C2.59 IOP

by Gunanti Mahasri

Submission date: 02-Sep-2021 11:03AM (UTC+0800)
Submission ID: 1639844840
File name: C2.59_IOP.pdf (615.77K)
Word count: 5310
Character count: 29005

Immune response and parasitic infestation on Pacific white shrimp (*Lithopenaeus vannamei*) in immuno-probio circulation system (SI-PBR) in ponds

Immune response and parasitic infestation on Pacific white shrimp (*Lithopenaeus vannamei*) in immuno-probio circulation system (SI-PBR) in ponds

G Mahasri[1,2], P D W Sari[1] and Prayogo[1]

[1]Department of Fish Health Management and Aquaculture, Faculty of Fisheries and Marine, Universitas Airlangga, Campus C Mulyorejo 60115, Indonesia

E-mail: mahasritot@gmail.com

Abstract. The main causes of death of pacific white shrimp in aquaculture are diseases. One effort to control deseases by improving the defense ability of shrimp body against disesases and optimizing water quality during farming through the application of a new aquaculture technology called Immuno-Probiocirculation System (SI-PBR). This research aimed to analyze immune response on Total Haemocyte Count (THC) and Differential Haemocyte Count (DHC), parasitic infestation on pacific white shrimp in many ages, survival rate of pacific white shrimp during farming period for 90 days in SI-PBR. The results of this research showed that the lowest parasitic infestation (*Zoothamnium penaei*) is 12.46 % that happened on 90-days-old shrimp in SI-PBR pond, while the highest infestasion is on the shrimp not given SI-PBR, reaching 54.65 %. In addition, the immune response (THC and DHC) also increased. The highest survival rate discovered in 90 days shrimp farming is 80% using SI-PBR. This is higher than the pond without SI-PBR, which is 22 %. Therefore, SI-PBR in shrimp farming in tradisional ponds is able to increase immune response, survival rate, and is also able to decrease parasitic infestation during 90 days of farming.

1. Introduction

Since late 1993, production of tiger shrimp has been declining drastically; only around 30 % of ponds are still operating till now, while the rest have become unproductive and idle [1]. The reality shows that increased production of vannamei shrimp is inhibited due to diseases caused by viruses, bacteria, fungi and parasites [2].

The main problems hampering revitalization efforts of shrimp ponds in Indonesia, particularly in East Java, are low level of growth and high number of shrimp death due to declining water quality and diseases/pathogens. White spot virus disease, also known as "white spot" (WSSV), is the main culprit and it has become a serious problem in shrimp farming activities. Moreover, traditional shrimp ponds farming activities are highly dependent on tidal conditions.

IMNV Infection on vannamei shrimp starts with the weakening of the shrimp body, decreased appetite, unbalanced swimming, and movement towards the surface. After that, around two segments on the base of the tail, and the base itself, become opaque white to reddish. Histopathologically, meat or muscle tissue, especially in the last part of the IMNV-infected segments, exhibits necrosis [3]. In addition, histopathology testing shows that IMNV-infected shrimp can be identified through lesions on skeletal muscles, including multifocal necrosis, haemolytic blockage, fibrocystic inflammation,

phagocytosis, and appearance of cytoplasmic body inclusion. IMNV appears to replicate in shrimp cytoplasmic muscle cells and exhibits a lack of conclusive evidence for protein incorporation.

Bacterial diseases that often emerge to infect shrimp in ponds and hatcheries include diseases caused by *Vibrio parahaemoliticus*, *Vibrio harveyii,* and others. Itami *et al*. [4], stated that shrimp infected by these vibrio bacteria will swim on the surface; their gills are dirty and their tails, fins and the anterior part of the body suffer many reddish wounds. This disease can cause death up to 86 %.

One of the parasitic diseases that can cause vannamei shrimp death both in ponds and hatcheries is Zoothamniosis. This disease is caused by *Zoothamnium penaei*. It causes shrimp to suffer difficulty in breathing, moving, searching for food Sindermann [5] and Foster *et al*. [6], and moulting. It also impedes growth, reduces economic values, and causes death up to 91 % [7]. In addition, this disease is a predisposing factor of secondary infection by bacteria and viruses.

