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THE EFFECT OF EM4 PROBIOTIC ADMINISTRATION FOR GROWTH AND SURVIVAL OF VANAME SHRIMP (*Litopenaeus vannamei*)

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ABSTRACT

Vannamei shrimp is a shrimp that has high economic value in Indonesia. The poor aquatic environment can reduce shrimp growth rates and stress and cause death, and efforts to improve water quality by giving Probiotics EM4. Probiotic EM4 contains beneficial bacteria that can improve shrimp's digestive system, decompose organic matter in the waters, control the growth of pathogenic bacteria, and improve water quality. The experimental parameters included total hemocyte count (THC), differential hemocyte count (DHC) consisting of hyaline cells, granule cells, and semi-granule cells, and phagocytic activity (AF). The study was carried out for 69 days using vanamei shrimp with PL 30. This study used four treatments and three replications, namely treatment P0 (without giving probiotic EM4), P1 (giving probiotic EM4 at a dose of 0.5 ml/L), P2 (giving probiotic EM4 at a dose of 1.5 ml/ L), P3 (giving probiotic EM4 dose of 2.5 ml/L). The study's results proved that the administration of probiotic EM4 on the maintenance medium had a significant effect ($P<0.05$) on THC, DHC, and AF. At THC, the highest value was at P3 with a value of 19.44 x 10 6 cells/ml, and the lowest was at P0 with a value of 11.01 x 10 6 cells/ml, then the DHC values such as hyaline cells ranged from 51.34 – 65.81%, granule cells ranged from 21.33 – 30.4%, semi-granular cells ranged from 12.84 – 18.24%. And then for the highest AF value at P3 with a value of 67.97% and the lowest P0 with a value of 49.34%.

INTRODUCTION

Vaname shrimp is one of the shrimp with high economic value in Indonesia. Vaname shrimp is also a shrimp that is very easy to cultivate. The market demand for vannamei shrimp is increasing every year. An increase in the amount of vannamei shrimp production in Indonesia is also followed by an increase in production in several regions. One of the provinces that have experienced an increase in the Province of West Nusa Tenggara (NTB). Vaname shrimp production in NTB in 2013 amounted to 56,960 tonnes to 75,808 tonnes in 2014,

Directorate General of Aquaculture (DJPB, 2015).

Water quality management is a significant factor in supporting the success of aquaculture by improving water quality, one of the efforts made by administering probiotics. Probiotics contain most of the microorganisms that can increase the decomposition of waste and can improve water quality. According to Akbar et al. (2013), the application of probiotics aims to increase the growth and survival of larvae so that they can help increase the amount of production. as well as the types of probiotics that can be applied to aquaculture activities, namely Probiotic EM 4 (Effective Microorganism 4).

EM4 Probiotics are Useful for increasing the bacteria that decompose organic matter, controlling the growth of pathogenic bacteria, improving water quality in ponds or ponds, and strengthening the body's resistance to fish/shrimp, so they are resistant to disease. According to this, research was conducted on vannamei shrimp (Litopenaeus vannamei) by administering EM4 (Effective Microorganism 4) probiotics to determine their effect on growth and survival. EM4 probiotics are mixed cultures in several very beneficial microorganisms.

METHODOLOGY

The biota used in this study were vannamei shrimp with PL 30 from PT. Superior seeds with a stocking density of 1 head/liter. The rearing containers were 12 80 L plastic buckets filled with 60 L water. In rearing, the feed was given ad libitum method as much as 6% of body weight, and the feed was given at 07.00, 11.00, 15.00, 19.00, and 22.00. The feed used in this study was Feng Li Gold FL1 of the crumble type, with a protein content of 40%.

The composition of the treatments in this study was treatment P0 (without giving EM4 probiotics), P1 (giving EM4 probiotics at a dose of 0.5 ml/L), P2 (giving EM4 probiotics at a dose of 1.5 ml/L), P3 (giving EM4 probiotics at two doses). ,5 ml/L) . The study was carried out for 69 days, during which maintenance was carried out for 60 days, and continued with a challenge test for nine days using V. parahaemolyticus bacteria, which were injected into the back of the shrimp as much as 10 6 CFU/ml per shrimp, data were collected on parameters of the immune response and the number was calculated. Dead shrimp as shrimp survivors at the end of the challenge test (Azhar, 2018). The variables studied included the total hemocyte count (THC), differential hemocyte count (DHC), phagocyte activity (AF), and water quality parameters.

