

**EFEKTIVITAS BDELLOVIBRIO TERHADAP SISTEM IMUN UDANG PUTIH  
(LITOPENAUES VANNAMEI) YANG DIINFEKSI BAKTERI VIBRIO HARVEYI**

***Effectiveness of Bdellovibrio Against the Immune System of White Shrimp  
(Litopenaeus vannamei) Infektion with Vibrio harveyi Bacteria***

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**ABSTRAK**

Udang putih (*Litopenaeus vannamei*) merupakan biota perairan yang termasuk dalam komoditas unggulan budidaya perikanan. Bakteri *Vibrio harveyi* dapat menyebabkan kematian pada udang vaname hingga 80%. *Bdellovibrio* predator sangat dianggap sebagai sumber antibiotik alternatif dan dilaporkan berpotensi mengendalikan patogen udang seperti *Vibrio cholerae* dan *Vibrio parahaemolyticus*. Tujuan penelitian ini adalah menganalisis pengaruh pemberian *Bdellovibrio* terhadap daya tahan tubuh udang vaname yang terinfeksi bakteri *Vibrio harveyi*. Penelitian ini bersifat eksperimental dengan menggunakan rancangan acak lengkap (RAL) yang terdiri dari 5 perlakuan dan 3 ulangan. Penelitian ini bersifat eksperimental dengan menggunakan rancangan acak lengkap (RAL) yang terdiri dari 5 perlakuan dan 3 ulangan. Perlakuan dalam penelitian meliputi P1(+) (Tidak diberikan *Bdellovibrio* dan diuji dengan *Vibrio harveyi*), P2 (-) (Tidak diberikan *Bdellovibrio* dan diuji dengan NaCl), P3 (Diberikan *Bdellovibrio* 30 ppm dan diuji dengan *Vibrio harveyi*), P4 (Diberikan *Bdellovibrio* 30 ppm dan diuji dengan *Vibrio harveyi*), P4 (Diberikan *Bdellovibrio* 30 ppm dan diuji dengan *Vibrio harveyi*), P4 (Diberikan *Bdellovibrio* 50 ppm dan diuji dengan *Vibrio harveyi*), dan P5 (Diberikan *Bdellovibrio* 70 ppm dan diuji dengan *Vibrio harveyi*). Pada penelitian ini diperoleh nilai perlakuan terbaik yaitu perlakuan P5, nilai THC sebesar 16,94 x 10<sup>6</sup> sel/ml, nilai DHC pada sel hialin sebesar 59,33%, sel granular 24%, sel semi granular 16,67%, nilai AF 64,82%, nilai TBC 5,98 x 10<sup>8</sup> CFU/ml, nilai TVC 1,43 x 10<sup>7</sup> CFU/ml, nilai FCR 1,23, laju pertumbuhan panjang spesifik 19,4% /hari, laju pertumbuhan bobot spesifik 9,96%/hari, nilai panjang absolutnya adalah 8,73 cm, berat mutlak 61 gram dan nilai SR 77,78%. Dari penelitian ini disimpulkan bahwa pemberian *Bdellovibrio* pada media pemeliharaan dengan dosis berbeda mampu meningkatkan daya tahan tubuh dan kelangsungan hidup udang putih yang diuji dengan bakteri *Vibrio harveyi*.

**ABSTRACT**

White shrimp (*Litopenaeus vannamei*) is an aquatic biota that is included in the leading commodity of aquaculture. The *Vibrio harveyi* bacteria can cause up to 80% death in white shrimp. The predatory *Bdellovibrio* is highly regarded as an alternative source of antibiotics and has been reported to have the potential to control shrimp pathogens such as *Vibrio cholerae* and *Vibrio parahaemolyticus*. The aim of this study was to analyze the effect of giving *Bdellovibrio* on the immune

system of vaname shrimp infected with *Vibrio harveyi* bacteria. This research is experimental using a completely randomized design (CRD), which consists of 5 treatments and 3 replications. This study was experimental using a completely randomized design (CRD), which consisted of 5 treatments and 3 replications. Treatments in the study included P1 (+)(Not given *Bdellovibrio* and tested with *Vibrio harveyi*), P2 (-) (Not given *Bdellovibrio* and tested with NaCL), P3 (Given 30 ppm *Bdellovibrio* and tested with *Vibrio harveyi*), P4 (Given *Bdellovibrio* 50 ppm and tested with *Vibrio harveyi*), and P5 (Given *Bdellovibrio* 70 ppm and tested with *Vibrio harveyi*). In this study, the best treatment value was obtained, namely P5 treatment, the THC value was  $16.94 \times 10^6$  cells/ml, the DHC value in hyaline cells was 59.33%, the granular cells were 24%, the semi-granular cells were 16.67%, the AF value 64.82%, TBC value  $5.98 \times 10^8$  CFU/ml, TVC value  $1.43 \times 10^7$  CFU/ml, FCR value 1.23, specific length growth rate 19.4%/day, specific weight growth rate 9.96%/day, the absolute length value is 8.73 cm, the absolute weight is 61 grams and the SR value is 77.78%. From this research, it was concluded that administration of *Bdellovibrio* in the rearing media at different doses was able to improve the immune system and survival of White shrimp tested with *Vibrio harveyi* bacteria.

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<b>Kata Kunci</b>	<i>Udang putih, Bdellovibrio, Vibrio harveyi, Sistem imun</i>
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## INTRODUCTION

White shrimp (*Litopenaeus vannamei*) or what is often called white shrimp is one of the crustacean aquatic biota which has high economic value, so many people carry out intensive scale white shrimp cultivation activities. According to data from the Central Statistics Agency (2017), the export value of vannamei shrimp in 2015 reached 145,007.9 tons. White vaname shrimp is a food source that is rich in protein at a cheaper price, thus encouraging people to increase consumption of white vaname shrimp as a nutritional requirement for health. Vaname shrimp cultivation has several advantages including high productivity, fast growth, high survival with high stocking density, resistance to disease (Cuzon et al., 2004; Jaspe et al., 2011; Mauladani et al., 2020), and tolerance to a wide salinity range (0.5-45 ppt), and requires relatively low protein, namely around 20-35%, low FCR value (Fao et al. al., 2004). Because of these advantages, vaname shrimp cultivation has great potential for development.

It is very important to control water quality in intensive system cultivating vaname shrimp to ensure the success of cultivation. Intensive system cultivation is known to use high density and use optimal feed, so it is susceptible to changes in water quality, this condition can trigger problems in cultivated vaname shrimp. Problems still experienced by farmers are diseases caused by *Vibrio harveyi* infection, such as eye lesions in shrimp, vasculitis (inflammation of blood vessels) and *Vibrio parahaemolyticus* which can cause lysis of blood cells in the host's body (Wachid et al., 2022), the deadly disease that still often appears in this cultivation activity is WFD

(White Feces Disease) which is caused by the bacteria *Vibrio* spp., which infects several organs such as intestines and hepatopancreas, then the symptoms that shrimp will experience if they are infected with WFD disease will cause several symptoms, namely abnormal growth, white feces, decreased appetite, and low survival rate (Mastan et al., 2015).

This vibriosis will attack its host in unfavorable water conditions. Poor water conditions can reduce the level of immunity in vaname shrimp. The shrimp immune system is still relatively simple, this immune system is different from the fish immune system in general which has a specific (adaptive) and non-specific (innate) immune system. The shrimp immune system only has a non-specific immune system, which means it has the ability to block all types of foreign objects that harm the body. The shrimp immune system works without basic memory, vaname shrimp only rely on hemocytes to attack foreign objects during infection (Rivera et al., 2019). The non-specific immune system is very vulnerable to bacterial diseases, the immune system can be more active if immunostimulants are given (Junaidi et al., 2020).

One alternative prevention of vibriosis is the administration of probiotic bacteria. Probiotic bacteria are live microbes that can increase the utilization of feed nutrients, improve the immune system, improve the quality of the host's living environment (Verschuere et al., 2000), increase shrimp survival, and suppress the population of *Vibrio* sp bacteria (Widarnani *et al.*, 2008). Antibacterials that can be used as an alternative that can suppress the population of *Vibrio* sp bacteria in preventing the spread of disease and can improve the immune system of shrimp is the predatory *Bdellovibrio*. Cao et al. (2019) revealed that the predatory *Bdellovibrio* is highly regarded as an alternative source of antibiotics and has been reported to have the potential to control shrimp pathogens such as *Vibrio cholerae* and *Vibrio parahaemolyticus*. Starr and Nancy (1966) also revealed that *Bdellovibrio bacteriovorus* is an unusual bacteria and is a predator and parasite on other bacteria. The initial predatory phase involves an apparent "selection" of a susceptible host, followed by violent contact between one or more highly motile *Bdellovibrio* cells and a much more massive host cell. After that, all or some of the host cells become round bodies so that the final result of this predatory and parasitic action is the complete breakdown of the host bacteria and an increase in the number of *Bdellovibrio* cells.