Smith *et al*. [8], wrote that the efforts to improve the immunity of shrimp body in both hatcheries and ponds can be performed using immunostimulants. Furthermore, Mahasri [9] reported that immunization using immunogenic membrane protein from *Zoothamnium penaei* could increase survival rate of shrimp up to 93 %. In addition, isolation of immunogenic membrane proteins can be conducted using SDS-PAGE, ELISA, and Western Blotting methods. The results showed that 7 proteins were discovered and 3 of them were immunogenic, namely membrane proteins MP38, MP48 and MP67.

Generally, crustacean group has non-specific immune system because it lacks the ability to memorize antigens. In the event of bacterial, viral, or fungal infection, the shrimp's hard cuticle is the first physical defence inhibiting the entry of pathogens. If the pathogens pass through this external defence, the internal defence of the shrimp body becomes the second defence performed by its cellular and humeral responses [10].

Immunity system in shrimp is different from that of vertebrates. What plays a role in the immunity system of shrimp is the defence mechanism through haemocytes and plasma proteins [11]. Haemocytes are cells that possess functional devices such as macrophages, granulocytes, and natural killer cells (NK cells) in vertebrate animals [12]. Haemocytes play an important role in the immune system of crustacean body by expelling foreign particles through phagocytosis, encapsulation, and nodular aggregation [13]. The marker parameters for the evaluation of shrimp defence system involve humeral and cellular immunity, which include haemocyte counts [14], prophenoloxidase (proPO) enzyme and phenoloxidase (PO) enzyme activities [14,15]. The immune response is characterized cellularly by increased haemocytes in shrimp and marked molecularly by increased plasma proteins, which can be detected and characterized by SDS PAGE electrophorus, as well as increased phenoloxidase activity as the parameters of increasing immune system in shrimp. Haemocytes have an important role in the internal immune system of shrimp. Shrimp has 3 types of haemocytes, namely hyalin that plays a role in the process of phagocytosis and granular and semi granular cells that are important in the process of activation of phenoloxidase.

Shrimp farming using Imuno-Biocirculation System (SI-PBR) is an effort to increase the harvest yield by applying immunization and biological filter with milkfish. This system is a combination of immunostimulant use in shrimp seeds before the stocking to increase the resistance of shrimp. The immunostimulant used is an immunostimulant obtained from immunogenic membrane protein of *Zoothamnium penaei* in order to improve the immunity of shrimp and to prevent the ectoparasitic diseases [9]. In addition, this system also uses probiotics to maintain water quality. Gunarto *et al*. [16], reported that utilization of probiotics, using various types of bacteria that decompose organic materials, on tiger shrimp farming has been widely implemented to increase shrimp life up to 86 - 93%.

The purpose of the use of filter is to balance the biomass of plankton and reduce nitrite and ammonia compounds, because milkfish is able to accumulate compounds in shrimp, so there is no decay inside the ponds. According to Mahasri [9], immuno-probiorecirculation system (SI-PBR) was able to laboratorically increase tiger shrimp life from 40 % to 82 %.

Based on the background of the problem, the application of SI-PBR method needs to be studied to increase shrimp production in traditional ponds and to analyze immune response (THC and DHC), ectoparasitic infestation, and survival rate (SR) of vannamei shrimp (*Litopenaeus vannamei*) nurtured in ponds using immuno-probiocirculation system (SI-PBR).

2. Materials and method

2.1 Research methodology and design

The research method used in this research was field experimental method (in ponds) to discover the values of independent variables, either one or more (independent) variables, without making comparison or links with other variables. It was expected that this research would be able to achieve the objective of analyzing immune response and survival rate of vannamei shrimp in ponds with immuno-probiocirculation system, using the following treatments:

K1 → Reservoir plot and biological filter of milkfish and Recirculation
K2 → Shrimp control group nurtured in the pond without Immuno-Probio-circulation system (SI-PBR)
K3 → Shrimp group kept in the pond using Immuno-probio-circulation system (SI-PBR)

2.2 Materials and equipments

The main materials in this study were 1000 healthy juvenile (40-days-old) vannamei shrimp and membrane protein of *Zoothamnium penaei*, as a substance for developing immunostimulant, that had been laboratorically tested by Mahasri [18]. The main materials for protein isolation and characterization include saline, ethanol, gradient Percoll solvent, pepsin, HCl, EDTA, KCl, KH_2PO_4, Na_2HPO_4, trypsin, sodium citrate, $NaHPO_4$, H_2NO_3, $NaHCO_3$, glucose, phenol red 0.5 %, NaOH, 0.22 μm filter, bovine serum albumin, dextrose, EtOH, proteinase, Forward ERIB1 5' primary pair – ACCTGGTTGATCCTGCCAG-3' (2-20) and Reverse ERIB10 5 '-CCTCCGCAGGTTCACCTACGG-3' 2079- 2059), 400 μM DNTP, 3 μm of MgCl2, yellow and blue dye, agarose, TAE buffer, sybrsafe, 100 bp and 1 bp of DNA ladder, loading dye, tris-HCL, 2-mercaptoethanol, sodium dodecyl sulphate (SDS) bromophenol blue, glycerol, SDS loading buffer, polyacrylamide, stacking gel, ammonium persulfate (APS), TEMED, and glycine.

