 20 kg (15 kg of bran and 5 kg of concentrate) is made for five days of feeding. Every 4 kg of standard feed was added with nanochitosan according to the treatment. Nanochitosan obtained from PT. Semarang Agrotechno Aesthetics Laboratory. The addition of nanochitosan for each treatment included P0 (4000 g standard feed), while for P1, P2, P3, and P4 were respectively composed of (3990 g standard feed + 10 g nanochitosan), (3980 g standard feed + 20 g nanochitosan), (3970 g standard feed + 30 g nanochitosan), (3960 g standard feed + 40 g nanochitosan). The feeding ducks were given in the form of semi-wet mash (400 g of feed formulated with nanochitosan mixed with 135 ml of water) and given for eight weeks.

Calculation of Research Variables

Data collection begins with weighing the final weight of the pengging ducks. Slaughter is carried out using the Kosher method. Next, surgery and isolation of the visceral organs was carried out. Variables measured included ventriculus weight, intestinal, liver, pancreas, spleen and heart. Visceral organ weights were obtained by weighing the organs using a digital balance, with a sensitivity of 0.1 g. Measurement of visceral organ weight (%) is obtained by dividing the visceral organ weight by live weight multiplied by 100% (Azizah & Sjofjan, 2022).

Data Analysis

The research data were analyzed to determine the pattern of distribution and homogeneity. The results of data analysis in the study showed a normal and homogeneous distribution pattern for all variables, then continued with Analysis of Variance (ANOVA) with a significance level of 5% using Statistical Product of Service Solution (SPSS) version 26.0 (Santoso, 2018).

RESULT AND DISCUSSION

The results of the analysis of variance with a significance of 5% showed no significant differences in ventriculus weight, intestinal, liver, pancreas, spleen, and heart between the treatment and the control (P> 0.05). This shows that nanochitosan as a feed additive has no significant effect on the weight of visceral organs, which include the ventriculus, intestines, liver, pancreas, spleen, and heart. The results of research with feed additive nanochitosan treatment obtained the same results as the results of research conducted by Lestari *et al*. (2020) with feed additives in the form of moringa leaf flour at levels of 0, 2.5, 5, 7.5, 10%.

These two feed additives did not have a significant effect on the size of the visceral organs in Pengging ducks, which means they did not cause changes in the size of the visceral organs. Tomasi *et al*. (2019) stated that the weight of the visceral organs in several vertebrate animals is fixed (isometric). It was further stated that the increase in digestive and metabolic performance by feed ingredients is closely related to the increase in energy products and metabolite products but does not correlate strongly with the weight of visceral organs. Energy and metabolite products are used for maintenance, not used for increasing size and adding biomass. Based on these results, it proves that the nanochitosan feed additive does not increase the size of the visceral organs but functions to maintain the size of the visceral organs so that they remain in isometric conditions. The result of data analysis that done to respective research variables were shown in Table 1.

Table 1. The average percentage of the visceral organs weight of pengging ducks after being treated with nanochitosan for 8 weeks.

Variable (%)	Concentration of nanochitosan (g/kg feed)				
	0	**2.5**	**5**	**7.5**	**10**
Ventriculus	2.77 ± 0.25	2.92 ± 0.28	2.94 ± 0.17	2.90 ± 0.11	3.13 ± 0.18
Intestinum	3.50 ± 0.32	3.92 ± 0.38	3.90 ± 0.35	3.81 ± 0.16	4.08 ± 0.26
Liver	1.89 ± 0.18	1.90 ± 0.12	1.98 ± 0.14	2.00 ± 0.09	2.11 ± 0.07
Pancreas	0.19 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.20 ± 0.01
Spleen	0.09 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.10 ± 0.01
Heart	0.58 ± 0.07	0.61 ± 0.08	0.62 ± 0.03	0.60 ± 0.02	0.67± 0.06

Annotation: Mean values without superscript within same column indicating no significant difference between treatment group (p>0.05). Data was shown as mean value±standard deviation (SD)

The results of the analysis of variance with a significance of 5% showed that nanochitosan had no significantly different effect on ventriculus weight (P>0.05). Research Wasilewski *et al.* (2015) stated that the percentage of normal ventriculus weight in ducks ranged from 2.68% - 3.25% of live weight. From the results of the study showed that the average ventriculus weight was still within the normal range. The average ventriculus weight between the different treatments was not significant, it was suspected that the addition of nanochitosan did not interfere with protein digestion in the ventriculus and protein in the feed would be used more to support egg production than growth because ducks were in the egg production phase.

