

Addition of® Bioimmunity to Feed to Increase Immunity of Vannamei Shrimp (*Litopenaeus vannamei*) Raised in Cement Tubs

Aulia Wanda Devania*, Esti Handayani Hardi, Sulistyawati, Ricky Djauhari

Faculty of Fisheries and Marine Sciences, Mulawarman University, Samarinda
Faculty of Agricultural, Forestry and Fisheries, Palangka Raya University, Palangka Raya

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Abstract

The purpose of this study is to determine the use of BIOIMMUNE® made from Sour Eggplant (*Solanum ferox*) and Lempuyang (*Zingiber zerumbet*) extracts mixed into pellet feed on the survival rate and immunity of vannamei shrimp (*L. vannamei*). The shrimp used are ± 10 g/head and are kept in a concrete tub with a density of 20 fish/repeat. This study used a complete randomized design (RAL) with 3 treatments repeated 4 times with BIOIMMUNE® doses of 0 mL/kg (P0), 40 mL/kg (P1) and 60 mL/kg (P2). The results showed that the survival rate of the three treatments reached 100%, the highest total hemocyte cells were P2 (3.73×10^7 cells/mL) and the lowest P0 (1.46×10^7 cells/mL), the highest granular cells at P1 (59.6%), the highest semi-granular cells (34.6%) and the highest hyaline at P0 (41.6%). The average water quality in this study was 27.6 °C, pH 7.50, DO 5.20 ppm, salinity 16.4 ppt and NH₃ 0.16. In conclusion, the administration of Bioimmunity® did not differ significantly in the survival rate but gave a significantly different result in increasing vannamei shrimp immunity. Immunity increases at a dose of 60 mL/kg of feed.

Keywords: Bioimmune®; Immunity; Vannamei Shrimp (*Litopenaeus vannamei*); Survival

1. Introduction

Shrimp is one of the fishery export commodities that plays an important role in Indonesia's foreign exchange income. Foreign exchange obtained from the fisheries sector is around 34% from shrimp exports (Syafri, 2020). Nur'aini et al. (2019) support this by stating that the introduction of vannamei shrimp (*Litopenaeus vannamei*) to Indonesia in 2001 has shown rapid development. The export volume which reached 145,092 tons in 2010 increased to 162,410 tons in 2013 (Inayah et al., 2015). One of the potential areas in vannamei shrimp cultivation is East Kalimantan Province, especially the Mahakam Delta area which has an area of 110,000 ha of aquaculture centers. However, the continuous expansion of pond areas has the potential to cause environmental problems, such as the conversion of mangrove forests into ponds, which causes damage and decrease in mangrove forest area. To minimize changes in the ecological function of mangrove forests as a habitat for coastal biodiversity, it is necessary to develop alternative cultivation systems, one of which is the use of cement tanks (Fahrany et al., 2018).

Vannamei shrimp only have a nonspecific immune system to defend themselves against pathogens, making them more susceptible to disease, especially in new environments. One of the efforts that can be made to overcome this is to increase the body's

defense system (immunity) through the administration of immunostimulants. Immunostimulants are substances that can increase the activity of immune cells in shrimp (Ismawati et al., 2019). The immunostimulants used must be environmentally friendly, leave no residue, and do not cause resistance, so natural-based ingredients (herbal) are needed. Some of these ingredients include sour eggplant (*Solanum ferox*) and lempuyang (*Zingiber zerumbet*), which are known to increase the immunity of vannamei shrimp (Rahardjo et al., 2022).

One of the herbal immunostimulant products that has been developed and marketed is Bioimmun.® This product functions as an antibacterial and immunostimulant with active ingredients from acidic eggplant extract (*S. ferox*) and lempuyang (*Z. zerumbet*). Bioimmunity® contains alkaloid compounds, flavonoids, steroids, and carbohydrates that have the potential to increase the resistance of the fish body to changes in water quality and pathogenic infections (Bioperkasa, 2016). Syakirin (2022) reported that the addition of acidic eggplant extract to feed can increase the growth of fish length and weight, while increasing the population of good bacteria in the intestine that play a role in food digestion.

