Biosaintifika 10 (3) (2018) 553-558

Biosaintifika

Journal of Biology & Biology Education

http://journal.unnes.ac.id/nju/index.php/biosaintifika

Utilization of Snakehead Fish Meat for Increasing Kidney Function in Rat Experiencing Physiological Stress

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DOI: http://dx.doi.org/10.15294/biosaintifika.v10i3.16254

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History Article

Received 6 October 2018
Approved 19 November 2018
Published 31 December 2018

Keywords

Stress; Supplement; Kidney; Snakehead Fish; Channa Striata; Wistar Rat

Abstract

Physiological stress for a long time can lead to impaired kidney organ function. Snakehead fish meat are known to contain important nutrients such as albumin, glutamine, glycine, cysteine, and various minerals that can improve kidney function. This study was conducted to examine and analyze the effect of snakehead fish supplementation in feed towards body weight, kidney weight, diameter and number of glomerulus in Wistar rats with physiological stress. This study used 20 male Wistar rats divided into 5 groups consisted of 1 control group (P0) and 4 treatment groups (P1: 5%, P2: 10%, P3: 15%, P4: 20%). The treatments were performed with 4 times repetition. This study used Completely Randomized Design (CRD). The variables measured were body weight, kidney weight, diameter and number of renal glomerulus. The data obtained were analyzed using Analysis of Variance (ANOVA) at 5% significance level and continued with Duncan test with 5% significance level. The results of this study were: the supplementation of snakehead fish with 20% of concentration can increase the body weight; concentration 10; 15; and 20% can increase the weight of the kidney; concentrations of 5%, 10%, 15%, and 20% can increase the glomerular diameter in Wistar rats with physiological stress. The supplementation of snakehead fish with concentration of 5% to 20% did not affect the number of glomerulus of Wistar rats with physiological stress. Results of this study is useful as information for people who study the mechanism of kidney repair due to physiological stress.

How to Cite

Sunarno, S., Sokmawati, H., & Rahadian, R. (2018). Utilization of Snakehead Fish Meat for Increasing Kidney Function in Rat Experiencing Physiological Stress. *Biosaintifika: Journal of Biology & Biology Education*, 10(3), 553-558.

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p-ISSN 2085-191X
e-ISSN 2338-7610

INTRODUCTION

Proper nutrition intake can improve the functioning of the body's defense system, so as to lower the physiological stress level and susceptibility to a disease. Essential nutrients play an important role in the growth and development of animals. However, unbalanced and unsuitable nutrients can result in the body's lack of nutrients (*undernutrition*) or malnutrition. Malnutrition can lead to changes in body composition, such as the amount and distribution of fluids, fats, minerals, and proteins, especially muscle protein. Malnutrition conditions can also trigger a decrease in the body's total potassium level, especially in cell, causing metabolic disorders in organs such as the kidneys, muscles, and pancreas. Abnormalities of organs often occur such as the upper alimentation system (mouth, tongue, neck), gastrointestinal system (liver, pancreas), heart, kidney, endocrine system, so that malnutrition conditions must be handled quickly, precisely, and meticulously (Sunarno *et al.*, 2016). Malnutrition accompanied by excessive physical activity can cause physiological stress in the body that has the potential to trigger the occurrence of various disorders of the organs of the body.

The kidneys are one of the body's organs that are susceptible and quickly experience structural and functional disturbances or histological changes due to long-term physiological stress. This histological condition has a correlation with a very high burden of kidney function, kidneys only weighing 0.4% of body weight but get 600ml/minute of blood flow and filter blood approximately 200 liters per day (Marieb, 2010).

Physiological stress for a long time may lead to impaired renal function or structural changes in the kidney so that the necessary supplement of animal material containing nutrient-rich to prevent or overcome the disorder. Snakehead fish known by the scientific name of *Channia striata* has abundant nutritional content. Santoso (2009) stated that snakehead fish meat contains 3.37% protein, 2.17% albumin, 32.39% glutamine, 6.61% cysteine, 9.69% glycine, 3.43% Zn, 2.34% Cu, and 0.81% Fe.

Based on the background of the research that has been described, this study aimed to find out the influence of snakehead fish meat supplementation in the feed towards the body weight, weight of the kidney as well as the diameter, and the number of glomerulus in Wistar rats with physiological stress triggered by nutritional deficiencies and excessive physical stress. The results of this study were expected to provide important information about the potential of snakehead fish as feed supplement ingredients and its function to the improvement of histological structure in Wistar rats due to physiological stress as an effect of nutritional deficiency and excessive physical stress.

