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# Effect of Treatment Duration Infection Myxobolus Spora Orally on Koi Fish's (Cyprinus Carpio L) Sugar Blood Level

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***Abstract***

*According to data from the Ministry of Marine and Fisheries (2010) the value of koi fish exports reached 200 billion in 2009. Koi fish farming is inseparable from the presence of disease. One of the diseases that attack the koi fish is Myxobolusis caused by Myxobolus spores. Disease infection in koi fish can be categorized as a stressor that causes the occurrence of stressful conditions that cause increased blood sugar levels of koi fish. Increased blood sugar levels are secondary effects of stress and greatly affect the health of fish. This study aims to determine the effect of long treatment of Myxobolus spores infection orally on blood sugar levels of koi fish (Cyprinus carpio L). This study was an experimental study with Completely Randomized Design (RAL) using 7 treatments and 4 replications respectively. The dosage used for Myxobolus spore infection is 120 spores / fish. The main parameters observed were the amount of blood sugar in the blood of koi fish infected with Myxobolus while for supporting parameters including water quality, among others temperature, pH, and dissolved oxygen were measured daily, in the morning and afternoon. The examination data are blood sugar value of koi fish with ANOVA test followed by Duncan test. The results showed that the blood sugar content of koi fish before infected was 35.25 mg / dl. The results of data analysis of blood sugar calculations analyzed with Anova can be seen that there is a significant difference ($p < 0.05$) for long treatment of Myxobolus spores infection. Based on the Duncan test, it is known that the highest blood sugar of koi fish obtained at 96 hours of treatment is 85 mg / dl and the lowest blood glucose on the 24 hours treatment time is 35,25 mg / dl.*

***Keywords:*** *Blood sugar, Koi fish (Cyprinus carpio), Myxobolus, Blood.*

## 1. INRODUCTION

Koi fish farming is one of the introduced freshwater ornamental fish commodities that are excellent and are expensive group fish. According to data from the Ministry of Marine Affairs and Fisheries (2010) the value of koi fish exports reached 200 billion in 2009. According to the statistics of East Java Fishery and Fishery Department, koi fish production in East Java Province from 2005 to 2009 was 216,920, 147,633, 185,100 , 375,000, and 446,800 tons. Koi fish farming is inseparable from the presence of disease that can diturb the production results so that the need for increased vigilance and l attention to disease in cultivation process. One of the diseases that attack koi fish is Myxobolusis caused by *Myxobolus* parasite. According to Kepmen. No. 26 / KEPMENKP / 2013 that *Myxobolus* is included in the list of *Quarantine* Disease Pests (HPIK) class I. [17] reported in Indonesia *Myxobolus koi* cause serious problems in koi fish culture (*Cyprinus carpio*) with mortality rate reachs 60-90%. Disease infection in koi fish can be categorized as a stressor that causes the occurrence of stressful conditions that cause increased blood sugar levels of koi fish. Increased blood sugar levels are secondary effects of stress and greatly affect the health of fish. Blood sugar is one of the important components in the blood that serves as a major source of fuel supply and an essential substrate for cell metabolism, especially brain cells.

Increased blood sugar levels are secondary effects of stress [11] and greatly affect the health of fish. Therefore, it is necessary to do research to know the effect of long treatment of *Myxobolus* spore infection orally to blood sugar level of koi fish (*Cyprinus carpio* L). This information is useful to know the level of stress experienced by fish during *Myxobolus* spore infection, thus opening the possibility of prevention path so that the disease does not spread.

## 2. RESEARCH METHODOLOGY

### 2.1. Place and Time

This research was conducted in November 2016 until April 2017 at Laboratory of Faculty of Fisheries and Marine Universitas Airlangga.

### 2.2. Research Materials

**A. Materials Research**

The materials used in the study were koi fish (*Cyprinus carpio* L.), *Myxobolus* spores, anticoagulants Ethylene Diamine Tetra Acetatic Acid (EDTA), aquadest and NaCl Physiological 0.9.

**B. Research Tools**

The tools used in this research are aquarium, measuring cylinder, plastic bucket tub, dropper dropper, haemocytometer, Erlenmeyer 100 ml, micro pipette, microscope, glass object, glass cover, syringe (syringe), plastic bag, and aeration stones, sechio sets, water thermometers, DO meters, pH meters, glucose kits and glucose strips.

