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Submission date: 22-Feb-2022 05:22PM (UTC+0800)
Submission ID: 1768235078
File name: C.67.pdf (1.25M)
Word count: 5808
Character count: 29607

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To cite this article: Gunanti Mahasri 2017 *IOP Conf. Ser.: Earth Environ. Sci.* **55** 012009

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Development of Spore Protein of *Myxobolus koi* as an Immunostimulant for Prevent of Myxobolusis on Gold Fish (*Cyprinus carpio* Linn) by Oral Immunisation

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Abstract. Production of Gold fish (*Cyprinus carpio* Linn) in Indonesia has always increased from 2013 to 2015 year by year with increasing average 2% per year. The amount of production was respectively 571.892 tonnes, 1129.273 tonnes, and 1186.674 tonnes. There were almost no problems to sale of gold fish because it had a good enough prospect. The aims of this research were Isolation of spore protein of *Myxobolus koi* by using SDS-PAGE to analyze immun respons and survival rate gold fish that immunized with spore protein of *Myxobolus koi*. The method of this research used experimental method, and belonged to 4 treatments that are: Control, the group of gold fish not immunized with protein spore of *Myxobolus koi* neither infected by *Myxobolus koi* (T1). The group immunized and infested by spore of *Myxobolus koi* (T2). The group which immunized and not infested by *Myxobolus koi* (T3), and The group only infested by *Myxobolus koi* (T4). The dose of immunostimulant was 5 ml in 1 kg of food. The result showed that there were two bands of whole spore protein with molecule weight (MW) 150 kDa and 72 kDa and one band of crude protein *Myxobolus koi* with molecule weight 73 kD and the optical density point was 0.132 on the first day and increased to 0.769 on the 56 th day. The result also showed that the immun respons and survival rate increased from 27% to 86% in chellence test. The protein spore of *Myxobolus koi* can used to develop material for immunostimulant and to prevent the myxobolusis.

1. Introduction

One of the diseases that plagues often become parasiter is a Protozoan disease caused by Myxobollus koi called myxobolusis. This disease started from 2009 was included into quarantine disease pest (HIPK) class I, because the disease causes illness and death of fish. The disease is called Myxobolusis which cause serious problems on the goldfish, koi and lead to the death up to 60-90% with prevalence reaching 100%. Furthermore it is said that in 1974 and 1978 Myxobolus attacked has occurred in Indonesia that led to the deaths of up to 100% of the koi fish mainly on stadia seeds.

The fish that attacked by the myxobolusis disease showed in difficulties to breathe because it found the existence of a nodule or cyst or nodule on the Gill filaments. In Blitar reported by farmers that in 2010 has occurred an outbreak myxobolusis on CARP koi in size 3-5 cm with mortality reached up to 90%.

While in 2002 goldfish mass death has occurred in Kulon Progo and Sleman, caused by a parasite *Myxobolus* sp. and *Henneguya* so that the losses experienced by the fish farmers were quite

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big. *Myxobolus* sp. was also found in Ngrajek, Magelang Regency in 2006 with prevalence reaching up to 91%. Followed the gold fish ponds koi in Blitar prevalence reaches up to 86% in 2010 [1]. The prevention efforts and counter measures against myxobolusis have been done using a disinfectant as well as other chemicals, but not yet able to meet the target, even cause resistance and residues in the body of the fish. It is necessary to look for the alternative prevention efforts which not cause a negative impact. One of the efforts that have already begun to be developed at this time is vaccination that can be done by immersion or injection.

The characterization of protein can be done by using SDS-Page. By using this method we know its molecular weight protein wether suitable for vaccine or not. Itabashi et al[2] and Mahasri[3] have managed to detect a protein core and anti-Zoothamnium arbuscula with cytoplasmic protein spasmin-1 on spasmonema. Immunoblotting analysis of results showed that the protein has a molecular weight of antigennya 68 kDa, 55 kDa and a 71 kDa. Chavda *et al* [4] suggested the results of the analysis of SDS-PAGE electrophoresis of Myxobolus cerebralis spores which infect fish Catla catla retrieved six expressions Ribbon protein with molecular weight 130 kDa and 60 kDa as well as four other 7 kDa protein Ribbon up to 45 kDa. Asri et al [5] showed the result of Myxobolus spores protein isolation koi which infects fish Cyprinus carpio with a method electrophoresis SDS-PAGE was obtained by two Ribbon proteins with molecular weight 70.22 kDa and 22.83 kDa. More Insariani *et al* [6] put forward the results of the insulating surface glycoproteins M. koi using the method of polymerase chain reaction (PCR) obtained a protein with molecular weight of 12 kDa, 25 kDa and 27 kDa.