The results of the research by Anggara et al. (2021) show that the addition of Bioimmune® by 20 mL/L can increase the growth of vannamei shrimp up to a weight of 15.64 g and a length of 4.2 cm with a feed

*) Corresponding author: Auliawandadevania28@gmail.com

conversion of 1.4. However, most of the Bioimmune® effectiveness tests so far have focused on fish, while in-depth studies on the effects of Bioimmune® on shrimp, especially vannamei shrimp in terms of immune response, are still very limited. In fact, the difference in the immune system between fish and shrimp where shrimp only have a nonspecific immune system makes the results of research on fish not necessarily applicable directly to shrimp. Therefore, this study is important to test the effectiveness of Bioimmune® in increasing vannamei shrimp immunity and ensuring its potential use in sustainable aquaculture systems.

2. Methods

This research began to be carried out for 30 days from January 22 – February 20, 2023. Vannamei shrimp farming was carried out at the Benur Idaman Hatchery, Muara Badak Ulu Village, Muara Badak District, Kutai Kartanegara Regency, East Kalimantan and observation of vannamei shrimp hemocytes was carried out at the Aquatic Microbiology Laboratory of FPIK UNMUL.

2.1 Research Tools and Materials

The tools used are 3 cement tubs with a size of 2 m x 2 m x 1 m, Digital scales, Beaker glass, 1 mL syringe, Microtube, Neubaur counting chamber, stereophone box, pipette, glass objects, microscope, Anco, Shelter, Tarpaulin, Waring (1 m x 1 m), Water quality measuring device (pH meter, DO meter, Thermometer, and refractometer), Scrub, Water pump machine, and Large Basin.

The ingredients that need to be prepared are vannamei shrimp (*Litopenaeus vannamei*), Bioimmune®, vannamei shrimp haemolymph, evergreen protein feed 30%, Methanol, Giemsa 7%, Alcohol 70%, EDTA solution 0.1%, and Aquades.

2.2 Research Procedure

2.2.1. Shrimp Preparation

The vannamei shrimp (*Litopenaeus vannamei*) used with a size of ± 10 g/head as many as 240 fish, came from the UPTD Central Brackish Air and Sea Water Seed Center, South Balikpapan District, Balikpapan City which was sent using land transportation, in good health. Shrimp were acclimatized for 1 week at the study site.

During the acclimatization, the shrimp were fed 4 times a day in the morning (08.00 WITA), during the day (13.00 WITA), in the afternoon (16.00 WITA), and at night (20.00 WITA). In addition, the acclimatization container is also aerated to support oxygen conditions as needed, at least 4kg/l (Wahyuni et al., 2022).

2.2.2. Container Preparation

The container used is a cement tub measuring 2m x 2m x 1m. One cement tub contains 1 treatment which is divided into 4 replicates using warping. The warping is 12 pieces with a size of 1m x 1m and each is equipped with 2 aerators and 4 shelters (diameter = 10-15 cm, length = 20 – 30 cm). The sorting container uses 2 styrofoam boxes measuring 80 cm x 40 cm.

2.2.3. Feed Preparation

The feed used is PV commercial pellet feed, shrimp feed with 30% protein content. Feed is given as much as 5% of the body weight of the shrimp and mixed with diluted Bioimmune® (stock solution). The Bioimmune® (40 mL and 60 mL) were diluted with 100 mL of water and became a stock solution (0.4 mL/kg feed and 0.6 mL/kg feed). Mixing Bioimmune® with feed is carried out when the shrimp will be fed by letting it sit for 10 – 20 minutes. Bioimmune® doses are administered according to the treatment.

2.2.4. Research Design

The design used in this study is a Complete Random Design (RAL) using 3 treatments, each of which is repeated 4 times. The treatment given to the shrimp to be stocked is P0 (Dose of 0 mL/kg of feed), P1 (Dose of 40 mL/kg of feed) and P2 (Dose of 60 mL/kg of feed).