The material for biological filter was 3000 milkfish with a length of 10-15 cm. The materials for ponds preparation were: 1,500 kg of dolomite lime, 150 kg of urea fertilizer, 75 kg of TSP fertilizer, and 2 ampoules of vitamin.

The equipments used were 3 ponds (each was 500 m^2), 2 sets of production facilities, 1 8-DHIM generator. To isolate protein, the tools used were haemocytometer, micrometer microscope, autoclave, centrifuge tube, swinging rotor, water bath sonicator and 1 set of electrophoresis equipment for SDS-PAGE [17].

2.3 Research procedure

2.3.1 Preparation of ponds

The ponds for the application of SI-PBR consisted of a reservoir plot, which was also used as recirculation plot with milkfish as biological filter, and shrimp nurturing plots. Each of these plots was connected to two 8 cm pipes for recirculation. Recirculation was run by a water pump with a size of 20 hp and 8 cm. The design of SI-PBR-Plus ponds can be seen in Appendix 1.

2.3.2 The making of immunostimulant materials from Zoothamnium penaei protein

The immunostimulant development material used in this study was the immunostimulant material produced by Mahasri [18]; The stages in the making of the material were: 1) in vitro farming and isolation of Zooid immunogenic proteins from *Zoothamnium penaei*, 2) characterization and purification of immunogenic proteins using SDS-PAGE, 3) determination of protein concentrations, and 4) testing of immunostimulant material on vannamei shrimp (*Litopenaeus vannamei*) in traditional-plus pond.

2.3.3 Application of SIBR-Plus
This stage was the stage of direct implementation of vannamei shrimp farming using Imuno-Probiocirculation System (SIBR-Plus) in traditional-plus pattern on ponds. This stage was started by re-checking biorecirculation and immunostimulant plots. The biofilter used was milkfish. in one cycle (90 days) of shrimp nurturing period, the activities conducted included data collection of the circulation plot, water quality, and shrimp health and growth as supporting data on the success rate of SI-PBR implementation. The area of shrimp nurturing plot was 500 m^2 with a dense stocking of 50 shrimp per m^2 and 3000 milkfish. Circulation was done 2 times every 24 hours, namely at 06.00 to 08.00 for 2 hours, and 24.00 - 02.00 for 2 hours.

2.3.4 Examination of ectoparasitic infestation on Vannamei Shrimp
Observation of ectoparasitic infestation was conducted natively by scrapping the entire surface of the shrimp body [19]. The result was placed on top of an object glass, given a drop of water, and examined using a microscope with 100× magnification. Parasitic infestation was calculated with a percentage of positive shrimp on the number of shrimp examined.

2.3.5 Total haemocyte count (THC)
Haemocytes were taken at the ventral portion of the second abdominal segment using 1 ml syringe containing 0.2 ml of cold Alsever modified solution (AS 19.3 mM; 239.8 mM Na citrate, 182.5 glucose NaCl, and 6.2 mM EDTA; pH 7.2) as an anticoagulant [20]. Furthermore, calculation of the number of haemocytes was performed using Haemocytometer seen under the microscope with 1000× magnification and was calculated with a hand counter. Total haemocyte count was conducted at the age of 30, 60, and 90 days in the ponds using the following formula [21]:

$$\text{PTH } /\text{mm}^2 = \frac{(C)(D)(100)(10)}{(S)(4)} \qquad (1)$$

Notes:
C = Number of countable cells
D = Dilution factor
S = Number of countable 1 mm boxes
PTH = Total haemocyte count /mm^2

2.3.6 Differential Haemocyte Count (DHC)
Blood was dripped on the object glass and blood smear was made by giemsa staining method [22]; therefore the cell type could be identified. Differential haemocyte count was aimed to discover the number, type, and percentage of haemocyte cells. The haemocytes analyzed were classified according to the method by Owens and O'Neill [23]. The number of granular haemocytes cells (DHC) was calculated up to 100 cells and its percentage was searched for [9]. Calculation of DHC was conducted at the age of 30, 60, and 90 days in the ponds.

