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Physiological Studies of Snakehead Fish (*Channa gachua*) Maintained in Controlled Containers

Rani Rehulina Tarigan*, Maheno Sri Widodo, Yuni Kilawati

Faculty of Fisheries and Marine Sciences, Universitas Brawijaya, Jl. Veteran, Malang 65145, Jawa Timur Province, Indonesia

Abstract : The purpose of this study was to determine the physiological study of snakehead fish (*C. gachua*) maintained in controlled containers with different substrates. This study was conducted in January to April 2016 in Fish Reproduction Laboratory, University Brawijaya. The study employed experimental method through a completely randomized design (CRD) with four treatments and three replications. The fish used are snakehead fish (*C. gachua*) with the size of 10-20 cm. Maintenance treatment consisted of sand, paralon, loster and gravel substrates. The main parameter to observe was the survival and physiological responses (*cortisol*, blood sugar levels, blood picture leukocytes, erythrocytes, hemoglobin and *hematocrit*) and survival rate (SR) of the fishes. The results showed that the snakehead fish experience stress as they were moved from the natural water to the controlled containers. The fishes kept in the controlled containers decreased their *cortisol* and blood sugar, and also experienced changes in blood profile including erythrocytes, leukocytes, hemoglobin, and *hematoktrit*. The best treatments for the fishes in the controlled containers were sand, *loster*, PVC pipe, and gravel as the substrates maintenance with the survival of 55%, 46 %, 42% and 25%, respectively.
Keywords: cortisol, blood sugar, blood picture, snakehead fish (*Channa gachua*).

Introduction

Snakehead fish (*C. gachua*) is one of the fish that are naturally available and abundant in nature, living in the rivers and dams. This fish is categorized wild fish usually caught from the waters of rivers and swamps[1]. This fish is from the family *Channidae*, and for *gachua* type has the smallest size of all types of snakeheads, and it has a complete lateral line scales almost a straight line from the top corner until mid overculum i-shaped tail fins and scales *stenoid*.

In Indonesia, snakehead fish spread in almost all regions, namely Java, Sumatra, Bangka, Singkep, Madura, Bali, Lombok, Flores, Ambon and Halmahera[2]. The snakehead fish species is also able to live at an altitude of 0-700 masl[3]. The snakehead fish (*C. gachua*) can live in a variety of ecosystems and nocturnal. These fish live in an area flooded with water with a depth of less than 20 cm and stagnant waters with low oxygen, turbid and muddy waters[4].

The snakehead fish is consumed ranging in size from small to large size, and can be consumed both in the form of fresh fish and already marinated[5]. The snakehead fish extract contains of albumin which is able to regenerate damaged cells; as a producer of Albumin, the snakehead fish has high nutritional value and can be used as an alternative to get cheaper albumin[6].

In order to fulfill the demand of snakehead fish (*C. gachua*), it is usually relies on natural catchment in nature, and it is necessary to maintain its sustainability through fish farming[7] to protect the resource from extinction[8]. Thus, it requires further information and study on the existing level of stress on the fish being cultivated on the farming sector. The review includes the cortisol hormone, blood sugar and blood picture (leukocytes, erythrocytes, hemoglobin, hematocrit and differential leukocyte), so that through the process of adaptation to the particular time, we understand the condition of the fish which is ready to be cultivated and may grow and develop at the optimal rate as its natural habitat.

Methods

Experimental Design

Initial research was conducted through experiment method with four treatments and three repetitions. Data obtained from the research results were statistically analyzed using analysis of variance (ANOVA) in accordance with the design used namely complete randomized design (CRD). The treatments used in this initial study were the addition of the substrate on maintenance media of sand, pieces of PVC pipe, gravel and loster (bricks with holes made of concrete).