The function of the receptor cell responses of fish is portrayed by some parameters of immune cells to produce a response form. MHC (Major Hstocompatibility Complex) is one of the molecules that are instrumental in the immune system of the fish. If antigen (vaccine) enters the host cell or body fish then the antigens presented by MHC, antigen will be captured by receptors on T helper cells (2), and helper T cells (2) going to secrete cytokines, namely IL-2, IL-4, IL-6 and aimed at the differentiation and proliferation of B cells, differentiation of B cells and plasma cells will produce memory cells. Next the cell plasma will synthesize specific antibody binds antigens so as to prevent the movement of antigen and eases the process of phagocytosis.

Anderson [7] argued that the immunostimulant is injected into the body of the fish will get into the kidneys and can increase the Adaptive immune response against the vaccine. In the front part of the kidney, macrophages and neutrophil antigens or vaccines will feed that goes into the body of the fish through the process of phagocytosis and then carried towards the thymus for T cell activation triggers. T cells expressing these antigens to special receptor known as the major histocompatibility complex or Major histocompatibility complex (MHC) antigen, then these are carried towards spleen, splenic release will occur in sitokinin form the B cells that cause an increase of the immune cells as a whole. B cells most do poliferasi or reproduce themselves and partly differentiated body cells into plasma B cells and memory as the humoral immune system. Based on that background the purpose of this research is to develop spore protein of *Myxobolus koi* as immunostimulant material to prevent the death of carp (*Cyprinus carpio Linn*).

2. Research Methodology

2.1. Time and Location

This research was carried out from March to November 2013, with the location of the research in the laboratory of Dry of the Faculty of Fisheries and Marine, Airlangga University and the laboratory of molecular biology of the Faculty of Sciences the university of Brawijaya, Malang.

2.2. Research Methods

This research is descriptive research experiments of laboratory, with the purpose to analyze immune response (an overview of fish blood)) of koi fish that vaccinated with protein *Myxobolus koi* as a sub unit material of vaccine to suppress the death of koi fish due to myxobolusis.

2.3. Research Materials

Carps were tested by using 4 aquariums with a capacity of 5 lt of water. Fish stocking density was 5 fish/aquarium with fish size 7-10 cm. Fish used for treatment were healthy fish marinated first in Methylene blue 3-5 g/m3 of water for 5 minutes to clear the organism that clings to the body of the fish. The treatment given is: T1 = 20 goldfish injected with a solution of PBS and without infection spores m. Koi (control), T2 = 20 carps applied with protein whole spores m. koi and infected with 80 spores/fish m. ko, T3 = 10 goldfish vaccinated with whole protein spores m. koi without spores infected m. Koi and T4 = as many as 20 carps without the vaccinated whole protein spores m. koi. Granting of immunostimulant protein from whole spores of Myxobolus koi done in person with a dose of 1 ml protein/kg feed, which refers to research conducted by [8].

The main materials for the isolation and characterization of a protein used are physiological NaCL, ethanol, solvents of percoll gradient, pepsin, HCL, EDTA, KCL, Na2HPO4, KH2PO4, trypsin, sodium citrat, NaHPO4, H2), NaHCO3, glucosa, phenols red 0.5%, NaOH, filter 0.22 um, bovineserum albumin, dextrosa, ETOH, proteinase, ForwardERIB1 primer pairs 5 '-ACCTGGTTGATCCTGCCAG-3'(2-20) and Reverse ERIB10 5'-CCTCCGCAGGTTCACCTACGG-3 ' (2069-2070), 400 UM DNTP, 3 um Mgso4 , yellow and blue dye, agarose, TAE buffer, sybrsafe, 100 DNAladder bp and bp 1, loading dey, tris-HCL, 2-mercaptoethanol, sodium dodecyl sulfate (SDS), bromophenol blue, glycerol, SDS loading buffer, polyacrylamide, stacking gels, ammonium peroxidaisulphate (APS), glycine and TEMED. Sedangkan peralatan utama yang digunakan adalah Haemocytometer, rubber pollicemen, microscope micrometer, autoclaf, tabung centrifuse, swinging rotor, water bath sonicator dan 1 set peralatan SDS-PAGE electrophoresis [9].