2.2.5. Maintenance

Each tub is given a partition using a warping which contains 4 replicas with a size of 1 x 1 m and contains 20 shrimps/replicas. Feeding is carried out 4 times a day, namely in the morning at 08.00 WITA, in the afternoon at 13.00 WITA, in the afternoon at 16.00 WITA and in the evening at 20.00 WITA. Feed is given to shrimp through anco to make it easier to check shrimp when eating. Cleaning is carried out every 3 days to clean organic dirt at the bottom of the pond. Water changes are carried out once a week to avoid an increase in ammonia. Cleaning of the remaining molting is carried out daily to avoid settling at the bottom of the tub. Maintenance is carried out routinely throughout the study.

2.2.6. Survival Rate Calculation

Monitoring of vannamei shrimp survival is carried out every 3 days, at the same time as feeding and water quality checks. The calculation of the survival rate of vannamei shrimp was only carried out 1x, namely on the 30th day at the end of the study.

2.2.7. Calculation of Total Hemocyte Cells

Shrimp hemolymph was collected at the base of the pleopod in the abdominal segment near the genital hole using a 1 mL syringe that had been moistened with an anticoagulant solution (EDTA 10%) with a ratio of

1 : 1. Hemolim is placed inside a sterile microtube and stored in a cool box. The calculation of shrimp hemocytes is carried out by dripping hemolym into the counting chamber and counting the number of cells per ml under a light microscope with a magnification of 40 times. The calculation of the total number of hemocytes (THC) was carried out using a haemocytometer with the Campa-Cordova et al. procedure. (2002) namely in one large box at the top and bottom right and the big box at the lower left and middle right and middle boxes. A circular hemocyte cell shape that has a cell nucleus in the middle.

2.2.8. Differential Calculation of Hemocytes

Shrimp hemolymph collection was carried out at the base of the pleopod in the abdominal segment near the genital opening using a 1 ml syringe that had been moistened with an anticoagulant solution (EDTA 10%) with a ratio of 2 : 1. Hemolim is placed inside a sterile microtube and stored in a cool box. The manufacture of hemocyte differential preparations is carried out by means of hemolysis dripped on the glass of the object and made a review, then dried in the air. Next, the preparation is fixed with methanol for 10 minutes, then dried in the air again. The preparation is soaked in a 7% Giemsa dye solution for 20 minutes, then washed with aquades and left to dry. The differential calculation of hemocytes is calculated by grouping hemocyte cells into 3 cell types (granular, semi-granular and hyaline) under a microscope with a magnification of 1000 times. The total number of hemocyte cell types calculated is 100 cells (Martin and Graves, 1995). Granular is rounded with full granules, and semi-granular is slightly oval granular and does not meet the cytoplasm, whereas hyaline is spherical with a faded color without granular.

2.2.9. Water Quality Measurement

Water quality measurement parameters include temperature, DO, pH, salinity, and ammonia. Water quality was measured in the morning at 07.00 WITA and 16.00 WITA.

2.3 Data Collection and Analysis

Samples will be tested for data taken once every 10 days. The data to be tested are as follows:

2.3.1. Survival Rate

The calculation of Survival uses the following formula (Effendi, 2002):

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

SR = Survival rate (%)

Nt = Number of live shrimp at the end of rearing (tail)

No = Number of shrimp at the beginning of rearing (tail)

2.3.2. Hemocyte Total Test

Calculation of total hemocytes using the following formula (Cordova et al. 2002):

$$\frac{\text{TSH}}{\text{Jumlah sel dihitung}} \times \frac{\text{(salt/mm3)}}{\text{Volume dihitung}} \times \text{faktor pengenceran} \times 10^4 =$$

TSH : Total Salt Hemosite

2.3.3. Hemocyte Differential Observations

The number of hemocytes is calculated up to 100 cells and the percentage of each type of hemocyte cell is determined and then calculated by the following formula (Marti and Graves 1985):

$$DH = x 100 = \frac{\text{Jumlah Tiap Jenis Hemosit}}{\text{Total Hemosit}}$$

DH = Hemocyte Differential

The data obtained were survival rates, hemocytes, and differential hemocytes. The data was then analyzed for homogeneity and analyzed using multiple fingerprint analysis (ANOVA Single Factor) at a confidence level of 95%. Results that show a real difference will be followed by the Duncan Test. Supporting data such as water quality and feed weight are tabulated in the form of a table.