Total hemocytes (THC) were observed by taking 0.1 ml of hemolymph from the base of the fifth leg with a 1 ml syringe containing 0.2 ml of the anticoagulant. The anticoagulant and hemolymph mixture was homogenized for 5 minutes. The initial drop of hemolymph is discarded, then dripped on the hemocytometer. Vaname shrimp's total hemocytes were counted using a hemocytometer, and the number of cells per ml was counted using a light microscope with a magnification of 40x (Arifin & Supriyono, 2014). The formula used is as follows:

$$\text{THC} = \text{Rata-rata jumlah sel hitung} \times \frac{1}{vol\ Haemocytometer} \times \text{FP}$$

Information :

FP: dilution factor

Hemocyte differential observation (DHC) was carried out by taking 0.1 hemolymph from the base of the fifth leg with a syringe filled with 0.2 ml of the anticoagulant. Then the hemolymph and anticoagulant mixture was homogenized for 5 minutes, placed on a glass

object, and a review of the hemolymph was made, then air dried and fixed with 100% methanol for 15 minutes. After fixation, it was air-dried and stained by soaking in 10% Giemsa solution for 15 minutes. The colored reviews are then washed using running distilled water and then allowed to dry. The preparations were observed under a microscope with 40x magnification and then differentiated by type: granular and hyaline cells (Ekawati et al ., 2012). The percentage of each hemocyte cell is calculated by the formula (Bunga R Tampangallo et al ., 2012) :

$$\text{Persentase jenis sel hemosit} = \frac{\text{Jumlah tiap sel hemosit}}{\text{Total hemosit}} \text{x } 100\ \%$$

Phagocytic activity (AF) was observed by taking 0.1 ml of hemolymph and putting it into the efendorf, then adding 25 µl of Staphylococcus aureus bacterial suspension (10 7 cells/ml) and mixing evenly and incubating for 20 minutes. The mixture is then taken up to 5 µl to be prepared on the smear preparation. Then fixed with methanol for 5 minutes, air dried, and soaked for 15 minutes in Giemsa solution. The preparations were washed with distilled water and then dried. Observations were made under a light microscope using 400x magnification. Phagocytic activity is calculated based on the percentage of cells carrying out phagocytic processes with the formula:

$$\text{AF} = \frac{\text{Jumlah sel yang melakukan fagositosis}}{\text{Jumlah sel fagosit}} \text{X } 100\%$$

RESULT

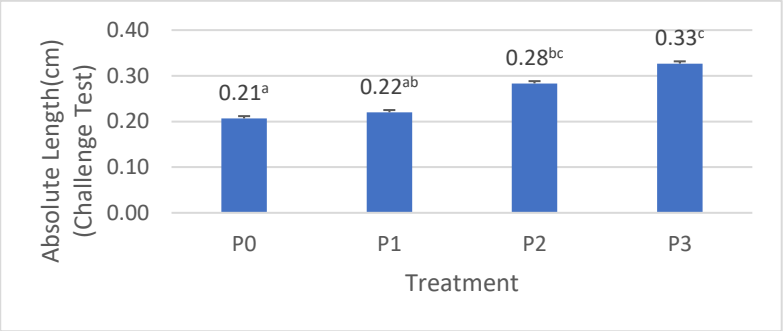

Figure 1. Total hemocyte count (THC)

The results of the analysis of variance (ANOVA) showed that after a challenge test was carried out on vannamei shrimp given EM4 probiotics, the results were significantly different ($P<0.05$).

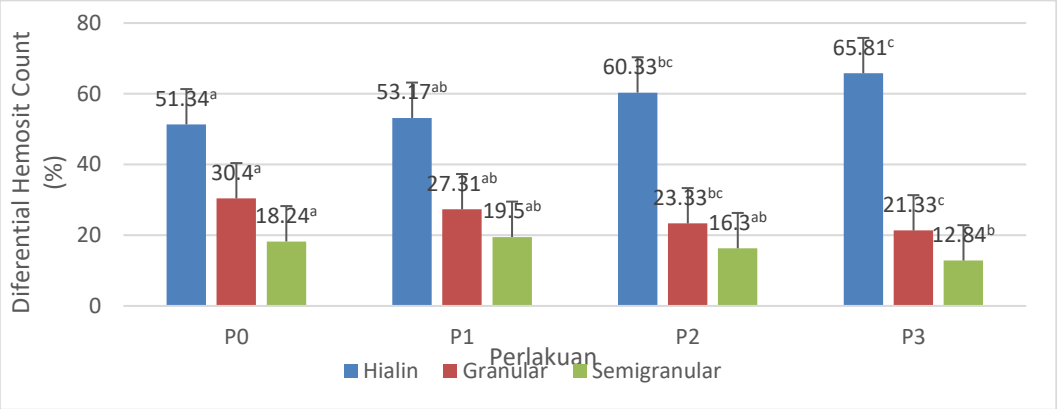

Figure 2. Differential hemocyte count (DHC)