## 3. RESEARCH METHODS

This study was an experimental study with Completely Randomized Design (RAL) using 7 treatments and 4 replications respectively. The dose used for *Myxobolus* spore infection is 120 spores / fish. This is in accordance with the statement of [8] who reported that the highest damage to the tissue of koi (*Cyprinus carpio*) infected by *Myxobolus* orally, the worst damage was found in koi fish with the highest infection dose of 120 spores / fish. The duration of treatment was carried out for 7 days (7x24 hours) after the fish was infected with Myxobolus spores. Observations were made daily, each treatment was as follows:

A: 5 fish + dose 120 spores *Myxobolus* / fish for 24 hours after treatment
B: 5 fish + dose 120 spores *Myxobolus* / fish for 48 hours after treatment
C: 5 fish + dose 120 spores *Myxobolus* / fish for 72 hours after treatment
D: 5 fish + dose 120 spores *Myxobolus* / fish for 96 hours after treatment
E: 5 fish + dose 120 spores *Myxobolus* / fish for 120 hours after treatment
F: 5 fish + dose 120 spores *Myxobolus* / fish for 144 hours after treatment
G: 5 fish + dose 120 spores *Myxobolus* / fish for 168 hours after treatment

### 3.1. Research procedure

**A. Preparation and Treatment of Fish During Research**

The aquarium was cleaned and chlorinated (Cl) 0.125 ppm as disinfectant, then each aquarium was filled with pre-deposited water taps. Koi fish that have been obtained from the pond in Kemloko Village, Nglegok District, Blitar Regency, East Java, were taken to the Wet Faculty of Fisheries and Marine Laboratory of Airlangga University Surabaya using a sealed plastic bag with the addition of oxygen. Before the koi fish used for the research done acclimatization for 3 days. This is in accordance with the statement [8] reported that before the koi fish used for research conducted acclimatization for 3 days. *Myxobolus* infected fish is examined nodules in its gills and picked up the nodule and placed on the container. The nodules are then broken down with tweezers, added with aquadest and diluted and observed under a microscope to determine the amount to be given according to the desired dosage, then

indirectly used nodules are immersed in Phosphat buffer solution and stored in refrigrator. Healthy koi fish are infected using *Myxobolus* exposure at a dose of 120 spores / fish. *Myxobolus* spores are administered orally on day 1 by removing the gills of the fish to open the mouths of the fish and then injected with *Myxobolus* spores and water quality control in the morning and evening. Fish blood collection was observed at 24 hours, 48 hours, 72 hours, 96 hours, 120 hours, 144 hours, 168 hours.

**B. Calculation of Spore Density**

Spore calculations were counted using a haemocytometer and to facilitate calculation of the hand counter. Spore calculations on blocks A, B, C dam D start from the left side of the box to the right and count the spores that are in the line or that are close to the inner boundary line of the box. The next step sums the calculations on the A, B, C and D blocks in the upper and lower calculation areas of the haemocytometer and then calculates the spore density (spores / ml) using the Big Block calculation formula [19].

$$\text{Spore density (spores / ml)} = \frac{nA+nB+nC+nD}{4x10^{-4}}$$

Keterangan:
nA,nB,nC,nD = number of spores in blocks A, B, C and D
4 = number of blocks calculated
$10^{-4}$ = volume of each block

**C. Examination of Blood Sugar**

**1) Blood Collection**

Taking fish blood is done by means of Spuit filled with EDTA (Etylene Diamine Tetraacetic Acid) so that the blood taken does not freeze. Furthermore, fish are prepared and taken blood by blood sampling is spuit inserted on the linea lateralis with $45^o$ slope, then blood is taken with a syringe slowly, so that obtained the desired blood. Place of fish blood collection can be done at linea lateralis area, dorsal ventralis, caudal peduncle and heart.

**2) Blood Sugar Examination**

Measurement of blood sugar in koi fish is done by taking blood koi fish on the linea lateralis using a syringe. Blood that has been obtained and then touch the part of the line that there is an arrow on the side edge of the Easy Touch glucose test strip that has been installed on the Easy Touch Glucose kit. Blood will immediately seep until the end of the strip and the beep. Wait a moment until the numbers appear on the screen. The number that appears is the result of blood sugar contained in blood [18].

**D. Experimental Design**

The experimental design used in the study was the amount of blood sugar in the blood of koi fish infected with *Myxobolus* is Completely Randomized Design (RAL) because in this study only has one source of diversity that is the duration of treatment. Completely randomized design has a single source of diversity ie treatment in addition to random influences, so the result of the difference between treatments is only due to the effect of random treatment and effect alone [10].