3. Results and Discussion

Research on the application of Bioimmunity® to the maintenance of vannamei shrimp (*Litopenaeus vannamei*) in cement tubs that lasted for 30 days showed that the use of Bioimmunity® had a positive effect on increasing the immunity of vannamei shrimp (*Litopenaeus vannamei*).

3.1 Survival Rate

The results of observation of the survival rate of vannamei shrimp (*L. vannamei*) at 30 days of maintenance in cement tanks at all treatments showed the same results. It can be seen in the image below that each treatment has a 100% percentage.

Figure 1. Vannamei Shrimp Survival Rate Bar Chart



Based on the results of variegated fingerprint analysis (ANOVA), the survival rate of vannamei shrimp (*L. vannamei*) showed no significant difference ($P > 0.05$), both without treatment (0 mL/kg feed) and those treated (40 and 60 mL/kg feed). Therefore, it can be seen that the administration of Bioimmunity® does not have a real effect on the survival rate of vannamei shrimp (*L. vannamei*).

During the research process in the concrete tank, the maintenance environment and vannamei shrimp were given controlled treatment such as water quality management and regular feeding. This is in line with Purnamasari (2017) who stated that water quality management and good feeding are one of the factors that cause the high survival rate of vannamei shrimp. Then, supported by the explanation of Amri (2013) stating that vannamei shrimp has a high level of resistance to survive in environments that have low water quality and are resistant to disease attacks. In addition, the stocking applied to vannamei shrimp does not exceed the normal limit, which is as much as 20 e kor/m². Normally, vannamei shrimp (*L. vannamei*) will grow well in a density of 60 – 150 heads/m² (Briggs et.al., 2004). In line with Effendi (2004), he explained that stocking density is one of the determining factors in the survival rate because stocking density will determine the high intensity of maintenance, avoid fighting for feed because vannamei shrimp are known as greedy aquatic biota and avoid cannibalism. With enough space for vannamei shrimp to be kept, the needs of shrimp can be met equally.

Although there is no real influence between P0, P1, and P2 but a percentage of 100% has shown excellent results. According to Widigdo (2013), shrimp can be said to have a good survival rate if it reaches > 50 - 70% and is included in the low category if < 50%.

3.2 Total Sel hemosit

Vannamei shrimp that were given a Bioimmune® treatment and those that were not given a treatment both provided an improvement. Of the three treatments, the highest total hemocyte cells were found in the second treatment (60 mL/kg feed) in week 4. In all three treatments on day 0, treatment 1 (40 mL/kg feed) had the highest yield, but a rapid improvement was found in P2 (60 mL/kg feed) on the last day of maintenance. The results can be seen in re

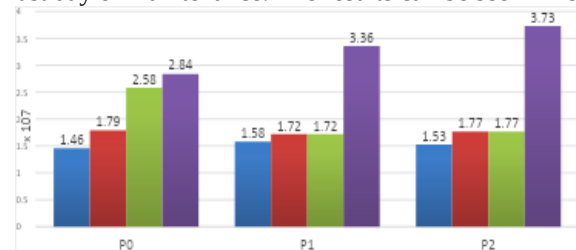


Figure 2. Stem Diagram of the total research results of Vannamei Shrimp hemocytes

Based on the results of variegated fingerprint analysis (ANOVA), the total hemocytes of vannamei shrimp (*L. vannamei*) had a significantly different effect ($p < 0.05$) on the total number of vannamei shrimp hemocytes with the highest total number of hemocytes obtained in treatment 2 (60 mL/kg feed). Based on the results of Duncan's follow-up test, it was known that the highest total number of hemocytes was at P2 (3.73 x10⁷ cells/mL) and the lowest total number of hemocyte cells was at P0 (1.46 x10⁷ cells/mL). This indicates that the administration of Bioimmune® as an immunostimulant can increase the total hemocytes of vannamei shrimp. According to Yeh et.al (2009) that normal vannamei shrimp will have a total of hemocyte cells between 1.80 x 10⁷ – 9.28 x 10⁷ cells/mL.

