BIOENCAPSULATION ARTEMIA WITH *Bacillus subtilis* AND SODIUM ALGINATE ON TOTAL HEMOCYTE AND SURVIVAL RATE OF *Litopenaeus vannamei* INFECTED WITH *Vibrio parahaemolyticus*

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**Abstract**

Disease prevention in shrimp farming can be done by increasing the shrimp's immune response to disease. One way is to use immunostimulants. Immunostimulants that can be used are *B. subtilis* and sodium alginate which are proven to increase the immune response of shrimp. This study aimed to determine the effect of bioencapsulation of Artemia with the combination of *Bacillus subtilis* and sodium alginate on total hemocyte and the survival rate of white shrimp post-larvae infected with *V. parahaemolyticus*. This study used a completely randomized design (CRD) with 5 treatments and 4 replications. Total hemocytes and survival rate were measured at H0, H7 (seven days post-bioencapsulation), H9 (one-day post-infection), and H16 (seven-days post-infection). The results of this study showed that the total hemocytes H7 and H9 in the P3 treatment were significantly different (p<0.05) from other treatments. The survival of white shrimp treated with immunostimulants showed that P3 had the highest survival rate and was significantly different (p<0.05). The conclusion of this study is that the bioencapsulation of Artemia with the combination of *B. subtilis* and sodium alginate can increase total hemocyte and the survival rate of white shrimp post-larvae infected with *V. parahaemolyticus*.

**Keywords:** *Bacillus subtilis*, Bioencapsulation Artemia, survival rate, sodium alginate, total hemocyte count

1. **INTRODUCTION**

White shrimp is one of the essential commodities in local and export markets, so the production of white shrimp is expected to continue to increase. The volume of shrimp production in 2020 is more than 900 thousand tons. The target in 2024 is 2 million tons (KKP, 2021). The high demand for white shrimp encourages intensive cultivation.
Intensification can be negative effect, one of which is bacterial disease, mostly caused by *Vibrio* spp. (Kurniawinata et al., 2021).

Strategies to prevent disease in shrimp are carried out by using antibiotics, but antibiotics can have a negative impact, namely causing accumulation of harmful residues and developing resistant pathogens (Singer et al., 2019), so that immunostimulants can be an innovative approach to shrimp health management because it can increase the immune response of shrimp to pathogenic microorganisms (Barman et al., 2013).

There are many immunostimulants that can be used, such as bacteria, algae derivatives, animal derivatives, nutritional factors, and hormones/cytokines (Barman et al., 2013). One type of bacteria that can be used as an immunostimulant is *Bacillus subtilis*. *Bacillus subtilis* is a gram positive bacterium. The bacterial cell wall is composed of peptidoglycan. Peptidoglycan is a bacterial polymer that can enhance the shrimp's immune response (Pan et al., 2015).

The addition of *B. subtilis* to white shrimp infected with the pathogen *V. Parahaemolyticus* shows that *B. subtilis* can increase the immunity of white shrimp (Vogeley et al., 2019). Latest research shows that using *Lactobacillus lactis* alone is less able to protect organisms from pathogens. The best result that can increase the immune response is the use of a combination of *L. lactis* bacteria and sodium alginate (Loh et al., 2021) so that in this study a combination of bacteria and sodium alginate will be used.

Sodium alginate can be used as an immunostimulant because it has been shown to increase the immune response of shrimp (Santos et al., 2019). Alginate found in the cell walls of brown algae consists of salts of calcium, magnesium, potassium, and sodium alginate (Rasyid, 2005). Alginate is found in Sargassum's cell wall, a polysaccharide type with immunomodulatory activity. The polysaccharide content can activate the response immune non-specific in white shrimp (Santos et al., 2019).

The method of giving anti-microbial agents to shrimp can be done in various ways, one of which is bioencapsulation. Bioencapsulation is an effective method to provide antimicrobial compounds to shrimp post larvae (Sivagnanavelmurugan et al., 2015). Bioencapsulation is the delivery of a substance into a living organism which is then used as food for the target organism. Live organisms that can be used as vectors for the distribution of antimicrobial materials are Artemia (Santos et al., 2019).

Artemia is a crustacean that is used as the best natural live food that can be stored and used in the hatchery process. Artemia has a non-selective filter feeder that can absorb all particles in the maintenance media. Bioencapsulation of Artemia with immunostimulant gives a good effect by increasing the stimulation of the immune response in white shrimp post-larvae (Rudtanatip et al., 2019).

The distribution of antimicrobials through Artemia bioencapsulation has been widely carried out (Sivagnanavelmurugan et al., 2015; Rudtanatip et al., 2019; Santos et al., 2019; Loh et al., 2021). Bioencapsulation of Artemia with a combination of *B. subtilis* and Sodium alginate for white shrimp post larvae has not been carried out, so this study needs to be carried out to determine the effect of bioencapsulation of Artemia on total hemocytes and survival rate on white shrimp post-larvae infected with *Vibrio parahaemolyticus*.