Research Procedures

Snakehead fish (*C. gachua*) from the natural catchment coming from the sources of sand and Dampit in Malang were measured for the length and severity and then acclimatized. The containers used for the maintaining the fish were aquarium with the size of 50x40x30 cm filled with water to a height of 25 cm. Each of the containers was filled with the snakehead fish (*C. gachua*) to the density of 8 fish / aquarium. Each aquarium was given aeration and homogeneous gas temperature controller. The substrates used were stone, sand, pieces of PVC pipe and loster. After the mixture of the substrates, the observation was conducted to see of the stress response, blood picture and survival of the fish. During maintenance of the fish would be fed with natural food such as worms as well as artificial feed in the form of pellets.

Test Parameters

Test parameters during the study included survival rate (SR), measurement of cortisol, blood sugar, erythrocytes, leukocytes hematocrit and hemoglobin were done every day during the maintenance. Supporting test parameters included the water quality which was observed every morning and evening during the maintenance including the water temperature, pH and dissolved oxygen (DO), while ammonia, nitrate and nitrite were observed once a week.

Result and Discussion

Survival Rate (SR)

The survival rate of the snakehead fish (*C. gachua*) treated with the addition of sand substrate, loster, PVC pipe and gravel were found to have survival rate of 55%, 46%, 42% and 25%, respectively (see Table 1), and it means the survival rate of the fish according the statistical analysis showed significant ($P> 0.05$).

Stress Response

Stress response of the snakehead fish (*C. gachua*) with the treatment of the addition of substrates like sand, loster, PVC pipe, and gravel were shown through cortisol and blood sugar (Table 2 and Figure 1). Cortisol and blood sugar of the snakehead fish (*C. gachua*) decreased during the study.

Blood Picture

Blood picture of the snakehead fish (*C. gachua*) with the treatment of the addition of substrates like sand, loster, PVC pipe, and gravel included erythrocytes (Table 2), leukocytes (Table 3), hematocrit (Table 5) and hemoglobin (Table 4). Statistical analysis showed differences in the blood picture snakehead fish (C.

gachua) significantly affected the survival rate (P> 0.05) and not significantly affected the survival rate (P <0.05)

Discussion

The low survival of snakehead fish (*C. gachua*) during the treatment because the fish were not able to adapt in particular period to the new environment, despite all environmental parameters were in accordance with the needs of the fish. Survival is an opportunity to live in a certain time which is influenced by abiotic and biotic factors.

Table 1. Survival (%) and variance of snakehead fish

Treatments	**Survival**			**Total**	**Mean**	**Deviation standard**
	1	2	3			
Sand	4	4	5	13.00	4.33	0.58
PVC pipes	3	4	4	11.00	3.67	0.58
Loster	3	4	3	10.00	3.33	0.58
Gravel	2	2	2	6.00	2.00	0.00

Variance	**db**	**JK**	**KT**	**F stat**	**F table 5%**	**F table 1 %**
Treatment	3.00	8.67	2.89	11.56	4.07	7.59
Random	8.00	2.00	0.25	**		
Total	11.00	10.67				

Information: *significant difference

The best treatment in this study was treated by using the addition of sand substrate with the survival rate by 55%, then the addition of loster and PVC pipe substrate by 46% and 42%, and the addition of gravel substrate was with 25% survival rate. The snakehead fish (*C. gachua*) are able to survive in extreme aquatic environments; even in the dry season when the marshes dried the snakehead fish are able to survive by burying themselves in the mud[9]. For the fish' response to stress, there were series of chemical changes (pH, pollution and nitrogen), biochemical (mucus and *osmoregulation*), physiological (temperature, lighting, handling or maintenance) and morphological (skin, scales) that would affect the survival of the fish. The survival is influenced by environmental conditions naturally. Every organism has the ability to adapt to environmental changes within certain tolerable limits. If the environment changes occur outside the tolerable range, sooner or later the organisms affected by the changes will die[10].