**E. Parameter of Research**

The main parameters observed were the amount of blood sugar in the blood of koi fish infected with *Myxobolus* while for the supporting parameters include water quality, among others temperature, pH, and dissolved oxygen measured daily, morning and afternoon.

**4. DATA ANALYSIS**

**4.1. The Examination Data Are Blood Sugar Value of Koi Fish with ANOVA Test Followed by Duncan Test**

**Results and Discussion**

**Research result Myxobolus Examination**

Koi fish infested with the *Myxobolus* parasite can be identified by the presence

of nodules in the gills of fish containing thousands of spores so that the operculum can not close completely. Images of koi fish infested with *Myxobolus* can be seen in Figure 1. The koi fish infested with Myxobolus in their intestines are spores after oral spore *Myxobolus* infections. Images of *Myxobolus* spores in the intestine can be seen in Figure 2.

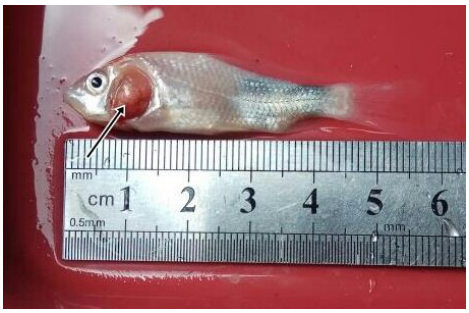


***Figure 1.*** *Nodules in the gills of koi fish.*

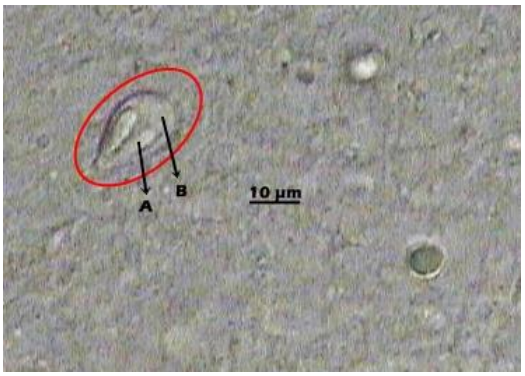


***Figure 2.*** *Myxobolus spores in the digestive tract (intestine) of koi fish (1000x magnification). Description: A; polar filament, B; polar capsule.*

**Blood Sugar Examination**

Graph of comparison of the average blood sugar level of each treatment can be seen in Table 1.

***Table 1.*** *Table average blood sugar levels.*

| Treatment | Number of blood sugar infected with *Myxobollus* (mg / dl) and Deficiency Standard |
|---|---|
| P0 (Control) | $35,25^{a}\pm2.500$ |
| P1 (24 Hours After Treatment) | $41,75^{b}\pm9.069$ |
| P2 (48 Hours After Treatment) | $57,5^{c}\pm1.291$ |
| P3 (72 Hours After Treatment) | $66,5^{d}\pm4.359$ |
| P4 (96 Hours After Treatment) | $85^{e}\pm1.826$ |
| P5 (120 Hours After Treatment) | $65,75^{d}\pm2.217$ |
| P6 (144 Hours After Treatment) | $59,75^{c}\pm1.708$ |
| P7 (168 Hours After Treatment) | $57,5^{c}\pm1.291$ |

Description: Super letters Different scripts in the same column show significantly different effects
($p<0.05$)

