Analysis of Blood Glucose Levels and The Development of Ectoparasite Infestation on Pacific White Shrimp (*Litopenaeus vannamei*) Which Were Given Crude Protein *Zoothamnium penaei* at High Stocking Densities

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Abstract. The main constraint in pacific white shrimp culture with high stocking density (super intensive), can cause a decrease in water quality and shrimp were stress and are susceptible to disease. The purpose of this study was to analyze stress levels (blood glucose levels) and the development of ectoparasite infestations. In this experimental study, used 2 ponds with an area of 2000 m² per pond, stocking densities in pond I were 150 individuals/m² and 350 individuals/m² in pond II, with a maintenance period of 90 days. The results showed that the shrimp was stressed during rearing, with blood glucose levels of white shrimp at a high stocking density of 350 individuals/m² ranging from 43-54 mg/dL significantly different from blood glucose levels at stocking density of 150 individuals/m² (p<0.05). The ectoparasites found were the genera *Zoothamnium*, *Epistylis* and *Vorticella*. The development of the infestation of the three genera showed an increase starting on the 30th, 60th and 90th days, the degree of infestation from mild to moderate, but on the 90th day there was a significant difference (p<0.05), with a moderate degree of infestation in the stocking density of 100 individuals/m² and weight at stocking density 350 individuals/m².

1. Introduction
Shrimp cultivation technology with a super intensive pattern, namely shrimp cultivation using a high stocking density of more than 150 individuals/m². The use of high stocking density also causes the content of organic matter such as ammonia from feed residues and excretion from shrimp to be higher. This causes water quality to decrease so that shrimp a decreased immune response and stress. Besides causing high organic matter, it will also increase competition for food, oxygen and a place to live. High organic matter can cause a decrease in water quality, which results in shrimp cause stress and decreased immune response [1]. This will cause shrimp stress which is characterized by increased glucose levels, decreased body resistance, so that shrimp will be susceptible to disease and decrease shrimp survival.

Efforts to increase the body's defense of shrimp that have not been widely developed is to provide immunostimulants, or what is called immunization. The addition of this immunostimulants is intended to increase the activity of the body's defense cells, so that shrimp are resistant to disease-causing infections (pathogens). Efforts to increase the shrimp's body defense during rearing can be done by giving immunostimulants through feed.[2]. found immunogenic membrane proteins with Molecular
Weights MP38, MP48 and MP67 from Zoothamnium penaei. Protein has been shown to increase the activity of the body’s defense cells and the survival rate of vaname shrimp up to 91% and reduce blood glucose levels, total haemocyte count (THC) and differential haemocyte count (DHC). Vannamei shrimp (Litopenaeus vannamei) treated with Zoothamnium penaei Crude Protein [3]. said that, feeding with the addition of Zoothamnium penaei crude protein was able to increase shrimp body defense (THC and DHC) and survival from 21% to 72% in vaname shrimp reared in ponds.

Ectoparasites are often found attacking white shrimp, among others, are caused by protozoa of the Ciliata class. namely Zoothamnium sp., Vorticella sp. and Epistylistis sp. [4,5]. This protozoa can cause shrimp mortality in culture up to 100% in hatcheries, with prevalence and intensity reaching 67%. [6].

Base on above the crude protein of Zoothamnium penaei will be develop as material for immunostimulant for prevent zoothamniosis on white shrimp in ponds. The purpose of this research is want to know the the immune respons and survival rate of white shrimp (Litopenaeus vannamei) that immunized by the immunogenic membrane protein Zoothamnium penaei

2. Materials and Method
The materials used in this study were Pacific white shrimp stadia Post Larva (PL-11), 2 pond plots measuring 200 square meters. The Zoothamnium penaei crude protein used in this study is the production by Mahasri [7]. which has been characterized by SDS-PAGE, ELISA and Immunoblotting. The Crude protein of Zoothamnium penaei that used in this study is the production of Mahasri [7]. This research was conducted on 2 groups of shrimp, namely:

P1 :  is a group of Pacific white shrimp that cultured with stocking densities 100 individuals/m²
P2 :  is a group of Pacific white shrimp that cultured with stocking densities 350 individuals/m²

Shrimp rearing is carried out for 90 days, with a stocking density of 100 and 350 shrimp per square meter. Before being spread, the seeds are immunized with crude protein Zoothamnium penaei by dipping for 15 minutes, at a dose of 0.03 µg/L [8]. Shrimp Blood Glucose levels examination by using Monitoring System Easy Touch [9]. Examination of ectoparasites in pacific white shrimp was carried out natively, on the rostrum, gills, body surface, swimming legs and walking legs.

