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The Potential of Spirulina Powder as Feed Additive on Hepatic Histomorphometry in Peking Ducks (*Anas platyrhynchos*)

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Abstract. Providing standard feed to support and improve the growth, productivity, health, and digestibility of livestock still requires feed additives, one of which is the natural feed additive spirulina flour. Spirulina flour was one of the natural feed additives chosen because it contained bioactive compounds, had no side effects on livestock, and substitute for antibiotics for livestock, so it could increase the growth, productivity, health, and digestibility of Peking ducks. The aim of this study was to analyze the effect of spirulina flour feed additive on Peking duck liver histomorphometry. This research used a Completely Randomized Design (CRD) with 5 types of spirulina flour concentration treatments (0%, 2.5%, 5%, 7.5%, and 10%) and 5 replications including 5 ducks in each replication. The treatment groups included P0, P1, P2, P3, and P4. Measurement variables included liver weight, hepatosomatic index, hepatocyte and central vein diameter, and sinusoid width. The data obtained were analyzed by the Analysis of Variance (ANOVA) test with a confidence level of 95%. The results showed that the addition of spirulina flour as a feed additive had no effect on liver weight, hepatosomatic index, hepatocyte diameter, central vein diameter, and sinusoid width. The conclusion of this research was that added spirulina flour (*Spirulin*a sp.) feed additive potentially maintained the histomorphometry of the Peking duck liver organ.

Keywords: *Spirulina* sp; feed additive; histomorphometry; Peking; liver

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INTRODUCTION

Indonesia is a tropical country that has great potential in the poultry farming sector, one of which is broiler ducks. The need for duck meat production in the last three years, namely 2020, 2021, and 2022, reached an average of around 42,923.17 tons (Central Statistics Agency, 2022). The potential for duck cultivation in Indonesia is very promising, because of the high number of enthusiasts and the need for animal food sources. Duck farming plays a role as a source of animal protein in the form of eggs and meat, as well as a source of income for its entrepreneurs. The demand for duck products has also increased along with the growing population and the increasing awareness and knowledge of the importance of consuming nutritious food (Hutahaean et al., 2022). One of the meat-producing ducks that are widely cultivated by poultry farmers in Indonesia is the Peking duck (*Anas platyrhynchos*). Peking ducks produce meat with complete nutritional content. The nutritional composition contained in Peking duck meat includes 21.4% protein, 8.2% fat, and 1.2% ash, with a metabolic energy content of 159 kcal/kg. The complete nutritional content of Peking duck meat is influenced by maintenance factors, namely management of feeding with a balanced nutritional composition. Feed ingredients with balanced nutrition can provide optimal physiological effects for poultry (Simanullang et al., 2015).

The feed commonly used by breeders is standard feed. A standard duck feed contains cereals, cereal by-products, vegetable protein, animal protein, minerals, vitamin supplements, and additives. The feed for ducks is provided according to their physiological age and production level. (Naik et al., 2023). Standard feed is a mixture of several ingredients that function to meet livestock energy needs. The nutritional content of standard feed is still less than optimal in supporting and improving growth, productivity, health, digestibility, and nutrient absorption performance in livestock, so the use of standard feed for poultry still requires feed additives to balance feed use (Nurmila et al., 2019, Astati et al., 2020).

Feed additives are non-nutritional ingredients added to feed with the aim of increasing optimal growth, health, feed consumption, and meat and egg productivity in livestock (Hartini et al., 2022). Feed additives that are starting to be widely used by breeders are natural feed additives in the form of extracts or other ingredients from plants that contain bioactive compounds because they do not have side effects for livestock and consumers who consume livestock products (Delima et al., 2018). A natural ingredient that has the potential as a feed additive for Peking ducks to help improve physiological performance, growth, and productivity is Spirulina sp (El-Hady & El-Ghalid, 2018).