The results of the analysis of variance (ANOVA) showed that after a challenge test was carried out on vannamei shrimp given EM4 probiotics. The results were significantly different for the values of hyaline cells, granule cells, and semi-granules (P<0.05).

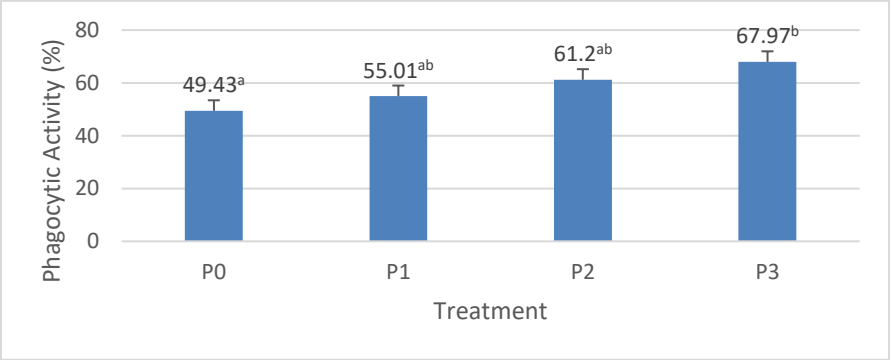

Figure 3. Phagocytic activity (AF)

The results of the analysis of variance (ANOVA) showed that after a challenge test was carried out on vannamei shrimp given EM4 probiotics, the results were significantly different from phagocyte activity (P<0.05).

DISCUSSION

From the observation of the hemocyte dots, the highest value was found in the P3 treatment, which was 19.44 x 10 6 CFU/ml, followed by the P2 treatment, which was 14.56 x 10 6 CFU/ml, then the P1 treatment, which was 12.04 x 10 6 CFU /ml, the lowest was found in treatment P0, namely 11.01 x 10 6 CFU/ml. The results of the ANOVA test showed a significantly different effect on all treatments. Hemocytes have a role in the animal defense system, especially in invertebrates with no adaptive immune system. Hemocytes participate in cellular immunity, including phagocytosis, encapsulation, and nodule formation (Chen & Kang, 2021). administration of EM4 probiotics to vannamei shrimp rearing media in this study showed that the treatment given EM4 probiotics was able to provide an increase in THC compared to the control treatment and did not interfere with the growth and health of vannamei shrimp. From Figure 18. It shows that applying higher EM4 probiotics can also increase the THC value of vannamei shrimp. Jannah et al . (2018) research results showed that vannamei shrimp given Lactobacillus sp. THC values ranged from 1.7 to 9.65 x 10 6 cells/ml.

The increase in THC value after applying EM4 probiotics protects vannamei shrimp from pathogen attacks. Besides that, the content of Lactobacillus sp. and Actinomycetes sp. plays a

role in stimulating the immune system, suppressing particular pathogens that are only present in several pathogenic strains, decomposing organic matter, and making it an antibiotic compound. Research by Valeriano et al . (2017) mentioned that Lactobacillus sp. benefits the host's health by providing Lactobacillus coevolution with the host's immune system. Setyowati (2018) Actinomycetes sp. can decompose organic matter and produce antibiotic compounds that are toxic or toxic to bacteria.

DHC observations aim to indicate the reaction that occurs against the defense of the shrimp body against the entry of foreign particles (pathogens) that enter the shrimp body. In the DHC observations, three types of cells were observed. The cells are distinguished from each cell granule in the cytoplasm. These cells consist of semi-granular cells, hyaline cells, and granular cells. Hyaline cells have a phagocytosis function in shrimp immunity, then semigranular cells and granular cells release the prophenoloxidase system and cytotoxic activity (Bagus, 2021). in the shrimp body protection system is still relatively primitive because it does not have memory cells that recognize and provide against foreign particles. This is consistent with Arthropods having a primitive immune system; shrimps are highly dependent on specific immune systems to overcome pathogenic agents. Prophenokoxidase is a basic level of defense. Low activity of this enzyme will result in lower shrimp resistance. Prophenoloxidase Activating Enzyme (PPA) is a protein found in the granular cells of hemocytes. PPA is activated by β 1,3-glucan and Lipopolysaccharide, which triggers Prophenoliksidase in inducing hyaline cells to carry out the phagocytosis step (Hidayat, 2017).