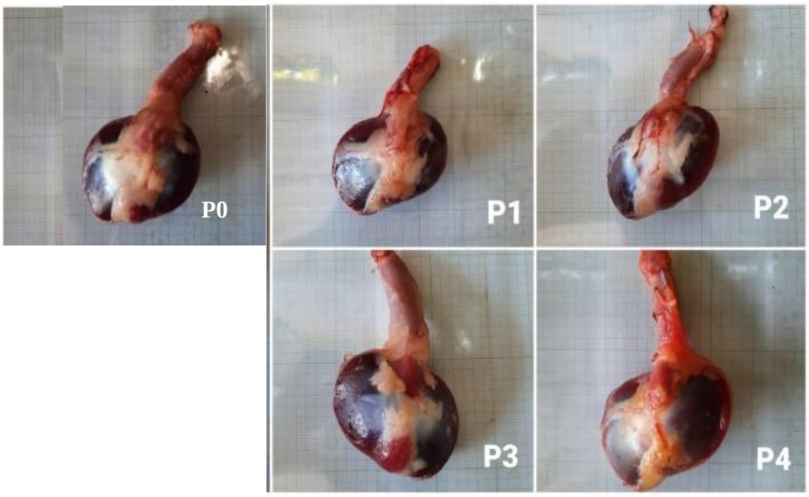

Figure 1. Ventriculus comparation of each grup treatment (P0) control without nanochitosan (P1) nanochitosan 2,5 g/kg feed (P2) nanochitosan 5 g/kg feed (P3) nanochitosan 7.5 g/kg feed (P4) nanochitosan 10 g/kg feed.

Firdamayanti *et al*. (2019) explained that nanochitosan will dissolve at an acidic pH in the proventriculus before reaching the ventriculus through the mechanism of releasing a hydrogen atom proton from the primary amine group so that the solution becomes positively charged. The presence of a negatively charged protease into the solution results in the formation of nanochitosan-protease crosslinks. Junianto *et al*. (2021) stated that the cross-linking that occurs between nanochitosan and protease involves an amine group (-NH_2) in nanochitosan with a hydroxyl group (-OH) in protease. Ekaputri *et al*. (2018) stated that nanochitosan cross-links can increase the catalytic activity of protease enzymes that help in protein digestion. The activity of this enzyme has no potential to interfere with protein digestion in the ventriculus, and the results of protein digestion will be used to support egg production, so that the weight of the ventriculus does not change significantly.

The results of the analysis of variance with a significance of 5% showed that nanochitosan had no significantly different effect on intestinal weight ($P>0.05$). The average intestinal weight between the different treatments was not significant, it was suspected that nanochitosan as a feed additive did not interfere with the digestion process and absorption of nutrients from digestion in the intestine so that the nutrients could be used to support the egg production process.

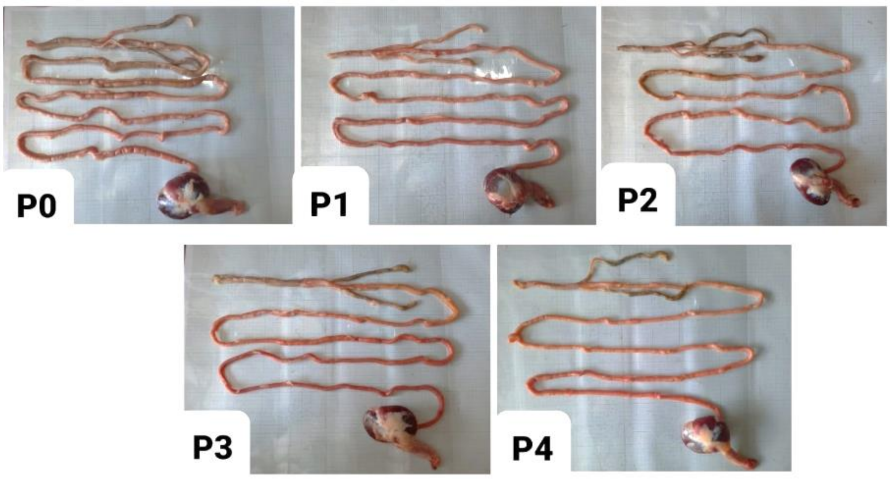

Figure 2. Intestine comparation of each grup treatment (P0) control without nanochitosan (P1) nanochitosan 2,5 g/kg feed (P2) nanochitosan 5 g/kg feed (P3) nanochitosan 7.5 g/kg feed (P4) nanochitosan 10 g/kg feed.