While the main equipments used are the Haemocytometer, rubber pollicemen, microscope tube micrometer, autoclaf, centrifuse, swinging rotors, water bath sonicator and 1 set of tools the SDS-PAGE electrophoresis [9].

2.4. Work Procedures

2.4.1. *Samples preparation of spores of Myxobolus koi*

The fish stricken by myxobolusis are washed with aquadest so that the dirt that clings to the body of the fish is gone, and then nodule myxobolus stuck in gills is taken by using a scalpel and tweezers slowly so that the nodules containing spores are not destroyed. The nodules already taken are then placed in Petri disc and given PBS to taste. Nodules are cut several pieces to remove spores by using a scalpel then added aquadest, included in the test tubes and centrifuged at the speed of 2500 rpm for 15 minutes to separate the cells of Myxobolus. Supernatan formed is then discarded and sediment plus aquadest, centrifuged again up to 4000 rpm with speed solidified for 10 minutes. Moreover pellet is calculated by haemocytometer, then added as much as 2 ml PBS and stored at 4oC in the freezer.

2.4.2. *Isolation of whole proteins of spores of Myxobolus koi*

Spores that have been calculated are given PBS to taste then centrifuged at speed 5000 rpm for 10 minutes. Pellets are added buffer lisis 500 µl of Lysis then sonicated in ice (1 minute sonication ½ minute break), carried out repeatedly 10 times. The results of it and then is vortexed (½ minute vortex I minute break) in ice, carried out repeatedly 15 times. The results of a vortex with a speed of 12000 rpm centrifuged for 5 minutes, the supernatan formed were collected and then carried out the analysis of SDS-PAGE. Determination of the concentration of the whole protein Myxobolus spores used Bio-Rad Protein Assay and read using UV-Visible Spectrophotometer with a wavelength of 600 nm.

2.4.3. *Analysis of whole proteins of spores of Myxobolus koi with SDS-PAGE*

The purpose of this activity is to find out the pattern of the molecular weight of each fraction protein. Analysis of the protein is done with a method electrophoresis SDS-PAGE gel separating composition of 12.5% and 5% stacking gel. This is done by electrophoresis method: Running a gel is created and inserted into the plate glass. After hardened on the top put stacking gel prepared.

A total of 10 µ g samples that added Laemly buffers with a comparison of 2:1 is done boiling at 100oC for 5 minutes, put well located in the stacking gel. As a marker used protein with molecular weight in the range 10-180 kDa (New England Bio-Labs). Then do a running on the chamber has filled Electrode Buffers 1 x with 100 Volts, 40 mA. The running process is stopped after a blue marker reaches the bottom plate of the gel. Next gel inserted into the washer solution consisting of 25 ml methanol, acetic acid, 3.7 ml Aquades 100 ml. and Rocked above the shaker for 30 minutes. Repeated washing is done with a solution that is similar to the reduction of the composition of ethanol and acetic acid addition half of previously for 30 minutes. Subsequent washing with a solution of 10% glutaraldehyde and aquadest for 30 minutes. After the washed gel stained with silver nitrate (AgNO3) for 15 minutes, then do the washing with aquades twice each for 2 minutes. Given the color development solution consisting of formaldehyde 3.7%, zitronsauce 5% and aquades. After the tape is visible then the reaction is stopped by adding acetic acid 10%. The results of the gel that has a Band-Band protein looks ready documented.