Cortisol is a hormone which is involved in General Stress Syndrome (GAS) and instrumental in helping the body to cope with stress. Cortisol has metabolic effects that increase the concentration of blood glucose by using protein and fat deposits, which means that the increase of deposits of glucose, amino acids, and fatty acids are available to use when needed, for example in a stress condition[11]. Fish primary response to stress is in the form of nervous (anxiety) and hormonal changes which include an increase in corticosteroid and catecholamine and changes in neurotransmitters[10]. In Rainbow trout fish, normal cortisol of the fish is of 0-30 ng/mL[12]. At the beginning of the maintenance, cortisol levels of each treatment were equal. High levels of cortisol were allegedly due to transportation and initial pisciculture stress conditions on the fish were still high. The factors that cause stress may come from the physical and psychological stimulations. Physical stress caused by exposure to stressors that are harmful to the body tissues, e.g. exposure of the cold or heat, lowering oxygen, infection, injury.

The increased level of cortisol fish indicates the high level of stress and handling of water quality during the study[13]. The decreased blood cortisol occurred in every treatment, such sand substrate of 1,750 decreased to 188.16 nmol/L, PVC pipe of 1,750 to 251 nmol/L, loster of 1,750 to 262 nmol/L and gravel of 1,750 to 281.68 nmol/L. Based on the data obtained by extrapolating data was then performed to determine the relationship of time with the lowest cortisol values. Images of the equation results can be seen in Figure 1.

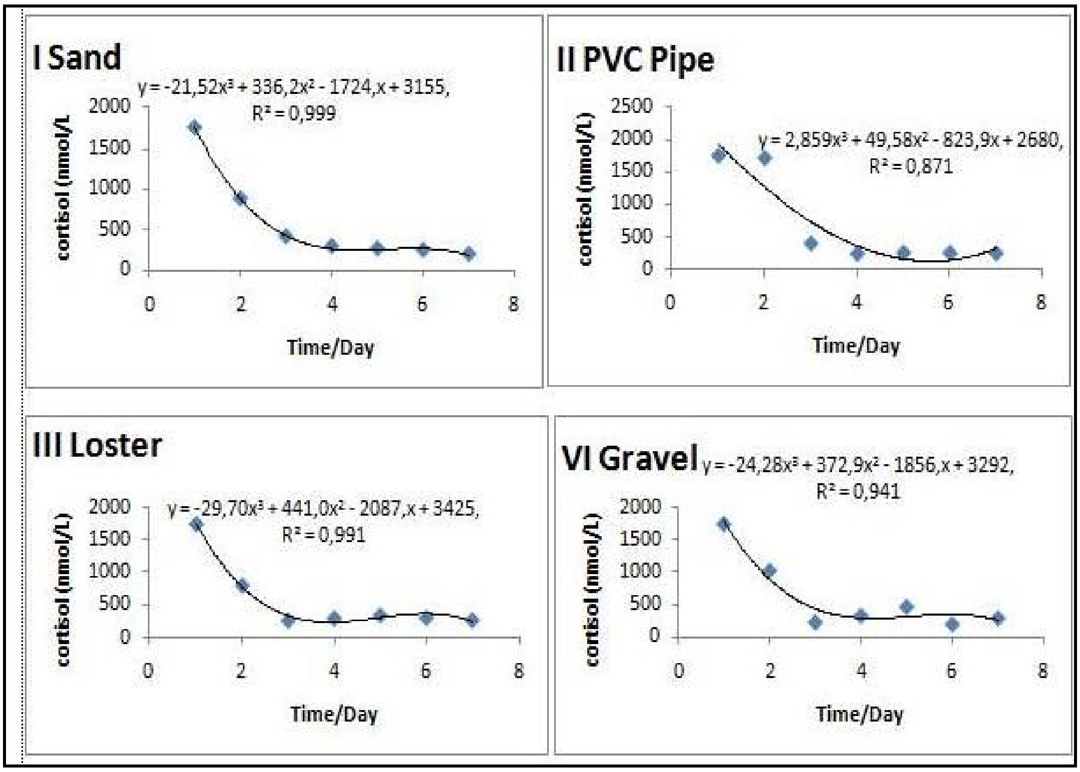

Figure 1. Graph of decreased cortisol during the maintenance with different treatments