Based on DHC observations of semi-granular, granular and hyaline cells. It was found that hyaline cells with the highest values ranged from 51 - 65%, while the total values for granular cells ranged from 21.33 - 30.4%, and for semi-granular cells, obtained values ranged from 12.84 - 18.24%. According to Darwin & Sidik (2016), the percentage of hyaline in vannamei shrimp generally ranges from 60-93% of the total hemocytes, while the percentage of granulocytes in average vannamei shrimp ranges from 17-40%, and the average value of semigranular cells in vannamei shrimp it ranges from 13 – 49%. The height of hyaline cells has a relationship with phagocytosis activity. Phagocytosis is the role of white blood cells as the body's defense system. Phagocytosis is the process by which cells engulf foreign substances. Most foreign substances that enter the tissue are eliminated through phagocytosis. Phagocytosis of macrophages is widely used as an immunological measure in assessing the health or function of the body's defense system (Handayani, 2018). Research conducted by Foysal et al. (2020) reported that a combination of two or more probiotics, including Lactobacillus species, could improve the performance of the immune system of aquatic hosts. The immune system itself is the primary protection against pathogenic microbes. From the research results, the high amount of hyaline is also suspected because of the steps of the shrimp's body in defending the body from attacks by Vibrio parahaemolyticus. This proves that giving EM4 probiotics can improve the shrimp's immune system.

From the results of research on phagocytosis activity (AF), it was found that if the addition of EM4 probiotics to the maintenance media were compared to the control treatment, the higher the dose of EM4 probiotics given would increase the AF value. From Figure 3. The highest value was obtained in the P3 treatment (2.5 ml/L), which was 67.9%, then followed by the P2 treatment (1.5 ml/L), which was 61.2%, then the P1 treatment (0, 5 ml/L), which is equal to 55.01%, and the lowest is in treatment P0 (control) which is equal to 49.43%. From the results of the ANOVA test, it was shown that the administration of EM4 probiotics to the rearing media gave significantly different results on shrimp phagocytic activity; treatment P0 did not provide significantly different values to P1 and P2 but was

significantly different from P3, treatment P1 did not show significant results. Significantly different from P2 and P3, treatment P2 did not give significantly different results from P3. The increase in AF value in the treatment given EM4 probiotics shows that EM4 probiotics can increase the response of shrimp to pathogen attacks that enter the shrimp body. One of the steps of the shrimp body in self-defense from pathogen attack is the process of phagocytosis. A phagocytosis is a form of cell defense. Phagocytosis begins with the attachment and ingestion of microbe particles in the phagocyte cells. In the phagocytosis step, semi-granular and hyaline cells play a significant role (Tampangallo et al ., 2013).

In the EM4 probiotic, Saccharomyces cerevisiae contains β-glucan, which will be used as an immunostimulant. Immunostimulants have a working system of maximizing the immune system by optimizing the activity of phagocytic cells. The ability of phagocytosis activity can increase during infection; if the presence of infection triggers a cellular non-specific protection system, it is expected to withstand disease attacks. According to Ammas (2013), immunostimulants are compounds that can maximize body protection, both non-specific and specific, and non-specific induction occurs as well as humoral and cellular defense mechanisms. These immunostimulants can be in the form of biological or other synthetic compounds such as unicellular agar, agar extract, LPS, β-glucan, vaccines, and vitamins A, B, and C (Ammas, 2013).

CONCLUSION

The observation of the vannamei shrimp's immune system showed that giving EM4 probiotics was able to provide increase the shrimp's immune system. The highest THC value was obtained in the P3 treatment of 19.44 x 10 6 cells/ml. Then the DHC values such as hyaline obtained ranged from 51.34-65.81%; granular cells obtained ranged from 21.33-30.4%, and semi-granular cell values obtained ranged from 12.84-18.24%. The highest AF value obtained was in the P3 treatment at 67.97%.

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