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Ectoparasite infestation and survival rate of pacific white shrimp (*Litopenaeus vannamei*) that immunized with crude protein *Zoothamnium penaei* in intensive ponds

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Ectoparasite infestation and survival rate of pacific white shrimp (*Litopenaeus vannamei*) that immunized with crude protein *Zoothamnium penaei* in intensive ponds

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Abstract. The aim of research was to determine ectoparasite infestation and survival rate of *L. vannamei* immunized with *Zoothamnium penaei* crude protein in intensive ponds. This research was an experimental study to determine the effect of using feed and added crude protein *Z. penaei* to *L. vannamei*. The sample used was 10,000 (PL-11) *L. vannamei*. The collected data of ectoparasite infestatiton were analyzed by descriptive and variance analysis. There was a significant difference between the pacific white shrimp immunized with crude protein *Z. penaei* on the 1th, 30th, 60th and 90th days of maintenance ($p< 0.05$). The highest ectoparasite infestation occurred in *L. vannamei* aged 60 days, and 30 days respectively. An increase of ectoparasites infestation occurred on shrimp aged 30 to 60 days in the ponds, though there was no significant difference ($P> 0.05$). On 90 days of maintenance, there was an increase of ectoparasites infestation and it was significantly different from the infestations in shrimp aged 60 days in ponds ($p<0.005$). The survival rate of pacific white shrimp immunized with crude protein was the age of 90 days in the pond (79%). crude protein *Z. penaei* could suppress ectoparasite infestation and *vannamei* shrimp mortality in intensive ponds.

1. Introduction

Lithopenaeus vannamei is one of the economically important shrimp that import from America. Since the end of 1993, the *Penaeus monodon* Fab. mortality rate had been relatively high and due to this circumstance many ponds collapsed and production dramatically declined year by year. So that the white shrimp is the one alternative to be develop and some attempts have already been made by the Indonesian Government to change to other species is *L. vannamei*, to increase the export value significantly. But actually the pathogen infection happen earlier. One of protozoan pathogen that could attack on shrimp was *Zoothamnium penaei* and cause zoothamniosis.

Zoothamniosis was the one of protozoan disesases that caused *Z. penaei*. It could cause death particularly at secondary infection. Zoothamniosis was a parasitic disease in shrimp caused by *Z. penaei*, a Ciliate normally lived in poor water quality. The distribution of *Z. penaei* include the brackish water ponds in Indonesia, Thailand, Malaysia, India, Japan etc. Breathing disturbance, mobile, food searching and moulting issues were clinical symptoms of infected shrimp. The incidence of zoothamniosis in the field has always been elevated so far. Zoothamniosis caused 100% mortality of the shrimp when

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attacked in the hatchery [1]. A shrimp infected by *Z. penaei* would be a indication that white-brownish parasite emerges on the surface of the body so that it emerges as algae, gill was turbid and dirty, anoxia, reducing activity, immobile and moulting failure. These were found on all stages of the shrimp. According to Sindermann [2] said that the prevalence of *Zoothamniosis* in the lower oxygen ponds was 80%, the adult shrimp was 89% [3]. The prevalence in China increased year by year due to season that increased in summer season (August – October). Observation and research about zoothamniosis had been done on clinical symptom, and the agent identification. The histopatology had not been done until now.

To controll of the zoothamniosis had been done with the circulation system, antibiotic, but optimal result had not been obtained. The curative treatment on to zoothamniosis in the hatcheries and in the ponds using antibiotic could relatively be better,but it caused the shrimp resistant and gave residu in the shrimp. Rukyani [4] said that to increase the shrimp defense in both the hatchery and ponds immunostimulant could be used. The non-specific defences of shrimp was more dominant than the specific defense [5, 6].

The body defense system of invertebrate (including the shrimp) was dominated by the haemocyte, of which scattering and increasing of the total haemocyte was assumed as the form of the cellular immunity respons in the body of the shrimp [7, 8]. Some protein was needed to conquer the foreign materials (agent) that entered to the shrimp body [5] to do the phagocytosis, encapsulation, nodulation, activate the prophenol oxidation system, anti-microbe and anti-toxic.