Blood glucose is the main source of fuel providers and essential substrate for the metabolism of the cells in the body, especially the brain cells that carry out brain function in a sustainable manner. If the fish stress increases glucose, it might be followed by death. In order to maintain stable blood glucose levels in the body, the homeostasis should be maintained by the liver through metabolism of glucose[14]. The body's response to the changes in fish habitat can be seen from the amount of blood sugar that exists in fish. Based on observations conducted during the maintenance, blood sugar levels in the snakehead fish body decreased, and it became normal on the day three. A decrease in blood sugar levels occurred in every treatment by addition of the substrates, namely sand substrate of 110 mg/dL decreased to 8 mg/dL, PVC pipe of 128 mg/dL to 33 mg/dL, *loster* of 120 mg/dL to 29 mg/dL and gravel of 105 mg/dL to 28 mg/dL (Figure 2). Blood glucose level of common carp is 111 mg/dL; normal Rainbow trout fish has 41-151 mg/mL[12]. Increased levels of glucose in the blood plasma of fish during stress may be caused by the action of catecholamine in the center of glycogen in the liver and tissues[15]. A decrease in blood glucose during maintenance can be seen in Figure 2.

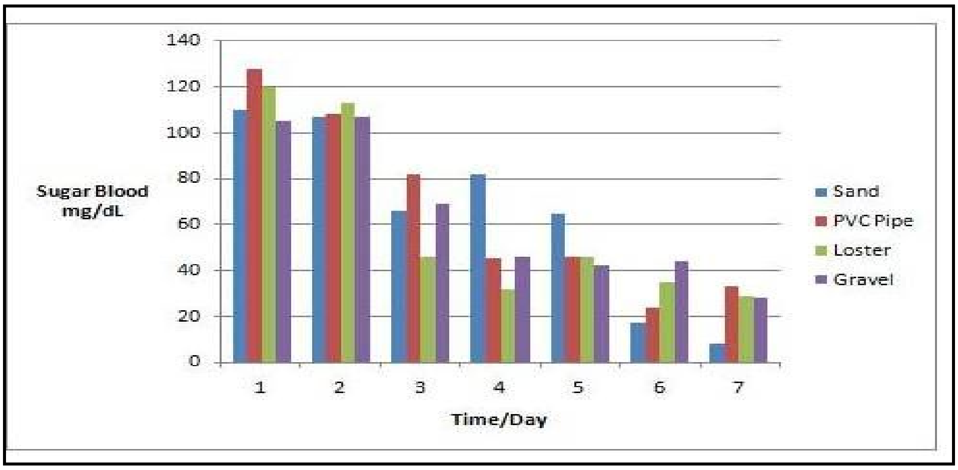

Figure 2. Graph of decreased blood glucose during the maintainence with different substrates

It is suggested that the mechanism of blood glucose changes in performance during stress is the occurrence of the stressor that will be received by the organs of the receptor, and then the information is delivered to the hypothalamus of the brain through the nervous system to cortisol which will stimulate the enzymes involved in *gluconeogenesis* resulting in increased blood glucose[16]. At the same time, brain hypothalamus secretes CRF (corticoid releasing factor) which regulates the pituitary gland to secrete ACTH (*Adenocorticotropik hormone*), MSH (*melanophore-stimulating* hormone) and β-End (β-endorphin). These hormones regulate the secretion of the cortisol hormones from *interrenal* cells. Cortisol will impede the enzymes involved in *gluconeogenesis* resulting in an increase in blood glucose from non-carbohydrate sources.