2.4.4. *Observation on the blood picture (immune response)*

Observation on blood picture of the fish is done as complementary parameters for analyzing immune response koi fish being exposed with crude protein spores Myxobolus koi. The parameters of the image of blood is observed counts (differensial) leukocytes by way of making the preparations of blood review [8]. The making of preparations is carried out by placing the blood review a drop of blood on a glass object first, object second pair of glasses with glass objects against angle 27o first, then pulled up to touch the blood, the blood is allowed to spread along the edge of the glass, and then the second object glasses of the second object is pushed along a surface of glass objects first so that it forms a thin layer of blood evenly.

Furthermore preparations were drained at room temperature then fixed with absolute methanol for 3 minutes and dried at room temperature back before tinged with Giemsa dye 10% for 15 minutes, then wash it back with aquadest preparations to reduce excess color and dried at room temperature. Once it is observed under a microscope with 1000x zoom. Leukocyte percentage calculated by way of observing 10 viewpoint and each type of Leukocyte that counted was grouped and presented according to type, in percent (%).

2.4.5. *Determination of Survival Rate (SR)*

Determination of level of survival rate is performed as the supporting parameter for analyzing immune response of koi fish in each treatment. The level of survival rate is expressed in the form of a percentage of the number of koi fish that lives up to the 14th day post treatment trial against the total number of fish kept. Fish survival rate is calculated by using the formula:

$$SR = \frac{Nt}{No} x100\%$$

Description:
SR = survival Rate
NT = the number of fish that live at the end of the observation
No = the number of fish that lived in the early test challenge

Data obtained consists of qualitative and quantitative data. Qualitative data is character data of protein in the form of protein molecules that Spore is shown as an image (a protein Band) results from elektroferesis of Gel SDS. While quantitative data is data analysis results description of blood and Survival Rate were analyzed using the statistical test ANOVA (analysis of Variance) and if there is a difference, it is continued by Duncan's Multiple Range Test) with a confidence level of 5%.

3. Results and Discussion

3.1 The Characterization of Protein Spores with SDS-PAGE

Identification of the results showed that the morphology of spores observed in accordance with the identification of Alvin and Matt [10] is spore M.koi. Spores are elliptical-shaped or Cone, had two twin polar capsules located in the anterior portion, pinkish white nodules, irregular round nodules form and located in the gills. While the results of the counting of the number of spores collected from carp (Cyprinus carpio) is 8 x 10^8 spores. The morphology of the spores found in the goldfish can be seen in Figure 1.

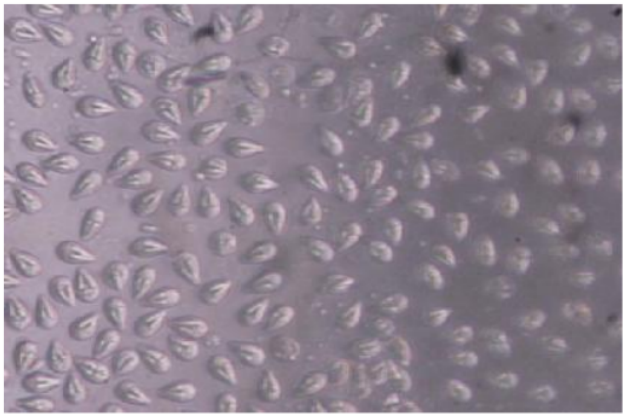

Figure 1. Myxobolus spores on Goldfish (400X Zoom)

3.2 Myxobolus Spores Protein by SDS-PAGE

Analysis results of Myxobolus koi spores protein with SDS-PAGE can be seen in Figure 2. that shows that 2 bands of soluble protein are found with molecular weight 150 kDa and 72 kDa and a Band of protein crude of Myxobolus koi with molecular weight 73 kDa.

3.3 Examination an Overview of White Blood Cells

Examination results of blood overview of Goldfish that is applied by Myxobolus koi spores protein are presented in table 1, which shows that the highest number of erythrosit occurred at the treatment K1 i.e. 88.21% and at lowest of at K2 i.e. 30,43%. While the highest number of leukocytes occurs at the K2 treatment i.e. 71.57% K2 and clearly different from treatment K3that is 63,08%.

