

## Non-Specific Immune Potentiating activity of Multivitamins in Catfish (*Clarias sp.*)

Cahyono Purbomartono<sup>1\*</sup>, Ulfatun Rizkiyah<sup>2</sup>, Dini Siswani Mulia<sup>2</sup>, Arief Husin<sup>2</sup>

<sup>1</sup>Department Aquaculture, Faculty of Agriculture & Fisheries, Universitas Muhammadiyah Purwokerto

<sup>2</sup>Department Biology, Faculty of Teacher Training and Education, Universitas Muhammadiyah Purwokerto

\*corr-author: cpurbomartono@yahoo.com

### ABSTRACT

Fish farming is well developed because many people like to consume fish. The main problem in cultivation is an attack of a disease. Generally, the disease in fish is MAS (Motile Aeromonas Septicemia) which is caused by *Aeromonas hydrophila*. Prevention of diseases in fish can be done by increasing immunity through the addition of multivitamins in feed. This study aims to determine the ability of multivitamins against non-specific immune responses in African catfish (*C. gariepinus*) as well as to know the optimum dose. The study used an experimental method with a completely randomized design using 3 treatments, 1 control, and 4 individual replications. The treatment using a multivitamin through feed with a dose of T1 = 2 g kg<sup>-1</sup>, T2 = 2.5 g kg<sup>-1</sup>, T3 = 3 g kg<sup>-1</sup> and T0 = control. The parameters observed were phagocytic activity, and differential leukocytes of monocytes and lymphocytes. Data were analyzed using Analysis of variance (ANOVA) at the 95% confidence level. If there is a real difference, then proceed with the Duncan Multiple Range test Test (DMRT) at a 95% confidence level. The results showed that the addition of multivitamins through feed on African catfish had a significant effect ( $p < 0.05$ ) on phagocytic activity and differential leukocyte of monocytes, but had no significant effect ( $p > 0.05$ ) on differential leucocytes of lymphocyte. The optimum dose for increasing non-specific immune responses in African catfish is T2 at a dose of 2.5 g kg<sup>-1</sup> of feed. Based on research, the addition of multivitamins through the feed can potentially be used in fish farming to increase immunity.

**Keywords:** multivitamins, catfish, immune non-specific

### INTRODUCTION

African catfish (*Clarias gariepinus*) are freshwater fish that have been commercially cultivated in Indonesia. The cultivation of African catfish is growing rapidly because it has prospects of high economic value (Bachtiar, 2006). This is proven by the increasing demand in the market so it has an impact on development in Indonesia.

African catfish cultivation is carried out intensively by utilizing limited land and high stocking density. The main problem in intensive cultivation is the increased levels of organic matter in water caused by feces. Feces will accumulate in water which can increase the concentration of ammonia which is toxic to fish (Effendi, 2003). According to Floyd *et al.* (2015), ammonia in water can make fish susceptible to bacterial infections and inhibit

growth. The high stocking density and ammonia in water are a cause of disease due to decreased water quality. This causes the fish to become stressed and thus vulnerable to disease (Afriyanto & Liviawaty, 2006). Prevention of diseases in African catfish can be done using vaccines and antibiotics. However, vaccines are specific to certain pathogens, have limited availability, and are expensive. Even though antibiotics have long been used in the prevention of fish diseases, the fact shows the use of antibiotics that are not following the dose can cause pathogenic organisms to become resistant and accumulate in fish and environmental pollution (Lengka *et al.*, 2013). Likewise, the administration of antibiotics causes the emergence of pathogens that are resistant to disease (Marentek *et al.*, 2013). Therefore, the current use of antibiotics has been very limited so there is a need for alternatives in prevention that are more environmentally friendly. One of them is by increasing fish immunity by administering immunostimulants (Syahida *et al.*, 2013)

According to Raa (2000), immunostimulant is a material that can increase non-specific immunity. At present, the use of immunostimulants was developed as a method of disease control in fish and shrimp culture. Rawung & Manoppo (2014), said that immunostimulants are added to feed can increase fish resistance to disease infections through increasing non-specific immunity. Immunostimulants that can be applied to fish are plant or animal extracts, polysaccharides, bacterial components, and multivitamins. This study uses a multivitamin added to fish feed. The addition of multivitamins in this study was based on the various roles of multivitamins in increasing fish immunity and growth. Among these multivitamins are vitamin B complex, C, E, and A (Sakai, 1999). According to Esmaeli & Khara (2013), folic acid can increase growth, FCR, Survival Rate, and the number of lymphocytes and neutrophils in *Oncorhynchus mykiss*. Likewise, the addition of vitamin C in the diet can increase the non-specific immune response of the mud grouper, *Epinephelus coioides* (Johnny *et al.*, 2007).