The influence of stress conditions the fish then causes the secretion of hormones from the adrenal *gradula* which increase the blood sugar so that the excess secretion of these hormones may suppress the inflammatory response[10]. Liver functions as glycogenic as it is stimulated by enzyme, the cells of the liver (hepatic) to produce glycogen (animal starch) of the glucose concentration of carbon hydrate foodstuffs. The liver also serves to change the amino acids that are absorbed by the blood. In the pancreatic islets of Langerhans, cell A produces the glucagon hormone. This hormone will stimulate the formation of glucose from glycogen through the process of glycogenolysis and inhibit the formation of glycogen from glucose. Glucose in the form of glycogen is derived from the final results that enter the Krebs cycle, and the cells do not undergo catabolism into *pyruvate* by glycolysis. By anabolic through *glikoneogenesis* process is stored in the liver. If your blood glucose decreased, or the amount of glucose entry into cells is not sufficient, the glycogen reserves will be used for the metabolism.

Erythrocytes in fish serve to bind oxygen[17]. Erythrocytes or red blood cells are the highest number of sizes and shapes between one and the other species. In *elasmobranchi* fish, there are 19.7 x 13.8 µm red blood cells, and there are oval-shaped cells for some species with the diameter of 11-14 with the cell's volume and nucleus ratio of 3.5-4 μm^3. It is stated that the red blood serves as the respiration gas transportation[18]. Red blood cells also serve as the tools which most widely distribute oxygen throughout the body's tissues. Molecules of oxygen are carried in the hemoglobin molecule in the cell. The decrease of erythrocyte may be due to a foreign object into the body and also the rupture of blood vessels. Erythrocytes have many constituent, and when each component experiences such disorder, it may cause damage on the cell, and the red cells cannot function properly. The level of damage may occur at the level of the membrane of the cell itself, precisely the different proteins under the membrane which plays a role in maintaining membrane. Erythrocyte cell damage may cause the disruption of transportation of the substances that the body needs[19]. The results of analysis of erythrocytes variance during the study are shown in Table 2.

Table 2. Erythrocytes in Snakehead Fish ($x10^3$ cells/ml) during the Maintainence

Day	**Erythrocytes in Snakehead Fish (x103 cells/ml)**			
Treatment	**Sand**	**PVC Pipe**		**Gravel**
1	317.76±2.08a	321.67±2.89a	330.33±1.53b	338.67±3.21c
2	249.55±1.15a	272.76±3.79b	264.00±3.61a	248.33±5.77a
3	257.33±2.52b	261.67±3.06c	258.67±3.51b	251.33±3.21a
4	267.33±2.52b	255.33±1.15a	251.33±2.52a	267.67±2.08b
5	271.00±3.46c	265.00±2.00b	266.00±1.73b	246.33±1.53a
6	333.33±2.89b	411.00±3.61c	309.33±2.08a	312.33±3.51a
7	258.67±3.51a	263.00±2.65a	265.33±2.52b	285.67±2.08c

Information: the numbers on the same line followed with the same letter are not significantly different (P>0,05).

Table 3. Leucosite in Snakehead Fish (x10^4 cells/ml) during the maintainance

Day-	Leucosite in Snakehead Fish (x104 cells/ml)			
Treatment	Sand	PVC pipe	Loster	Gravel
1	86.87±1.53b	77.00±1.73a	88.00±2.00b	78.00±2.00a
2	78.00±2.00b	68.00±1.73a	77.67±2.52b	79.67±3.06b
3	57.33±2.52ns	54.67±2.52ns	51.67±1.53ns	54.00±3.61ns
4	48.67±2.52b	39.33±1.53a	36.00±2.00a	36.00±2.00a
5	45.67±1.53c	36.33±2.52a	38.00±2.00b	35.33±0.58a
6	29.67±2.08a	28.33±2.52a	25.33±1.53a	33.00±1.00b
7	27.00±2.00ns	27.67±2.52ns	28.00±2.00ns	24.67±2.52ns

Information: the numbers on the same line followed with the same letter are not significantly different (P>0,05).

