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Application of Snakehead Fish to Increase Liver Function of Rats after Experiencing Physiological Stress

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Abstract

Physiological stress due to nutritional deficiency accompanied by excessive activity can cause problems of energy deficiency required by the body. Energy shortage can affect the body weight, as well as the weight and histology of the liver. This study was conducted to examine and analyze the effect of snakehead fish meat supplementation in feed toward body weight, weight and hepatic histology in animals experiencing physiological stress. This study used 20 male Wistar rats consisting of 5 treatments with 4 replications. The treatment in this study consisted of control (P0) and treatment (P1: 5%, P2: 10%, P3: 15%, P4: 20%) groups. This research used Completely Randomized Design (CRD). The variables measured in this study were body weight, hepatic weight, central venous diameter and hepatocytes in the test animals. The data obtained were analyzed using Analysis of Variance (ANOVA) at 5% significance level and continued with Duncan test with 5% significance. The result of data analysis showed that fish snakehead fish supplementation in feed gave a significant effect to body weight, weight and histology of animal liver. Concentrations of snakehead fish supplements can increase the body weight, hepatic weight, and hepatic histology which was indicated by higher central venous and hepatocyte diameter values compared to other controls and treatments. The conclusion of this research is the provision of snakehead fish supplement (*Channia striata*) can increase the body weight, hepatic weight, central venous and hepatocytes diameter of Wistar rats with the most influential concentration of 20%. This evidence is useful in studying the mechanism of liver repair due to stress

How to Cite

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INTRODUCTION

Weight loss and hepatic impairment are major health problems for animals. This case is common in animals with physiological stress. Liver is very potential to decrease the weight and disturbance because it is one of the organs that are vulnerable to physiological stress. Physiological stress due to nutritional deficiencies accompanied by excessive activity can lead to increased production of free radicals, hormonal balance disorders and cause problems of deficiency of energy required by the body. Free radicals, hormonal regulatory disorders of metabolism, and lack of energy can cause hepatic histopathologic disorders (Silva *et al.*, 2015).

Hepatic disorder can trigger the occurrence of hepatocyte degeneration of the hepatic tissue lining cells. The disorder can be caused by dietary deficits, feed deficits, prolonged hypoxia, intoxication and tissue aging. Degeneration of untreated hepatic cells can lead to a hepatocyte necrosis (Suarsana *et al.*, 2013). The results of Erlangga and Nurcahyani (2013) study on mice treated with physiological stress in the form of noise exposure with intensity 85-90 dBA for 6 hours/day, 8 hours/day, 12 hours/day for 21 days showed a damage to hepatocyte cells in the form of necrosis.

Structural or functional disorders of the liver due to physiological stress can be improved with dietary supplements containing essential nutrients and antioxidants (Charlton, 2006). The principle of hepatic histopathologic recovery is the maintenance of hormonal balance, increase antioxidants in the body, ensure the availability of nutrients needed by the body to support energy-oriented metabolic processes, and the formation of new tissues (Silva *et al.*, 2015). Various feed supplements have been recognized by the society to support the hepatic histopathology improvement. The purpose of extending the feed supplement is to ensure the availability of nutrients that can support hepatic tissue repair, such as albumin, glutamine, cysteine, and glycine (Chotechuang, 2010). Wide selection of feed supplement for the repair and recovery of hepatic histopathology in animals due to physiological stress has been undertaken by several researchers and the choice of other ingredients to be tested is available.

The results of study by Silva *et al.* (2015) showed that 50 g (10%) daily protein supplementation has not been effective to improve the nutritional status, liver function, reduce the risk of complications and improve life expectancy. Mahesh *et al.* (2009) has conducted a study using rat as test animal that were experiencing physiological stress and proved that honey supplementation has not been effective in protecting liver against oxidative damage caused by physiological stress induction. The results of Charlton (2006) study demonstrated that 10% concentration brain-chain amino acid supplementation in patients with hepatic dysfunction can reduce the risk of hepatic dysfunction and improve liver function. The results of these studies become the basis for further research development and the use of other supplements for improvement or enhancement the liver function due to physiological stress.

Indonesia has a wide range of freshwater fisheries resources. Various types of fish both wildlife in nature and which are cultivated were in considerable amount and one of them is the snakehead fish. The search for dietary supplements derived from freshwater fish is mostly done by researchers. Freshwater fish have been known to contain many bioactive compounds or essential nutrients such as albumin, glutamine, cysteine, and glycine. The example of feed supplements that have such potential are sourced from snakehead fish or *Channa striata* meat (Ningrum and Abdulgani, 2014).