However, the use of *Bdellovibrio* in improving the immune system of vaname shrimp and helping fight harmful bacteria, namely *Vibrio harveyi*, is very rarely heard and reported. Therefore, this research will optimize the use of *Bdellovibrio* by finding the best dose to help improve the immune system of vaname shrimp to fight *Vibrio harveyi* bacteria.

## **METHODS**

### **Place and Time**

This research will be carried out for 45 days, starting from February 2023 – April 2023. This research will take place at the Fish Production and Reproduction Laboratory, Fish Health Laboratory, Aquaculture Study Program, Faculty of Agriculture, Mataram University.

## Materials and Time

The tools used in the research are stationery, atoclave, petri dish, DO meter, container, hot plate, tube needle, jerry can, glass preparation, camera, micropipette, microscope, ruler, pH meter, test tube rack, refractometer, siphon hose, scoop, aeration set, digital scale, small jar and feed container

The materials used in the research were sea water, anticoagulants, *Vibrio harveyi* bacteria, *Bdellovibrio* bacteria, hemolymph, label paper, methanol, feed, syringes, TCBS agar, TSA agar, and white shrimp.

## Research Methods

The experimental method is a method that will be carried out by researchers by implementing a Completely Randomized Design (RAL). The aspect observed was the effectiveness of *Bdellovibrio* Powder mixed into water media with different doses on vaname shrimp with 5 (five) treatments and 3 (three) repetitions and using 2 (two) control treatments and 3 (three) repetitions, to maintenance media, 15 experimental units were obtained.

Table 3. Treatment plan implemented in the study

No	Treatment	Information
1	P <sub>1</sub> (Kontrol+)	+ Bacterial infection
2	P <sub>2</sub> (Kontrol-)	+ NaCl 0,9% infection
3	P <sub>3</sub>	<i>Bdellovibrio</i> 30 ppm + Bacterial infection (Andayani et al, 2020)
4	P <sub>4</sub>	<i>Bdellovibrio</i> 50 ppm + Bacterial infection (Andayani et al, 2020)
5	P <sub>5</sub>	<i>Bdellovibrio</i> 70 ppm + Bacterial infection (Andayani et al, 2020)

## Procedure

### Preparation of Containers and Maintenance Media

The maintenance containers used in this research were 12 buckets with a capacity of 40 liters of water. To avoid disease, the bucket is sterilized with 1 ppm chlorine, then the bucket is rinsed and dried for 24 hours. The bucket is cleaned with detergent as a disinfectant (Haliman and Adijaya, 2006). Then the buckets are placed in place and filled with 20 liters of sea water equipped with aeration, one for each bucket to fill the water with oxygen. To prevent shrimp from jumping out, the bucket is closed using a bucket lid. Then during the research there was no provision of shelter in buckets and each bucket was given a label according to the treatment.

### Preparation of Test Animals

The test animal used in this research was PL 15 vaname shrimp obtained from STP Sumbawa. Arianto et al., (2018) stated that the acclimatization process is an adjustment to different environmental conditions (from the hatchery to cultivation waters) so that it does not cause stress to the fry. Temperature acclimatization by immersing a plastic bag containing shrimp fry in a closed position until there is steam in the plastic bag which indicates that the temperature inside the plastic bag is the same as the temperature of the research environment.

### **Administration of *Bdellovibrio***

The probiotic application of *Bdellovibrio* resulting from the culture is given every week starting from before the fry are sown. Distribution of *Bdellovibrio* was carried out by placing it directly into the rearing container according to the experimental dose in each research container. Probiotics are taken using a 10ml syringe to determine the dose to be given for each treatment (Susilowati et al., 2017).

### **Feeding and Water Changes**

The feed used in this research was commercial pellets with a protein content of 40%. Feeding of vaname shrimp is given 4 times, namely at 07.00, 12.00, 17.00, 22.00, in accordance with Restricted feed, which is given according to the shrimp's body weight at 5% of the shrimp biomass given during rearing (Jannah et al., 2018). Water quality management during shrimp rearing is carried out by siphoning off 10% of the total container volume. Water injection is done once a day in the morning.

### **Preparation of Test Bacteria**

The *Vibrio harveyi* bacteria used were cultured first using TCBS (thiosulfate citrate bile salt sucrose) media for 24 hours. Next, bacterial colonies were taken with a loop and serially diluted using liquid SWC (sea water complete) media up to 106. These bacteria were ready to be used for challenge tests (Fuandila et al., 2019).

### **Challenge Test**

The challenge test was carried out after treatment, namely on day 35, with the aim of understanding the effect of applying the probiotic *Bdellovibrio* to vaname shrimp on *V. harveyi* bacterial infection. The infection is applied directly to the water medium with. The bacterial density of *V. harveyi* applied was 106 CFU/ml (Fuandila et al., 2020). The challenge test was carried out for 9 days, data on immune response parameters was taken and the number of dead shrimp was calculated as shrimp survival data at the end of the challenge test (Azhar, 2018).

### **Research Parameters**

#### ***Total Haemocyte Count (THC)***

Total hemocyte count or total hemocytes is the number of blood cells in the shrimp's body. Observation of total hemocytes was carried out on day 45 after a 10day challenge test. Total hemocytes were observed by taking 0.1 ml of shrimp haemolymph from the base of the fifth leg using a syringe containing 0.2 ml of anticoagulant. The mixture of hemolymph and anticoagulant was homogenized for 5 minutes. The first drop is discarded and the second drop is dripped into the hemocytometer. Total shrimp hemocytes were counted using a haemocytometer with the help of a microscope at 40x magnification (Ismawati et al., 2019). The calculation formula is as follows:

#### **Total Hemosit**

$$= \frac{\text{Jumlah sel yang teramati}}{\text{Jumlah kotak teramati}} \times 25 \times \frac{1}{\text{volume haemocytometer}} \times \text{Faktor pengencer}$$

#### ***Differential Haemocyte Count (DHC)***

DHC observations were carried out on the 45th day after a 10 day challenge test. DHC observations were carried out with a syringe filled with 0.2 ml of anticoagulant and shrimp hemolymph was taken from the legs of the five test shrimp using the same syringe. The mixture of haemolymph and anticoagulant was

homogenized for 5 minutes then pressed on a glass object. Hemolyte was made on a glass slide and air dried then fixed with 100% methanol for 15 minutes. After that, the fixed hemolyme was dried again and stained by soaking in 10% Giemsa solution for 15 minutes. The stained hemolyte was washed in flowing distilled water for 30 seconds and allowed to dry again. The preparations were observed using 40x magnification and differentiated according to their type, namely hyaline and granular cells (Ekawati et al., 2012). The percentage of each hemocyte cell is calculated using the formula:

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

### Phagocytic Activity (AF)

AF was calculated based on the method of Anderson and Siwicki (1993). AF was measured at the end of treatment, namely on day 45 after the challenge test. AF was observed by taking 50 µl of hemolyte into a microtube, then adding 50 µL of *Staphylococcus aureus* bacterial suspension (107 CFU mL<sup>-1</sup>) mixed evenly and incubated for 20 minutes. Next, 10 µL of the mixture was taken to make smear preparations. This preparation was fixed with methanol for five minutes and dried, then soaked in Giemsa solution for 15 minutes. The preparation was then washed in running water and dried. Observations were carried out using a microscope with 400x magnification. Phagocytic activity was calculated based on the percentage of phagocytic cells that showed phagocytic activity. Phagocytic activity can be calculated using the formula:

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

### Survival Rate (SR)

The fish survival rate (SR) is calculated from the percentage of the number of fish alive at the end of the rearing period compared to the number of fish at the initial stocking time. Survival rates can be calculated using the formula (Huisman 1987) :

$$\text{SR} = \frac{N_t}{N_o} \times 100\%$$

Information:

SR : Survival Rate (%)

N<sub>t</sub> : Population at the end of rearing (tail)

N<sub>o</sub> : Population at the start of rearing (tail)

### Data Analysis

Observation data was analyzed using analysis of variance (ANOVA) with SPSS. At the 5% significance level in understanding the effect of treatment on research. If the data shows a real influence, then further analysis is carried out with the Least Significant Difference test (BNT).