Leukocyte is an important component and acts as the body's immune system, so that when the fish is under stress or infected with the disease, the number of leukocyte will increase. On the third and the seventh day, there was not significantly difference of the leukocyte, while on the first, second, fourth and fifth, the best treatment was the treatment of sand substrate (Table 3). The range of white blood in fish of the sand treatment from 29.67 to 86.87 x10^4 cells/mm^3, while the observation of PVC pipe substrate treatment was 27.67 to 77.00 x10^4 cells/mm^3, loster substrate treatment of 25.33 to 50.88 x10^4 cells/mm^3, and gravel treatment ranging from 24.67 to 79.67 x10^4 cells/mm^3. These results are far away from the normal range, which is 20-150 X10^3 cells/mm^3 [20]. White blood cells have some of the successful fights against foreign objects into the body. The increase of white blood cells is a response of self-protection against the foreign cells coming into body. Leukocytes also contribute to maintaining a healthy body that is *detoxination* of protein before the damage occurs in the body[21].

Hematocrit is a measurement result stating comparison of the red blood cells to the volume of blood. Results of analysis of variance to hematocrit during the study are shown in Table 4.

Table 4. Hematocrit (%) in snakehead fish during the maintenance

Day-	Treatments			
Treatment	Sand	PVC pipe	Loster	Gravel
1	31.33±1.53b	30.33±2.52a	26.33±0.58a	26.33±2.52a
2	24.33±0.58b	26.33±2.52b	23.33±1.53b	18.33±2.52a
3	20.67±2.08b	20.33±2.08b	13.33±2.31a	22.33±3.51b
4	27.00±1.73b	23.67±1.53a	25.67±2.08a	30.67±1.53c
5	15.00±3.00ns	15.00±1.73ns	14.33±2.08ns	19.33±2.52ns
6	14.67±2.08a	13.33±1.15a	17.00±1.73b	18.67±1.15c
7	20.00±1.00b	16.33±1.5a	19.67±1.53b	21.33±1.53b

Information: the numbers on the same line followed with the same letter are not significantly different (P>0,05).

The range of hematocrit in the snakehead fish (*C.gachua*) during the maintenance in all treatments ranged from 13.33 to 31.33%. The range of hematocrit in healthy fish is 35 to 40%. The decrease in hematocrit value may be used as guidance about the low content of protein, vitamin deficiencies or an infection in the fish.

Hemoglobin is the iron-containing protein in red blood cells that function as a carrier of oxygen from the lungs throughout the body. Hemoglobin levels vary in number depending on the species of fish, pH, blood, environmental conditions and oxygen partial pressure (PO2). If oxygen partial increased as it is inside the capillary, then the oxygen binds to hemoglobin; otherwise, if oxygen partial, according to the network then the oxygen will be released by hemoglobin[18]. Results of analysis of variance hematocrit during the study are shown in Table 5.

Table 5. Hemoglobin Hb/100ml in snakehead fish during the maintenance

Day-	Hemoglobin in snakehead fish (Hb/100ml)			
Treatment	**Sand**	**PVC pipe**	**Loster**	**Gravel**
1	13.33±1.53b	11±1.53a	14±2.52b	15.33±1.53c
2	12.67±2.52b	12±2.65b	12±1.53b	8.00±1.00a
3	7.00±0.58ns	8.33±0.58ns	7.67±1.00ns	7.00±1.53ns
4	7.00±1.73ns	8.33±3.00ns	7.67±2.52ns	7.00±1.73ns
5	4.33±1.53a	5.33±1.15b	4.67±0.58a	5.67±1.15a
6	4.76±2.08ns	4.33±0.58ns	5.67±0.58ns	6.00±2.00ns
7	3.00±1.00ns	5.33±1.53ns	4.00±2.00ns	3.33±1.53ns

Information: the numbers on the same line followed with the same letter are not significantly different (P>0,05).