Junianto *et al*. (2021) stated that the amine group ($-NH_2$) in nanochitosan can cross-link with the hydroxyl group (-OH) in protease. Costa-Silva *et al*. (2021) stated that cross-linking between nanochitosan and enzymes can increase the surface area of enzymes so that the chances of interactions between enzymes and substrates increase. Yan *et al*. (2021) added that nanochitosan has a positively charged amine group ($-NH_2$) which can form bonds with the negatively charged bacterial cell wall. Bonilla *et al*. (2019) stated that the bond between the positive charge of nanochitosan and the negative charge of the bacterial cell wall can cause changes in cell shape, cell wall leakage, and cytoplasmic discharge. The growth of the population of beneficial bacteria can increase the digestibility of feed so that the absorption of nutrients in the small intestine can be optimized. Sunarno *et al*. (2021) stated optimal absorption of nutrients can be beneficial for increasing livestock productivity, especially egg production in layer phase ducks. Based on this, the level of nanochitosan given does not interfere with the work of the intestine and the nutrients absorbed will be utilized to support egg production. This causes the intestinal weight does not change significantly.

The results of the analysis of variance with a significance of 5% showed that nanochitosan had no significantly different effect on liver weight (P>0.05). Kusmayadi *et al*. (2019) stated that the percentage of normal liver weight is between 1.88% -3.67% of live weight. The average liver weight in the study was still within the normal range. The results of the study with the treatment of feed additive nanochitosan gave the same results as those of Lestari *et al*. (2020) with the addition of moringa leaf meal (0, 2.5, 5, 7.5, 10%), where the size of the visceral organs (liver) in Pengging ducks did not change in size after the addition of these two types of feed additives, but had differences in terms of chemical quality of eggs. The chemical quality of the eggs which included protein content of egg white, egg yolk cholesterol, egg white weight, egg yolk weight, and egg weight after nanochitosan treatment were better than moringa leaf flour. This condition is related to the non-toxic nature of nanochitosan and can inhibit fat accumulation so that it can improve liver function.

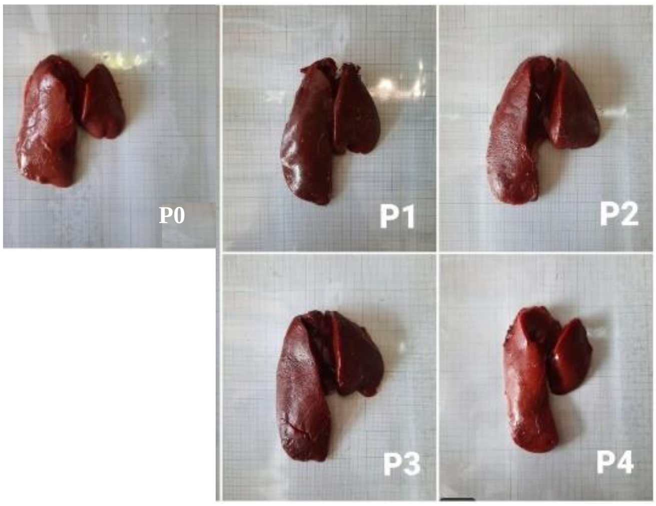

Figure 3. Liver comparation of each grup treatment (P0) control without nanochitosan (P1) nanochitosan 2,5 g/kg feed (P2) nanochitosan 5 g/kg feed (P3) nanochitosan 7.5 g/kg feed (P4) nanochitosan 10 g/kg feed.

El-Fattah *et al.* (2013) stated that administration of nanochitosan could reduce liver damage due to its non-toxic and antioxidative properties. Jiang *et al*. (2018) stated that nanochitosan can reduce the workload of the liver due to the presence of excess fat. Miyazawa *et al*. (2018) stated that nanochitosan can inhibit fat accumulation in the liver that is fed a high-fat diet, because it can form bonds with fat which causes inhibition of fat absorption and reduces low density lipoprotein (LDL) so that it has the effect of improving liver function. Based on this, the given nanochitosan level did not potentially interfere with normal liver function so that the liver weight did not change significantly.