3. Results and Discussion
3.1 Results
The results showed that the shrimp were positively infested with the genus Zoothamnium, Vorticella and Epistylistis from the Ciliata class. on shrimp, both exposed and immunized with Zoothamnium penaei crude protein and reared with stocking densities of 150 and 350 individuals/m². The results of the blood glucose levels and ectoparasite infestation examination can be seen in Table 1 and Table 2.

| Maintenance (days) | Blood Glucose of Shrimp (mg/dL) ± SD |
|--------------------|--------------------------------------|
|                    | Stocking Densities (100 individuals/m²) | Stocking Densities (350 individuals/m²) |
| 30                 | 29.81a ± 2.71                          | 47.46a ± 3.61                          |
| 60                 | 33.16b ± 3.73                          | 51.13b ± 4.82                          |
| 90                 | 34.07b ± 3.23                          | 66.33b ± 4.67                          |

Note: Different superscripts in different columns and rows indicate a significant difference (p<0.05)

In Table 1 it show that the highest shrimp blood glucose levels occurred in shrimp reared for 90 days is 66.33 mg/dL and the lowest on 90 days is 29.81 mg/dL. The results of statistical analysis showed a significant difference (p<0.05) between the blood glucose levels of the shrimp immunized with the
Zoothamnium penaei crude protein that reared with a stocking density of 100 and 350 individuals/m² at 30, 60 and 90 days.

Table 2. Ectoparasite Infestation Examination Results in Shrimp

| Maintenance (days) | Blood Glucose of Shrimp (mg/dL) ± SD |
|--------------------|--------------------------------------|
|                    | Stocking Densities (100 individuals/m²) | Stocking Densities (350 individuals/m²) |
| 30                 | 4.67ᵃ ± 2.31                          | 13.54ᵈ ± 3.06 |
| 60                 | 11.73ᵇ ± 3.12                         | 23.43ᵉ ± 2.79 |
| 90                 | 13.25⁷ᵇ ± 3.25                        | 25.37ᶠ ± 3.12 |

Note: Different superscripts in different columns and rows indicate a significant difference (p<0.05)

In Table 2 show that the highest ectoparasites infestation in shrimp that reared for 90 days is 34.37 zooid and the lowest on 30 days is 4.67 zooid. The results of statistical analysis showed a significant difference (p<0.05) between ectoparasite infestation in shrimp that immunized with the Zoothamnium penaei crude protein and reared with a stocking density of 100 and 350 individuals/m² at 30, 60 and 90 days.

The results of the inspection of water quality parameters can be seen in Table 3.

Table 3. Results of Water Quality Inspection During Shrimp Rearing

| Parameters              | Average Maintenance Water Quality | Normal Range |
|-------------------------|-----------------------------------|--------------|
|                         | Stocking Densities (100 individuals/m²) | Stocking Densities (350 individuals/m²) | |
| Temperature (°C)        | 27 - 28                           | 28 - 29      | 27 - 32 |
| pH                     | 7.8 - 8.1                          | 7.1 - 7.4    | 7.5 - 8.5 |
| Dissolved Oxygen (DO)  | 5.2 - 5.7                          | 3.0 - 3.2    | >4 - 7 |
| Amonia (mg/L)          | 0.7 - 0.9                          | 1.09 - 2.19  | <1 |
| Salinity (ppt)         | 21 - 23                           | 21 - 23      | 16 - 30 |

is under normal conditions.

3.2 Discussion

The addition of Crude protein Zoothamnium penaei 0.03 g/L which was done by dipping before stocking the fry, and reared at high stocking density, gave different effects (p<0.05) on shrimp blood glucose levels. Shrimp reared at a stocking density of 350 individuals/m² was more than normal and higher than that at a stocking density of 100 individuals/m², although on the 60th and 90th days there was also an increase, but still at normal levels, namely 34 mg/mL. [10]. The high blood glucose level of shrimp is due to the use of high stocking density in shrimp rearing, which can cause water quality to decrease and shrimp experience stress. According to Fendja;ang et al [11] which states that the higher the stocking density, the higher the blood glucose level of the shrimp and the longer the rearing time will be. Furthermore, according to Rustam [12] stress can be caused by the environment, feed and cultivation with high stocking densities. Stress causes an increase in blood glucose levels.