Hemoglobin in the snakehead fish (*C.gachua*) decreased during the maintenance. The range of hemoglobin was 3.00 to 15.3 Hb/100ml during the maintenance. The lower the value of hematocrit, the lower the hemoglobin content in the blood. Hemoglobin level in fish is associated with a set amount of erythrocytes as hemoglobin is contained in erythrocytes. Red blood cell reserve is likely not perfect yet, so the ability of the hemoglobin to bind oxygen is not optimum, so fish may experience oxygen deficiency[10].

The quality of water during the study was in accordance with the criteria pisciculture for snakehead fish, and it can be seen in Table 6.

Table 6. Level of water quality during the maintenance

Parameters	**Treatments**			
	Sand	**PVC pipe**	**Loster**	**Gravel**
Temperature	26-27 ^{0}C	26-26 ^{0}C	26-27 ^{0}C	26-27 ^{0}C
pH	7.9-8.4	7.3-8.5	7.9-8.1	7,5-8,2
DO	52-6.8 mg/l	5.1-6.5 mg/l	5.4-6.6 mg/l	5.4-6.8 mg/l
Nitrate	0.3-0.7 mg/l	0.3-0.8 mg/l	0.4-0.7 mg/l	0.5-0.6 mg/l
Nitrite	12.5-25.0 mg/l	18.8-31.3 mg/l	12.5-29.2 mg/l	12.5-29.2 mg/l

Tropical fish are able to grow well at a temperature ranging from 25 to 32 ^{0}C. Snakehead fish (*C.gachua*) are able to live at temperature ranging from 13 to 35 ^{0}C. It is stated the effect of temperature is indirectly affects the metabolism, solubility of gases, including oxygen and a variety of chemical reactions in the water[22]. The majority of aquatic biota is sensitive to changes in pH and common pH is likely about 7 to 8.5[21]. Moreover, it is stated that the dissolved oxygen levels in the water are usually less than 10 mg/l[21]. Dissolved oxygen in the water is a very important factor in supporting the survival of fish and also essential factor in the metabolism. The need of oxygen of each organism differs according to type, age, size and activity of the organism. The utilization of oxygen by organisms depends on the species, size, temperature, nutrient levels and other factors[23].

Nitrites are usually found in small portion in natural waters. The level is higher than nitrate as nitrite would not be stable if there is oxygen. Nitrite is an intermediate form between ammonia and nitrate (*nitrification*) and also between nitrate and nitrogen gas (*denitrification*) (Effendi, 2003). Nitrates are not toxic for aquatic organisms. Nitrates are not toxic to the aquatic container[21], and it is suggested that the nitrate concentrations in shrimp farming be less than 100 ppm. Nitrate will be toxic at concentrations above 300 ppm.

According to the research on the physiological study of the snakehead fish maintained in controlled containers, the following concluding remarks are drawn:

1. Snakehead fish maintained in controlled containers undergo changes in terms blood profile including erythrocytes, leukocytes, hemoglobin, and hematocrit during the maintenance; this is in order to maintain body homeostasis condition

2. The best treatment for the maintenance was by addition of substrates namely sand, *loster*, PVC pipe, and gravel with survival rate of 55%, 46%, 42% and 25%, respectively.

It is preferable for pisciculture of snakehead fish in controlled containers by other and proper treatments. Based on the results of the study, the best treatment for the pisciculture of snakehead fish is by addition of sand substrate.