Spirulina flour feed additives contain bioactive compounds of flavonoids, alkaloids, saponins, tannins, and steroids. Giving the right dose of spirulina as a feed additive can have an influence on the performance of the digestive organs, one of which is the liver (El-Shall et al., 2023). Structural and functional changes in the liver organ can be observed using the histomorphometer method (Latifah et al., 2022). This study aims to analyze the effect of spirulina flour feed additives on Peking duck liver histomorphometry. The results of this study can enhance the knowledge of Spirulina's potential as a feed supplement to improve liver health in ducks, particularly Peking ducks. Spirulina contains high nutrients and bioactive compounds that are safe for the structure and function of duck liver, as well as supporting metabolism and energy availability in duck livestock. This research provides new insights into duck nutrition, particularly in maximizing the use of natural ingredients beneficial for duck health. Furthermore, this study opens opportunities for further research on other natural feed ingredients with similar benefits.

Another benefit of this study is that poultry farmers can use Spirulina as a natural feed ingredient to reduce dependence on antibiotics or other chemicals in livestock feed. The use of Spirulina can support the implementation of more environmentally friendly farming practices and help reduce the risk of antibiotic resistance issues.

METHODS

Research Design

The research design used in this study was a Completely Randomized Design (CRD) with 5 types of treatment and 5 repetitions. Each treatment consisted of 5 male peking ducks and each replication contained 1 male peking duck placed in each cage unit. Feed treatment with spirulina flour supplementation consisted of five concentrations, including P0 (standard feed + 0% spirulina flour (control)), 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

This research variable consists of an independent variable and a dependent variable. The independent variable in this study was the concentration of spirulina flour (0%, 2.5%, 5%, 7.5%, and 10%). The dependent variables in this study were liver weight, hepatosomatic index, hepatocyte diameter, central vein diameter, and sinusoid width.

Experimental Animal Preparation Stage

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 experimental animal used in this study was a 1 day old male Peking duck (*A. platyrhynchos*) obtained from a breeder. District Secang, Kab. Magelang, Central Java. Newly arrived ducks are placed in a collective cage, and then given a sugar solution of 30 g/liter of water so that the ducks' body condition remains optimal, and their initial weight is measured. Acclimation was carried out for one week in a collective cage with controlled environmental temperature conditions.

Experimental Animal Rearing Stage

Maintenance is carried out in controlled environmental conditions. Temperature and humidity were measured using a thermogyrometer every morning and evening. The cage is routinely cleaned every five days. At the age of 1-21 days, the litter is routinely turned every two days and at the age of 22-39 days, the litter is routinely changed every two days to prevent an increase in ammonia levels. Body weights were measured in the morning and before feeding to minimize stress and then recorded. Weighing during the acclimation period is carried out every day, while at the age of 8-39 days, it is carried out every five times. Feeding is carried out routinely twice a day, namely in the morning (07.00 WIB) and in the afternoon (16.00 WIB). The test animals during the acclimation period were given standard B-11 feed and drinking water ad libitum. Treated feeding is given at the age of 8-39 days on a limited basis in the morning and evening by mixing the feedstock with sufficient water in the feed container, then stirring from bottom to top and from right to left side repeatedly until a mixture of feed ingredients is formed. Homogeneous. Drinking water is provided ad libitum with a mixture of Vita stress electrolyte preparations to prevent stress in ducks. Vaccination is carried out twice, namely at 7 and 14 days of age, by dropping it into the eyes. The first vaccination uses the ND HB1 vaccine and the second vaccination uses the ND La Sota vaccine.

Isolation Stage and Making Histology Preparations of The Liver Organ

The organ isolation process was carried out on the 40th day by cutting three channels in the ventral neck. Organs are removed by slicing the abdomen to the right and left to the thorax, then opening the front of the chest and removing the liver. The liver was then weighed using a digital scale with an accuracy of 0.01g, and washed using physiological saline. The right lobe of the liver was cut to a size of 3x2 cm to be used as a sample for making preparations and placed in a 10% Buffered Neutral Formalin (BNF) fixative solution. Organs that have been isolated are then prepared using the paraffin method and Hematoxylin-Eosin (HE) staining.