## RESEARCH METHOD

This research material used a multivitamin and catfish. Catfish bought from the Purbalingga fish market then maintained in the Laboratory of Zoology of the Biology Department for acclimatization. During acclimatization, the fish are fed commercial pellets. After the fish are stable, familiar with pellet feed and no one dies, then the research begins. This study used an experimental method with a completely randomized design, consisting of 3 treatments and 1 control each with 4 replications. The treatment was the addition of a multivitamin into the feed at a dose of T1 2.5, T2 5, T3 7.5 g kg<sup>-1</sup> feed and control, with 4 individual replications. The fish are kept in a bucket of 4 fish. The research was conducted for 15 days with 3% feed by weight of biomass with a protein content of 31% given in the morning and evening. Data were analyzed using one-way ANOVA and Duncan's multiple range test (5% level of significance), if differences were detected, using SPSS software (version 21.0).

## RESULT AND DISCUSSION

The results showed the addition of a multivitamin through the feed can increase the immunity in catfish. The addition of multivitamins in catfish can significantly ( $p < 0.05$ ) increase phagocytic activity (Table 1) and monocyte percentage (Table 2), but not significantly ( $p > 0.05$ ) to the percentage of lymphocytes (Table 3).

Increased phagocytic activity is thought to influence the presence of vitamin C contained in a multivitamin. Johnny *et al.* (2007) in their report revealed that vitamin C can enhance the immune response of non-grouper mudfish. Immunostimulant works by

increasing the activity of phagocytic cells to eliminate foreign particles or pathogens (Raa, 2000). Hastuti (2012) states, increased immune can be known from phagocytic cell activity. Cell phagocytes function to carry out phagocytosis of foreign objects that enter the host.

**Table 1. Phagocytic activity of catfish after treatment with multivitamins**

Parameter	Phagocytic activity			
	day-0	day-4	day-8	day-12
I0	68.27 ± 4.74 <sup>a</sup>	76.35 ± 9.68 <sup>a</sup>	93.05 ± 3.78 <sup>a</sup>	97.73 ± 3.35 <sup>a</sup>
T1	68.03 ± 4.46 <sup>a</sup>	79.17 ± 1.89 <sup>a</sup>	99.71 ± 2.77 <sup>b</sup>	99.38 ± 1.60 <sup>a</sup>
T2	51.75 ± 29.82 <sup>a</sup>	88.99 ± 1.52 <sup>b</sup>	98.74 ± 1.02 <sup>b</sup>	99.97 ± 2.71 <sup>a</sup>
T3	68.27 ± 4.74 <sup>a</sup>	87.42 ± 1.98 <sup>b</sup>	98.77 ± 3.21 <sup>b</sup>	99.37 ± 1.22 <sup>a</sup>

The phagocytic index is significantly different in catfish. According to Rustikawati (2012) phagocytes is the most powerful and most important part of the immune system as well as immediately fighting the invasion of micro-organisms after entering the body. Multivitamins added to the feed in this study contained vitamin E. Vitamin E is known to increase immunity, as reported by Milad *et al.* (2001), the addition of vitamin E and selenium produced significant differences in index and activity phagocytosis. Supplementation of vitamin E as an antioxidant can protect the integrity of phospholipids in cell membranes from oxidative reactions. This affects the function of immune cells, especially T-cells in interacting with pathogenic compounds, and at the same time increases T-cell proliferation. Increased phagocytosis index shows the ability of African catfish against foreign bodies entering the body increases due to the addition of multivitamins into the feed which has immunomodulatory activity. Besides containing vitamin E, the added multivitamin also contains vitamin C. This addition of vitamin C can increase the non-specific immune response of the mud grouper, *Epinephelus coioides*.

The addition of a multivitamin through feed significantly increases the differential leukocytes of catfish in the percentage of monocytes (Table 2) but not in the percentage of lymphocytes (Table 3).