METHODS

Research Design

The experimental design used in this study was a Completely Randomized Design (CRD). A total of 20 male Wistar rats were divided into 5 (1 control and 4 treatment) groups namely P1, P2, P3, P4 and P5 with 4 times repetition of treatment. The observed research variables included, body weight, kidney weight, glomerular number, and glomerular diameter.

Acclimation and Conditioning Physiological Stress Test Animal

The animals were acclimatized for 7 days in individual cages. During the acclimation process, rats were fed with commercial pellets and water *ad libitum*. The animals were maintained under controlled environmental condition to make the rats experiencing stress by fasting and only being given water *ad libitum* followed by swimming in a bucket for 10 minutes (Sunarno *et al.*, 2013; Sunarno *et al.*, 2015, Sunarno *et al.*, 2018a).

Procedure of Feed Supplementation in Animal Test

Administration of snakehead fish as feed supplement was done for 14 days after acclimatization process for 6 consecutive days. In this study, snakehead fish supplementation consisted of 5 concentrations, *i.e.* 0%, 5%, 10%, 15%, and 20% (group 1 to 5 respectively). During the treatment, Wistar rats were given water *ad libitum*. Weight weighting was performed at the beginning of treatment (after acclimatization) and one day after the end of treatment.

Data Collection and Analysis

Observation of kidney profile was done after making the renal histological preparations with Hematoxylin-Eosin staining. The variables observed include diameter and number of glomeruli. In one preparation was observed as many as 4 field of view kidney weight, as well as the number and diameter of glomerulus obtained were tested by Analysis of Variance (Anova) with a significance of 5%.

If it is found that the treatment gives a real effect on the observed variables followed by Duncan test with 5% significance.

RESULTS AND DISCUSSION

The analysis of experience physiological stress triggered by deficiency and excessive physical activity after supplementation of snakehead fish meat in feed can be seen in Table 1.

The results of further analysis of the body weight using *analysis of varian* (Anova) with a significance of 5% showed significantly different results (P<0.05). The results of further tests with the Duncan test at 5% significance showed that there was a significant difference between P4 and control, but not significantly different from P1, P2, and P3. Other data show that P1, P2, and P3 are not significantly different from controls. Sequentially, the average body weight of P4, P3, P2, P1, and control are 290; 280; 270; 260; and 257.5 g.

The increase in body weight of rats in P4 treatment which was significantly different compared to the other groups was thought to be due to the nutrient in P4 group containing more protein albumin, glutamine, cysteine, and glycine as well as Zn, Cu, and Fe than control and other treatments. Sunarno (2015) stated that snakehead fish meat contains amino acid 32.39% glutamine, 6.61% cysteine, and 9.69% glycine per 100 g of meat. Snakehead fish meat supplementation in P1, P2, and P3 groups could not increase the body weight of test animals. This can be due to insufficient amount of nutrients such as protein albumin, glutamine, cysteine, glycine, and minerals Cu, Zn, and Fe. These conditions can result in less energy production in the body which ultimately affects the ability of cell repair so that it is not optimal in increasing body weight. Leeson (2010) stated that body weight is influenced by the content and composition of proteins and amino acids in feed. It was further stated that protein and amino acids with unbalanced concentrations could disrupt the mechanism of cell repair so that animal body biomass did not increase.

The results of kidney weight analysis with ANOVA at 5% significance showed that supplementation of snakehead fish meat in feed gives a significantly different effect on this variable (P<0.05). The results of further tests with the Duncan test at 5% significance showed that the average kidney weight in P2 and P3 was significantly different from P1, P4, and P0 (control). Other data showed that kidney weight of P4 was significantly different from P0 but not significantly different from P1, while kidney weight of P1 was not significantly different from P0 (control). Sequentially, the average kidney weight of P4, P3, P2, P1, and control were 0.88; 0.94; 0.95; 0.84; and 0.82 g. The data of this study showed that supplementation of snakehead fish meat mixed into feed with a concentration of 10; 15; and 20% could increase the kidney weight of rats that experienced physiological stress with the highest values found in P2 and P3, respectively 0.95 g and 0.94 g and the next is P4 of 0.88 g. Kidney weight in Wistar rats with physiological stress can be increased by utilizing snakehead fish meat which contains lots of protein albumin, glutamine, cysteine, and glycine as well as Zn, Cu, and Fe (Sunarno *et al.*, 2018b; Nugroho, 2009).