Research conducted by [9] in intensive ponds and traditional ponds in Gresik Regency, it was proven that there were several infested ectoparasites, namely *Zoothamnium*, *Epistylis* and *Vorticella*. The infestation degree of the three ectoparasite species in *vannamei* shrimp in intensive ponds was included in the heavy category of 76.56 zooids and in traditional ponds it was in the moderate category of 43.78 zooids. Then the research conducted by [10] at Gampong Pande Banda Aceh proved that each class of shrimp attacked ectoparasite with the highest prevalence value reaching 100% and the highest intensity value reaching 135 parasites/ind found in shrimp measuring 16-20 cm.

Based on that statement, the crude protein of *Z. penaei* would be developed as material for immunostimulant to prevent zoothamniosis on white shrimp in ponds. The purpose of this research was to know the the immune respons and survival rate of *L. vannamei* that immunized by the immunogenic membrane protein *Z. penaei*.

2. Material and methods

2.1. Research materials

The materials of this study were 50,00 Pacific white shrimp (PL-11), 2 pond plots measuring 200 square meters, 200 sick juvenile pacific white shrimp, 1,000 shrimp infested by parasites and two ponds for maintenance. The *Z. penaei* crude protein used in this study was the production of [11] which had been characterized by SDS-PAGE, ELISA and Immunoblotting.

2.2. Culture of Zoothamnium penaei

The culture of *Z. penaei* was done by cohabitation, through contaminating of one infected and 15 healthy shrimps. The shrimps which used in this research gets from the ponds in Bangil District, East Java. The *Z. penaei* that using in this Research were the result of the cultivation in the laboratories, two hundred healtly white shrimps 60 days old which 25– 30 gram in weight were gets from ponds Bangil, Pasuruan, East Java.

2.3. Shrimp Immunization By Crude Protein Z. penaei

The Crude protein of *Z. penaei* that conducted in this research was the production of [11]. This research was conducted on 2 groups of shrimp, namely:

P1 : was a group of pacific white shrimp that were not immunized with *Z. penaei* crude protein

P2 : was a group of pacific white shrimp immunized with *Z. penaei* crude protein

Shrimp rearing was carried out for 90 days, with a stocking density of 100 shrimp per square meter. Before being spread, the seeds were immunized by soaking for 15 minutes, at a dose of 3 ml per 5000 shrimp in 1 liter of water [12]. Data on ectoparasite infestation and survival of Pacific white shrimp were collected then analyzed with ANOVA, and if there was a difference, continue with the Duncan test [13].

2.4. Ectoparasite Infestation Examination in Vannamei Shrimp
The ectoparasite infestation observation was performed natively using [13], by scraping the entire shrimp surface. The scrapings were arranged on a slide, provided water and examined with a magnification of 100X under a microscope. The percentage of shrimp which were positive for the number of shrimp examined was determined for parasitic infestations.

2.5. Calculation Survival Rate of Vannamei Shrimp
Survival of *L. vannamei* was measured as the percentage of shrimp that was alive during the maintenance of the total amount of shrimp being reared, which was conducted at the end of maintenance, namely at the time of harvest at 90 days in the pond. The formula using to calculate based on Effendi [15] is:

$$SR = \frac{No \times 100\%}{Nt}$$

Information:
SR = Survival rate / survival (%)
Nt = The total of live shrimp at the end of the research
No = The total of live shrimp at the beginning of the research