## RESULT

### Survival Rate (SR)

The survival rate in this study is presented in graphical form in Figure 6. With the results obtained, the highest survival value was in treatment P5 at 77.7%, followed by P4 at 75.5% and then P3 at 73.3, then P2 with a score of 62.2% and the lowest score is P1 with a score of 60%.

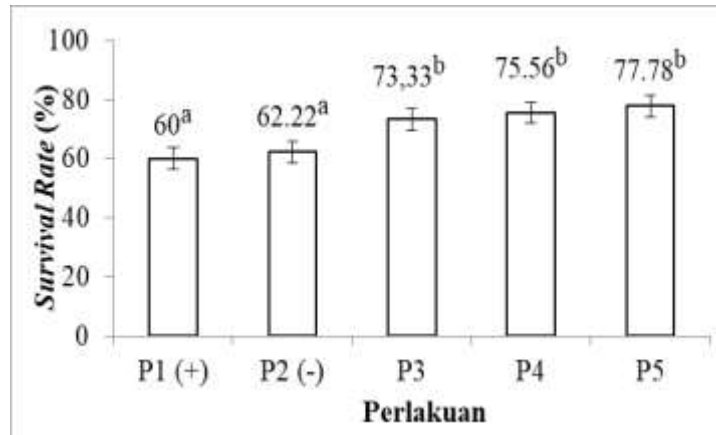


Figure 6. Average Survival Rate (SR)

Based on the results of statistical tests using the One-way Annova test and Duncan's advanced test, the Survival Rate (SR) values showed that all treatments using *Bdellovibrio* were significantly different ( $P < 0.05$ ) to P1 and P2, but P2 and P3 had the same values. not significantly different ( $P < 0.05$ ), while P3, P4 and P5 have values that are not significantly different.

### Total Haemocyte Count (THC)

The total hemocyte count in this study is presented in graphical form in Figure 7. With the results obtained, the number of hemocytes (THC) is descriptively the highest hemocyte value, namely at P5 at  $16.94 \times 10^6$  cells/ml, followed by P4 at  $15.08 \times 10^6$  cells/ml, after that P3 was  $14.80 \times 10^6$  cells/ml, then P2 was  $12.75 \times 10^6$  cells/ml and the lowest was P1 was  $11.69 \times 10^6$  cells/ml.

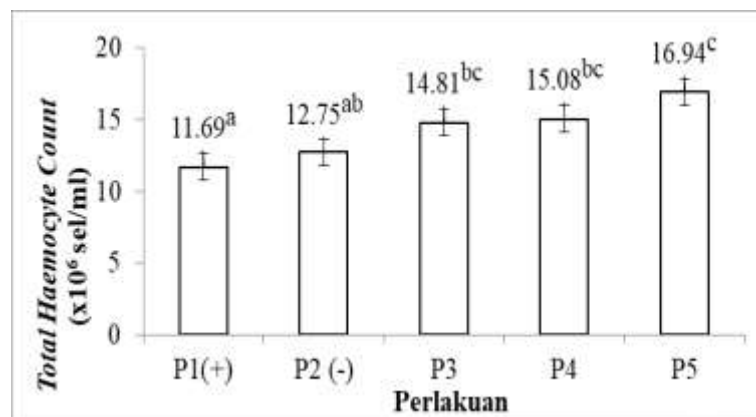


Figure 7. Average THC value of Vaname Shrimp

Based on the results of statistical tests using the One-Way Annova test and Duncan's advanced test, the THC value after treatment with *Bdellovibrio* in water media showed significantly different results ( $P < 0.05$ ) with P1 Control+), P1 and P2 were not significantly different ( $P > 0.05$ ), P2 and P3 are not significantly different ( $P > 0.05$ ), P3 and P4 are not significantly different ( $P > 0.05$ ), P4 and P5 are not



significantly different ( $P>0.05$ ), P1 and P2 are significantly different from P5 ( $P<0.05$ ).

### Differential Hemocyte Count (DHC)

The Differential Hemocyte Count in this study is presented in graphical form in Figure 8. With the results obtained, the DHC count was calculated descriptively, showing that the highest hyaline value was at P5 at 59.3% followed by P4 at 56.6% then P3 at 55.0%, after that P1 50.6% and the lowest amount of hyaline was found in P1 at 48.6%. In granular cells, the highest value was in treatment P1 at 36.6%, followed by P2 at 34.0%, then P3 at 28%, then P4 at 27.3% and the lowest was P5 at 24%. Likewise, semi-granular cells showed the highest value in treatment P3 at 17%, followed by P5 at 16.6%, then P4 at 16%, then P2 at 15.3% and the lowest value at P1 at 14.6%.

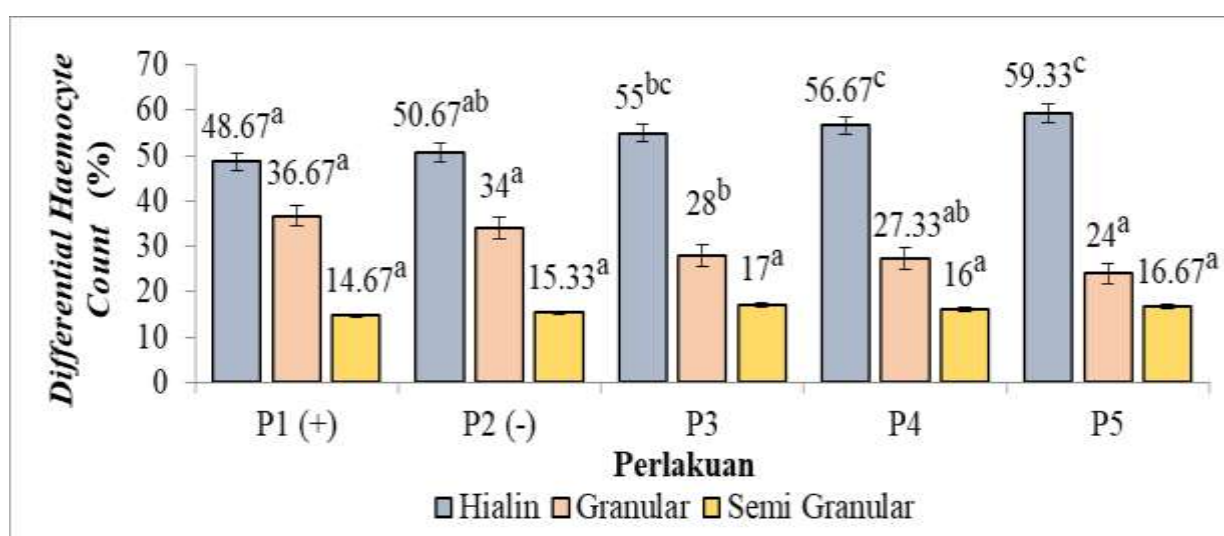


Figure 8. Average DHC value of Vaname Shrimp

Based on the results of statistical calculations from the One-Way Anova test and Duncan's further test, it showed that the value of hyaline cells in the treatment given *Bdellovibrio* in water media was significantly different ( $P<0.05$ ) from P1 (Control +), furthermore P1 and P2 were not significantly different ( $P<0.05$ ), P3, P4 and P5 were not significantly different ( $P>0.05$ ). The granular cell values showed that the control treatments P1 and P2 were significantly different ( $P>0.05$ ) from the treatment given *Bdellovibrio* in water media, P3 and P5 were significantly different ( $P<0.05$ ) while P4 and P5 were not significantly different, and so did with P3 and P4 not significantly different ( $P<0.05$ ). Meanwhile, the semigranular value showed no significant difference ( $P<0.05$ ) for all treatments.

### Phagocytic Activity (AF)

Phagocytosis activity in this study is presented in graphical form in Figure 9. With the results obtained, the number of AF was calculated descriptively, that the highest value of phagocytosis activity was found in P5 at 64.8%, then followed by P4 at 64.08%, then P3 with a value of 61.8%, then P2 is 53.39% and the lowest value is P1 of 51.36%.