Table 1. The results of the determination of the Blood of Goldfish exposed by Spore Proteins After 21 days of maintenance.

Treatments	Description of gold fish blood after 21 days of maintenance (%)	
	Erythrosit	Leukosit
control, not infected and not exposed by protein (K1)	88.21	11.79
Infected by Myxobolus and exposed with Spore Protein dose 600 µl/fish (K2)	28.43	71.57
Infected by Myxobolus and not exposed with Spore Protein dose 600 µl/fish (K3)	34.92	63.08
Not infected by Myxobolus and exposed with Spore Protein dose 600 µl/fish (K4)	59.63	41.23

Description: different superscript on the same column indicates the existence of the real difference ($p < 0.05$)

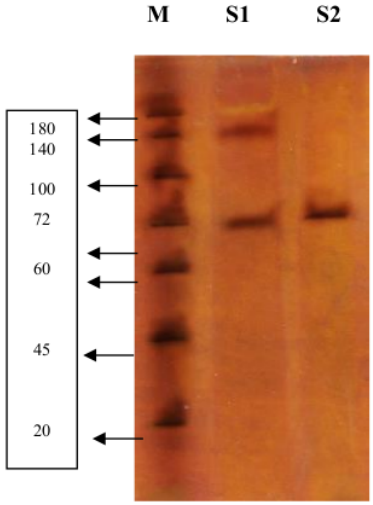

Figure 2. Profile of protein with SDS-PAGE method. M = marker 20-180 kDa, S1 = samples of whole protein, S2 = sample of solubel protein.

3.4 Determination of Differensial Leukocytes of Carps exposed by Protein Spores.

The results of the examination of white blood cells (leukocytes) can be seen in table 2, which indicates that the highest percentage of lymphocytes occurring on protein without the exposure of infection treatment Myxobolus koi (K2) 59.40%, then followed the treatment K3, K1 and K4 40, consecutive 58.20; 56.30 and 53,42%. The highest percentage of monocytes in the K2 's treatment of 21.20%, then followed in the treatment of 20,40% K3, K4 40 treatment of 19.23% and lowest occurred on treatment K1 of 13.20%. The highest percentage of neutrophils in the treatment of 20.20% K1 and K2 treatment occurs at the lowest (8, 14%) i.e. precisely on the treatments being with pprotein. While the highest percentage basophils occurs at the treatment i.e. K1 1.50% and lowest on K3 's treatment of 0.40%.

Table 2. The image of Blood white (Leukocytes) in Carp (*Cyprinus carpio*)

TREATMENT	KINDS OF LEUKOCYTES (%)				
	LIMPHOCYITE	MONOCYTES	NEUTROPHILS	EOSINOPHILS	BASOPHILS
K1	52.40	16.40	20.20	8.60	1.50
K2	59.40	21.20	8.14	10.55	0.71
K3	58.20	20.40	9.60	11.40	0.40
K4	54.00	19.23	14.30	11.26	1.21
NORMAL	**52.00**	**18.00**	**20.00**	**8.00**	**2.00**

3.5 Determination of Survival rate of Carp Exposed by Spore Protein.

Survival rateof koi fish is expressed in percentage and calculated on day fourteen presented in table 3. In the table 3. the test shows that in M. Koi spore protein protection test, there is a noticeable difference ($p < 0.05$) between the treatment of average survival rate between being and not being with spores of Myxobolus koi after 21 days of maintenance.

Table 3. The Results of The Determination of Survival Rate of Koi Fish Exposed by Spore Protein After 21 days of Maintenance

Treatment Group	Survival rate of Carps after imminized
Control, not infected and not exposed with Protein (K1)	96.00^{a} ± 2.24
Infected by Myxobolus and exposed with Dipapar spore Protein doses 600 µl/fish (K2)	86.00^{b} ± 6.52
Infected by Myxobolus and not exposed by spore Protein dose 600 µl/fish (K3)	27.00^{c} ± 3.54
Not Infected by Myxobolus and exposed by spore Protein dose 600 µl/fish (K4)	98.00^{a} ± 2.94

Description: different Superscript on the same column indicates the existence of the real difference ($p < 0.05$)

3.6 Examination of Water quality on maintenance media

The results of examination of water quality of maintenance of water quality parameters: temperature, pH, dissolved oxygen, nitrite, nitrate and ammonia can be seen in Table 4.