3. Result and discussion
3.1. Results
The results showed that the shrimp were surely infested with *Z. penaei*, which occurred in both exposed and unexposed *Z. penaei* crude protein and the examination of the parasite infestation of shrimp showing to crude protein appeared that the most elevated infestation was 16.53% in shrimp aged 90 days, while those that were not exposed showed a higher number of 61.76%. The lowest infestation occurred in shrimp exposed to crude protein, namely 7.65% at the aged 30 days in ponds, and 18.85% in shrimp that were not exposed. Clinical symptoms of infested shrimp indicate that when swimming, the tail didn't even look like a fan, the shrimp swam on the surface and in groups,all over the body and gills there were parasites that stick to brownish white, There was an empty digestive tract, the surface of the body and the gills were cloudy like moss. Although safe shrimps appeared smooth, translucent, cleaned (not attacked by zoothamniosis) and there was no color change on the entire surface of the body and gills, shrimp were active swimming and like a fan opened the tail. According to the identification results of [16], which had a peristome in the shape of a circle surrounded by cilia, within there was a contractive vocole and many food vocabulary, necleus, and had a stalk on the posterior side, the Zoothamnium zooid identification results found.

Table 1.The *Zoothanium penaei* infestation in pacific white shrimp

Day of Farming (Day)	Infestation of Pacific Shrimp *Zoothamnium Penaei* (%)	
	Not exposed to *Z. penaei* crude protein (P1)	Exposure to crude protein *Z. penaei* (P2)
30	18.85	7.65
60	31.26	14.46
90	61.76	16. 53

Table 1 displays the effects of the Zoothamnium penaei infestation test on vannamei shrimp. Table 1. showed that the highest parasite infestation in pacific white shrimp occurred in non-immunized and parasite-infested shrimp, namely 61.76% which was positively infested with parasites, which was very significantly different from the shrimp group that was given protein and parasite infestation, namely 16.53%.

3.1.1. Determination of Survival Rate of Vannamei Shrimp
The survival rate of pacific white shrimp was calculated at harvest time, which was on the 90th day.The results of calculating the survival rate of pasific vannamei shrimp showed that the survival rate of the exposed pacific white shrimp to crude protein *Zoothanium penaei* reached 71.34%, while those who were not exposed to crude protein were 26.78% (Table 2).

Table 2. The determination of survival rate for pacific shrimp

Treatment	Survival Rate (%) (90 Days)
P1: Not Immunized with Crude Protein	26.78
P2: Immunized with Crude Protein	71.34

The probability of survival of *L. Vannamei* that was immunized before being processed and raised in the ponds is 71.34, higher than those that were not immunized, which is just 26.78 percent at the end of the 90-day maintenance period.

3.1.2. Water Quality Measurements
The results of water quality checks for 90 days of maintenance could be seen in Table 3.

Table 3. The average pond water quality measurements per 90 days of shrimp treatment

Treatment	Average parameter	Range Normal Value
Temperature (^{o}C)	28 – 29	27 – 32
Salinity (ppt)	18 – 19	16 – 30
pH	7.8 – 8.5	7.5 – 8.5
Dissolved oxygen (ppm)	4.8 – 6.3	>3 – 7
Ammonia (ppm)	0.07 – 0.08	<0.1

Table 3 shows that the quality of pond water for 90 days of maintenance of pacific white shrimp using immunostimulants on average was still in the normal range, so that it was in accordance with the requirements for maintaining pacific shrimp.

3.2. Discussion
Crude protein *Z. penaei* added to shrimp feed from the time the fry would be stocked, start before 30 days until they were 90 days old in the pond, could reduce *Z. penaei* infestation, and increase the survival of *L. vannamei* when harvested, which was 90 days old.

The results also showed a reduction in *Z. penaei* parasite infestation along with the increasing age of shrimp in ponds (Table 1). *Z. penaei* infestation, whether exposed or not to crude protein Z, started to occur in pacific white shrimp aged 30 days or older. Penaei. The highest infestation, however, occurred in pacific white shrimp that was not exposed to crude protein, 71.34%. Meanwhile, infestations at the age of 30 , 60 and 90 days of pacific white shrimp exposed to crude protein all reported lower numbers relative to those not exposed to protein.