Results Observation Stage

Variables for observing the results include liver weight, hepatosomatic index, and liver histology observations consisting of measuring the diameter of hepatocytes and central veins, and the width of the sinusoids. The body weight of the ducks after slaughter and the weight of the isolated liver were weighed using a digital scale in grams and then recorded. Hepatosomatic index was obtained by measuring liver weight (g) divided by body weight (g) × 100. Observations and measurements of hepatocyte diameter were carried out using a photomicrograph with 400x magnification and analyzed with an Optilab Camera. Diameter measurements are carried out by dividing the cross-section into perpendicular lines based on horizontal lines (x) and vertical lines (y) if the hepatocyte cross-section is round. If the cross-section of a hepatocyte has an oval shape, the diameter of the hepatocyte is measured by dividing the crosssection of the hepatocyte perpendicularly based on the closest distance (y) and the furthest distance (x). Measurement uses the formula $[(x+y)/2]$. Observations were made in 5 fields of view on each preparation, 5 hepatocytes were taken from each field of view for diameter measurements.

Data Analysis

Analysis of the data obtained was carried out descriptively qualitatively and quantitatively. Qualitative descriptive analysis was carried out in the form of analyzing images based on the histological structure of the liver organ. Quantitative analysis takes the form of calculating and measuring the dependent variable by carrying out statistical calculations using the SPSS version 25.0 application. Quantitative data was first tested for normality with the Test of Normality (Shapiro-Wilk), then continued with a homogeneity test with the Test of Homogeneity of Variance (Levene Statistics). Normally and homogeneously distributed data were then analyzed using parametric statistical tests One Way Analysis of Variance (ANOVA) at a confidence level of 95%.

RESULTS AND DISCUSSION

The results of screening for spirulina flour phytochemical compounds were carried out to analyze the active secondary metabolite content in spirulina flour which is shown in Table 1. Test results show that spirulina flour contains phytochemical compounds, including flavonoids, alkaloids, tannins, saponins, and steroids. Sunarno et al. (2023) stated that spirulina flour contains phytochemical compounds, which include flavonoids, alkaloids, saponins, tannins, steroids, triterpenoids, and glycosides.

The phytochemical compounds contained in spirulina flour are known to be able to maintain the structure and performance of liver cells because they have high antioxidant activity. High levels of antioxidants function as scavengers which function to inhibit, interrupt, and stop the production, chain reactions, and effects of free radicals by donating hydrogen atoms. This antioxidant potential is able to prevent degenerative damage or decreased function of liver cells. Liver cell damage can occur if there is exposure to free radicals, toxic substances, and excessive intake of nutrients, causing an imbalance in the cells. Phytochemical compounds in spirulina with high antioxidant activity are known to be able to maintain cell structure in the liver organ when an imbalance occurs in the cells, including flavonoids, alkaloids, saponins, tannins, and steroids (Fithriani et al., 2015).

Kintoko et al. (2018) stated that the phytochemical content in Spirulina platensis can bind free radicals, thereby preventing degenerative cell damage in the liver. The results of this research are supported by Fithriani et al. (2015) who stated that high antioxidant activity can have a hepatoprotective effect. This effect is thought to be able to prevent or repair liver damage from exposure to toxic substances that enter the sinusoids via the hepatic artery and portal vein.

Results of analysis of the effect of spirulina flour feed additives on liver weight, hepatosomatic index, hepatocyte diameter, central vein diameter, and sinusoid width Peking duck (*A. platyrhynchos*) are shown in Table 2. The results of the ANOVA test on liver weight, hepatosomatic index, hepatocyte diameter, central vein diameter, and sinusoid width showed no significant differences between treatments (P>0.05). These results provide evidence that the phytochemical compounds contained in spirulina flour feed additives are able to work synergistically and not antagonistically on the liver organs of Peking ducks.