2.3.7 Determination of survival
Based on a research conducted by Nuhman [24], observation on survival was performed through direct observation by observing live shrimp at the beginning until the end of the study. The formula used to measure it is as follows:

$$SR = \frac{Nt}{No} \times 100\% \qquad (2)$$

Notes: SR = Survival rate (%)
Nt = Live shrimp at the end of the research
No = Live shrimp at the beginning of the research

3. Results

3. 1 Analysis of Zoothamnium penaei infestation on Vannamei Shrimp

Examination result of parasitic infestation on vannamei shrimp showed that there were shrimps positively infested with *Zoothamnium penaei*; both in the pond with and without SI-PBR. Parasitic infestation on the shrimp nurtured using SI-PBR was 17.34 % on 90-days-old shrimp, while on the shrimp pond nurtured without SI-PBR, the highest level of infestation was 54.65 %. The lowest parasitic infestation on the shrimp was found on the shrimp nurtured using SI-PBR, which was 7.68%, after 30 days in the pond. The shrimp infected by parasites showed several clinical symptoms: there were objects looking like fibres attached to the tails, their swimming and walking legs became brownish white, their tail did not look like fans when swimming, they swam clustered on the surface, their digestive tracts were empty, and their body surface and gills seemed turbid like containing moss. Healthy shrimp (not infested by zoothamniosis) looked clear, transparent, and clean and there was no discoloration on the entire surface of the body and gills, they actively swam, and their tail opened like fans. The examination result of *Zoothamnium penaei* infestation on vannamei shrimp can be seen in table 1.

Table 1. Examination Result of *Zoothamnium penaei* on Vannamei Shrimp.

Age of Shrimp Nurturing (Day)	Infestation of *Zoothamnium penaei* on Vannamei Shrimp (%)	
	Shrimp nurtured using SI-PBR	Shrimp nurtured without SI-PBR
30	7.68	14.76
60	9.24	24.74
90	17.34	54.65

3.2 Analysis of immune response (THC and DHC) of vannamei shrimp seeds

Examination of immune response of the seeds (logs/PL 30) stock could not be conducted because the seeds were too small, so there was difficulty in taking blood sample. The calculation result of total haemocyte count (THC) on the vannamei shrimp nurtured in SI-PBR pond at the age of 30, 60, and 90 days is presented in table 2. The highest THC was found on 60-days-old shrimp nurtured in the pond using SI-PBR, reaching 61.23 x 10^6 cells/mL, while the lowest was found on 30-days-old shrimp in the pond without SI-PBR, which was 29.34 x 10^6 cells/mL.

Table 2. Determination of Total Haemocyte Count (THC) of Vannamei Shrimp.

Age of Nurturing (Day)	Total Haemocyte Count (THC) of Vannamei Shrimp (10^6 cells/ml)	
	Shrimp Nurtured using SI-PBR	Shrimp Nurtured without SI-PBR
30	29.34	21.56
60	61.23	36.35
90	48.96	32.54

Table 3 shows the differential haemocyte count (DHC) of vannamei shrimp nurtured in the ponds with and without SI-PBR.

Table 3. Differential Haemocyte Count (DHC) of Vannamei Shrimp.

Age of Nurturing (Day)	Differential Haemocyte Count (DHC) of Vannamei Shrimp (%)	
	Shrimp Nurtured Using SI-PBR	Shrimp Nurtured without SI-PBR
30	25.32	11.23
60	19.73	15.84
90	15.54	10.79

The results showed that the highest DHC was found in 90-days-old vannamei shrimp nurtured in the pond using SI-PBR, which reached 25.32 %, while the lowest was found in 90-days-old shrimp nurtured in the pond without SI-PBR, which was 10.79 %

3.3 Determination of Survival Rate (SR) of Vannamei Shrimp

The survival rates of vannamei shrimp are shown in table 4.

Table 4. Survival rates of vannamei shrimp.

Treatment	Survival Rate (%)
Shrimp Nurtured in the pond using SI-PBR	80
Shrimp Nurtured in the pond without SI-PBR	22

Table 4 shows that the survival rate of 90-days-old vannamei shrimp in the pond that used SI-PBR reached 80 % at harvest, higher than the shrimp nurtured in the pond without SI-PBR, which reached only 22 % at the end of nurturing period.

