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**APLIKASI DAN EVALUASI UJI LAPANGAN IMUNOSTIMULAN DARI
PROTEIN MEMBRAN IMUNOGENIK *ZOOTHAMNIUM PENAEI* UNTUK
MENEKAN TINGGINYA KEMATIAN UDANG *VANNAMEI*
(*LITOPENAEUS VANNAMEI*) DI TAMBAK**

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SUMMARY

**APPLICATION AND EVALUATION FIELD STUDY OF IMMUNOSTIMULANT
FROM PROTEIN MEMBRANE IMMUNOGENIC OF *ZOOTHAMNIUM PENAEI*
TO DECREASE THE MORTALITY OF WHITE SHRIMP
(*Litopenaeus vannamei*)**

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White shrimp (*Litopenaeus vannamei*) is one of species of Crustacean import from Amerika and entry in Indonesia since 1998. After observed through any kind technology application and try out in field (pond) for 3 years. So that The government has been already done effort to change with other species that was *Litopenaeus vannamei*, to developed in Indonesia, but there is many factors to influence to develops as specially is diseases that can make 100% of the shrimp mortality in three days after infection. Zoothamniosis is one of the shrimp diseases which can cause death particularly at germ stage. Clinical symptom of infected shrimp is respiration disturbance, mobile, seeking food and moulting difficulties. So far the occurrence zoothamniosis is still high in the field.

The purpose of this research as follow to find immunogenic membrane protein as immunostimulant development material for *Penaeus monodon* Fab to Zoothamniosis. The special purpose of this research are as follows: (1) Identification and cultivation of *Zoothamnium penaei*, (2) to analyse immunogenic membrane protein of *Zoothamnium penaei*, which can impede pathological changes of skin and gill of *Zoothamnium penaei* from infected *Penaeus monodon* Fab (3) to find immunogenic membrane protein of *Zoothamnium penaei* which can protected and can increase survival rate (SR) of *Penaeus monodon*, (4) to find immunogenic membrane protein of *Zoothamnium penaei* which can increase immune response (Total Haemocyte/THC and Total Differential Haemocyte/DHC) and can be used for immunostimulant development material of *Penaeus monodon* Fab.

This research was designed to use explorative experiment and experimental laboratory methods which used completed random sampling design. Collected data was analyzed with analysis of variant for examination of *Survival Rate* (SR), *Total Haemocyte Count* (THC) and *Differensial Haemocyte Count* (DHC) for treatment of doses and ages. The research divided into 2 steps: (1) Identification, cultivation and determination infection level and *Penaeus monodon* Fab gill and skin histopathological appearance by zoothamniosis, Analysis of membrane protein by SDS-PAGE, polyclonal antibody production from rabbit, antibody titer examination by ELISA and characterization of immunogenic membrane protein by Western Blotting, (2) Immunogenic membrane protein protection test on Survival Rate level and immune response (THC and/or DHC level).

The result of research showed that 60 days olds of *Litopenaeus vannamei* Fab from field infected by *Zoothamnium penaei*. Cultivation and determination of infection level showed that the end of cultivation during 26 days appeared all *Penaeus monodon* Fab were severe *Zoothamnium penaei* infection. Gill and skin scrapings were got 4.91×10^8 *Zoothamnium penaei* zooids.

Zoothamnium penaei infection on shrimp 60 days olds which cultivated during 7 days and infected with *Zoothamnium penaei* zooid at dose of 0, 25, 50, 75 and 100 occurred 2 days post infection. The cultivation of shrimp from 30 days old showed that the 1st and 2nd days post infection, shrimp has been slightly infected and 3rd day was moderate infection. Then, from 4th until 7th days post infection was severe infected by 157.7143 zooids. Whereas shrimp 60 days old showed moderate infection at 1st day and 2nd day post infection, the next 3 until 7 days post infection was heavily infected by 173.5714 ($p < 0.01$). Infection level on shrimp was grouped into several levels among other things: slight infection, moderate infection, heavy infection and no damaged level. *Zoothamnium penaei* infected shrimp was appeared by white-brownish parasite attachment at the body surface so that appeared muddy, gill was turbid and dirty, anoxia, decreasing activity, immobile and moulting failure. The survival rate of shrimp 60 days old by *Zoothamnium penaei* zooid doses infection (50, 75 and 100) which is 100%, 94%, and 90% for 60 days old, respectively.

There was seven *Zoothamnium penaei* membrane proteins found in several molecules weight 38 kDa, 48 kDa, 67 kDa, 71 kDa, 77 kDa, 98 kDa and 104 kDa by SDS-PAGE. In fact through immunogenicity test ELISA and Western-Blotting only three clear bands appeared which is 38 kDa, 48 kDa and 67 kDa, respectively, whereas the other proteins were not appeared. This circumstance caused by low concentration of high weight molecules and uncomplicated structure same.

There is significantly difference of protection among immunogenic membrane proteins (MP38, MP48, and MP67) ($p < 0.05$). The survival rate of shrimp at 60 days old which immunized by membrane proteins (MP38, MP48, and MP67) at dose of 5 µg/ml of 300 µl intramuscularly injection and 600 µl by dipping on shrimp at 30 days old during for 10 minutes, then is both challenged with 80 *Zoothamnium penaei* zooids and the highest survival rate was shrimp at 60 days old which immunized with MP38 was 96% that higher than MP48 and MP67 which is 94% and 93%, respectively.

Immune response (increasing THC and DHC) that raised by each protein indicated the presence very significantly difference among treatment ($p < 0.01$). The highest level of Total Haemocyte occurred in immunized shrimp with MP38 protein was 69.9140 cells/ml that higher than MP48 and MP67 from immunized shrimp with 68.9529 cells/ml and 66.5940 cells/ml, respectively. While the highest level of DHC was resulted in immunization with MP48 membrane protein which is 29.71% that higher than MP38 and MP67 which is 27.6560% and 27.7380% and there were not significantly difference both.

By protection test, MP38, MP48 and MP67 membrane proteins were proved that those proteins have ability to decrease damaged level of shrimp skin and gill at 30 days old as well as 60 days old and significantly difference among membrane proteins ($p < 0.01$). MP38, MP48 and MP67 membrane proteins could decline skin damaged level from heavy level to slight level at 30 days old as well as 60 days old. Moreover, to those proteins could decrease gill damaged level from moderate level to be slight level at both age.

MP38, MP48 and MP67 *Zoothamnium penaei* immunogenic membrane proteins could enhance survival rate, immune response (increasing THC and DHC) and decrease shrimp skin and gill damage levels and those proteins can be developed as immunostimulant material for *Penaeus monodon* Fab to zoothamniosis, although still needed deeply observation.