Table 4. The results of examination of water quality of maintenance during studies

No.	Parameter	Treatment			
		K1	K2	K3	K4
1.	Temperature (Celsius degree)	30	30	30	30
2.	Dissolved Oxygen (ppm)	5	5	4.0	4.0
3.	pH	8	8	8	7.5
4.	NH3	0.2	0.2	0.3	0.3
5.	NO2	0.3	0.3	0.4	0.4

Table 4 indicates that the water quality parameters during maintenance in optimal conditions for the maintenance of the fish, although in media exposure with the maintenance of the protein and the presence of infection with myxobolus koi showed a decline, but still in the normal range.

The results of the analysis of the whole protein spores M. koi used SDS-PAGE 2 band which seem obvious, namely protein with molecular weight (BM) 150 kDa and 72 kDa and one band of crude protein Myxobolus koi 73 kDa molecular weight .The results showed that proteins found have a high molecular weight. Other studies conducted by Chavda et al. (2010) shows the results of the SDS-PAGE electrophoresis analysis of Myxobolus cerebralis spores obtained expressions band protein with molecular weight 130 kDa and 60 kDa as well as four bands other protein with BM 7 kDa to 45 kDa. Protein molecular weight differences on both the research allegedly because of differences of origin, preparation techniques and sampling technique of insulation used. immunogenic proteins are proteins that have an molecular weight between 20,000-100,000 Dalton. A molecule has munogen properties when its weight is more than 10 kDa. Based on these statements, whole spore protein molecular weight of Myxobolus koi of the analysis result by SDS-PAGE BM has more than 10 kDa, but to ascertain the nature of immunogenic, it needs to do test again with imunoblotting so that potential of immunogenic properties of whole spore proteins of Myxobolus koi can be utilized as a candidate of vaccine in tackling myxobolusis.

Fish have a defense system of the body to fight various diseases. Fish immune defences consists of non-specific and specific defences. Non-specific defenses consist of skin, scales and

mucus which is the body leading the defense in the face of the onslaught of various microorganisms that enter. Specific defences on a fish that comprise the macrophage cells, Natural Killer cells and leukocytes. Specific defence system takes time to identify antigens before able to give a response [11].

According to Guyton [12], leukocytes or white blood cells that make up the immune system is the most active units because it acts against various infectious diseases and foreign materials. Leukocytes are found in the bone marrow (mieloid tissue) and partly on the lymph tissue, then remained stored in the bone marrow and will increase in number when required. Leukocytes of fish composes of granulocytes (neutrophils, eosinophils, and basophils) and agranulosit (lymphocytes and monocytes).

Changes in the total number of and types of leukocytes can be used as an indicator of the presence of certain infectious diseases that occur in fish. Leukocytes in blood components is one that serves as a specific body defenses that will neutralize and destroy pathogens through phagocytosis. Lymphocytes is one form of leukocytes. The observations of lymphocytes in the table 1 showed the highest percentage in the treatment of K2 (vaccinated by whole spore protein M. koi and not infected by 80 spores/fish of M. koi) of different real 59.40% with treatment K3 (exposed by whole spore protein of M. koi and infected by M. koi) and K1 (control). but the treatment did not differ markedly with K4 treatment (without exposed with whole spore protein of M. koi and infection with 80 spores/fish of M. koi). Normal lymphocyte percentage of the fish ranges between 60-70% [13].

An increased number of lymphocytes in vaccinated and infected fish with spores of M. koi is the body's defense system response of fish over the entry of pathogens. This is in accordance with the opinion of Bastiawan et. al. [14] lymphocytes functions as antibody producer for immune to interference from disease. Lymphocyte cells essentially consists of two populations i.e. B cells and T cells. B cells have the ability to transform into plasma cells i.e. cells which produce antibodies and memory cells. T cell has important role for immune (cytotoxic T cells) and control the immune response (suppressor T cells). After the binding of an antigen with antigen receptor cell lymphocytes, lymphocyte cells will then divide and differentiate into effector cells and memory cells.