3.4 Examination of water quality

Table 5 shows the water quality during 90 days of nurturing period in the ponds.

Table 5. Water quality in the Ponds during 90 Days of Nurturing Period.

Parameter	Average Parameter/Quality of Water during Shrimp Nurturing	Normal Range
Temperature (°C)	27 – 29	27 – 32
Salinity (°/oo)	26 – 26	16 – 30
pH	7.7 – 8.3	7.5 – 8.5
Dissolved Oxygen (ppm)	4.1 – 6.2	>3 – 7
Ammonia (ppm)	0.8 - 0.96	<1

Table 5 shows that the water quality in the ponds for 90 days of shrimp nurturing was generally within the normal range, thus it was in accordance with the nurturing requirements of vannamei shrimp.

4. Discussion

Results showed that shrimp farming using SI-PBR system was able to reduce parasitic infestation (particularly *Zoothamnium penaei*), improve the immune response (based on the increase in THC and DHC), and increase the survival of vannamei shrimp. Parasitic infestation occurred during nurturing both with and without SI-PBR, but infestation on the shrimp with SI-PBR was lower and showed a declining trend at the age of 30, 60, and 90 days. Table 1 also shows that parasitic infestation always

increased with increased nurturing period, starting from 7.68 %, 9.24 %, and 17.34 % on the shrimp nurtured using SI-PBR system. It also happened on the shrimps without SI-PBR in its nurturing, which were 14.76 %, 24.74 % and the highest was at the age of 90 days during the nurturing that reached 54.65 %.

Table 1 and 2 show that the immune response (THC and DHC) of vannamei shrimp nurtured in the pond using SI-PBR increased, starting from the 30th day of the nurturing, from 21.56 x 10^6 cells/mL to 29.34 x 10^6 cells/mL. While in 60-days-old shrimp, there was an increase in THC from 36.35 x 10^6 cells/mL to 61.23 x 10^6 cells/mL and at the age of 90 days, it increased from 32.54 x 10^6 cells/mL to 48.96 x 10^6 cells/mL. Table 1 shows that vannamei shrimp nurtured with and without SI-PBR experienced an increase during the nurturing period until the age of 60 days and then it decreased at the age of 90 days (harvest).

DHC of the vannamei shrimp (table 3) nurtured using SI-PBR also increased from 11.23 % to 25.32 % in shrimp aged 30 days, from 15.84 % to 19.73 % in shrimp aged 60 days, and from 10.79 % to 15.54 % in shrimp aged 90 days.

The increased immune response in both THC and DHC, along with the increase in survival rate of vannamei shrimp (between 22 % and 81 %), were indicators that nurturing using SI-PBR caused the shrimp to have higher immunity, lower parasitic infestation, and higher survival rate. Existence of immunostimulant was able to provide protection to the shrimp nurtured in the ponds, especially against parasitic infestation particularly *Zoothamnium penaei* since the immunostimulant entering the body of the shrimp would stimulate the activities of haemocyte cells in the shrimp as an effort to fight the pathogens. This is in accordance with Van de Braak in Mahasri [9], who stated that immunostimulant-activated haemocyte cells will perform phagocytic activities on the shrimp by hyalin (granular) and semi-granular cells.

Soderhall and Cerenius [25], said that the immune system in shrimp is still primitive, unlike the systems in fish and mammals that have immunoglobulin, so immunoglobulin in shrimp is replaced by prophenoloxidase activating enzyme (PPA). PPA is a protein located in granular haemocyte cells. it can be activated by lipopolysaccharides and β 1,3-Glucan, which will stimulate prophenoloxidase to change into phenoloxidase. This transformation produces a compound similar to Opsonin Factor protein that can induce hyalin cells to perform the process of phagocytosis. Van de Braak [26], also supported this statement and added that haemocyte cells will degranulate and some proteins would be released for the immune responses, such as: increased haemocytes, trapping activity, and phagocytosis. In addition, immunogenic membrane proteins will stimulate haemocytes to release proPO and protein-binding PPA, thus causing haemocyte cells to increase their activities to perform trapping and phagocytosis from the agents of diseases, which in this case is *Zoothamnium penaei*. Table 1 shows that parasitic infestation on the shrimp nurtured using SI-PBR exhibited a low tendency in the shrimp aged 30, 60, and 90 days in the pond, or equal to 7.68%, 9.24%, and 17.34%. Meanwhile, parasitic infestation on the shrimp nurtured in the pond without SI-PBR showed higher tendency, which were 14.76%, 24.74%, and 54.65%.