The results of Aisyatussofi and Abdulgani (2013) showed that the protein content in snakehead fish meat was 25.2% and 6.2% of them was albumin. Furthermore, the flesh of snakehead fish is stated to has protein content which is useful to repair damaged body cells. Ningrum and Abdulgani (2014) stated that snakehead fish meat is a source of albumin and this type of protein has a positive correlation with antioxidant activity that serves to protect the body tissue from damage cause by free radicals due to physiological stress. It is further stated that albumin is a protein with a single chain polypeptide with a molecular weight of 66.4 kDa and consists of 585 amino acids. The albumin molecule has a sulfhydryl group (-SH) that can serve as a radical binder, so that, albumin can function as an antioxidant. Sunarno *et al.* (2015) stated that every 100g of snakehead fish meat contains 32.39% glutamine, 6.61% cysteine, and 9.69% glycine. Proteins and amino acids such as glutamine, cysteine, and glycine have been known to play an important role in repairing body tissues function that have decreased due to the physiological stress (Sunarno *et al.*, 2013).

The study of snakehead fish meat as a dietary supplement for improvement or enhancement of hepatic weight and body weight due to physiological stress has not been done yet. One

type of fish that is popular in the society and empirically has been used for years by the ancestors to maintain health is a snakehead fish. Based on that reason, this research will be done by using snakehead fish meat as a dietary supplement and test its effect on body weight, liver weight and histopathology of the liver in the test animals which are experiencing physiological stress.

This study is useful in studying the mechanism of liver organ repair due to physiological stress by supplementation of snakehead fish meat in feed. Utilization of snakehead fish meat can be a solution of various case related to disorders of the structure and function of organs due to physiological stress

METHODS

Research Design

The experimental design used in this study was CRD (Completely Randomized Design) consisted of 5 treatments with 4 replications. Treatment in animal experiments was divided into: P0: Control (feed without addition of snakehead fish meat supplement); P1: feed with the addition of 5% snakehead fish meat supplement); P2: feed with the addition of 10% snakehead fish meat supplement; P3: feed with the addition of 15% snakehead fish meat supplement; and P4: feed with the addition of 20% snakehead fish meat supplement. Observed research variables which were hepatocyte diameter, central venous diameter, hepatic weight and body weight.

Procedure of Feed Preparation with Snake Head Fish Supplementation

Snakehead fish meat supplementation was made by adding the processed snakehead fish meat to the rat feed according to the required percentage. The preparation of feed supplements of snakehead fish flesh follows the procedures performed by Irwanda *et al*. (2012). The procedure for preparing feed supplements begun with cutting of body parts separated by head and tail. The flesh of the body was separated from the skin and the vertebrae or spines. Meatwas cleaned and cut into size like dice. Then, the pieces of meat were blended until homogeneous, so the homogenate was obtained.

Homogenates that have been determined by weight were then mixed with the weighed rats feed as needed repeletting. Before mixing, the rat feed in the tray given warm water with needed volume. Then the mixture was homogenized until obtained a caly feed. The homogenates of snakehead fish meat were then mixed into the feed with the required concentration (0%, 5%, 10%, 15%, and 20%)

Feed that has been homogeneously mixed will be made into pellets using a grinder. The pellets were dried by oven at 60 ^{0}C for 2 days to obtain a moisture content about 10%. Dry pellets containing snakehead fish meat were further used as a treatment in Wistar rats that had undergone a physiological stress conditioning.

Acclimation of Testing Animals

Test animals in the form of Wistar white rats as many as 30 individuals were acclimated in individual enclosures. During the acclimation process, male white rats were fed with commercial pellets in the form of BR-2 pellets and drinking ad-libitum. The acclimation process in Wistar rats was performed for one week.

Physiological Stress Treatment on Test Animal

After receiving acclimation treatment in individual cages, male white rats were conditioned to stress by not being fed for a week and only given an ad-libitum drink followed by swimming activity in tap water for 10 minutes with closed bucket position. The choice of stress model was based on the consideration of easier, simple and practical intensity monitoring. The duration of swimming time was determined based on the maximum ability of rats to be unable to swim or nearly drown (Hairrudin and Helianti, 2009). This treatment was arranged for one week in a row (Suarsana *et al.*, 2013).