Vannamei shrimp (*L. vannamei*) is one of the classes of Crustaceans that do not have a specific immune system. Saha (2011) stated that shrimp have a non-specific immune system that can recognize and detect foreign objects that enter the shrimp's body and can destroy them. Therefore, the non-specific immune system has an important role in shrimp immunity ((Johansson et.al., 2000). An increase in the total number of hemocyte cells means an increase in the health status of the shrimp because hemocytes can form phagocytosis cells to defend themselves from the attacks of microorganisms. Hemocytes can be a quantitative parameter in measuring the stress response and ability of shrimp to be able to fight foreign bodies as well as some responses to infections that are affected by total hemocytes so that low hemocytes can cause susceptibility to pathogen attacks. Conversely, if the total number of hemocyte cells is high, vannamei shrimp can defend themselves from pathogenic attacks (Tenriulo et.al., 2014).

3.3 Hemocyte Cell Differential

Shrimp hemocytes consist of 3 types of cells, namely granular, semi-granular, and hyaline (Hartinah et al., 2014). An increase in hemocytes is accompanied by an increase in hemocyte cell types. Immunostimulants can stimulate hemocyte cells (granular) to carry out the body's defenses. From the results of the study, it was found that the differential hemocytes of vannamei shrimp that were maintained for 30 days were dominated by granular cells. This shows that the addition of immunostimulants from Bioimmune® provides good results against the immunity of vannamei shrimp (*Litopenaeus vannamei*). The results can be seen in Figure 3.

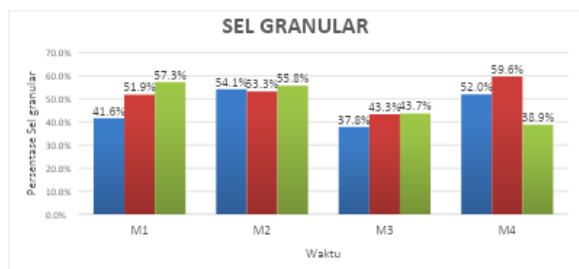


Figure 3. Vannamei Shrimp Granular Cell Stem Diagram

Vannamei shrimp granular cells were highest on day 30 (59.6%) at P1 (40 mL/kg feed) and lowest on day 20 (37.8%) at P0 (0 mL/kg feed). Based on the results of differential fingerprint analysis (ANOVA), the differential cells of vannamei shrimp (*L. vannamei*) had a significantly different effect ($p < 0.05$) on granular cells. According to Owens and O'Neill (1997) the percentage of normal granular cells is in the range of 17 – 40% and normal hyaline is in the range of 60 – 93%. This shows that the addition of immunostimulants from Bioimun® gives good results against vannamei shrimp immunity. Supamattaya (2000) stated that granular cells tend to process phenoloxidase enzymes which play an important role in the defense system during pathogen attacks.

Darwantin (2016) explained that the increase in granular cells is the result of immunostimulants entering the shrimp's body. Immunostimulants can stimulate hemocyte cells (granular) to carry out the body's defenses. Shrimp that do not have specific immunity will have different body defense mechanisms than other animals that have specific immunity or that have immunoglobulins. Soderhall and Cerenius, (1992) explained that immunoglobulins in shrimp are replaced by Prophenoloxidase Activating Enzyme (PPA) which is a protein present in granular cells. Just like immunostimulants that activate leukocytes in fish, immunostimulants also activate hemocytes in shrimp and will stimulate prophenoloxidase into phenoloxidase which will later produce a kind of Opsonin Factor protein that can induce hyaline cells to increase their activity when attacked by a foreign body in their body (Johansson and Soderhall, 1989).

Van de Braak (2002) than Smith, et al. (2003) also supports the statement that the haemocyte cells will degranulate, and some proteins will be released for the sake of the immune response, such as: increased haemocyte cells, and increased snare activity and phagocytosis. In addition, immunostimulants will stimulate haemocytes to release proPO and protein-binding PPA as signaling molecules, resulting in haemocyte cells increasing their activity as defense cells by recognizing foreign bodies entering the body of vannamei shrimp.