If immunostimulant enters the body of the shrimp, it will increase the number of haemocytes (THC) and differential haemocyte cells (DHC). This is an indication of increased immunity of vannamei shrimp [9]. Furthermore, Itami [27] supported the theory by stating that providing immunostimulant may prevent infection of diseases within the host body and increase phagocyte activities of haemocytes and proPO enzymes. This statement was also supported by Van de Braak [26] who wrote that immunostimulant substances entering the shrimp body would produce antibodies capable of neutralizing pathogens, so pathogens cannot infect the shrimp, resulting in lower infestation (infection) by pathogens.

Mahasri [9], stated that the immunogenic membrane protein of *Zoothamnium penaei* can increase the life of vannamei shrimp from 22 % to 81 % in the shrimp aged 90 days old (end of nurturing). In addition, it was also reported that the immune response of tiger shrimp also increased, marked by the increase of THC and DHC since the immunogenic membrane protein of *Zoothamnium penaei* had a

high molecular weight, greater than 1000 Da. Proteins with high molecular weight and high level of immunogenity must have a complex structure.

The increase in THC and DHC can be used as an indicator for pathogenic infection in the host body. Infection will cause inflammation, which is a non-specific body defence mechanism caused by several factors such as parasites, bacteria, fungi, viruses and nonliving agents Soderhall and Cerenius [25] THC in the shrimp given immunostimulant increased starting from the age of 30 days until 60 days, but decreased at the age of 90 days. shrimp immune system increases along with the increased age, but at certain age it decreases back. The high level of THC indicated the added crude protein could improve immune response in shrimp since high THC is an indicator of increased body resistance. This is in accordance with the statement by Soderhall and Cerenius [25] who asserted that increase in immune response on invertebrates is indicated by an increase in THC. Increased THC and DHC can be used as indicators of the immune system reaction in shrimp in the presence of pathogens. The increase in DHC (granular haemocyte cells) was presumed to be due to the lack of memory cells in shrimp immune system, so they were not able to detect pathogenic substances they had been exposed to. Therefore, it can be concluded that the immunostimulant was able induce the immunity mechanism in shrimp body. However stimulating the hematopoietic organ to produce granulocytes to fight pathogens needs time [9]. Granulocytes destroy pathogens by swallowing them, so they have to migrate to parasite-infested areas first.

Water quality during nurturing also affects infestation and immune response of shrimp. Based on the results, the water quality was in optimum condition for shrimp. good water condition of made opportunistic pathogens unable infect shrimp, thus the shrimp stayed in good health and immune system. Maintenance of water quality causes shrimp to live well and prevent diseases from emerging so the shrimp can grow well.

Mahasri [28], stated that probiotic is a material containing various types of bacteria that can play a positive role in decomposing organic materials in ponds and helping metabolism when mixed in shrimp feed; while biofilter, a role that can be played by milkfish (*Chanos chanos Forsk*) or seaweed, can be used to balance biomass (plankton) in the pond water. Furthermore, water circulation is necessary to be implemented in shrimp farming activities to maintain water quality.

application of probiofilter technology can result in high quality and disease-free shrimp harvest. the ponds Water remain in good condition, so diseases do not emerge during farming and the shrimp can grow well and healthy.

it appears that shrimp nurtured using SI-PBR reached 80 % of survival rate, while those without the addition of crude protein only reached 22 %. it indicated the crude protein given was able to improve defence system in the shrimp, and good quality of pond water during the research caused the shrimp to stay in good condition, resulting in increased growth rate of the shrimp.

5. Conclusions

There was an increase in immune response caused by provision of immunostimulant, probiotics, biological filter, and circulation in SI-PBR, resulting in a decrease in *Zoothamnium penaei* infestation on vannamei shrimp. Provision of immunostimulant improved immune response (increased THC and DHC) on vannamei shrimp nurtured in the ponds. The immune response of vannamei shrimp increased for 60 days and started to decrease after 90 days in the ponds. Provision of immunostimulant, probiotics, biological filter, and circulation of SI-PBR were able to improve the survival rate of vannamei shrimp from 22 % to 80 % in 90 days of nurture in the ponds.