Treatment of Snake Fish Meat Supplement on Test Animal

The commercial BR2 pellet feed that has been added with snakehead fish meat was administered to the test animals for 14 consecutive days, starting on the 8th day until the 21st day. The treatment duration of pellet feeds supplemented with snakehead fish meat was based on the time required by hepatic tissue to regenerate (14 days) (Michalopoulos, 2007). In addition, during this level of treatment, rats were also given an ad libitum drink.

Liver Preparation on Animal Test

All the rats were sacrificed by the dislocation in the neck. The mouse's body surgery procedure was performed from the posterior body axis toward the anterior collateral. The liver organ was separated from the rat body by cutting and continued liver insulation. The liver organ was fed into a physiological salt (NaCl 0.95%), placed on a dry tissue, followed by weighing with

an analytical scale and then stored in a BNF fixative solution at least 24 hours before being used for preparing histologic preparations.

Histological Preparation Processing Procedure with Hematoxylin-Eosin Staining

Histologic preparations of the liver were prepared by paraffin method. Histologic preparations of the liver were further processed by Hematoxylin-Eosin staining (Sunarno, 2015).

Measurement of the Liver Histologic Variables

Histologic preparations of Wistar mouse hepatic were observed under a light microscope. Each preparation was observed in 5 field of view, each field of view was observed 10 liver cell images. Histopathological changes were observed in the form of central venous diameter and hepatic hepatocytes

Data Analysis

The data obtained were tested for distribution pattern and homogeneity and continued with Analysis of Varian (ANOVA) test followed by Duncan Multi Range Test (DMRT), each with 5% significance.

RESULTS AND DISCUSSION

The results of the analysis on body weight, hepatic weight, central venous diameter, and hepatocytes of Wistar rat which experienced physiological stress after treatment are presented in Table 1.

The analysis result of snakehead fish meat supplement on body weight in rats with physiological stress showed significant different result (P <0.05). The provision of snakehead fish supplements in the feed affects the control body weight of controlled rats, P1, P2, P3, and P4, which each weighing 257 g, 260 g, 270 g, 280 g, and 290 g. Duncan test results with significance of 5% to body weight of rats showed that there was no significant difference between P1 and P0; P2 and P0; P1 and P2; P1 and P3; and P2 and P3. Other results indicated a significant difference between P4 and P0; P4 and P1; but there was no significant difference between P4 and P2; and P4 and P3 (Table 1; Figure 1).

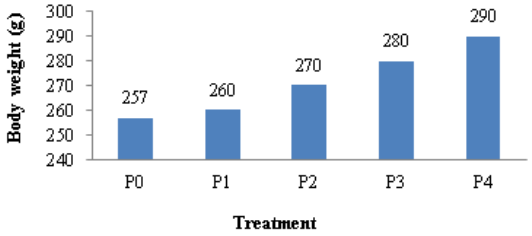

Figure 1. Wistar rat body weight histogram with physiological stress after being fed with snakehead fish meat supplement

The analysis result of snakehead fish meat supplement on hepatocyte diameter of mouse showed significantly different result (P <0.05). Further test results with Duncan test on 5% significance showed that there was no real difference between P1 and P0; P2 and P0; P1 and P2; P3 and P1; P3 and P2; there was a real difference between P3 and P0; P4 and P0; P4 and P1; P4 and P2; and P3 and P3. Table 1; Figure 2)

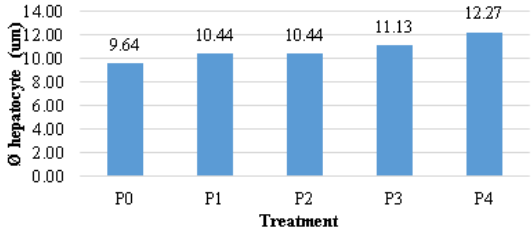

Figure 2. Histogram of hepatocyte diameter of Wistar rat experiencing physiological stress after being fed with snakehead fish meat supplement.