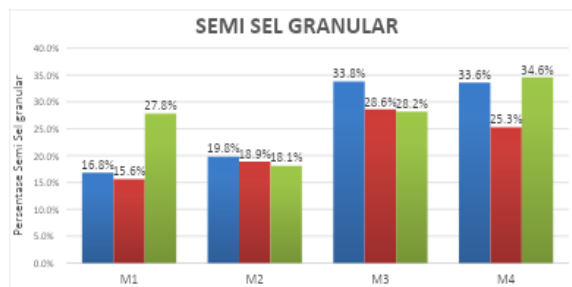


Figure 4. Vannamei Shrimp Granular Semi Cell Stem Diagram

Semi-granular cells of vannamei shrimp were highest on the last day (34.6%) at P2 (60 mL/kg feed) and lowest on day 0 (15.6%) at P1 (40 mL/kg feed). The results can be seen in Figure 4. Based on the results of variegated fingerprint analysis (ANOVA), the differential semi-granular hemocyte cells of vannamei shrimp (*L. vannamei*) exerted a noticeably different effect ($p < 0.05$) on vannamei shrimp semi-granular cells. The normal percentage of semi-granular cells is 13 – 49% (Johansson et.al., 2000). Semi-granular cells are the protectors of hyaline cells, so hyaline cells will not develop into semi-granular cells and there will be a percentage decrease in semi-granular cells because the main role when there is a pathogen that attacks is hyaline cells Van De Braak (2002).

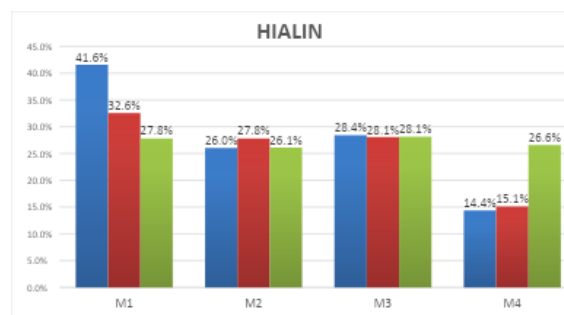


Figure 5. Hialin Shrimp Vannamei

Vannamei shrimp hyaline was highest on day 0 (41.6%) at P0 (60 mL/kg feed) and lowest on the last day (14.4%) at P0 (40 mL/kg feed). The results can be seen in Figure 5. In hyalin cells, based on the results of multiple fingerprint analysis (ANOVA), the number of vannamei shrimp hyaline cells (*L. vannamei*) did not differ significantly ($p > 0.05$).

Although hyaline cells show lower than average numbers, semi-granular cells show normal results. So, if the hyaline cells are lower than the semi-granular cells, it means that the hyaline has matured (developed) into a semi-granular cell. This indicates that the shrimp farming environment is in good condition due to the low chance of infection or pathogen attack from the outside, so that hyaline cells

do not work to bind and develop into semi-granular cells. Anderson and Siwicki (1995) stated that in the event of an infection, blood cells will migrate to the infected area of the pathogen and will produce more hyaline. In addition, this also indicates that immunostimulants are able to stimulate hemocytes well because the number of granular cells as a form of defense is in the normal range.

3.4 Water Quality

Water quality measurements are carried out every 3 days in the morning and ammonia (NH₃) every 7 days. The results of the measurement of temperature, pH, DO, salinity are measured three times and for ammonia (NH₃) can be seen in Table 1.

Table 1. Water quality of vannamei shrimp

Water Quality Parameters	Week 1				Average
	1	2	3	4	
Temperature (°C)	26,3	25,4	26,3	26,3	27,58
	28,6	28,6	28,8	28,9	
pH	7,45	7,46	7,42	7,48	7,50
	7,47	7,80	7,78	7,80	
OD (ppm)	4,2-5,9	4,4-4,7	4,3-4,8	4,3-4,9	5,20
Salinitas (ppt)	17	17	16	16	16,40
Ammonia (NH ₃)	0.3	0.3	0,1	0.2	0,16