2. RESEARCH METHOD

2.1 Sodium Alginate Extract and Culture of *B. subtilis* and *V. parahaemolyticus*

Sodium alginate was extracted from *Sargassum polycystum* seaweed taken from Talango, Madura. The seaweed was dried and sodium alginate was prepared according to the method of Setyoaji et al., (2019).
Bioencapsulation of Artemia

Commercial A. franciscana cysts from Great Salt Lake Utah, USA. Artemia cysts were hatched in seawater for 24 hours (instar II) (Widodo et al., 2016). The bioencapsulation of Artemia process was carried out for 8 hours in a beaker glass with a density of 15 nauplii/mL (Loh et al., 2021). The bioencapsulated doses were sodium alginate 0.4 g/L (Inmanuel et al., 2012) and 10⁶ CFU/mL B. subtilis (Vidal et al., 2018).

White Shrimp Post Larvae Treatment

White shrimp post larvae (PL 8) came from hatcheries in Sidayu, Gresik, Indonesia. Maintenance of white shrimp post larvae at a density of 5 shrimp/Liter (Sivagnanavelmurugan et al., 2015). Feeding pellets (200-300 µm) were carried out three times a day at 07.00, 12.00, and 17.00 ad libitum during the rearing period. Feed treatment with Artemia bioencapsulated was given at 14.00 as many as 60 nauplii/larvae/day (SNI, 2009) for seven days of treatment.

Survival Rate Count

The survival rate of white shrimp post larvae was observed until day 16th, then the survival rate was recorded at the beginning of

2.4 Research Design

The research method used is an experimental method using a Completely Randomized Design (CRD) with 5 treatments and 4 replications.

In this study, the treatments used were K- (Artemia without bioencapsulation and without infection), K+ (Artemia without bioencapsulation and V. parahaemolyticus infection), P1 (Artemia bioencapsulation with B. subtilis and infection), P2 (Artemia bioencapsulation with sodium alginate and infection), P3 (bioencapsulation of Artemia with the combination of B. subtilis and sodium alginate and infection).

2.5 Total Hemocyte Count

Total hemocytes in white shrimp post larvae were calculated based on the whole shrimp body. White shrimp post larvae was put into a 1.5 mL eppendorf tube containing 0.4 mL of anticoagulant (Trisodium citrate, NaCl, EDTA, aquades). The white shrimp post larvae was ground and homogenized (Tampanagallo et al., 2018), then the suspension was taken using a pipette and dripped on a hemocytometer. Performed under a light microscope with a magnification of 400x. Total hemocytes were calculated based on Abdollahi-Arpanahi et al. (2018).

2.6 Survival Rate Count

On day 8th, white shrimp post larvae were challenged with V. parahaemolyticus by immersion method at a density of 10⁶ CFU/mL LD₅₀ (Garcia-Bernal et al., 2018). Immersion was carried out for 2 hours and then transferred to clean seawater, while in treatment (K-) only water was changed (Joseph et al., 2015).
rearing (H0), after treatment (H7), one day after infection (H9), and seven days after infection (H15). The survival rate calculations based on Loh et al. (2021).

3. RESULTS AND DISCUSSION

3.1 Total Hemocyte

There are three types of total hemocytes in shrimp, namely hyaline, granular and semi-granular. In shrimp, hemocytes are key in the immune mechanism. Hemocyte cells can activate prophenoloxidase, phagocytic activity, encapsulation, and nodule formation (Madani et al., 2018).

Table 1. Total hemocytes of white shrimp post larvae

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total Hemocyte (10^6 cell/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>H0</td>
</tr>
<tr>
<td>K-</td>
<td>6.38 ± 0.21a</td>
</tr>
<tr>
<td>K+</td>
<td>6.34 ± 0.12a</td>
</tr>
<tr>
<td>P1</td>
<td>6.38 ± 0.18a</td>
</tr>
<tr>
<td>P2</td>
<td>6.33 ± 0.17a</td>
</tr>
<tr>
<td>P3</td>
<td>6.31 ± 0.15a</td>
</tr>
</tbody>
</table>

Table 1, H0 is the initial hemocytes of white shrimp post larvae which ranged from 6.31 x 10⁶ cell/mL to 6.38 x 10⁶ cell/mL. After being given Artemia bioencapsulation treatment up to H7, it showed an increase in total hemocytes compared to the control treatment. This indicates that the shrimp's immune response increases. Increased immunity can be caused by the peptidoglycan content in the cell wall of B. subtilis bacteria (Ramadhani et al., 2015) and the polysaccharide content in sodium alginate from S. polycystum (Santos et al., 2019).

Peptidoglycan and polysaccharides can work as Pathogen Related Molecular Patterns (PAMPs) which are molecules that can induce innate immunity in shrimp. The introduction of PAMPs by the shrimp immune system creates the necessary solutes to trigger both humoral and cellular responses in shrimp (Maliwat et al., 2021).