Kidney weight in P4 treatment was lower and significantly different than P2 and P3. This condition is thought to be due to the metabolic energy of albumin, glutamine, cysteine, and glycine contained in more snakehead fish meat was used for homeostasis maintenance. This results in lower kidney weight in P4 treatment than P2 and P3. Sunarno *et al.* (2018) stated that metabolism with the availability of protein and excess amino acids has the potential to increase nitrogen waste as the by product of these metabolism, such as ammonia and urea. This condition results in energy requirements for higher maintenance of

Table 1. The average value of body weight, kidney weight, and number and diameter of glomerulus after treatment

Parameter	Snakehead fish supplement in feed (%)				
	P0	P1	P2	P3	P4
Body weight (g)	257.5^{a}±26.3	260.0^{a}±21.6	270.0^{ab}±8.16	280.0^{ab}±8.16	290.0^{b}±14.1
Kidney weight (g)	0.82^{a}±0.041	0.84^{ab}±0.03	0.95^{c}±0.03	0.94^{c}±0.06	0.88^{b}±0.01
Glomerular diameter (μm)	63.75^{a}±1.5	67.75^{bc}±1.89	69.00^{bc}±0.82	70.50^{c}±1.91	66.75^{b}±3.30
Number of glomerulus	26.00^{a}±2.65	28.00^{a}±5.51	28.00^{a}±3.51	31.00^{a}±4.58	29.00^{a}±3.61

Note: Number followed by different *superscript* letter in the same row show a real difference (P<0.05). P0, P1, P2, P3 and P4 are sequentially treated with concentration 0%, 5%, 10%, 15%, and 20%

homeostasis and ultimately results in lower kidney weight as in P4 treatment.

The results of the analysis of the mean diameter of glomeruli with ANOVA at a significance of 5% showed that the snakehead fish meat supplementation in feed affected this variable. The results of further tests with the Duncan test at 5% significance showed that the average glomerular diameter in P3 treatment was significantly different from P4 and P0 (control), but not significantly different from P1 and P2. Other data showed that the mean renal glomerulus in P1, P2, and P4 were not significantly different but the average glomerular diameter in each treatment was significantly different from the control. Sequentially, the mean diameter of the glomerulus in treatment P4, P3, P2, P1, and P0 (control) were 66.75; 70.50; 69.00; 67.75; and 63.75 µg

The data of this study showed that supplementation of snakehead fish meat mixed with feed with concentrations of 5; 10; 15; and 20% could increase the glomerular diameter of rats with physiological stress with the highest value found in treatment P3, which was 70.50 µg. Glomerular diameter can be increased by utilizing snakehead fish meat which contains a lot of albumin protein, glutamine, cysteine, and glycine as well as Zn, Cu, and Fe. There is an increase in the average glomerular diameter at P1, P2, P3, P4 which is significantly different from P0 (control) presumably because nutrients include albumin protein, glutamine, cysteine, and glycine as well as Zn, Cu, and Fe play a role in maintaining, supporting integrity, and repairing body tissues.

Hamid and Azmi (2010) stated that albumin is a protein that has an important role to prevent the inflammation, maintain cells' integrity, and repair tissues or organ due to physiological stress. Salim (2011) stated thatalbumin functions in the process of recovery and increase in organ biomass, including kidney. Albumin at optimal levels also plays a role in increasing body weight. Maulidah (2015) stated that albumin functions as a plasma buffer (maintains physiological pH levels) and prevents folic acid degradation.

Based on histological observations (Figure 1), it was showed that the glomeruli of the test animal were in normal conditions. The normal glomerulus has some features, including no adhesion, atrophy, and glomerular hypertrophy. Lazuardi (2011) stated that normal kidneys do not have some features such as *polymorphonuclear cells*, parenchymal cell cariolysis, endothelial cell cariolysis, narrowing gap between Bowman's capsule and renal medulla, glomerular atrophy and hypertrophy and narrowing contour of duct lumen. Fahrimal *et al.*, (2016) stated that in normal kidneys, there were no tubular and glomerular degeneration. Oliviera *et al.* (2010) stated that glutamine has an important role to maintain metabolic function and the integrity of body tissues including the tissues in the kidneys. In addition, glutamine together with the amino acids (glycine and cysteine) act as precursors of endogenous antioxidants (glutathione) which can protect tissues from free radicals formed during physiological stress.