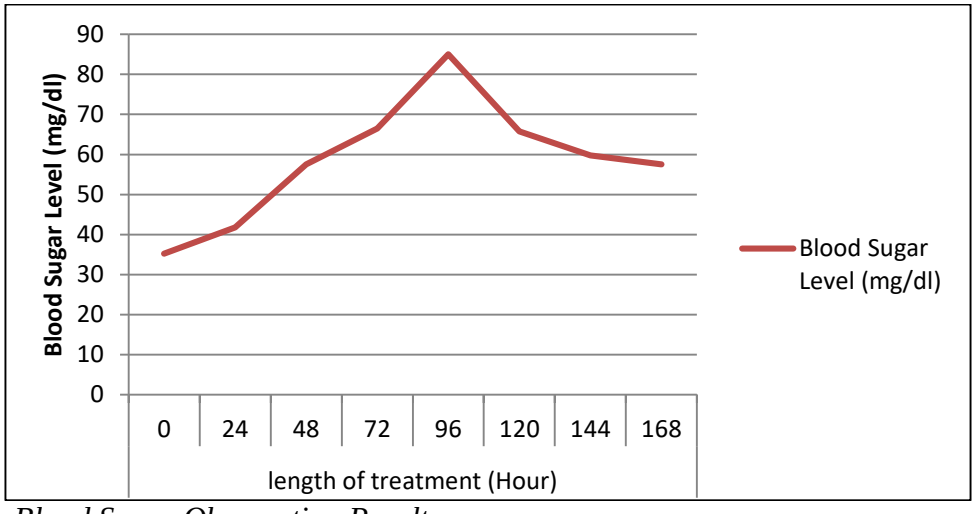


***Figure 3.*** *Blood Sugar Observation Results.*

Table 1 shows the average blood sugar levels in koi fish increased and decreased. At the time before the study of blood sugar levels of koi fish averaged 35.25 mg / dl. The treatment of 24 hours to 96 hours of blood sugar levels of koi fish continues to increase before falling on the treatment duration of 120 hours. The results of data analysis of blood sugar calculations analyzed with Anova can be seen that there is a significant difference ($p < 0.05$) tehadap long treatment of *Myxobolus* spores infection. Based on the Duncan test, it is known that the highest blood sugar yield of koi fish is obtained at 96 hours of treatment which is 85 mg / dl and the lowest blood glucose in the 24 hours treatment time is 35,25 mg / dl. Fish behavior during maintenance can be seen in Figure 3.

**Water Quality**

Water quality measurement data at the time of maintenance showed temperature range 250 C - 260 C, pH range 7, 46 - 7,49, ammonia 0,56 mg / l and dissolved oxygen ranged from 7,97 - 8,13 mg / l. Water quality measurements during maintenance indicate that the aquarium water quality conditions are in normal condition for the survival of koi fish. Water quality data can be seen in table 5.3.

**Discussion**

Based on the result of the research, the effect of the long treatment of *Myxobolus* spores infection orally on the blood sugar level of koi fish is known that blood sugar level of koi fish before injection of spore is 35,25 mg / dl. The blood sugar level is relatively normal based on [5] that is 25-54 mg / dl. Koi blood sugar levels continued to increase after infected *Myxobolus* spores for 24 hours to 96 hours. Increased blood sugar levels occur due to *Myxobolus* spores infection. According to [14] *Myxobolus* can infect Cyprini fish at 8-12 hours. *Myxobolus* infected koi fish shows behavioral changes from the start of 48 hours after fish infection begins to inactivate swimming and decreases appetite. This is different in koi fish on treatment 24 hours after infected fish still looks healthy. Changes in koi fish behavior on treatment 96 hours after infected fish began to swim towards the surface waters to the treatment 168 hours after infected fish look limp and tend to be bottom waters.

Changes in fish behavior can be triggered by stressors [7]. *Myxobolus* became the biological stressor causing changes in fish behavior after oral infection. *Myxobolus* becomes a pathogenic agent that can infect and cause stress. Changes in behavior begin to show when fish have started to stress characterized by reduced appetite, fish body activity increased until long fish activity decreased, swimming at the bottom of the aquarium, and swirling unbalanced [9]. According to [1] another impact of stress is a change of behavior (liveliness) of fish. Fish in stressful situations require the supply of energy from sugar in the blood to deal with stress [6]. Stress will increase blood sugar levels to above normal, according to [5] normal blood sugar levels ranging from 25-54 mg/dl.

In [4] has been stated that *Myxobolus* infestation occurs when spores are released in the waters consumed by the host and enter the intestine. When after the splitting is found *Myxobolus* spores on the koi fish intestine so that the fish has been infected *Myxobolus*. The spores in the intestine release the polar filaments to attach to the cell after the internal part of the spore (sporoplasm) changes its shape to ameboid and penetrates into a cell in the intestine to reach the target organ or as a passenger in the white blood cell [3]. Spores that develop will damage internal organs and breathing in koi fish because the gills form new nodules [4].

*Myxobolus* spores that penetrate will cause damage to the intestine such as haemorrhage, inflammation, necrosis and *Myxobolus* cyst found on the intestinal

tissue of koi fish. The tissue damage will then be responded by the body by forming the body's defense system with the leukocytes migrating to the wound area and lyse the pathogen organism or foreign substances [13]. Damage to the intestinal tissue will increase the activity of leukocytes, platelets and erythrocytes in response to infections that occur. Cortisol, blood sugar, hematocrit and white blood cell counts are stress indicators in fish [15]. When fish experience stress, the number of erythrocytes and hematocrit tends to decrease, while blood sugar, cortisol, and lymphocyte levels increase significantly (Tavares -Diaz et al., 2001).