The results of the analysis of variance with a significance of 5% showed that nanochitosan had no significantly different effect on pancreatic weight (P>0.05). Sunder *et al*. (2015) stated that the percentage of normal pancreatic organ weight ranges from 0.18% -0.23% of live weight. The average weight of the pancreas in the study was still within the normal range. Saraswati *et al*. (2022) in his research found that nanochitosan can accelerate the proliferation of cells in the islets of Langerhans in the pancreas, such as alpha cells and beta cells. Nanochitosan is also known to increase insulin secretion. Priyanka *et al*. (2022) stated that nanochitosan can protect pancreatic cells by increasing the activity of antioxidant enzymes or acting as a free radical scavenger. Hassan *et al.* (2021) stated that hydroxyl and amino groups in nanochitosan can be used as hydrogen donors for peroxyl radicals and react with unstable free radicals, thus protecting cells from damage. Pancreas under normal conditions, especially acin cells will produce enzymes needed for the digestive process in the duodenum. Based on this, the levels of nanochitosan given did not potentially interfere with the normal work of the pancreas so that the weight of the pancreas did not change significantly.

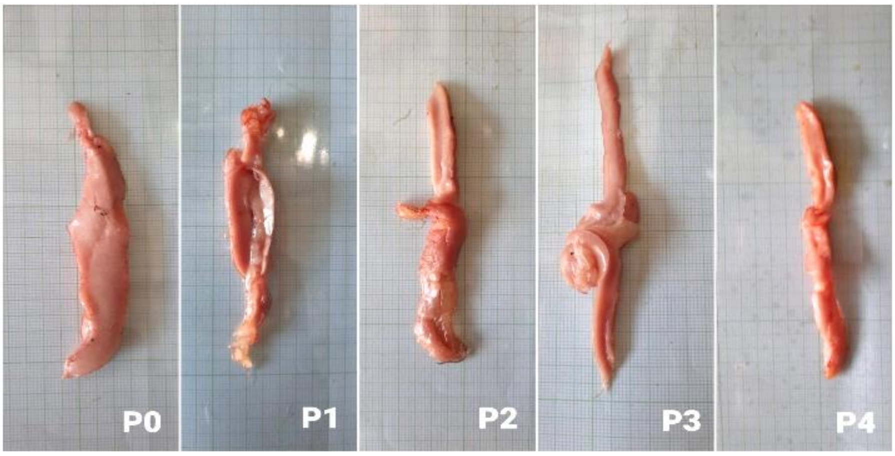

Figure 4. Pancreatic comparation of each grup treatment (P0) control without nanochitosan (P1) nanochitosan 2,5 g/kg feed (P2) nanochitosan 5 g/kg feed (P3) nanochitosan 7.5 g/kg feed (P4) nanochitosan 10 g/kg feed.

The results of the analysis of variance with a significance of 5% showed that nanochitosan had no significantly different effect on spleen weight (P>0.05). Nova et al. (2020) stated that the percentage of normal spleen weight ranges from 0.08% -0.16% of live weight. The average value of the spleen weight in the study was still within the normal range. The average spleen weight between different treatments did not significantly indicate that the nanochitosan content given in the feed did not interfere with spleen performance.

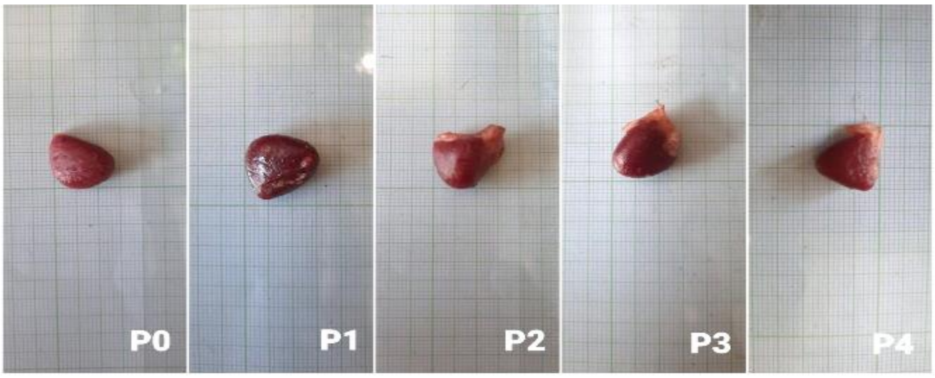

Figure 5. Spleen comparation of each grup treatment (P0) control without nanochitosan (P1) nanochitosan 2,5 g/kg feed (P2) nanochitosan 5 g/kg feed (P3) nanochitosan 7.5 g/kg feed (P4) nanochitosan 10 g/kg feed.