The administration of spirulina flour as a feed additive to the liver weight of Peking ducks showed that the results were not significantly different between treatments (P>0.05). Data in Table 2. showed that the average liver weight value of male peking ducks in this study was in the normal range, namely 36.75-40.57 g with percentages ranging from 1.88-2.69%. Kusmayadi et al. (2019) stated that the percentage of normal duck liver weight is between 1.88-3.67% of live weight. Feeding with spirulina flour feed additives is given in amounts that suit the nutritional needs of Peking ducks so that it does not cause the liver organ to work hard in the metabolic process and does not cause an increase in organ weight. Bondar et al. (2023) in their research stated that spirulina is a functional ingredient that is not toxic and can improve liver performance by optimizing the function of blood vessels in the liver, and the distribution of oxygen from the liver throughout the body. Liver weight is related and can be used to calculate the HSI value.

Table 1. Screening analysis of phytochemical compounds of Spirulina flour.

Phytochemical Compounds	Test Results	Description
Flavonoids	+	A yellow precipitate is formed
Alkaloids	+	A yellowish-white precipitate is formed
Saponin	+	A stable foam is formed
Tannin	+	Blue-black precipitation is formed
Steroids	+	A green ring forms

Note: + (contains compounds).

Table 2. Result analysis liver weight, hepatosomatic index, hepatocyte diameter, central vein diameter, and sinusoid width after administration of spirulina flour feed additives.

Treatment	Liver Weight (g/ekor)	Hepatosomatic Index	Hepatocyte Diameter (μm)	Central Vein Diameter (μm)	Sinusoid Width (μm)
P0	40.57 ± 7.41	2.46 ± 0.12	7.45 ± 0.47	52.00 ± 7.77	1.55 ± 0.30
P1	37.53 ± 3.14	2.52 ± 0.20	7.65 ± 0.22	54.58 ± 9.96	1.74 ± 0.30
P2	37.95 ± 4.22	2.57 ± 0.19	7.78 ± 0.23	56.14 ± 7.54	2.12 ± 0.56
P3	38.27 ± 2.02	2.52 ± 0.88	7.68 ± 0.22	60.61 ± 6.56	1.94 ± 0.38
P4	36.75 ± 4.21	2.47 ± 0.13	7.83 ± 0.26	62.04 ± 6.88	2.31 ± 0.43

Description: Data is presented in the form of mean ($\bar{x}$) ± standard deviation (SD). P0: Standard feed + 0% spirulina flour (control), P1: Standard feed + 2.5% spirulina flour, P2: Standard feed + 5% spirulina flour, P3: Standard feed + 7.5% spirulina flour, P4: Feed standard + 10% spirulina flour.