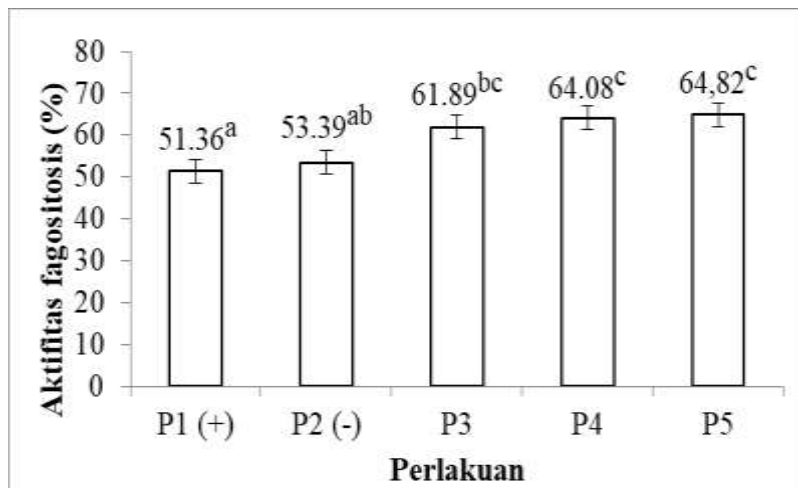


Figure 9. Average AF value for Vaname Shrimp

Based on the results of statistical tests using the One-Way Annova test and Duncan's advanced test, the phagocytosis activity value given *Bdellovibrio* in water media was significantly different ( $P < 0.05$ ) from the P1 (Control+) treatment. P1 and P2 are not significantly different but are significantly different ( $P < 0.05$ ) from P4 and P5. P2 and P3 are not significantly different ( $P < 0.05$ ), while P3, P4 and P5 are not significantly different ( $P < 0.05$ ).

#### Feed Conversion Ratio (FCR)

The Feed Conversion Ratio in this study is presented in graphical form in Figure 10. With the results obtained, it is calculated descriptively that the highest FCR value is found at P1 at 1.78, followed by P2 with a value of 1.75, then P3 with a value of 1.34, then P4 with a value of 1.27 and the lowest value is P5 with a value of 1.23.

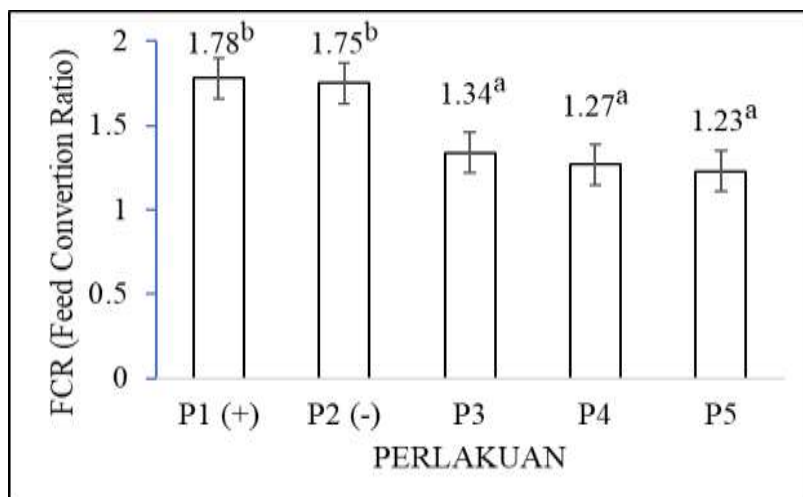


Figure 10. Average FCR Value of Vannamei Shrimp

Based on the results of statistical tests through the One-Way ANOVA test and Duncan's further test, it shows that all treatments using *Bdellovibrio* are P3, P4 and P5 have values that are not significantly different ( $P < 0.05$ ). but significantly different ( $P < 0.05$ ) to the P1 value (Control +) and P2 value (Control -).

### Specific Length Growth Rate (Length SGR)

The specific length growth rate in this study is presented in graphical form in Figure 11. With the results obtained, the highest value for specific length was in treatment P5 19.4%/day, followed by P4 with a value of 18.1%/day, then P3 with a value 16.1%/day, next is P2 (-) with a value of 13.7%/day and the lowest is P1 (+) with a value of 13.3%.

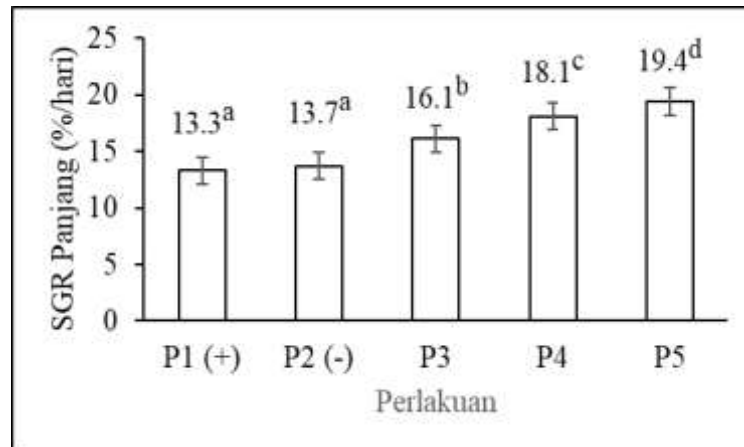


Figure 11. Average Specific Length Value of Vannamei Shrimp

Based on the results of statistical tests using the One-Way Annova test and Duncan's advanced test, the specific length values of control treatments P1 and P2 have values that are not significantly different ( $P < 0.05$ ) but are significantly different ( $P < 0.05$ ) with all treatments given *Bdellovibrio*, while the treatments given *Bdellovibrio* namely P3, P4 and P5 each had significantly different values ( $P < 0.05$ ).

### Absolute Longitudinal Growth

Absolute Length Growth in this study is presented in graphical form in Figure 12. The highest value obtained for Absolute Length was in treatment P5 with a value of 8.73 cm, followed by P4 with a value of 8.16 cm, then P3 with a value of 7.22 cm, then P2 with a value of 6.18 cm and the lowest value is P1 with a value of 5.98 cm.

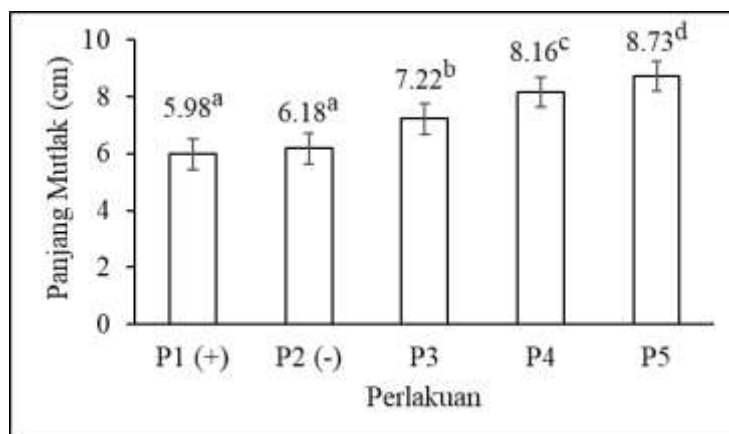


Figure 12. Average Absolute Growth Length of Shrimp

Based on statistical test results using the One-Way Annova test and Duncan's advanced test, the Absolute Length value shows that the control treatments, namely P1 and P2, have values that are not significantly different ( $P < 0.05$ ) but are significantly different from all treatments given by *Bdellovibrio*, P3 has a value that is

significantly different ( $P < 0.05$ ) from treatment P4 and P5, while P4 and P5 have a value that is not significantly different ( $P < 0.05$ ).

### Specific Weight Growth Rate

The specific weight growth rate in this study is presented in graphical form in Figure 13. With the results obtained, the highest value of weight SGR was found in treatment P5 with a value of 9.96%/day, followed by P4 with a value of 9.42%/day. then P3 with a value of 9.22%/day, then P2 with a value of 6.94%/day, and the lowest is P1 with a value of 6.79%.