Increased activity in the fish body will cause homeostasis in the body disrupted and cause stress [1]. Stress due to *Myxobolus* infection will increase glucocorticoids resulting in elevated blood sugar levels. Blood sugar is used to meet the physiological needs of the body and energy needs are high at the time of stress [16]. Increased blood sugar is a secondary response to stress-induced fish, following a primary response that increases the amount of stress hormones such as cortisol and catecholamines from internal cells [2].

Stress due to *Myxobolus* spore infection will be accepted by the brain as a stimulus. The brain through the hypothalamus will command chromafin cells to secrete catecholamines through sympathetic nerve fibers. Catecholamines will activate enzymes involved in the catabolism of glycogen and muscle deposits, thereby suppressing the secretion of insulin hormones, resulting in increased blood sugar [20]. At the same time the hypothalamus will secrete CRF (Corticoid Releasing Factor) which will regulate the pituitary gland to secrete ACTH (Adenocortico-Tropic Hormone), MSH (Melanophore-Stimulating Hormone) and p-End (p-Endorphin). The hormone will regulate the hormone cortisol from the interenal. This cortisol will bully the enzymes involved in gluconeogenesis resulting in an increase in the guladara derived from noncarbohydrate sources. The occurrence of protein catabolism to form sugars also produces acids amino, so that the amino acid in the blood has increased. Increased amino acids in the blood will activate the insulin back so it can transport sugar, so the sugar in the blood will decrease again [6].

Koi blood sugar continues to increase from 24 hours - 168 hours, [15] states that 6 to 48 hours after organisms are adversely affected by chemical changes in the blood (increased cortisol), where the condition is called general alarm reaction (GAR). The highest increase was obtained at 96 hours treatment duration of 85 mg / dl. Increased blood sugar in fish shows the amount of energy used to cope with stress caused by *Myxobolus* spores infection. At the time of treatment 120 hours blood sugar levels of koi fish decreased to 65.75 mg / dl. This is because the energy reserves or blood sugar has been widely used in times of stress due to *Myxobolus* spore infection so that blood sugar levels decreased. The decreased blood sugar is still above the normal blood sugar level in koi fish of 35.25 mg / dl. So the fish is still in a state of stress due to *Myxobolus* infected. Efforts to accelerate the return of blood sugar to normal levels after fish experience stress is necessary so that the growth of fish is not disturbed. During this stressful state fish will continue to maintain body homeostasis that began to change by continuing to remove sugar for energy needs, during the tempo of stress is still ongoing [16].

Water quality measurements show the water temperature of 250 C - 260 C. The temperature of these waters is a normal value for the maintenance of koi fish (*Cyprinus carpio* L) based on SNI 7734-2011. Temperature is one of the most important factors in regulating organism's life processes. Water temperature can affect fish life, because the higher the temperature the lower the oxygen solubility. Simultaneously, the increase in temperature also leads to increased metabolic activity of aquatic organisms so

that the need for oxygen will also increase [12]. In the results of the water quality inspection of koi fish maintenance showed good criteria with the value of DO 7,97 - 8,13 mg / l and the ammonia value 0,56 mg / l based on SNI 7734-2011 (Table 5.4). Good water quality is expected not to be a stressor for fish because the quality of the waters in accordance with the needs of living koi fish. Stressors are expected to originate only from oral *Myxobolus* spores infections resulting in elevated blood sugar levels in koi fish.

## 5. CONCLUSIONS AND RECOMMENDATIONS

The duration of *Myxobolus* spore infection treatment orally affected the blood sugar content of koi fish (*Cyprinus carpio* L) which increased from treatment 24 hours-168 hours. *Myxobolus* spore infection orally had a significant effect on koi fish (*Cyprinus carpio* L) exceeding normal blood sugar level of fish 35.25 mg / dl was highest 85 mg / dl at treatment duration 96 hours after infection and lowest 41.75 mg / treatment 24 hours after infection.

We recommend further research on the measurement of blood sugar levels of koi fish infected by *Myxobolus* spores using different doses and different treatments to cause clinical symptoms or nodules in the gills.