**Table 2. Differential leucocyte of monocyte in catfish after treatment with multivitamins**

Parameter	Percentage of monocyte (%)			
	Day-0	Day-4	Day-8	Day-12
T0	27.50 ± 1.000 <sup>a</sup>	27.00 ± 0.816 <sup>a</sup>	26.75 ± 0.500 <sup>a</sup>	25.50 ± 1.000 <sup>b</sup>
T1	27.50 ± 1.000 <sup>a</sup>	27.00 ± 1.155 <sup>a</sup>	26.50 ± 1.291 <sup>a</sup>	25.00 ± 0.816 <sup>ab</sup>
T2	26.50 ± 0.577 <sup>a</sup>	25.75 ± 1.258 <sup>a</sup>	26.25 ± 0.500 <sup>a</sup>	24.25 ± 0.500 <sup>a</sup>
T3	26.50 ± 0.577 <sup>a</sup>	25.75 ± 1.258 <sup>a</sup>	26.00 ± 0.000 <sup>a</sup>	24.75 ± 0.500 <sup>ab</sup>

Monocytes are phagocytic cells that are very important in non-specific immune responses. Monocytes are cells that can penetrate the walls of capillaries to enter tissues and differentiate into macrophages. Moyle & Cech (1988) reported that monocytes in peripheral blood circulation can increase due to infection or foreign bodies in the tissues or bloodstream. Monocyte percentage in fish as much as 0.1% of the total circulating leukocytes (Roberts, 1978). the increased percentage of monocytes in this research is thought to be due to a mild natural infection. this as stated by Maftuch *et al.* (2013), improves monocytes due to bacterial infection and then turn into macrophages to the site

of infection to carry out phagocytosis. monocytes are the main cells that play a role in the process of phagocytosis and kill pathogenic bacteria.

Conversely, the addition of multivitamin catfish did not cause a significant increase in lymphocytes (Table 3). Dellman and Brown (1989) in Taukhid *et al.* (2010) explained that the resistance activity of white blood cells causes the number of lymphocytes to decrease because these lymphocytes function to provide immune substances for body defense. Rustikawati (2012) explained that an increase in the intensity of natural infections by pathogens will trigger the need for lymphocytes. Lymphocytes are cells that specifically recognize and respond to antigens from outside and as mediators of cellular and humoral immunity. Cell B lymphocytes are cells that can produce antibodies and recognize extracellular antigens.

**Table 3. Differential leucocyte of lymphocyte in catfish after treatment with multivitamins**

Parameter	Percentage of lymphocyte (%)			
	Day-0	Day-4	Day-8	Day-12
T0	66.50±0.577 <sup>a</sup>	66.50±0.577 <sup>a</sup>	67.50±0.577 <sup>a</sup>	68.50±1.000 <sup>a</sup>
T1	66.50±0.577 <sup>a</sup>	66.50±0.577 <sup>a</sup>	67.50±0.577 <sup>a</sup>	68.75±0.957 <sup>a</sup>
T2	66.75±0.500 <sup>a</sup>	67.25±0.500 <sup>a</sup>	67.50±0.577 <sup>a</sup>	69.50±0.577 <sup>a</sup>
T3	66.50±0.577 <sup>a</sup>	67.00±0.816 <sup>a</sup>	67.25±0.500 <sup>a</sup>	69.25±0.957 <sup>a</sup>

Whereas if the percentage of lymphocytes increases, it is described by Rustikawati (2012), that an increase in the intensity of natural infections by certain pathogens will trigger the need for lymphocytes. Lymphocytes are not phagocytic but play an important role in the formation of antibodies (Bratawidjaja, 2006). This indicates the possibility of a mild infection that causes an increase in lymphocytes. Lymphocytes are cells that specifically recognize and respond to external and subsequent antigens as mediators of cellular and humoral immunity. Cell B lymphocytes are cells that can produce antibodies and recognize extracellular antigens. Cell T lymphocytes are intermediate immune cells, that recognize intracellular antigens and function to destroy microbes or infected cells (Abbas & Lichman, 2005).

## CONCLUSION

The addition of a multivitamin in catfish through the feed can increase immunity. It can significantly ( $p < 0.05$ ) increase phagocytic and monocyte percentage, but not significantly ( $p > 0.05$ ) to the percentage of lymphocytes.

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