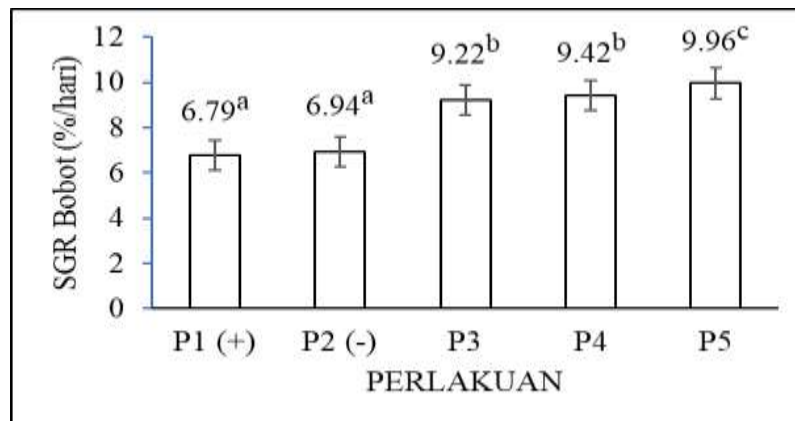


Figure 13. Average SGR Value for Vannamei Shrimp Weight

Based on statistical test results using the One-Way Anova test and Duncan's advanced test, the SGR Weight value in the control treatment, namely P1 and P2, has values that are not significantly different ( $P < 0.05$ ) but are significantly different from all treatments given by *Bdellovibrio*, in The treatments given by *Bdellovibrio* P3 and P4 had values that were not significantly different ( $P < 0.05$ ) but each P3 and P4 had values that were significantly different ( $P < 0.05$ ) from P5.

### Absolute Weight Growth

The absolute weight growth in this study is presented in graphical form in Figure 14. The highest results obtained from absolute weight were in treatment P5 with a value of 61 g, followed by P4 with a value of 59 g, then P3 with a value of 56.1 g, then P2 with a value of 42.9 g and the lowest value was in treatment P1 with a value of 42.1 g.

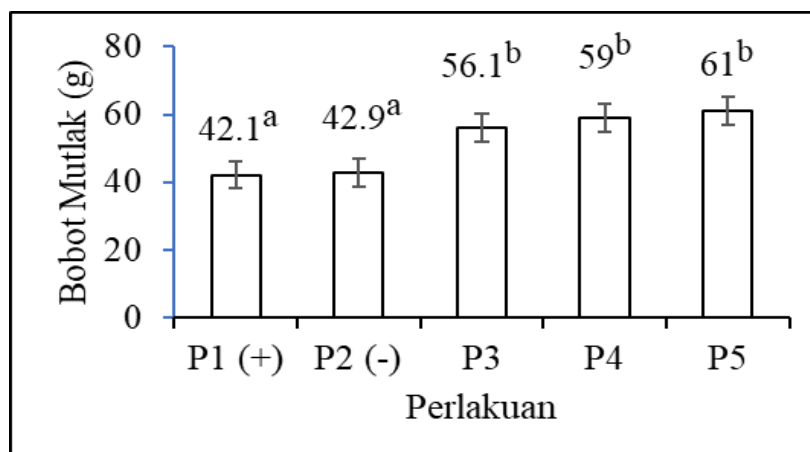


Figure 14. Average Absolute Weight Value of Vaname Shrimp

Based on the results of the Statistical Test via the One-Way Annova Test, it shows that the Absolute Weight values in the control treatments P1 and P2 have values that are not significantly different ( $P < 0.05$ ) but are significantly different from all treatments given by *Bdellovibrio*, namely P3, P4 and P5, The treatments given by *Bdellovibrio*, namely P3, P4 and P5, each had values that were not significantly different ( $P < 0.05$ ).

### Total Bactery Count (TBC)

The total bacterial count in this study is presented in graphical form in Figure 15. With the results obtained, P5 has the highest TBC value, namely  $5.98 \times 10^8$  CFU/ml, followed by P4 with a value of  $5.14 \times 10^8$  CFU/ml, then P3 with a value  $5.01 \times 10^8$  CFU/ml, then P2(-) with a value of  $1.76 \times 10^8$  and the lowest value is P1(+) with a value  $1.75 \times 10^8$ .

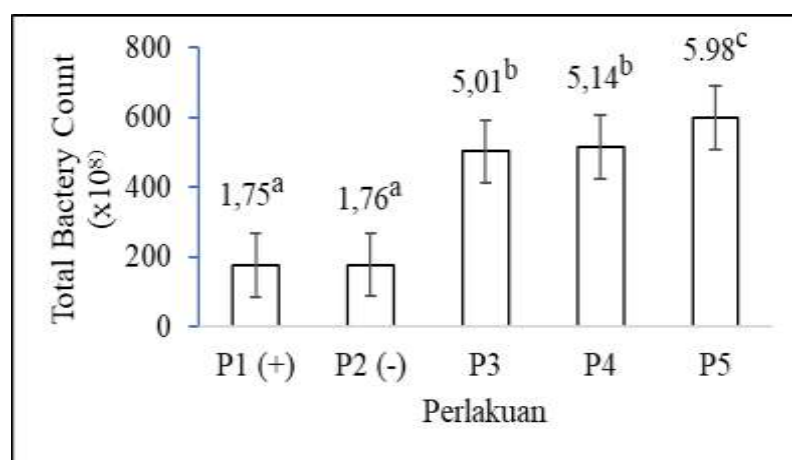


Figure 15. Average TBC Value of Vaname Shrimp

Based on the results of statistical calculations, the One-Way ANOVA test and Duncan's advanced test showed that the addition of *Bdellovibrio* to the rearing media at different doses had a significant effect ( $P < 0.05$ ) on the TBC value in the vaname shrimp rearing media tested with *Vibrio harveyi* bacteria. Treatments P1 and P2 were not significantly different ( $< 0.05$ ) but were significantly different from all treatments given *Bdellovibrio*, P3 and P4 had values that were not significantly different ( $< 0.05$ ) but were significantly different from P5.

### Total Vibrio Count (TVC)

The total Vibrio Count in this study is presented in graphical form in Figure 16. The highest value obtained was P1 with a value of  $3.26 \times 10^7$  CFU/ml, followed by P3 with a value of  $2.03 \times 10^7$  CFU/ml, then P4 with a value of  $1.73 \times 10^7$  CFU/ml, then P5 with a value of  $1.43 \times 10^7$  CFU/ml, and P2 with a value of  $1 \times 10^7$  CFU/ml.

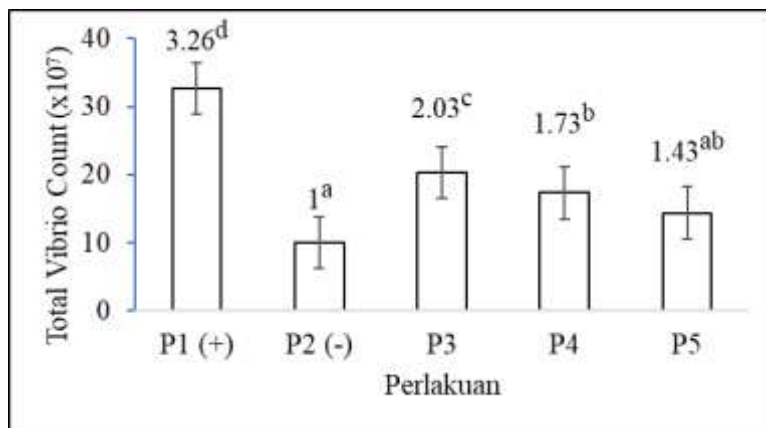


Figure 16. Average TVC Value of Vaname Shrimp

Based on the results of the One-Way Anova test and Duncan's further test, the addition of *Bdellovibrio* to the water media at different doses had an effect ( $p < 0.05$ ) on the TVC value of vaname shrimp tested with *Vibrio harveyi* bacteria. The graph above shows that the highest TVC value was in treatment P1 (+), namely  $3.26 \times 10^7$  CFU/ml and was different ( $P < 0.05$ ) from all treatments. P5 and P2 had TVC values that were not different ( $p > 0.05$ ), namely  $1.43 \times 10^7$  CFU/ml and  $1 \times 10^7$  CFU/ml respectively, followed by P4 and P5 which were not different ( $P < 0.05$ ) with values of  $1.73 \times 10^7$  CFU/ml and  $1.43 \times 10^7$  CFU/ml, and P3 which was different ( $P < 0.05$ ) from all treatments with value  $2.03 \times 10^7$  CFU/ml.

### Water Quality

The results of water quality measurements are presented in Table 4. The water quality parameter values in the media were measured during the research to determine the suitability of the waters as a suitable medium for rearing vaname shrimp during rearing. The water quality parameter values measured include DO, temperature, pH, ammonia and salinity.