6. References

[1] Department of Fisheries and Marine 2001 Type of shrimp disease on brackish water cultivation artikel 02/09/04 Mina diklat BPPP Belawan Medan

[2] Lightner D V 2002 *Prevalence and geographic distribution of mbv and other diseases in cultured Giant Tiger Prawns (Penaeus monodon) in the Philippines* (Hawaii : The Oceanic Institute, Honolulu)

[3] Lightner D V 2003 *Exclusion of specific pathogens for disease prevention in a penaeid shrimp biosecurity program* In: Lee, CS, O'Bryen P J (Eds) Biosecurity in Aquaculture Production Systems: Exclusion of Pathogens and Other Undesirables. The World Aquaculture Society (Baton Rouge, Louisiana) p 81–116.
[4] Itami T, Kondo M and Takahasi Y 1996 Enchancement of disease resistance of kuruma prawn (*Penaeus japonicas)* After Oral Administration of Peptidoglucan (Japan : National Fisheries University) p 59-65.
[5] Sindermann C J 1997 *Trans. Am. Microsc. Soc.* **98** 136 –138.
[6] Foster C A, Sharpie T G and Hawkins W E 1998 *J. Fish. Dis.* **1** 321-335
[7] Tonguthai K 1991 Diseases of the freshwater prawn *Machrobrachium rosenbergii*, AAHRI Newsletter Bangkok Thailand Article **4**(2)
[8] Smith V J, Brown J H and Hauton C 2003 *Fish Shellfish Immun* **15** 71 – 90.
[9] Mahasri G 2007 *Immunogenic membrane protein zoothamnium penaei as an immunostimulant development in Shrimp Windu (Penaeus monodon Fab.) To Prevent Zoothamniosis* (Surabaya : Universitas Airlangga).
[10] Van de Braak K 2002 *Haemocytic defence in black tiger shrimp (Penaeus monodon)* Disertation (Netherland: Wageningen University).
[11] Vargas-Albores 1996 *Dev Comp Immunol* **20** 299-306
[12] Raa J 2000 *The use of immunostimulant in fish and shellfish feeds*, In : LE Cruz Suarez, D Richie-Marie, M Tapia-Salazar, MA Olver-Novoa, R Civera-Cerecedo (Eds) Avences en Nutricion Acuicola, Merid, Yucatan, Mexico : 47-54.
[13] Rodríguez J, Le Moullac G 2000 *Aquacult* **19** 109-119.
[14] Le Moullac, G Soyez, C Saulnier, D Ansquer, D Avarre, J C Levy P 1998 *Fish Shellfish Immunol* **8** 621–629.
[15] Hernández-López J, Gollas-Galván T and Vargas-Albores F 1996 Activation of the prophenoloxidase system of the brown shrimp Penaeus californiensis Holmes. Comparative Biochemistry and Physiology **113C**: 61-66.
[16] Gunarto, Mansyur A dan Muliani 2009 Application of different probiotic fermentation doses on the cultivation of vaname shrimp (*Litopenaeus vannamei*) with intensif pattern. Center for Brackishwater Aquaculture Research, Maros, South Sulawesi.
[17] Rantam FA 2003 Metode Imunologi (Surabaya-Airlangga University Press).
[18] Mahasri 2015 Respon Imun Dan Kelulushidupan Udang Vannamei (*Lithopenaeusvannamei* Yang Dimunisasi Dengan Protein/Membran Imunogenik *Zoothamnium penaei*. *Article of Konggres and International Seminar IFS, Can Tho University, Vietnam.*
[19] Johnson 1986 *J. Parasitol* **72** 517–520.
[20] Van de Braak CBT, N Taverne, MHA Botterblom, WPW Van der Knaap and JHWM Rombout 2000 *Fish Shellfish Immuno* **10** 515-530.
[21] Blaxhall P and K Daisley 1993 *J. Fish. Biol***. 5** 1-8.
[22] Nash G L, Anutara and W Boonsirm 1993 Rapid Diagnosis of yellow head diseases in black tiger shrimp culture reesearch centre. AHHRI NEWSLETTER (Choroen Phokphand Feedmil Co. Ltd, Smuth Sakorn Thailand) p 87.
[23] Owens L and O'Neill A 1997 *Dis. Aquat. Org.* **31** 147-153.
[24] Nuhman 2008 *Scientific J. Fish. Sci.* **1**
[25] Sŏderhăll K and Cerenius L 1992 *Ann. Rev. Fish. Dis.* 3 – 23.
[26] Van de Braak CBT, MHA Botterblom, N Taverne, JHWM Rombout and WPW Van der Knaap 2002 The roles of haemocytes and the lymphoid organ in the clearance of injected Vibrio bacteria in Penaeus monodon shrimp *Fish Shellfish Immunol* **13**: 239-309.
[27] Itami T 1996 *body defense system of penaeid shrimp, seminar on fish physiology and prevention of epizootics* (Japan : Department of Aquaculture and Biology, Shimonoseki University of Fisheries) **7** : 59-65.