The factor causing hepatocyte diameter value in P3 was higher than P0, P4 was higher

Table 1. Body weight, hepatic weight, central venous diameter and hepatocytes Wistar rat which experiencing physiological stress after being fed with snakehead fish supplementation

Variable	Treatment				
	P0	P1	P2	P3	P4
Body weight (g)	257^{a}±26.3	260^{a}±21.6	270^{ab}±8.2	280^{ab}±8.2	290^{b}±14.1
Ø hepatocytet (µm)	9.6^{a}±0.7	10.4^{ab}±0.2	10.4^{ab}±0.8	11.1^{b}±0.4	12.3^{c}±0.3
Ø central venous 1 (µm)	132.4^{a}±39.5	125.2^{a}±29.1	133.5^{a}±44.9	231.3^{b}±20.8	238^{b}±85.6
Hepatic weight (g)	9.3^{b}±1.2	6.7^{a}±0.6	7.3^{a}±1.2	7.7^{ab}±1.2	8.3^{ab}±0.6

Description: numbers shown with the same superscript on the same line show insignificant difference (P> 0.05). P0: controlled rat; P1, P2, P3, and P4 are treatment of snakehead fish supplementation in feed with concentration of 5%; 10%; 15%; and 20% respectively.

than P0 and other treatment is metabolic changes. The change in metabolism is characterized by nutritional needs when recovery from increasing physiological stress conditions to support the continuity of physiological processes, thus causing the hepatocyte diameter of P3 which is higher than P0 and hepatocyte diameter of P4 which is higher than other treatments. The availability of substrate from snakehead fish containing albumin, glutamine, cysteine, and glycine will support the metabolic rate of P4 faster than other treatments, so the volume of hepatocytes is increased. The increased volume of hepatocytes has a correlation with increased distribution of substrate to the body tissues, especially muscle, so this can affect the enhancement of metabolic products and body weight (Ningrum and Abdulgani, 2014). Conversely when nothing changed in metabolism, hepatocyte diameter would not change too. The metabolic products in the body does not change so the body weight of mice does not increase. Flaring *et al*. (2003) suggested that the availability of albumin, glutamine, cysteine, and glycine protein in the body will support the enhancement in hepatocyte diameter, metabolic products, and increased body weight.

The analysis result of snakehead fish meat supplementation on diameter of central hepatic vein showed significant different result (P <0,05). In further test results with Duncan test on 5% significance, there was no real difference between P1 and P0; P2 and P0; P1 and P2; but there was a real difference between P3 and P0; P3 and P1; P3 and P2; P4 and P0; P4 and P1; P4 and P2, however, P4 and P3 was insignificantly different.

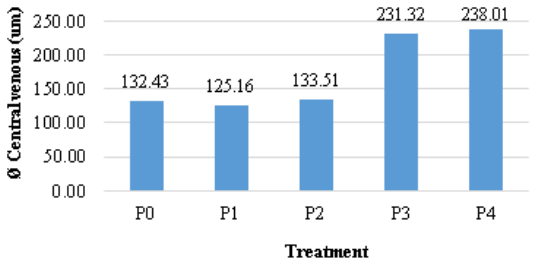

Figure 3. Histogram diameter central venous Wistar rat experiencing physiological stress after being fed with supplementation of snakehead fish meat

Factors that cause these conditions are metabolic changes which are indicated by changes in hepatocyte diameter. Provision of snakehead fish supplements in high concentration (P3 and P4) will increase the hepatocyte diameter. The enhancement in cell diameter will lead to an increase in hepatic central venous diameter. Wider central venous diameter allows the distribution of metabolic substrate (albumin, glutamine, cysteine and glycine) to the body tissues, especially the muscles become more numerous. This condition will affect the metabolic rate and metabolite products as well as energy in the tissues of the body (muscle)that will be larger and ultimately gives effect to the increase in body weight (Ningrum and Abdulgani, 2014).

Gibson (2005) stated that albumin, glutamine, cysteine, and glycine have an important role in cell and tissue improvement as well as increase the body weight. Nugroho (2009) stated that the addition of snakehead fish meat extract can increase levels of albumin, glutamine, cysteine, glycine and body weight in Wistar rats. Yuniarti *et al.,* (2013) stated that albumin protein plays an important role in tissue repair. Schade *et al*. (2009) stated that glutamine, cysteine, and glycine can increase the synthesis of nucleic acids and proteins and give the anabolic effect which needed to speed the recovery of the physiological state of the body. Flaring *et al*. (2003) stated that glutamine, cysteine and glycine can induce cellular anabolic effects by increased cell volume, activated protein kinases to support energy product metabolism, increased cell proliferation, accelerated tissue repair and increased body weight.