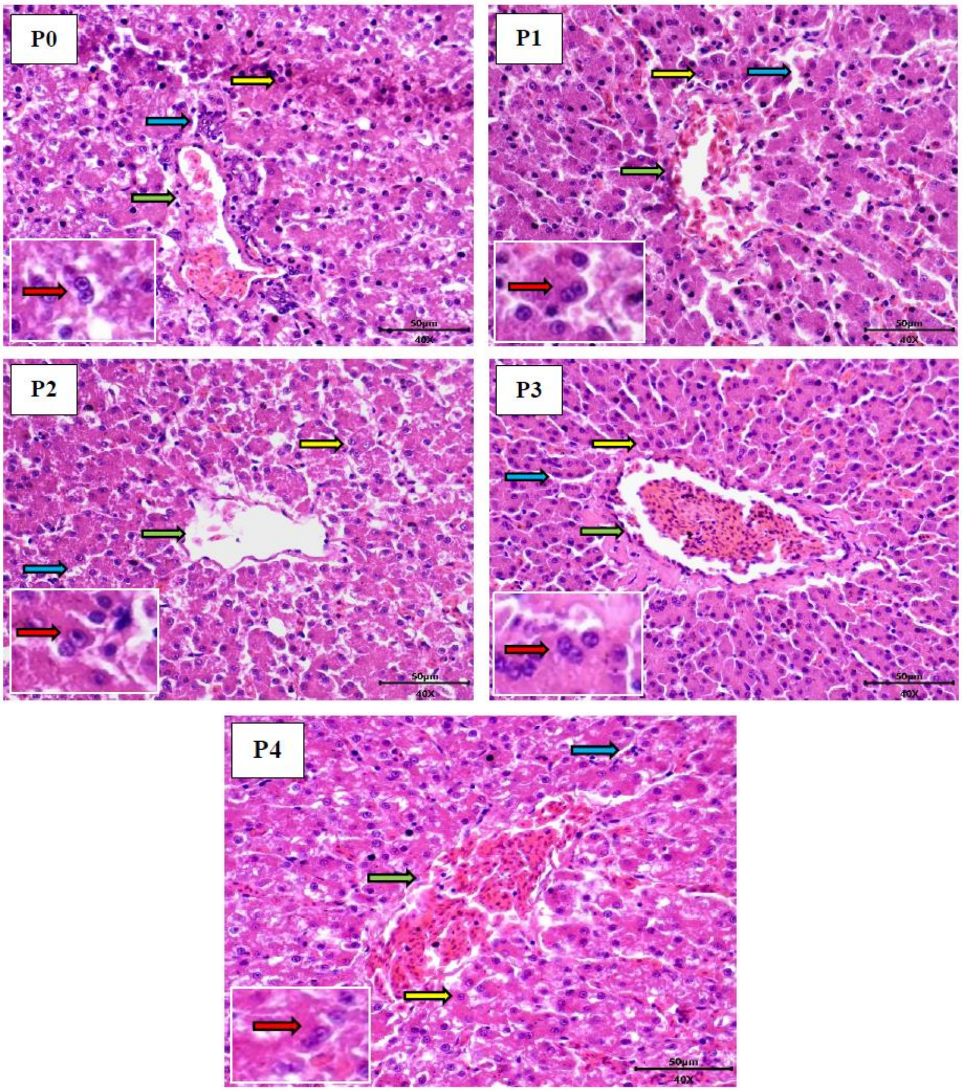

Figure 1. Histological structure of Peking duck (A. platyrhynchos) liver (HE staining, 400x magnification). Description: central vein (Green Arrow), sinusoids (Blue Arrow), normal hepatocytes (Yellow Arrow), binucleate cells (Red Arrow).

The administration of spirulina flour as a feed additive to the HSI value of Peking ducks showed that the results were not significantly different between treatments (P>0.05). Data in Table 2. shows that the average HSI value for male peking ducks in this study is in the normal range, namely 2.46-2.57. The research results of Latifah et al. (2022) using Peking ducks in the finisher phase showed that the HSI value ranged between 2.44-2.99. These results are in accordance with research by Sobrepeña et al. (2019) that the HSI value for peking ducks after a 30 day rearing period is 2.22-2.47. A normal HSI value is an indication that the cells that make up the liver are working well and are not experiencing any disturbances so that the liver cells can carry out the functions of nutrient metabolism, detoxification, and storing energy reserves in the form of glycogen optimally. Liver weight which is directly proportional to the HSI value is an indication of body weight and liver weight does not interfere with liver performance, so the cells are in normal condition (Malik et al., 2012).

Liver weight and HSI values were not significantly different between control and treatment. This evidence shows that the nutritional content of spirulina flour additives does not interfere with digestive performance, liver structure, and function, so that the nutrients resulting from digestion can be optimally absorbed by small intestinal cells. Liver weight and HSI values are an indication that the nutrients absorbed in the control and treatment are relatively the same, and the phytochemicals contained in spirulina flour are not cytotoxic. Fithriani et al. (2015) stated that the bioactive compounds contained in spirulina have antioxidant activity which is able to fight free radicals, thereby preventing cell damage to organs.