## REFERENCES


1. Barton, B. A., "Stress in fishes: A Diversity of Responses with Particular Reference to Changes in Circulating Corticosteroids1", *Integ. And Comp. Biol.*, 42 (2002) 517-525.
2. Barton, B. A., Ribas, L., Acerete, L., Tort, L., "Effects of chronic confinement on physiological responses of the juvenile gilthead sea bream, Sparus aurata L. to acute handling", *Aquaculture Research*, 36 (2005) 172-179.
3. Dana D., "*Beberapa Parasit dan Penyakit Ikan",* Fakultas Perikanan Institut, TFA, Aberdeen (1984).
4. Farmer, J. N., "The Protozoan Introduction to Protozoology", the C.V. Mosby Company, St. Louis, (1980).
5. Ghittino, P., "Tecnologia e patologia in acquacoltura", *Tecnologia. Torino, Tipografia Emilio Bono*, 1 (1983) 523.
6. Hastuti, S, "Respon Glukosa Darah Ikan Gurami (*Osphronemus gouramy*, LAC.) terhadap Stress Perubahan Suhu Lingkungan", *Jurnal Akuakultur Indonesia*, 2(2) (2003) 73-77.
7. Inglis, V., Roberts, R. J., Bromage, N. R., "Bacterial Diseases of Fish", *Institute of Aquaculture. Blackwell Science,* (1993) 196-210.
8. Insivitawati, E., Mahasri, G. dan Kusnoto, "Gambaran Darah Dan Histopatologi Insang, Usus Dan Otak Ikan Koi (*Cyprinus carpio koi*) Yang Diinfeksi Spora *Myxobolus koi* Secara Oral", *Jurnal Ilmiah Perikanan dan Kelautan*, 7(2) (2015).
9. Ismail, "Kiat Mengatasi Stres Pada Ikan, Buku Panduan Perikanan", *Mediatama*, (2015). ISBN.9797401448
10. Kusriningrum, R. S., "*Perancangan Percobaan*", Airlangga University Press, Surabaya, (2012).
11. Li, P., Ray, B., Delbert M., "Effect of Handling and Transport on Cortisol Response and Nutrient Mobilization of Golden Shiner (Notemigonus crysoleucas)", *Journal of The World Aquaculture Society*, 40(6) (2009).
12. Mahasri, G. dan Kismiyati, Buku Ajar Parasit dan Penyakit Ikan I (Ilmu Penyakit Protozoa pada ikan dan udang), Fakultas Perikanan dan Kelautan, Universitas Airlangga, Surabaya, (2015).
13. Mitchell, R. N., Kumar, Abbas Fausto, "Buku Saku Dasar Patologis Penyakit Robbins dan Cotran, Edisi 7. Penerbit Buku Kedokteran EGC. Jakarta. Hal (2006) 29-59.
14. Oumouna, M., Hoffman, R. W., "Susceptibility of rainbow trout (*Oncorhynchus mykiss*) and carp (*Cyprinus carpio*) to *Myxobolus cerebralis,* the agent of whirling disease", *Journal of cell and animal biology*, 5(14) (2011).
15. Porchaz, M. M, Cordova, L. R. M., Enriquez, R. R., "Cortisol and Glucose: Reliable Indocators of Fish Stress", *Pan American Journal of Aquatic Sciences*, 4(2) (2009) 158-178.
16. Rachmawati, F. N., Susilo, U., Sistina, Y., "*Respon fisiologi ikan nila Oreochromis niloticus, yang distimulasi dengan daur pemuasaan dan pemberian pakan kembali*", Prosiding Seminar Nasional Biologi Universitas Gajah Mada, (2010).
17. Rukyani, A., "Histopathological Changes in the Gill of Common Carp (*Cyprinus carpio* L.) Infected with the Myxosporean Parasite *Myxobolus Koi* Kudo", *Jurnal Perikanan Surabaya*, (1990) 337-341.

18. Samsisko, R. L. W., "*Respon Hematologis Ikan Kerapu Tikus (Cromileptes altivelis) Pada Suhu Media Pemeliharaan Yang Berbeda*", Fakultas Perikanan Dan Kelautan Universitas Airlangga Surabaya, (2013).
19. Satyantini, W. H. dan Masithah, E. D., "Diktat Penuntun Praktikum Budidaya Pakan Alami", Fakultas Perikanan dan Kelautan Universitas Airlangga, Surabaya, Hal. (2008) 28-49.
20. Wendelaar, B. S. E., "The stress response in fish", Physiol. Rev., 77 (1997) 591-625.