Table 1. Water Quality Measurement Results

No.	Treatment	P1 (+)	P2 (-)	P3	P4	P5	Mark Optimum
1	Ammonia (mg/L)	0,6	0,6	0,6	0,6	0,6	0,2 mg/L (Fendjalang <i>et al.</i> , 2016)
2	DO (mg/L)	5,5-6,4	5,5-6,4	5,5-6,4	5,5-6,4	5,1-5,8	>3mg/l (Citria, 2018)
3	pH	7,3-7,6	7,3-7,5	7,6-7,8	7,3-7,8	7,5-7,8	7,5 – 8,5 (Citria, 2018)
4	Salinity (ppt)	29-32	29-32	29-32	29-32	29-32	30-53 ppt (Citria, 2018)

5	Temperature (°C)	30-31	30-31	30-31	30-31	30-31	16-32°C (Citria, 2018)
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## DISCUSSION

### Survival Rate (SR)

Survival rate is a comparison of the total number of living things in a certain period of time. Based on observations, the survival rate (SR) value was used as the main parameter in determining the success rate in this study. This is in line with the statement from Ni'mah et al., (2021) which states that survival is one of the measurements carried out to determine the resistance of shrimp to environmental stress and disease. Based on Figure 6, the highest survival rate (SR) value was obtained at P5 (Bdellovibrio 70 ppm) with a value of 77.78%, while the lowest value was at P1 (Control +) with a value of 60%. The high survival rate value in P5 compared to P1 is thought to be because the administration of probiotics can help shrimp from attacks by pathogens which can cause death in shrimp. The survival rate value in this study is still relatively good if referring to the statement of Rosyida et al., (2022) which states that a good survival rate for vaname shrimp is when the SR value is > 70%, and is considered low if the SR value is <50%, followed by the statement of Iribarren et al., (2012) if probiotics are given capable of increasing the survival rate of cultivated biota, as well as reducing environmental burdens due to the accumulation of aquatic waste and strengthening the body's resistance to aquatic biota attacks by pathogens.

Based on Figure 6, the Survival rate value in this study has a value that continues to increase. It is thought that the higher the dose of Bdellovibrio bacteria given to the water media, the higher the Survival Rate value. This is confirmed by the statement of Burhanuddin & Gunarto, (2008) who stated that Vaname shrimp survival tended to increase in treatments using probiotics compared to controls (without use of probiotics). Amin & Hendrajat (2008) stated that administering commercial probiotics at a concentration of 0.5-1.5 mg/L/week in vaname shrimp rearing media resulted in 92.33%–94.33% higher survival compared to control treatment (without probiotics) with a survival of 86.33%.

### Total Hemocyte Count (THC)

Hemocytes are the main constituent of the immune system which carries out its role through the mechanisms of phagocytosis, encapsulation, nodulation, and as a medium for cytotoxicity against foreign material. The total number of hemocytes is an indicator of the shrimp's increased defense response. Total hemocytes in shrimp play a very important role in the shrimp body because hemocytes have a function as a defense system against pathogens. This is in line with the statement by Oktaviana et al., (2020) which states that hemocytes are part of the body's defense system in white shrimp which plays a role in the phagocytosis process, nodulation and encapsulation, as well as a high number of hemocytes indicate a good level of shrimp health.

Based on Figure 7, the highest THC value was obtained at P5 (Bdellovibrio 70ppm) with a value of  $16 \times 10^6$  cells/ml while the lowest value was at P1 (Control +) with a value of  $11.69 \times 10^6$  cells/ml. The high THC value in P5 is thought to be due to the effectiveness of probiotics which indirectly enter the shrimp's body as well as stimulation from the environment and nutrients in their body and Bdellovibrio is a bacteria that is beneficial for the health of vaname shrimp, thereby triggering an increase in shrimp hemocytes. This is in line with the statement of Ekasari et al., (2014) which states that probiotics contain various microorganisms, cell components

and metabolites that can act as immunostimulants. Microorganisms such as bacteria have cell walls consisting of lipopolysaccharide (LPS), peptidoglycan (PG), and  $\beta$ 1,3-glucan (B3) which are able to activate immunity in shrimp. Xu & Pan (2013) also stated that the total number of hemocytes in shrimp reared using probiotics was higher than shrimp cultivated without using probiotics.

Based on Figure 7, it can be seen that the THC value in this study continues to increase. It is suspected that giving *Bdellovibrio* at different doses has a real influence on the high value of shrimp hemocytes. The higher the hemocyte value in vaname shrimp, the better the shrimp's health level. This is in line with the statement Febriani et al., (2018) stated that a high number of hemocytes indicates good shrimp health. This hemocyte value is in line with the results of research by Vieira et al., (2016) which shows that the total hemocyte value of vaname shrimp given the probiotic strain *Lactobacillus plantarum* has an optimal value of 8.58 - 36.26 x10<sup>6</sup> cells/ml.

### **Differensial Hemosit Count (DHC)**

Observation of the DHC value is also an important indicator regarding the immune response in shrimp by hemocyte cells. In accordance with the statement by Rahim et al., (2020) which states that health conditions and modulation of the immune system in shrimp can be known through cellular and humoral parameters such as hemocyte differential. In DHC observations, three types of hemocyte cells in crustaceans (including shrimp) were observed. This type is divided based on the presence of cytoplasmic granules, namely hyaline, semi-granular, and granular, each of which has an important role in non-specific defense processes.

Based on Figure 8. The percentage value of hyaline cells obtained in this study ranged from 48.66% -59.33% of the total hemocytes, the number of granulocytes ranged from 24% - 34.66%, while the lowest percentage number was semi granular which ranged between 14.66 % - 17%. The range obtained is still classified as normal if we refer to the statement by Darwantin et al., (2016) that the percentage of hilain in normal baname shrimp is around 50% - 80%. Meanwhile, the granulocyte presentation in normal vaname shrimp ranges from 17 - 40%, and the normal value of semigranular cells in vaname shrimp ranges from 13 - 49%. The increase in DHC in the treatment given by *Bdellovibrio* is an indicator that the administration of *Bdellovibrio* has a good influence on improving the immune system in vaname shrimp, where *Bdellovibrio* is able to increase the population of good bacteria and indirectly enters the shrimp's body as one of the things that stimulates cell activities. hemocytes in shrimp to fight pathogens that enter the shrimp during cultivation, This is reinforced by the statement of Ekasari et al., (2014) who stated that probiotics contain various microorganisms, cell components and metabolites that can act as immunostimulants. Microorganisms such as bacteria have cell walls consisting of lipopolysaccharide (LPS), peptidoglycan (PG), and  $\beta$ 1,3-glucan (B3) which are able to activate immunity in shrimp. From the two types of cells between granular and semi-granular, it can be seen in this study that granular cells have higher values, this indicates that granular cells play a greater role than semigranular cells. In accordance with the statement of Suleman et al., (2019) which stated that phagocytic activity is closely related to the number of hyaline cells which has increased, because those carrying out the phagocytic process are hyaline cells and a small number of semi-granulocytes.

An increase in the number of hyaline cells is related to phagocytic activity, where phagocytosis is the first line of defense to ward off pathogens. These hyaline cells are activated by opsonic factors resulting from the activation of ProPO to become



PO in granular cells, so that they can phagocytose foreign material, both bacteria and viruses. According to (Supamataya 2000; Takwin, 2021) granular cells have more functions in the process of producing the enzyme phenoloxidase which plays an important role in the defense system during a pathogen attack. Granular and semi-granular cells will carry out degranulation, cytotoxicity and lysis of foreign material in the waters so that the number of hemotic cells in the hemolymph will decrease. Granulocytes are a cellular defense system that fights infection. These cells migrate to areas of the shrimp's body that are infected. Granulocytes contain granules in the cytoplasm and are blue when stained with Giemsa, granulocytes engulf pathogens by destroying them. Destroyed pathogens are released from granulocytes. If the phagocytic capacity of the granulocytes has been exhausted and the pathogen infection is increasing, then the granulocytes can be destroyed by the virus resulting in death of the host. These semi-granular cells can carry out the encapsulation process and play a small role in the phagocytosis process. Encapsulation is a defense reaction against particles in large numbers that cannot be phagocytosed by hemocyte cells.