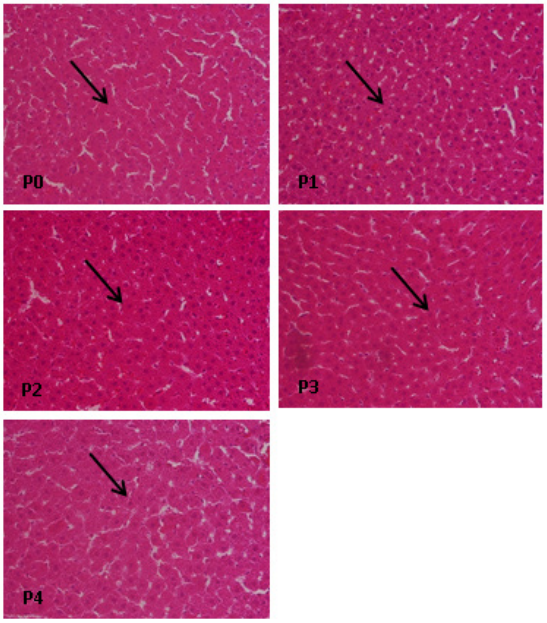

Figure 4. Image of Wistar hepatocyte histopathologic which experiencing physiologic stress after being fed with snakehead fish meat supplementation. Stained with hematoxylin-eosin at 1600x magnification. P0: control rat; P1, P2, P3, and P4 respectively were: treatment of snakehead fish

supplementation with concentration of 5%; 10%; 15%; and 20%; arrows indicate hepatocytes.

Histopathological analysis (hepatocytes) in hepatic tissue is shown in Figure 4. The result showed that there is a significant difference of hepatocyte hepatic diameter between P4 and control. The image of the liver tissue on the control and all treatments looks normal and did not find the degenerated cells. Observations of hepatocytes also found no pyknosis, DNA fragmentation, chromatin condensation or necrosis. Ningrum and Abdulgani (2014) stated that the degeneration of hepatocyte cells was characterized by the presence of pyknosis, swelling of cells triggered by excess water due to cell loss control in water regulation. Hepatocyte degeneration is also characterized by the expression of endonucleases that cause DNA fragmentation followed by chromatin condensation that triggers apoptosis.

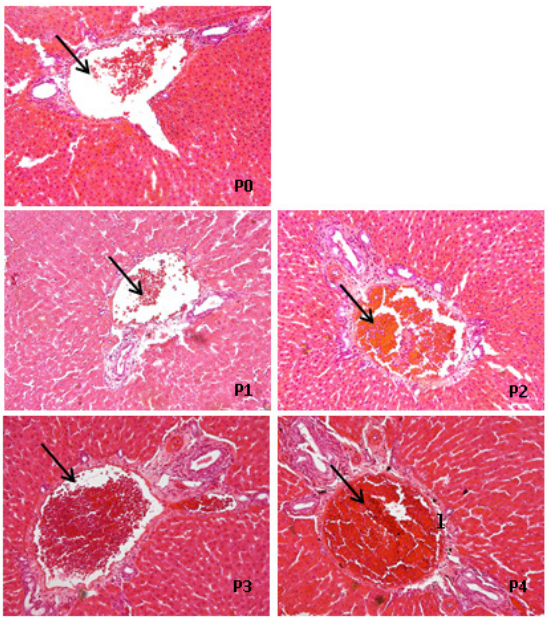

Figure 5. Histopathological image of central vein heist Wistar rats which experiencing physiological stress after being fed with snakehead fish meat supplementation. Stained with hematoxylin-eosin and being observed under 400x magnification. P0: control rat; P1, P2, P3, and P4 respectively are: treatment of snakehead fish supplementation in feed with concentration of 5%; 10%; 15%; and 20%; arrows indicate a central vein.

Histopathological analysis (central venous) of hepatic tissue is shown in Figure 5. The results showed a significant difference in hepatic central venous diameter between P4 and control. The central venous features of the liver on control and all treatments appeared normal, central venous damage and endothelial cells were not find in the degenerated central veins (Ningrum and Abdulgani, 2014).