The results exhibited that the crude protein *Z. penaei* had an immune response characterized by an increase in THC and DHC, a decrease in the infestation of pacific white shrimp parasites. The survival rate of pacific white shrimp, which increased from 26.78% to 71.34%, may also demonstrate the potential of this protein as an immunostimulating content. This implies that the *Z. penaei* protein is capable of preserving the healthy white shrimp that are kept in the pond. As an attempt to counter pathogens that entered the shrimp body during maintenance, crude protein that entered the shrimp body would stimulate the activity of haemocyte cells in shrimps. This was in agreement with [8,17] that hyalin (granular) and semi-granular cells could carry out phagocytic activity in shrimp by haemocyte cells activated by crude protein.

According to Sŏderhặll K, and Cerenius L [5], who claimed that the immune system was still rudimentary in pacific white shrimp and was substituted by Prophenoloxidase Activating Enzymes (PPA) as opposed to fish and mammals that had immunoglobulins in shrimp. The PPA was a protein that was found in hemocyte granular cells. Lipopolysaccharides and β 1,3-Glucans, which induce prophenoloksidase to become phenoloksidase, will further activate this PPA. A kind of Opsonin Factor protein that could induce hyalin cells to conduct the phagocytosis process would be generated as a result of this transition. Van de Braak [8] and Smith *et al*. [18] also endorsed the argument that haemocyte cells would degranulate and that certain proteins would be released for the benefit of the immune response, such as: increased haemocyte cells and increased phagocytosis and trapping activity. In addition, crude proteins will induce the release of proPO and protein-binding PPA by haemocytes, resulting in haemocyte cells increasing their activity against disease agents, in this case *Z. penaei*, to trap and phagocytosis. The incidence of zoothamniosis in immunized shrimps has been shown to be lower and substantially different from that of unimmunized shrimps. There were still *Z. penaei* infested shrimp, because this parasite is opportunistic, but it still grew but evolved for a long time in normal water conditions, so it did not cause shrimp disease. However, the state of these waters may not have increased the activity of the *V. parahaemoliticus*, so that the disease of the vannamei shrimp was not induced.

It will cause an increase in the number of haemocytes (THC) and differential haemocyte cells (DHC) if crude protein enters the shrimp body as an immunostimulant substance. This was a sign of the improved protection of the body against pathogenic infections[17]. In addition,[7] the hypothesis that vaccine administration could prevent disease infection in the host body and lead to increased activity of haemocyte phagocytes and proPO enzymes is also supported [7]. This argument was confirmed by [20] and [8] that antibodies that were able to neutralize *Z. penaei* infestation in pacific white shrimp will be triggered by the vaccine content entering the shrimp body.

Based on Mahasri [17], the survival of vannamei shrimp in shrimps aged 90 days (the end of maintenance) could be improved from 17 percent to 68 percent by crude proteins entering the body. In addition, the immune response of tiger prawns was also reported to have improved, marked by an increase in THC and DHC, since the immunogenic membrane protein *Z. penaei* had a large molecular weight greater than 1,000 Dalton in order to be immunogenic. Proteins with a high molecular weight and a high degree of immunogenicity must be organized in a complex way. Proteins that were immunogenic had a broad molecular weight of more than 1,000 Daltons and a complex structure, according to the opinions of [21] and [22].

It could therefore be argued that the immunostimulant content from the crude protein could induce the shrimp body's defense mechanism. It did take time, however, to stimulate the development of granulocytes by the hematopoietic organs to counter zoothamniosis attacks [17]. Through ingesting these pathogens, these granulocytes can kill pathogens, such that these granulocytes can migrate to parasite-infested areas. On the other hand, infestation and immune response in pacific white shrimp are also influenced by the quality of water maintenance, but based on the results of the analysis, it was shown that the quality of the water was in optimal conditions for shrimp survival.

4. Conclusion

Crude protein *Z. penaei* could suppress ectoparasite infestation and increase the survival of pacific white shrimp (*L. vannamei*) from 26.78% to 71.34% to 90 lines of maintenance.

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