The results of water quality measurements in this study were carried out every day at 08.00 - 09.00 showing that several quality parameters in rearing shrimp with a stocking density of 350 fish/m² were
at levels below normal. The range of pH, dissolved oxygen and ammonia, respectively 1.09 -2.19 and 3.0 - 3.2 and pH between 7.1 - 7.4, so that the maintenance water decreased. These three parameters also show as an indicator of the accumulation of organic matter in the water of the rearing media, which results in the shrimp being reared being stressed and experiencing a decrease in body resistance, making them susceptible to disease infections. According to McEwen and Wingfield [13] that to deal with stress, animals need the ability to provide enough energy in the tissues to face the allostatic load that can be obtained from glucose and protein.

Stress conditions on the shrimp have an impact on ectoparasite infestations that occur in shrimp reared at a stocking density of 350 individuals/m² which has a significantly different effect (p<0.05) on ectoparasite infestations. However, shrimp in rearing with a stocking density of 100 individuals/m² were also infected with ectoparasites, although in a lower condition, which was still in the moderate category as shown in Table 2. The ectoparasite infestation was good. Zoothamnium. Epistylis and Vorticella in shrimp began to be found in shrimp aged 30 days, both reared at 100 and 350 individuals/m². However, the highest infestation occurred in shrimp at a stocking density of 350 individuals/m², which was 25.37 zooids with a moderate level of infestation. (Table 2).

Based on this explanation, it can be seen that the administration of *Zoothamnium penaei* crude protein has an effect on the high and low blood glucose levels of shrimp ectoparasite infestations during maintenance for both 30, 60 and 90 days, which indicates a number that tends to be higher in shrimp reared at a stocking density of 350 individuals/m² than 100 individuals/m². This is in accordance with the results of research by Pahlevi *et al*. [3] which said that ectoparasite infestation in shrimp reared in ponds and given immunostimulants from the membrane protein *Zoothamnium penaei*, was lower than in shrimp that were not given the membrane protein.

Dipping shrimp in *Zoothamnium penaei* crude protein before being stocked in ponds, will enter the shrimp body, it will cause an increase in the number of haemocytes (THC) and differential haemocyte cells (DHC), which is an indication of increased defense of the shrimp body against pathogenic infections [2]. Furthermore, Itami *et al* [14] support the theoretical basis, which states that vaccine administration can prevent disease infection in the host's body and cause increased activity of haemocyte phagocytes and proPO enzymes. This statement is reinforced by Van de Braak [15] that the vaccine material that enters the shrimp body will produce antibodies that are able to neutralize *Zoothamnium penaei* infestations in shrimp.

According to [7] crude protein that enters the body can increase the immune response of tiger prawns, which is marked by an increase in THC and DHC, because the protein has a large molecular weight, which is greater than 1000 Dalton, so it is immunogenic. Proteins that have a high molecular weight, and have a high level of immunogenicity, must have a complex structure. According to Tizard [16] and Baratawidjaja [17] proteins that are immunogenic have a large molecular weight of more than 1000 Dalton and have a complex structure. Furthermore, the enzyme Prophenoloxidase Activating Enzyme (PPA). PPA is a protein that resides in the granular cells of haemocytes (shrimp blood cells). This PPA can be activated by crude protein that enters the shrimp body, which will stimulate prophenoloxidase to become phenoloxidase. As a result of this change, a kind of Opsonin Factor protein will be produced which can induce hyaline cells to carry out the phagocytosis process. Van de Braak [15] and Smith *et al*. [18] also support the above statement that the haemocyte cells will degranulate, and some proteins will be released for the benefit of the immune response, such as: increased haemocyte cells, and increased entrapment and phagocytosis activity. In addition, immunogenic membrane proteins will stimulate haemocytes to release proPO and protein-binding PPA, resulting in haemocyte cells increasing their activity to trap and phagocytose disease agents, in this case *Zoothamnium penaei*. It was proven that the prevalence of zoothamniosis in immunized shrimp was lower and significantly different from the prevalence in unimmunized shrimp. There are still shrimp infested with *Zoothamnium penaei*, because this parasite is opportunistic, so that in normal water conditions it still grows but develops for a long time, so it does not cause disease in the shrimp. However, these water conditions have not been able to increase the activity of *Vibrio parahaemolyticus* bacteria, so they have not caused illness in Pacific white shrimp.
4. Conclusion
The use of a stocking density of 350 individuals/m² can cause glucose levels to exceed normal, as an indicator of stress, but it does not cause a different effect on ectoparasite infestations in Pacific white shrimp fed with Crude protein Zoothamnium penaei before stocking, including in the moderate category, during the rearing period 90 day.