### **Phagocytic Activity (AF)**

Phagocytosis (AF) activity is a process where cells ingest foreign substances that enter the tissue and are eliminated through the phagocytosis mechanism. Based on Figure 9. Phagocytic activity shows an increase in the treatment given *Bdellovibrio* into the maintenance water medium compared to the control treatment. It is thought that the higher the dose of *Bdellovibrio* given, the more the AF value will increase. This *Bdellovibrio* is a bacterium that has predatory characteristics for pathogenic bacteria so that This can trigger an increase in good bacteria in the maintenance media and also help suppress the growth of pathogenic bacteria. This is reinforced by the statement by Duncan et al., (2018) that *Bdellovibrio* is a predatory bacteria that is able to prey on various bacteria. Gram-negatives include human pathogens and are often described as living antibiotics, their use as antibiotics to eliminate harmful bacteria and pathogens and as probiotics to help curb and control bacterial populations in the intestinal tract.

Based on Figure 9, the highest value of phagocytic activity was found in P5 (*Bdellovibrio* 70 ppm) with a value of 64.82% and the lowest value was in treatment P1 (Control +) with a value of 51.36%. The increase in phagocytic activity in the treatment given by *Bdellovibrio* is thought to be closely related to the increased immune system of white shrimp in response to pathogens that enter their bodies. One of the efforts of the vaname shrimp's body to defend itself from attacks by pathogens is to destroy these pathogens through the process of phagocytosis. In accordance with the statement of Ekasari et al., (2014), probiotics contain various microorganisms, cell components and metabolites which can act as immunostimulants. Microorganisms such as bacteria have cell walls consisting of lipopolysaccharide (LPS), peptidoglycan (PG), and  $\beta$ 1,3-glucan (B3) which are able to activate immunity in shrimp, followed by the statement by Duncan et al., (2018) that *Bdellovibrio* is predatory bacteria capable of preying on a variety of Gram-negative bacteria including human pathogens and are often described as living antibiotics, their use as antibiotics to eliminate harmful bacteria and pathogens and as probiotics to help curb and control bacterial populations in the digestive tract. intestines. The phagocytic activity value in this study is considered good if you look at the results of research from Wilisetyadi et al., (2022) who used EM4 probiotics in water media, obtaining phagocytic activity values ranging from 49.43% - 67.97%.

### **Feed Conversion Ratio (FCR)**

Feed conversion (FCR) is a measure that states the ratio of the amount of feed needed to produce 1kg of fish meat. FCR is an indicator to determine whether the amount of feed given during the rearing period is absorbed or utilized optimally by vaname shrimp. Based on Figure 10, the highest FCR value was obtained from treatment P1 (+) 1.78 while the lowest value was in treatment P5 (Bdellovibrio 70ppm) with a value of 1.23. The high FCR in P1 indicates that the feed given during rearing has not been absorbed optimally by the shrimp. In the control treatment, while the FCR value obtained at P5 was low, it can be seen that the administration of *Bdellovibrio* bacteria in water media had an influence on the feed conversion ratio (FCR) value compared to the treatment without administration. *Bdellovibrio* or control treatment. The treatment of *Bdellovibrio* bacteria in water media gave a lower value compared to the control, indicating that the work of *Bdellovibrio* bacteria can maintain water quality from pathogenic bacteria and is indirectly absorbed through feed and enters the shrimp's body so that it can help suppress pathogenic bacteria in the shrimp's digestive system. so that feed utilization is more optimal, followed by the statement of Cao et al., (2019) who said that *Bdellovibrio* is considered a probiotic that is good for food safety and the environment, which does not have cytotoxicity against fish cells, does not show hemolytic activity, does not show virulence for fish, shrimp and mice and can reduce ammonia, nitrite, sulfide and pathogenic bacterial populations in aquaculture water, *Bdellovibrio* can also act as a driver of microbial diversity intestine without pathogenicity and toxicity. From the research results of Simanjuntak et al., (2020) it was stated that the administration of probiotics can also influence absolute length growth and feed conversion ratio. From the results of their research it was also found that the lowest FCR value was in the treatment with the highest dose of probiotics. The FCR value in this study can be said to be good when compared with the results of research from Syadillah et al., (2020) which used the addition of *Lactobacillus* sp bacteria. with different concentrations on the growth of vaname shrimp with FCR values obtained ranging from 2.046 – 2.606.

### **Specific Length Growth Rate**

Specific length growth rate is the percentage rate of increase in length growth per day. Based on Figure 11. The highest value of Specific Length Growth Rate is found in treatment P5 (*Bdellovibrio* 70ppm) with a value of 19.4%/day while the lowest value is found in P1 (+) with a value 13.3%/day, the high value in P5 is because *Bdellovibrio* which is applied in water media is thought to be absorbed into the feed given so that it indirectly enters the digestive tract of vaname shrimp and forms colonies that are not pathogenic to the shrimp so that it can help the feed absorption process and increase the appetite of vaname shrimp. This is reinforced by the statement of Cao et al., (2019) who said that *Bdellovibrio* is considered a good probiotic for food and environmental safety, which does not have cytotoxicity against fish cells, does not show hemolytic activity, does not show virulence for fish, shrimp, and mice and can reduce ammonia, nitrite, sulfide, and pathogenic bacterial populations in aquaculture water, *Bdellovibrio* can also act as a booster of intestinal microbial diversity without pathogenicity and toxicity and followed by the statement by Defatri et al., (2015) who stated that growth in fish is not also caused by the frequency and ability of fish to optimize feed for metabolism, but also by the quantity and quality of feed which can influence it. The Specific Length value in this study is classified as good if referring to the research results of Wilisetyadi et al., (2022) which

obtained a specific Length growth value in the use of EM4 probiotics which ranged from 1.22 – 1.45%/day.

### **Absolute Length Growth**

Based on Figure 12, it can be seen that the highest absolute length value is in P5 (Bdellovibrio 70ppm) with a value of 8.73 cm, while the lowest value is in P1 (+) with a value of 5.98 cm. The high absolute length value in P5 is thought to be in line with the growth rate parameter. This specific length is because Bdellovibrio given at the highest concentration is able to optimize the growth of vaname shrimp. This is confirmed by the statement of Cao et al., (2019) who said that Bdellovibrio is considered as a good probiotic for food and environmental safety, which does not have cytotoxicity against fish cells, does not show hemolytic activity, does not show virulence for fish, shrimp and mice and can reduce ammonia, nitrite, sulfide and the population of pathogenic bacteria in water. aquaculture so that this can trigger the growth rate of vaname shrimp. The Absolute Length value in this study is classified as good if referring to the research results of Wilisetyadi et al., (2022) which obtained absolute length growth values in the use of EM4 probiotics which ranged from 3.98 – 4.94 cm.

### **Specific Weight Growth Rate**

Weight specific growth rate is the percentage rate of increase in weight growth per day. Based on Figure 13. The highest value of specific weight growth rate was found at P5 with a value of 9.96%/day while the lowest value was at P1 (+) with a value of 6.79%/day. The high specific weight growth rate at P5 is thought to be in line with the rate parameters. The specific length growth of Bdellovibrio can help increase good bacteria that can maintain water quality in the rearing media and also suppress pathogenic bacteria so that it can help increase the growth of vaname shrimp, this is reinforced by the statement of Cao et al., (2019) who said Bdellovibrio is considered a good probiotic for food and environmental safety, which does not have cytotoxicity against fish cells, does not show hemolytic activity, does not show virulence for fish, shrimp and mice and can reduce ammonia, nitrite, sulfide, and Pathogenic bacteria in aquaculture water can trigger the growth rate of vaname shrimp. From the research results of Widarnani et al., (2016), using Synbiotics obtained specific growth rate values ranging from 6.65% - 6.93%. So it can be said that in this research the use of Bdellovibrio has a relatively good specific growth rate value.

### **Absolute Weight Growth**

Based on figure 14, it can be seen that the highest absolute weight value is in P5 (Bdellovibrio 70ppm) with a value of 61 g, while the lowest value is in P1 (+) with a value of 42.1 g. The high value of Absolute Weight in P5 is in line with the specific weight growth rate parameter stated It is thought that Bdellovibrio can help increase good bacteria that can maintain water quality in the rearing media and also suppress pathogenic bacteria so that it can help increase the growth of vaname shrimp. This is confirmed by the statement of Cao et al., (2019) who said that Bdellovibrio is considered a good probiotic for food and environmental safety, which does not have cytotoxicity against fish cells, does not show hemolytic activity, does not show virulence for fish, shrimp and mice and can reduce ammonia, nitrite, sulfide, and the population of pathogenic bacteria in aquaculture water so that this can trigger the growth rate of vaname shrimp. From the research results of Wilisetyadi et al., (2022) in using EM4 Probiotics, absolute weight growth values were obtained which ranged

from 25.22 – 36.37 g. so that the daily weight growth value in this study can be said to be good.