Histomorphometric variables are used to determine changes in the size of the liver's histological structure, including hepatocytes, central veins, and sinusoids (Abdullah et al., 2023). Rusyn et al. (2022) stated that the liver is an organ that is vulnerable to exposure to toxic compounds carried by the blood. The liver is a vital organ in the animal body. Although it accounts for only about 2% of body weight, approximately 25% of the total cardiac output passes through the liver. Since the liver is the first organ to come into contact with blood from the digestive tract, it plays a crucial role in detecting and processing molecular signals and xenobiotics derived from the intestines, which can then influence the function of other organs. The cytotoxic effect of phytochemical compounds in additives can be determined based on the degree of change in the histological structure of the liver, by looking at the shape, size, and arrangement of cells (Guyton & Hall, 2008).

The results of the study in the form of average hepatocyte diameter showed no significant difference between treatments ($P>0.05$). Data in Table 2. shows the average value of liver hepatocyte diameter in male Peking ducks. The average value of this variable is in Table 2. is in the normal range, namely between 7.45-7.83 µm. Ojkic & Sellers (2019) state, the average diameter of broiler duck hepatocytes ranges from 3-8 µm. This condition is evidence that the use of spirulina flour feed additives does not increase the diameter of hepatocytes in the liver of male Peking ducks. The hepatocyte diameter which was not significantly different between the control and treatment is an indication that the levels of spirulina flour feed additives given to Peking ducks are safe and do not have cytotoxic potential. Pestana et al. (2020) stated that giving spirulina at a dose of 10% in poultry feed is still safe and does not affect digestive viscosity. Cardoso et al. (2018) stated that digestive viscosity is influenced by factors providing spirulina levels and the amount of drinking water consumed. Optimal viscosity will support digestive function and the cells that make up the liver.

The histological structure of hepatocytes between controls and treatments can be seen in Figure 1. These results show a normal hepatocyte structure with a round nucleus in the middle, the boundaries between each hepatocyte cell are clear, and there are no signs of damage such as cell degeneration, and there are binucleate cells. Latifah et al. (2022) stated that the normal histological structure of the liver is characterized by the presence of hepatocyte cells with one nucleus and some also have more than one nucleus in the middle, have transparent cytoplasm, are spread radially near the central vein, and appear sinusoidal. Clear with a regular layout.

Binucleate cells are cells that show the ability to regenerate liver cells after mild hepatocyte damage. The results of observations of the histological structure of the liver are shown in Figure 1. No hepatocyte necrosis was found, as well as normal HSI values. This explanation is supported by Isdadiyanto & Tana (2019) that the presence of binucleate cells in the liver organ indicates that liver cells have the ability to regenerate quickly and are a defense mechanism for liver cells if they experience minor damage. Hoehme et al. (2010) explained that hepatocyte damage can be caused because hepatocytes are unable to metabolize toxic materials such as exposure to free radicals carried by the blood through the sinusoids to the central vein. Damaged hepatocytes are usually accompanied by an increase in the diameter of the central vein.