Factors that led to the increase were suspected to be associate with the treatment of snakehead fish meat supplement with a concentration of 20%. The treatment of high concentrations of snakehead fish meat supplements will lead to an increase in availability of albumin, glutamine, cysteine, and glycine in plasma. Increased protein and some types of amino acids will cause the availability of proteins and intracellular compounds to be increased which ultimately affects the increase in cell volume. Schade *et al.* (2009) and Flaring *et al.* (2003) stated that albumin, glutamine, cysteine, and glycine proteins can increase cell volume and tissue weights. It is further stated that albumin, glutamine, cysteine and glycine are associated with anabolic processes (synthesis of nucleic acids and proteins), are involved in the recovery of physiologic conditions post-stress, cell or tissue repair and increased cellular volume and integrity (Dringen *et al.,* 2000; Roth, 2008).

Cells and hepatic tissue are tissues that have the ability to maintain cellular integrity from the effects of free radicals and free radical reaction chains formed during physiological stress (Andreas *et al.*, 2015). It is further stated that the liver has an enzymatic and non-enzymatic system to neutralize or deactivate free radicals or free radical reaction chains involving the activity of several enzymes, including superoxide dismutase, glutathione, and catalase. Finally, physiological stress does not trigger any damage or degeneration of cells or hepatic tissue as described in the evidence of this study.

The analysis results of snakehead fish supplementation on hepatic weights showed significantly different results ($P < 0.05$). In further test results with Duncan test on 5% significance, there was a real difference between P1 and P0; P2 and P0; but there was no real difference between P1 and P2, P1 and P3, P1 and P4; P2 and P3; P2 and P4; P3 and P4; P3 and P0; and P4 and P0 (Table 1). The mean hepatic weight of P1 and P2 treatment had lower values than controls, respectively of 6.7 g and 7.3 g (Figure 6).

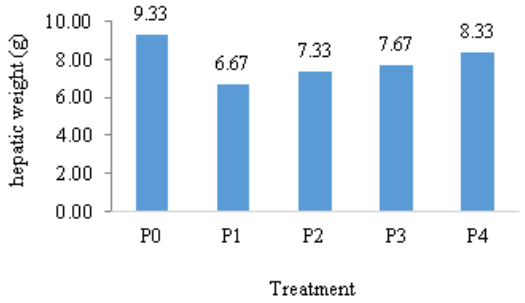

Figure 6. Hepatic weight histogram of Wistar rat with physiological stress after being fed snakehead fish meat supplement

Changes that occur in variables such as hepatocyte diameter, central venous diameter, and hepatic weights will affect the increase in body weight of rat as shown in the evidence as the results of this study. The metabolic changes associated with the improvement of several variables in this study are strongly associated with the important role of albumin, glutamine, cysteine, and glycine proteins in snakehead fish meat added to the feed.

Dringen *et al*. (2000) stated that albumin, glutamine, cysteine, and glycine are the precursor of amino acids for the synthesis of nucleic acids and proteins. As a major metabolite, albumin, glutamine, cysteine, and glycine have an influence on the various molecular pathways that induce cellular anabolic effects by increasing cell volume, activating protein kinases that are closely related to cell and tissue repair. Improved tissue, increased volume and cellular metabolite materials lead to increased tissue weights (Roth, 2008). The hepatic weight of the results of this study is an evidence of increased cellular processes and increased tissue weights due to the effects of nutrients (albumin, glutamine, cysteine, and glycine) in snakehead fish meat.

The results of research prove that feeding with snakehead fish meat supplementation affect the body weight, hepatic weight, central venous diameter and hepatocytes in Wistar rats that experiencing physiological stress. Histologic analysis showed that the liver tissue of all treatments appeared normal. These data provide evidences that physiological stress in this study may only have an effect on metabolic disorders, anabolic processes and decreased cell/ tissue size but do not trigger the degeneration or hepatic tissue necrosis. Based on this, the use of snakehead fish meat as a feed supplement with a concentration of 20% is very important to support the increase of body weight, hepatic weight recovery, and repair hepatic cells or tissues in animals, especially animals with physiological stress.

This study is useful in studying the mechanism of liver organ repair due to physiological stress with supplementation of snakehead fish meat in feed. Utilization of snakehead fish meat can be a solution of various cases related to structural disorders and organ function due to physiological stress.

CONCLUSIONS

Based on the results of this study, it can be concluded that feeding with snakehead fish meat supplementation can increase the body weight, hepatic weight, central venous and hepatocytes diameter in Wistar rats which experiencing physiological stress. The administration of snakehead fish meat supplementation by 20% concentration gave a better effect. The use of snakehead fish meat as a feed supplement especially in 20% concentration is highly recommended to increase body weight and improve the liver due to disorders triggered by physiological stress.

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