Monocytes of fish are round oval, located in the middle of the nucleus and the cytoplasm has no bergranula. There are monocytes in blood circulation and in the number of little limfonodus, embedded in bone marrow and lymph. Monocytes migrate from blood circulation towards to the tissue when it receives the appropriate stimulation with its reseptor. Immature monocytes leave the blood circulation, to and settled in the tissue, and then develop into macrophages (Feldman et al., 2000). The observations of each treatment are shown on monocytes Table 2. The highest percentage of monocytes in treatment (K2) of 21.20% followed by treatment of K4 and K3. An increase in the number of monocytes in treatment of K2, K4 and K3 when compared with controls.

This increase is due to the monocytes fagosits spores M. koi in the body of the fish. This is in accordance with the opinion of Ardelli and Woo [15] stating that the monocyte with macrophage will fagosit along with disease-causing agents that enter the body. Bastiawan et. al. [14] explains, the monocytes function as phagocytes against foreign objects which act as the agent of the disease. Further Kresno (2001) argued the role of monocytes is very important, as the cells phagocytes to destroy a variety of main pathogens and acts as an antigen presenting cells (APC) that function to present antigen to the lymphocytes cells.

Neutrophils in fish is formed in kidney organ in the front. The highest percentage of neutrophils in the treatment of the K1 (control) of different real 21.20% with treatment of the K2, K3 and K4 as shown in Table 2. A decrease in the percentage of neutrophils in the treatment of the K2, K3 and K4 when compared to control due to the increase in lymphocytes and monocytes neutrophils so decreased. Affandi and Tang [13] suggests that the percentage of neutrophils in the blood of fish ranged between 6-8% of total leukocytes. In addition neutrophils do not too plays a role in responding to an infection caused by a parasite. More neutrophils play a role in the infections caused by bacteria.

The observations of the number of eosinophils showed the highest percentage in treatment of K3 11.40% that tends to be decreased in treatment of K2, K1 and K3. Spore infection of M. koi

increases the number of eosinophils in the blood of fish. This is in accordance with the opinion of the Tizard [16] stating that eosinophils is one of the body's defense cells that are dominant in the blood and will increase sharply in number in case of an infection of parasitic diseases. Eosinophils formed on haematopoiesis process that occurs in the bone marrow before migrating into blood circulation. They contain a number of chemical substances include histamine, eosinophils peroxidase, Ribonuclease, deoksiribonuklease, lipase, and some amino acids are produced through a process of degranulation after they are activated. These substances are toxins against parasites and body tissues.

Observation of basophils on treatment indicated a small percentage compared to the other types of leukocytes. The highest percentage basophils in treatment of 1.5% K1 also tend to decline treatment K3, K2 and K3. Affandi and Tang [13] stated that the percentage basophils in the blood of fish ranged from 0.17-0.19% and measuring 8-12 µm. The existence of basophils in blood circulation has been observed only in a small number of species of fish. Even more rarely basophils are found on examination of blood compared to eosinophils.

4 Conclusion

Catfish (Clarias gariepinus) cultured in Moro Krembangan Surabaya, East Java, are mostly infected with Aeromonas hydrophila and *Saprolegnia* sp., It is caused by the environmental conditions that are unfavorable. The percentage of catfish (Clarias gariepinus) infected with *Aeromonas hydrophila* is 95% and infected with *Saprolegnia* sp. is 90%. The conclusions that can be presented from this research is that there are 5 profiles of spore protein bands of Myxobolus sp that are obtained spores with molecular weight. Whole spore proteins of Myxobolus koi is found with molecular weight 150 kDa and 72 kDa. This spore protein of Myxobolus koi can enhance the immune response and survival rate of koi fish from 10% up to 86% and can increase the body's defenses against infection of Myxobolus koi , so that it could be developed as immunostimulant.

Acknowledgement

Financial support for this research was provided by Ministry of Research Technology and Hightly Academic National. The authors wish to thank for Prof.H. Hery Purnobasuki, Drs.,M.Si.,Ph.D. Head of Research and Inovation Institute, Airlangga University.

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