The results of the study in the form of the average central vein diameter showed no significant difference between treatments ($P>0.05$). This condition is evidence that the use of spirulina flour feed additives does not increase the diameter of the central vein in the liver of male Peking ducks. The diameter of the central vein that does not increase indicates that the energy required for the process of distributing metabolic substrates from the liver to the body's tissues is balanced. Ningrum & Abdulgani (2014) in their research stated that the dilated central vein allows the distribution of metabolic substrates to cells to be greater, and has an effect on increasing body and organ weight. These results are supported by the average liver weight value (Table 2.), which is within the normal range, indicating that the metabolic rate of energy carried by the blood from the liver to body tissue is optimal. The liver is an organ with multiple functions, involved in bile secretion, as well as lipid, carbohydrate, and protein metabolism, along with various other metabolic functions. This organ can easily adapt to changes in feed and the environment (Kim et al., 2019). Due et al (2024) stated that the diameter of the central vein in the duck liver expands and contracts in line with energy requirements and body metabolism. The dilation of the central vein diameter occurs when the body requires more blood to deliver oxygen and nutrients to organs, particularly to muscles and metabolically active organs. This condition typically arises when physical activity increases, such as when ducks are actively moving, laying eggs, or undergoing rapid growth, all of which demand an increased blood supply to the liver and other body organs. Under such conditions, the body enhances blood flow to the liver to support higher metabolism, such as nutrient processing and detoxification, which can lead to the dilation of blood vessels, including the central vein. Conversely, the constriction of the central vein diameter can occur when the body does not require increased blood flow or during rest periods when metabolism is lower. This can also happen when the body experiences stress or a decrease in energy needs, such as when ducks are inactive or fasting. Vessel constriction helps regulate blood distribution efficiently, prioritizing blood flow to organs that require more oxygen and nutrients. Overall, this phenomenon is an adaptive mechanism of the body to ensure that blood, oxygen, and other nutrients are delivered to the organs that need them, based on the level of energy demand. This process is also influenced by environmental factors, dietary patterns, and the physical activity of the ducks (Peng et al., 2019).

The histological structure of the central vein between control and treatment can be seen in Figure 1. These results show that the administration of spirulina flour feed additives is still at a safe level so the central vein does not experience damage as indicated by the liver cells being arranged in radials, the endothelial cells around the central vein do not experience lysis, absence of congestion, and inflammation of cells in the area around the central vein. The research results of Hartoyo et al. (2020) stated that excessive metabolic processes can cause changes in transport function and cell membrane permeability due to damage to hepatocyte cells.

The administration of spirulina flour as a feed additive to the width of Peking duck liver sinusoids showed that the results were not significantly different between treatments ($P>0.05$). Data in Table 2. showed that the average value of the sinusoid width of male peking ducks in this study was in the normal range, namely 1.55-2.31 µm. The results of this study could be caused by several factors, including normal hepatocyte cells, no intracellular osmolarity, and the level of spirulina flour given had low toxicity. These factors indicate that the administration of spirulina flour feed additives does not cause negative effects on the sinusoids, such as permanent widening (Figure 1.). This statement is supported by Putri et al. (2018) that the widening of sinusoids can be caused by damaged hepatocyte cells because sinusoids are directly adjacent to hepatocytes. Foye et al. (2016) stated that if energy requirements increase, the blood supply in the liver increases, which is marked by the widening of the sinusoids.

The histological structure of sinusoids between controls and treatments can be seen in Figure 1. These results show that the sinusoids have an irregular shape, and spread throughout the liver, and there is no indication of permanent widening of the sinusoids. The research results of Hamodi et al. (2013) stated that the sinusoids in three types of birds, namely seagulls, Fischer's lovebirds, and Pearl chickens have the same shape, namely irregular. Sinusoids are special channels in the liver that are modifications of blood vessels, carrying metabolic blood from the hepatic artery and portal vein, so that they will experience direct contact with toxicants. The inner layer of the sinusoids is lined with endothelial cells and there are Kupffer cells (macrophages) which function to phagocytose incoming toxic materials and pathogens. Shetty et al. (2018) stated that liver sinusoid endothelial cells line the liver sinusoid capillary channels which function in the filtration process and maintain homeostasis of the liver organ. Based on Peking duck liver histomorphometric data (Table 2.), it shows that the administration of spirulina flour feed additives does not have a cytotoxic effect so it does not cause structural changes and decreased function in the liver organ.

CONCLUSIONS

The conclusion of this study is that Spirulina sp. feed additives did not have a significant effect on the liver histomorphometric parameters in Peking ducks, such as liver weight, hepatosomatic index, hepatocyte diameter, central vein diameter, and sinusoid width. Spirulina is safe to use in duck feed without causing negative effects on the liver structure and function. Further research is needed to evaluate the long-term effects of Spirulina sp. feed additives and their impact on other physiological systems in Peking ducks that were not explored in this study.

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