### **Relationship Between Length Growth and Weight**

Growth is the process of absolute increase in size, volume and mass due to cell enlargement and increase in cell number due to the process of cell division. Growth can be expressed quantitatively because growth can be known by looking at the changes that occur in the living creature in question. In this study, the growth of vaname shrimp that was observed was the length and weight of the vaname shrimp. Based on the results of the Specific Growth Rate Parameters, Length and Weight, the growth values for Length and body weight are directly proportional, but based on the data obtained, the weight growth of vaname shrimp in this study is more dominant than the Length growth, so this can be said to be positive allometric, it is assumed that weight growth is more dominant. because as vaname shrimp get older their growth will be more focused on fattening so their length will be hampered, this is in accordance with Desrita's (2018) statement that shrimp that are approaching maturity will grow in weight faster than their length growth. The addition of *Bdellovibrio* bacteria with different concentrations will increase the shrimp's appetite so that their weight growth will increase. Nadhif (2017), in his research stated that appetite and feed retention will be higher so that shrimp growth will be better.

### **Total Bacteria Count (TBC)**

Total Bacteria Count is a calculation of the number of all bacteria contained in the maintenance media. Based on Figure 15. The highest value is in P5 (*Bdellovibrio* 70ppm) with a value of  $5.98 \times 10^8$  CFU/ml, while the lowest value is in P1 (+) with a value of  $1.75 \times 10^8$  CFU/ml given *Bdellovibrio* at different doses in The rearing media is one of the things that causes high TBC values, but the high TBC values in this study did not cause death of shrimp or damage to water quality, this was due to bacteria in the water. dominated by *Bdellovibrio* bacteria, where Cao et al., (2019) stated that *Bdellovibrio* is considered a good probiotic for food and environmental safety, which does not have cytotoxicity against fish cells, does not show hemolytic activity, does not show virulence for fish, shrimp, and mice and can reduce ammonia, nitrite, sulfide, and the population of pathogenic bacteria in aquaculture water. According to Nayak (2010), the addition of probiotics can increase the immune system and the number of beneficial bacteria in the shrimp digestive tract and suppress the growth of disease pathogens, so that shrimp survival increases. The TBC value in this study can be categorized as good if referring to the research results of Susilowati et al., (2017) in using probiotics, the total population value of general bacteria in pond water was found to be in the range of  $10^6$  -  $10^8$  CFU/ml.

### **Total Vibrio Count (TVC)**

Total Vibrio Count is a calculation of the number of bacteria, especially *Vibrio* bacteria, which were used as test bacteria in this study. Based on Figure 16. The highest TVC value is in P1 (+) with a value of  $3.26 \times 10^7$  CFU/ml, while the lowest TVC value is in P2 (-) with a value of  $1 \times 10^7$  CFU/ml, but among the treatments given are *Bdellovibrio* bacteria. , P5 has the lowest TVC value with a value of  $1.43 \times 10^7$  CFU/ml, the high TVC value in P1 is thought to be due to the absence of bacteria. *Bdellovibrio* in the maintenance media so that this can trigger an increase in the TVC value in this treatment, while the low TVC value in P5 is thought to be in line with the TBC Parameters in P5 where the concentration of *Bdellovibrio* 70 ppm is able to

increase the number of good bacteria so this can cause suppression of Pathogenic bacteria, one of which is *Vibrio harveyi*, is in accordance with the statement by Duncan et al., (2018) that *Bdellovibrio* is a predatory bacteria that is able to prey on various Gram-negative bacteria including human pathogens. and is often described as a live antibiotic, its use as an antibiotic to eliminate dangerous and pathogenic bacteria and as a probiotic to help curb and control bacterial populations in the intestinal tract is continued with the statement by Kharisma & Manan (2012) that in white shrimp the abundance of bacteria exceeds 10<sup>4</sup> CFU/ml susceptible to vibriosis attacks and can cause mass death. If the total bacterial and total vibrio values exceed the threshold, it can cause mass deaths in vaname shrimp cultivation. The total value of bacteria and vibrio obtained exceeded the normal limit value, but did not cause mass deaths in shrimp because of the administration of *Bdellovibrio* which was able to suppress the growth of pathogenic bacteria which increased the population of good bacteria in the rearing media. The total value of vibrio obtained was lower than the total value of common bacteria. So that the research results obtained did not cause mass deaths. Jayadi (2016) who reported the total number of vibrio in the intestines of shrimp infected with *Vibrio* sp. amounting to 3.9 x 10<sup>7</sup> CFU/ml does not kill vaname shrimp.

### **Water Quality**

Water quality is a determinant of the optimal value that fish can tolerate in order to survive and grow. In this research, the water quality parameters measured include ammonia, dissolved oxygen, acidity, salinity and temperature.

Water temperature is the level of cold or hot water, in this research the tool used to measure it is a thermometer. Temperature measurements were carried out every 10 days, the temperature obtained ranged from 30 – 31°C, the temperature obtained was within the optimal value range for vaname shrimp, 16-32°C (Citria, 2018). Values that are below optimal can still be tolerated by vaname shrimp to survive. Temperature can also affect the metabolic rate of shrimp, resulting in a decrease in growth rate and water temperature can also affect the solubility of oxygen in the water and influence chemical and biological processes in the water.

The degree of acidity (pH) is a parameter that determines the acid level or basically waters. In this study, pH measurements were carried out every 10 days, the pH value obtained in this research was in the range of 7.3-7.8. The pH value obtained is included in the optimal value for the life of vaname shrimp, according to Citria, (2018) the optimum pH value for white vaname shrimp ranges from 7.5 – 8.5. A pH value that is too low will make vaname shrimp become soft due to calcium absorption not being able to take place properly, whereas if the pH value is high it will increase ammonia which is toxic to vaname shrimp.

Salinity is a water parameter that has an impact on biological processes and can directly influence the growth of vaname shrimp. In this study, salinity measurements were carried out every 10 days. In this study, the salinity value obtained was 29-32 ppt. According to Citria (2018), the optimum value of salinity in the growth and survival of vaname shrimp is 30 - 53 ppt. Changes in salinity that are too high can cause damage to the pancreas in small fry, besides that salinity is closely related to the availability of calcium.

Dissolved Oxygen (DO) is the amount of oxygen that is excessive in the cultivation water media. Dissolved Oxygen (DO) has a very important role in the metabolic processes of aquatic biota, especially vaname shrimp. The availability of DO is very influential in supporting shrimp development, life and growth. In this

research, DO measurements were carried out every 10 days. The DO values obtained ranged from 5.1 – 6.4 mg/l. According to Citria (2018), the optimum dissolved oxygen value for the life and growth of vaname shrimp is >3 mg/l.

Ammonia is a buildup of organic material that comes from uneaten food residue, shrimp waste that settles at the bottom of the water, and dead microorganisms. In this study, ammonia was measured every 10 days with a spectrophotometer, from the observations the ammonia was obtained at 0.6 mg/l. According to Fendjalang et al., (2016) a good ammonia value is <0.2 mg/l. This high ammonia value is thought to be in line with the growth of vaname shrimp so that vaname shrimp activity continues to increase due to the metabolic results released and the feed that needs to be given which causes high ammonia levels, however this can still be tolerated by white vaname shrimp because they still carry out daily feeding and additional water after siphoning and giving *Bdellovibrio* so that this can help maintain water quality so that vaname shrimp can survive.

## CONCLUSION AND SUGGESTIONS

### Conclusion

Optimal dose of P5 (*Bdellovibrio* 70 ppm) treatment in water media was able to improve the immune system of vaname shrimp, the THC value was  $16.94 \times 10^6$  cells/ml, the DHC value in hyaline cells was 59.33%, granular cells 24%, semi-granular cells namely 16.67%, AF value 64.82%, TBC value  $5.98 \times 10^8$  CFU/ml, TVC value  $1.43 \times 10^7$  CFU/ml, FCR value 1.23, specific length growth rate value 19.4%/day, specific weight growth rate 9.96%/day, absolute length value 8.73 cm, absolute weight 61 grams and SR value of 77.78%.

### Suggestion

The results of this research show a graph with a value that continues to increase, so further research is needed to obtain the optimal dose value for using *Bdellovibrio*.

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