Based on the results of the Analysis of Variance (ANOVA) showed that the administration of the combination of B. subtilis and sodium alginate had an effect on increasing the total hemocytes of white shrimp post-larvae (p<0.05). Total hemocytes on H0 were not significantly different (p>0.05) from the other treatments. On H7 after treatment with Artemia bioencapsulation and on H9 after being challenged with V. parahaemolyticus bacterial infection showed that P3 was significantly different (p<0.05) to the other treatments. On H15 after seven days of infection showed that K+ was significantly different (p<0.05) from the other treatments. The total hemocytes of white shrimp post larvae was on Table 1.

On H9 and H15 after V. parahaemolyticus infection, showed the lowest total hemocytes in shrimp that were not given immunostimulants. The decrease in total hemocytes is a form of shrimp immune response to pathogens. Hemocyte cells migrate to bacteria-infected organs (Pudgerd et al., 2021) then hemocyte cells will carry out a phagocytosis process that begins with attachment, ingestion, digestion, and destruction of microbes (Chifdhiyah, 2012). Hemocyte cells will be damaged and lysis after the phagocytosis process is complete so that the number of hemocytes will decrease (Song et al., 2003).

3.2 Survival Rate

The survival rate is a comparison value between the number of living organisms at the end of rearing and the

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number of organisms at the beginning of rearing, which is expressed in percent. Based on the results of the Analysis of Variance (ANOVA), showed that the administration of a combination of B. subtilis and sodium alginate had an effect on the survival rate of post-larvae white shrimp (p<0.05). Survival on H0 and H7 was not significantly different (p>0.05) from other treatments. The highest survival was at H9, namely, the K- treatment but not significantly different (p>0.05) from the P3 and P2 treatments, while at H15, the P3 treatment was the best treatment with an 89% SR which was significantly different (p<0.05) to all treatment. The survival rate of white shrimp post larvae is shown in Table 2.

Table 2. Survival rate of white shrimp post larvae

<table>
<thead>
<tr>
<th>Treatment</th>
<th>H0</th>
<th>H7</th>
<th>H9</th>
<th>H15</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-</td>
<td>100 ± 0a</td>
<td>100 ± 0a</td>
<td>98 ± 1.6b</td>
<td>95 ± 2.6c</td>
</tr>
<tr>
<td>K+</td>
<td>100 ± 0a</td>
<td>100 ± 0a</td>
<td>78 ± 6.7c</td>
<td>66 ± 6.9d</td>
</tr>
<tr>
<td>P1</td>
<td>100 ± 0a</td>
<td>100 ± 0a</td>
<td>92 ± 3.7bc</td>
<td>81 ± 5.3c</td>
</tr>
<tr>
<td>P2</td>
<td>100 ± 0a</td>
<td>100 ± 0a</td>
<td>90 ± 3.7bc</td>
<td>80 ± 1.6c</td>
</tr>
<tr>
<td>P3</td>
<td>100 ± 0a</td>
<td>100 ± 0a</td>
<td>96 ± 3.7bc</td>
<td>89 ± 2.6b</td>
</tr>
</tbody>
</table>

Table 2. The survival rate on H7 shows 100% results so the administration of Artemia bioencapsulation can increase the survival of white shrimp. On H9 and H15 after being infected with V. parahaemolyticus there was a decrease in the survival rate of white shrimp post larvae. The highest survival rate was in the P3 treatment using Artemia bioencapsulation with a combination of B. subtilis and sodium alginate. The high survival rate of vaname shrimp postlarvae is due to the polysaccharide content in the sodium alginate and the peptidoglycan content in B. subtilis can stimulate the shrimp’s immune system by increasing resistance to disease (Pan et al., 2015; Santos et al., 2019).

5. CONCLUSIONS AND SUGGESTIONS

Bioencapsulation of Artemia with the combination of 10^8 CFU/mL B. subtilis and 0.4 g/L sodium alginate can increase the total hemocytes and the survival rate of white shrimp post larvae infected with V. parahaemolyticus.

BIBLIOGRAPHY


Fu, S., Tian, H., Wei, D., Zhang, X. and Liu, Y. 2017. Delineating the origins of Vibrio...
an budidaya udang 

Litopenaeus vannamei) on growth -

Loh Kurniawinata, M.I., Sukenda, S., Joseph García-Bernal DOI 10.20473/jbp.v25

Jurnal ©, (J2023 and dietary probiotic Bacilli (and Hoseinifar, S.H. 2018. The effects of perch larvae, digestive enzyme production in climbing lactis nauplii bioencapsulated with Ting shrimp farming. phytoplankton in intensive pacific white


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SNI 7311. 2009. Produksi benih udang vannamei (Litopenaeus vannamei) kelas benih sebar. Badan Standardisasi Nasional. 16 hal.