[28] Mahasri 2004 Effectiveness of milkfish as biological filters suppress zoothamniosis on intensive cultivation national seminar review of the development of science and technology of fisheries, on 30 November, in Semarang.

Acknowledgments
The authors would like to express gratitude to the Dean of the Faculty of Fisheries and Marine in Airlangga University who had provided funds through RKAT for conducting this research.

C2.59 IOP

ORIGINALITY REPORT

13%	9%	11%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	pustaka.unpad.ac.id Internet Source	2%
2	P A Wiradana, G Mahasri, R E R Sari, U C Marwiyah, R Prihadhana. " Identification of white spot syndrome virus (WSSV) in pacific white shrimps ( ) from ponds postexposure to immunogenic membrane proteins ( ) ", IOP Conference Series: Earth and Environmental Science, 2019 Publication	2%
3	id.123dok.com Internet Source	1%
4	gfzpublic.gfz-potsdam.de Internet Source	1%
5	Haryanti, I G N Permana, Fahrudin, Sari Budi Moria, Ahmad Muzaki, K. Sugama. " The effect of feed supplement on growth, survival rate and immunity response of Pacific white shrimp ", Journal of Physics: Conference Series, 2020 Publication	1%

6	N D Takarina, A Rahman, T Siswanting, T J Pin. "Content of Cr, Cu, Pb, and Zn on Pacific white shrimp cultured in modern farm at BLUPPB, Karawang, West Java", Journal of Physics: Conference Series, 2018 Publication	1 %
7	A Rahman, N D Takarina, T Siswantining, T G Pin, T Soedjiarti. "Bioaccumulation of Copper (Cu) and Chromium (Cr) on export comodity vanamei shrimp from Karawang, West Java", Journal of Physics: Conference Series, 2018 Publication	1 %
8	ejournal-balitbang.kkp.go.id Internet Source	1 %
9	Muhammad Junda. " Development of Intensive Shrimp Farming, In Land-Based Ponds: Production and Management ", Journal of Physics: Conference Series, 2018 Publication	1 %
10	Harlina, Rosmiati, S Jafar, Sukmawati, Nurhidayah, Kamaruddin. " Effect of as bioactive compound in artificial diet on survival rate of tiger prawn ", IOP Conference Series: Earth and Environmental Science, 2019 Publication	1 %
11	dashboard.unair.ac.id Internet Source	<1 %

12	eprints.nottingham.ac.uk Internet Source	<1%
13	M H Fattah, S R Busaeri, M Kasnir, Siswanto. "Changes in Managerial Decision on Pond Management to Adapt to Climate Anomalies in the Coastal Area of Pare-Pare Gulf, District of Pinrang", IOP Conference Series: Earth and Environmental Science, 2017 Publication	<1%
14	Z Zainuddin, S Aslamyah, K Nur, Hadijah. "The Effect of Dosage Combination and Feeding Frequency on Growth and Survival Rate of Vannamei Shrimp Juveniles in Ponds", IOP Conference Series: Earth and Environmental Science, 2019 Publication	<1%
15	eprints.unm.ac.id Internet Source	<1%
16	Submitted to Universitas Airlangga Student Paper	<1%
17	Yeh, S.T.. "Effect of copper sulfate on the immune response and susceptibility to Vibrio alginolyticus in the white shrimp Litopenaeus vannamei", Fish and Shellfish Immunology, 200411 Publication	<1%
	pt.scribd.com	

18	Internet Source	<1%
19	e-journal.unair.ac.id Internet Source	<1%
20	www.fish.wa.gov.au Internet Source	<1%

Exclude quotes	Off	Exclude matches	< 10 words
Exclude bibliography	On		

C2.59 IOP

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9

PAGE 10

PAGE 11