References

1. Widodo, M.S, Marsoedi, T. Susilawati, A. Permana. Maturity Level and Somatic Index of Gonado at Dwarf Snake-head (*Channa gachua*) during January to December 2009. Journal of Basic and Applied Scientific Research, 2013, Vol.3(**3**)387-393.
2. Djuanda,T. Dunia Ikan. 1981. Armoco.Bandung
3. Fujaya, Y. Fisiologi Ikan Dasar Pengembangan Teknik Perikanan. 2004. Penerbit Rineka Cipta
4. Couternay, W.R. dan J.D.Williams. Snakesheads (Pisces, Channidae) A Biological Synopsis and Risk Assesment. 2004. U.S Geological Survey. Florida, p.70.
5. Firlianty. Potensi Albumin Ikan Famili *Channidae* Asal Kalimantan Tengah untuk Penyembuhan Luka. 2015. Dissertation. Universitas Brawijaya.
6. Sediaoetama, S. Ilmu Gizi Untuk Mahasiswa dan Profesi. 2004. First Edition. Jakarta
7. Makmur, S. Sudahkan Anda Tau? Pemanfaatan dan Pelestarian Ikan Gabus (*Channa striata* Bloch). 2006. Badan Riset Kelautan dan Perikanan Balai Riset Perikanan Perairan Umum. Palembang.
8. Murtidjo, B.A. Beberapa Metode Pembenihan Ikan Air Tawar. 2001. Kanisius Yogyakarta.
9. Muslim. Perikanan Rawa Lebak Lebung Sumatera Selatan. 2012. Penerbit Unsri Press. Palembang.
10. Irianto, A. Patologi Ikan Teleostei. 2005. Gadjah Mada University Press. Jogya 256
11. Guyton A.C. Text Book of Medical Physiology. 2000. 10th. Ed.USA. W.B. Saunders Co.
12. Schreck, C.B. and Petter, B.M. Methods for Fish Biology. 1990. American Fisheries Society Bethesda, Maryland,USA. 451-477
13. Jentoft S, Aastveit AH, Torjesenc PA, Andersen Ø. Effects of stress on growth, cortisol and glucose levels in non-domesticated Eurasian perch *Perca fluviatilis* and domesticated rainbow trout *Oncorhynchus mykiss* Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology, 2005, 141: 353–358
14. Piliang, W.G. & S. Djojosoebagio. Fisiologi Nutrisi, Volume I. 2000. Institut Pertanian Bogor ISBN 979-8212-06-1, Bogor.
15. Svobodova, Z., Vykusova, B., Modra, H., Jarkovsky, J., dan Smutna, M. Haematological and biochemical profile of harvest – size carp during harvest and post-harvest stroge. Aquaculture Research, 2006, 37, 959 – 965.
16. Hastuti, S., E. Supriyono., I. Mokoginta dan Subandiyono. Respon Glukosa Darah Ikan Gurami (*Osphronemus gouramy*, Lac.) terhadap Stres Perubahan Suhu Lingkungan. Jurnal Akuakultur Indonesia, 2003, 2(2): 73-77.
17. Affandi R dan Tang, U. M. Fisiologi Hewan Air. 2002. Uni press. Pekan Baru.
18. Bijayanti,R. 2005. Hematologi Ikan Teknik Pengambilan Darah dan Pemeriksaan Hematologi Ikan. Kedokteran Hewan Universitas Airlangga. Surabaya
19. Maryani. Penggunaan Senyawa Aktif batang Akar Kuning (*Arcangelisia flava* Merr) Sebagai Antibakteri Alami Terhadap Pengobatan Penyakit Infeksi Aeromonas hydrophilla pada Ikan Patin (Pangasius hypotalamus). 2014.
20. Alamanda, I. E., N. S. Handajani and A. Budiharjo. Penggunaan metode hematologi dan pengamatan endoparasi t darah untuk penetapan kesehatan ikan lele dumbo (*Clarias gariepinus*) di kolam budidaya Desa Mangkubumen Boyolali. Biodiversitas, 2007, 8(1) 34-38.
21. Effendi dan Zukesti. Peranan Leukosit Sebagai Anti Inflamasi Alergik dalam Tubuh. 2003. Bagian Histologi Fakultas Kedokteran Universitas Sumatera Utara. USU Digital Library.
22. Kordi. K. M. Ghufran, H., and A.B. Tancung. Pengelolaan Kualitas Air Dalam Budidaya Perairan. 2007. Rineka Cipta. Jakarta
23. Boyd C.E. Water Quality in Pond for Aquaculture. 1990. Auburn University Alabama. (US): Auburn University Agriculture Experiment Station.

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