Tufan & Arslan (2021) found that the addition of nanochitosan in the ration with a concentration of 100 mg/kg had no effect on the relative weight of the spleen. Vimal *et al*. (2013) stated that nanochitosan is nontoxic. Nanochitosan can increase circulating antibody serum production and enhance immune response in poultry. Miao *et al*. (2020) found that nanochitosan can enhance the immune function of poultry by increasing serum concentrations of poultry IgG, IgM, and IgA and cytokines, indicating that nanochitosan is very helpful for increasing the release of immunoglobulins. Based on this, the level of nanochitosan given did not potentially interfere with the work of the spleen so that the weight of the spleen did not change significantly.

The results of the analysis of variance with a significance of 5% showed that nanochitosan had no significantly different effect on heart weight (P>0.05). Ebruaja *et al.* (2020) stated that the percentage of normal heart weight in poultry ranged from 0.5% -1.42% of live weight. The mean value of heart weight in the study was still within the normal range. The average heart weight between different treatments was not significant, it was suspected that the nanochitosan levels given in the feed were non-toxic and did not interfere with heart performance.

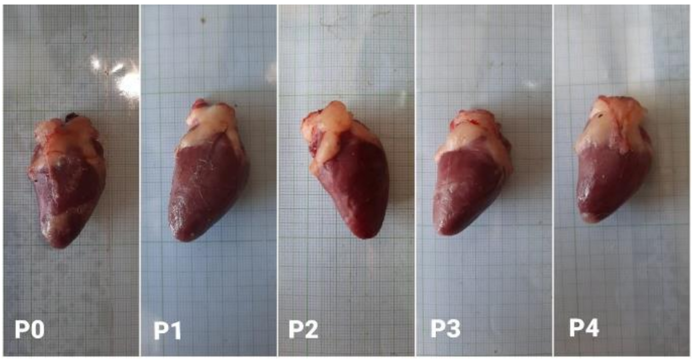

Figure 6. Heart comparation of each grup treatment (P0) control without nanochitosan (P1) nanochitosan 2,5 g/kg feed (P2) nanochitosan 5 g/kg feed (P3) nanochitosan 7.5 g/kg feed (P4) nanochitosan 10 g/kg feed.

Sunarno *et al*. (2021) stated that nanochitosan is a natural polysaccharide that is nontoxic or non-toxic. Yang *et al*. (2020) states that the heart is sensitive to toxins and anti-nutrients in feed. Sugiharto *et al*. (2021) stated one of the roles of the circulatory system is to circulate blood containing nutrients from the heart to the body's cells and then transported back to the heart, when the blood contains nutrients. toxins and anti-nutritional substances will cause excessive contraction of the heart, causing swelling of the heart. The results showed that the heart is still within its normal weight range, this is because nanochitosan is non-toxic. Based on this, the nanochitosan given in the feed has no potential to interfere with the performance of the heart so that the heart works normally and does not cause significant changes in the weight of the heart.

This research has a novelty, namely that the use of nanochitosan as a feed additive did not cause changes in the weight of the Pengging ducks visceral organs in the layer phase. The weight of the visceral organs in the layer phase is isometric, meaning that the nutrients from digestion are not used to support the growth and development of the visceral organs, but are used to support the reproductive performance of laying ducks. This study provides important data information for the development of further research on the effect of nanochitosan as a feed additive on visceral organ size in the grower phase, looking for a relationship between visceral organ size and the characteristics of anatomy, physiology, and behavior of breeding ducks. The results of this study can be used as initial data or comparative data on the biological effects of nanochitosan on visceral organ weight in layer-phase pengging ducks thereby increasing exploration of the use of nanochitosan products in various types of aquaculture poultry. This research can provide a reference for farmers that nanochitosan is safe to use as a feed additive and does not have a negative impact on visceral organs.

CONCLUSION

The conclusion of this study is that nanochitosan as a feed additive does not cause changes in the function of the visceral organs based on the indication of the weight of the visceral organs measured, namely the ventricles, intestines, liver, pancreas, spleen, and heart. It is necessary to conduct further research on higher levels of nanochitosan as a feed additive to obtain optimal effects on digestive processes, metabolism, reproductive performance, and egg productivity related to visceral organ size in Pengging ducks.

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