The table above shows that the temperature of vannamei shrimp media is at normal values. Pratama (2017) stated that good water quality will cause physiological processes in the shrimp body to run well, thereby supporting the growth and survival rate of shrimp. Tahe et al (2015) stated that the optimal temperature for vannamei shrimp cultivation is 27 – 32°C. High temperatures can usually increase the metabolic rate so that it can increase the appetite of shrimp. This is in line with Swan's (1997) statement that temperature can affect the activity, behavior, eating habits, growth, and reproduction of all aquatic organisms.

pH concentration affects the fertility level of the waters because it affects the life of the body of the renik. Acidic waters tend to cause death in fish as well as in pH that has a value that is too alkaline. At the pH of the water that is too low or too high, it can cause stress on shrimp and the softness of the shrimp skin and the low survival of the shrimp (Chakravarty et al., 2016). The degree of acidity (pH) in the table shows an average result of 7.50. Haliman & Adijaya (2005) stated that the optimal pH in vannamei shrimp maintenance is in the range of 7.5 - 8.5 so it can be concluded that the pH during maintenance is in the normal range. This low pH value can lower the pH of fish/shrimp blood called the acidosis process so that the function of the blood to transport oxygen also

decreases (Buwono, 1993).

Dissolved Oxygen is very important to determine the life and death of vannamei shrimp. The results of DO measurement during the maintenance period during the study were 4.2 – 4.9 mg/L with an average value of 5.20 mg/L. This result is in accordance with Supito (2017) who stated that the optimal DO in shrimp maintenance is around > 4 mg/L, so that during the maintenance of vannamei shrimp DO is supplied with normal limits.

Salinity is one of the water quality parameters that has an important role in the survival and growth of *L. vannamei*. Salinity plays a role in the process of osmoregulation and molting in shrimp. At high salinity (euryhaline) shrimp growth will be disrupted because the osmoregulation process is disrupted. The presence of osmolarity disorders causes the energy used for growth activities to decrease (Salsabiela, M., 2020). Based on the table above, the salinity measurement results during the maintenance period during the study were 16-17 ppt with an average value of 16.40 ppt. Vannamei shrimp will live optimally in the salinity range of 15 – 25 ppt.

At high concentrations, ammonia is toxic, causing a decrease in the supply of oxygen in large amounts and undesirable changes in aquatic ecosystems (Jang, Barford, Lindawati, & Renneberg, 2004). The results of ammonia measurements during the maintenance period had an average value of 0.16 ppm. Based on the results, the average value of ammonia is still within the tolerance limit for shrimp. This is supported by WFF Indonesia (2014), stating that the NH₃ tolerance limit for shrimp farming ranges from 0-0.5 ppm.

4. Conclusion

Based on the results obtained from the research on the application of Bioimmune® through feed to the growth and survival of vannamei shrimp (*Litopenaeus vannamei*) raised in cement tanks, it can be concluded that the addition of bioimmunity® through feed did not have a significant effect on the survival rate of vannamei shrimp (*Litopenaeus vannamei*) reared in cement tanks. Bioimmunity® is able to increase non-specific immunity of vannamei shrimp, as seen from the total number of hemocytes and the percentage of defense cells. In treatment with a dose of 60 mL/kg feed (P2), the total hemocyte cells (3.73 x 10⁷ cells/mL) and the percentage of semi-granular cells (34.7%) and hyaline cells (26.7%) were higher than other treatments. Meanwhile, the highest percentage of granular cells was obtained at a dose of 40 mL/kg feed (P1) of 59.5%. The Bioimmune® Dose of 60 mL/kg of feed is effective in increasing the total number of hemocyte cells, semi-granular cells, and hyalin cells, so that this dose has the potential to be applied in the vannamei shrimp cultivation scale to increase the immunity and health of shrimp optimally.

Based on the process during the study, the researcher suggested that the time of taking shrimp hemolymph should not be too far from the observation time to reduce the potential for high lysis. If you want to increase shrimp immunity, you can use a Bioimmune® dose of 60 mL/kg of feed. It is recommended to conduct further research on shrimp that have been affected by the disease to find out what is the best dose needed to increase the immunity of shrimp that are currently suffering from the disease.

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