5. References
[1] Gao, Y., H. Zhuliu, V. Hector, Z. Bo, L. Zhiwei, H. Jie, L. Jeong and C. Zhangjie. 2017. *Turkish J of Fisheries and Aquatic Scie*, 17 877 - 884.
[2] Mahasri, G. 2007. Protein membrane immunogen zoothamnium penaei sebagai bahan pengembangan imunostimulan pada udang windu (Penaeus monodon fabricius) terhadap zoothamniosis. Disertasi. Program Pasca Sarjana Universitas Airlngga Surabaya 284
[3] Pahlefi, R. C., Suwarno and Mahasri, G. 2017. Evaluasi Pemberian Crude Protein Zoothamnium penaei terhadap Laju Pertumbuhan, Respon Imun dan Kelulushidupan Udang Vaname (Litopenaeus vannamei) di Tambak. Jurnal Biosains Pascasarjana, Universitas Airlangga. 19 2
[4] Itabashi T, Mikami K and Asai H. 2003. *Res. Micro J*, 154 5 361 – 367
[5] Itabashi T, Terasaki T and Asai H. 2004. Novel Nuclear and Cytoplasmic Proteins Detected by Anti-Zoothamnium arbuscula (Protozoa) Spasmin 1 Antibody Mammalia Cell Are Dependent on the Cell Cycle, Biochem J, 136 5 651-657.
[6] Itami, T. 1994. Body Defense System of Penaeid Shrimp, Seminar on Fish Physiology and Prevention of Epizootics, Department of Aquaculture and Biology, Shimonoseki University of Fisheries, Japan, 7 59-65.
[7] Mahasri, G., M. Yusuf, R. Woro and M. B. 2019. The protection capacity of the crude and whole protein spores of Myxobolus koi as an immunostimulant material development in goldfish (Cyprinus carpio) for preventing Myxobolusis. International Conference on Fisheries and Marine Science IOP Conf. Series: Earth and Environmental Science 236 012084 : 1-10.
[8] Zang, X; C. Huang, X. Xu; C.L. Hew. 2002. *J. of Virol*, 83 1069 – 1074.
[9] Mahasri, G., Mukti, A.T., Nisa, M., Prakosa, G. C., and Satyantini, W. H. 2021. Ectoparasite infestation and survival rate of pacific white shrimp (Litopenaeus vannamei) that immunized with crude protein Zoothamnium penaei in intensive ponds. IOP Conf. Series: Earth and Environmental Science 679 1 - 6
[10] Lorenzon, S., P. G. Edomi, R. Giulianini, Muttulio and E. A. Ferrero. 2004. *The J of Expe Bio* 207 4205 - 4213.
[11] Fendjalang, S. N. M., T. Budiardi, E. Supriyono dan I. Effendi. 2016. *J Ilmu dan Teknologi Kelautan Tropis* 8 1 201 - 214.
[12] Rustam, H., K. Jusoff, S. T. Hadijah and Illmiah. 2013. *World Appl Scie J*, 26 82 - 88.
[13] McEwen, B. S., and Wingfield, J. C. 2003. The concept of allostatic in biology and biomedicine. Hormones and Behavior, 43 1 2 – 15.
[14] Itami T, Kondo M and Takahasi Y. 1996. Enchancement of Disease Resistance of Kuruma Prawn, Penaeus japonicus After Oral Administration of Peptidoglucon, National Fisheries University, Japan. 7 59 - 65
[15] Van de Braak, K. 2002. Haemocytic defence in black tiger shrimp (Penaeus monodon), Disertation, van Warenigen Universiteit, Germany.
[16] Tizard, I.R. 1988. Pengantar Imunologi Veteriner. Airlangga University Press, Surabaya
[17] Baratawidjaja, C. 2004. Immunologi Dasar, edisi 4. Balai Penerbit Fakultas Kedokteran, Universitas Indonesia, Jakarta
[18] Smith VI, Brown J.H., and Hauton. 2003. Immunostimulation in crustaceans : does it really protect against infection fish and shellfish Immunology. 15 : 71 – 90
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