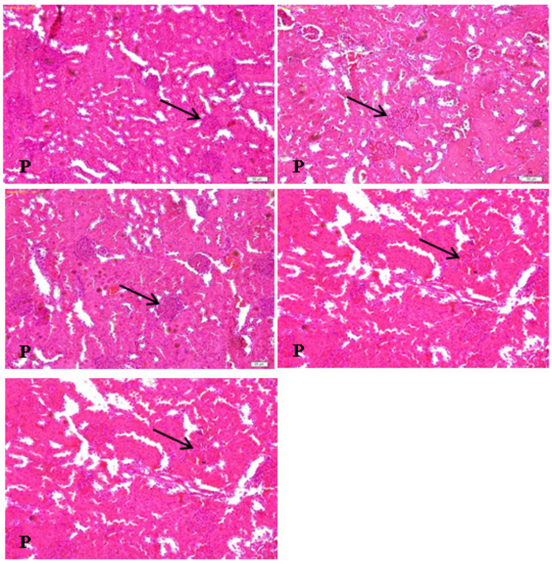

Figure 1. Histological picture of Wistar rats glomeruli by the influence of snakehead fish meat supplementation (P0, P1, P2, P3 and P4 respectively with concentrations of 0%, 5%, 10%, 15%, and 20%; arrows show glomerulus) in 400x magnification

Kidney glomerular diameter in this treatment was still in normal size, this was in accordance with the results of research conducted by Susilorini, *et al.* (2013), found that the lowest diameter of glomerulus was 50.23 µm and the highest was of 176.02 µm. This means that the protein and amino acid content in snakehead fish meat (albumin, glutamine, cysteine, glycine) used in this study is more available so that it can optimally support an increase in glomerular diameter in Wistar rats experiencing physiological stress. The results of the analysis of the number of glomerulus after supplementation of snakehead fishusing ANOVA showed that the amount of renal glomerulus in the treatment of P1, P2, P3, and P4 were not significantly different (P>0.05). Sequentially, the mean of the number of glomerulus in treatment P4, P3, P2, P1, and control were 29; 31; 28; 29; and 26.

The number of glomerulus which was not significantly different between the four treatment groups with control group showed that supplementation of snakehead fish did not increase the amount of glomerulus in Wistar rats with physiological stress. Referring to the previous discussion that the supplementation of snkaehead fish can increase kidney weight and glomerular diameter, the role of the supplement with albumin, glutamine, cysteine and glycine content is maintaining metabolic functions that guarantee the availability of energy in the body (Arifin, 2011). The results of other studies suggestted that the distribution of glutamine in the body in abnormal conditions will change, where glutamine will be taken to meet the needs of the kidneys other than the liver, intestines, and immune cells (Coeffier *et al.*, 2010). Under physiological stress, glutamine acts to protect body cells and tissues through several mechanisms, which include limiting activation of NF (nuclear factor), maintaining a balance between pro and anti-inflammatory cytokines, improving cell integrity and immune function, and increasing the expression of heat shock protein (Wernerman, 2008). Glutamine will increase glutathione levels in tissues by preventing activation of NF, increasing the capacity of antioxidants, and stimulating protein formation through DNA synthesis (Molfino *et al.*, 2010; Ziegler *et al.*, 2008).

Results of this study proved that supplementation of snakehead fish meat (*Channa striata*) mixed with feed in test animals experiencing physiological stress can increase body weight, kidney weight and glomerular diameter but not affecting the number of glomerulus. The supplementation of snakehead fish with a concentration of 20% can increase the body weight of test animals; concentration of 10; 15; and 20% are optimum in increasing the kidney weight; concentrations of 5%, 10%, 15%, and 20% can increase the diameter of the glomerulus in Wistar rats that experience physiological stress. The supplementation of snakehead fish meat with a concentration of 5%, 10%, 15% and 20% had no effect on the amount of glomelurus of Wistar rats experiencing physiological stress. Based on this, the use of supplements of snakehead fish meat in the feed is important to prevent, maintain, improve kidney histology in animals that experience physiological stress. The use of snakehead fish meat as a feed supplement can be recommended for use in animals that experience physiological stress with the aim of maintaining tissue integrity, as well as repairing body tissue from the influence of physiological stress, such as kidney organs that have a high level of vulnerability.

CONCLUSIONS

Based on the results of this study, it can be concluded that the supplementation of snakehead fish meat in feed can increase body weight, kidney weight, glomerular diameter, but it doesn't have any effect on the number of renal glomeruli in Wistar rats that experience physiological stress. Optimal concentration of snakehead fish supplementation in feed to improve rat body weight and kidney structure is between 15% and 20%.

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