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Immune Response of White Shrimp *Litopenaeus vannamei* that Injected with the Extract of Diatomae *Chaetoceros ceratosphorum*

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ABSTRACT

The haemocytes play an important and central role in shrimp immune defense system. Biological or synthetic compounds from such as bacterial cell walls, compound complex carbohydspeeds, nutrients, animals or plant extracts, bio marine extract, have been tested to stimulate the immune system of shrimp. The objective of this research was to investigate the haemocyte response of white shrimp *Litopenaeus vannamei* (11.9 ± 1.5 g) that have been injected with extract Diatomic *Chaeoceros ceratosphorum.* The research was conducted using 6 dosages of *C. ceratosphorum* extract with three replications. The immune defense factors such as total haemocyte count (THC) and differential haemocyte count (DHC) of shrimp that have been injected with extract *C. ceratosphorum* was increased significantly at 4 – 6 days post injection. The survival speed (SR) of shrimp that have been injected with extract *C. ceratosphorum* was not significantly difference among the treatment after 10 days.

KEYWORDS: shrimp immune response, extract *Chaetoceros ceratosphorum*, injection, *L. vannamei*

INTRODUCTION

Pasific white shrimp (*Litopenaeus vannamei*) is one of the special commodities on fishery plantation in Indonesia even Asia and there is as the commodity which is sufficiently influenced on society economic. The main problem as the failure cause of shrimp plantation is infection attack which is caused by parasit, virus, and bacteria. Infection is boundary factor which is very influenced to the benefit and development of shrimp plantation industry that is included *L. vannamei*. One of the efforts which is carried out for infection controlling on shrimp plantation is by preventing and controlling to the factors that are very influenced to the appeared infection internally as well as externally. The internal factor which can influence the appeared infection includes shrimp body immune, but the external factor includes water environmental quality and pathogen attack. Shrimp is part of *krustasea* which is characterized by a pair of appendixes and cuticila layer or eksoskeleton which covered all of body surface [1] that is functioned as the first resistance on preventing pathogen infection [2]. Then, the ristence to pathogen infection is internally through the selular and humoral responses. Most of krustasea resistance is based on the activity of blood cell or haemocyte [3]. This cell can lose the stranger particle that enters to shrimp body through the activities of fagositosis or enkapsulasi, rapid wound covering for preventing going out of hemolim, also preventing micro-organism sticks on the wound, and clotting [3]. The increasing and spreading of hemocit quantity is assumed as the immune response form of seluler on shrimp body [4][5].

Generally, there are three kinds of different haemocyte on the shrimp blood such as cells of hialin, semigranular, and granular [3]. Hialin cell is characterized with being not had sitoplasma which is as agranular and has the smallest size among haemocyte cell [6]. This cell can lose the stranger particle on aquatic krustasea body thorugh the activity of fagositosis or enkapsulasi. Semigranular cell is characterized by a number of small granular. This cell has the ability for knowing and responsing unsure particle or stranger molecule [7] and responsing polysaccharide of bacteri cell wall or β-glucan which comes from fungus or to be known as active cell in inkapsulasi [8]. However, granular cell has a great amount of granul and it is functioned on producing, saving, and secresing antimicroba compound. The increasing of defence on shrimp body to the infection can be carried out by the giving of imunostimulan [9]. The giving of imunostimulan is intsnded to activate the immune system of non spesific cell like microfag on vertebrata and hymocit [10]. Stimulan material is as the biological compound group or syntesis that can be taken from bacteri cell wall, carbohydspeed complex compound, vacsine, nutrition, extract from anaimal, sitokin, lektin, and sctract of medicine plant [11]. One of the natural materials that can be used as imunostimulan is mikroalga diatom which has the content of polysaccharide with wide structure range and can be exported as active biological content [12]. Polysaccharide is as imunogen such as a material that has the ability to genespeed imunohospes response to the stranger thing and the moving on spesific of antigen from generation to generation [13]. Polysaccharide can be in mere condition or as polysaccharide. Polysaccharide compound or its generation can be produced from the extraction process by

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using water. Two of them are the extracts of *Gracilaria tenuistipitata* [14] and *Sargassum duplicatum* [15]. These two extracts can increase the imunity of vannamei shrimp. Galaktosa as the product of hot water extraction on *Gracilaria tenuistipitata* can fasten the response parameter recovery of vannamei shrimp shrimp to the changes of temperature and *Vibrio alginolyticus* infection [15]. However, fucoidan which is extracted from *Sargassum polycystum* can decrease WSSV infection to the windu shrimp [16]. These two extracts comes from microalga, but microalga also has the potency which is not lost by macroalga, one of them is the diatome group of *Chaetoceros* sp.

Extract of *Chaetoceros mulleri* can increase life graduating and the growth of Cod fish larva [12], *Chaetoceros calcitrans* has positive influence to the *total haemocyte count* (THC), percentage of granulosit, fagositosis speed, and ocidative activity on the shell species hemosit of *Crassotrea gigas* and *Ruditapes phillipinarum* compared with the use of *Crassotrea gigas* dan *Ruditapes phillipinarum* [17]. The giving of *whole cell Chaetoceros ceratosporum* in the weft formula indirectly can increase the response of windu shrimp immune [18] Water fraction from the extract of *Chaetoceros ceratosporum* indicates the higher total increasing of haemocyte compared with the fraction of methanol, n-hexane, and ethyl-acetat [19]. The injection of hot water extract on *Chaetoceros ceratosporum* has the possibility to be going to increase the immune response of *L. vannamei*. The increasing of shrimp immune response can be seen on the activity change of haemocyte such as the increasing of THC (total haemocyte count), DHC (differencial haemocyte count), and life sustainability.

MATERIALS AND METHODS

Preparation of Chaetoceros *ceratosporum*extract

Microalga of *Chaetoceros ceratosporum* was obtained from Gondol Research Big Department of Marine Plantation (Balai Besar Riset Budidaya Laut, BBRBL). General culture was conducted in Laboratory of Natural Weft on Plantation Department of Saltish in Situbondo and based on the method of Haryanti *et al*. [20] which is started from indoor until 2,000 ml of volume and then being continued with outdoor scale. Extraction of *Chaetoceros ceratosporum* followed the procedure of Hayashi *et al*. [21]. Powder of *Chaetoceros ceratosporum* was dissolved into deionized hot water (1:5) and to be let during 1until3 hours, being filtered with nylon mesh. Obtained filtered was centrifuged on 800 *g* during 10 minutes or was vacuum evaporate-speed or being dried. Obtained extract was known as Hot Water Extract (HWE). Obtained extract (HWE) was rehidspeed by solution of *phosphat buffer saline* (PBS), and then it was entered to bottle and saved in the temperature of 4 ºC.

Research design

White shrimp of *L. vannamei* that was used was obtained from the dyke of Saltish Water Plantation Department in Situbondo with the averaged weight of 11.9 g. Shrimps were breeder in 18 plastic places with the capacity of 60 l included 40 l sea water of $34^{o}/_{oo}$, each place consisted of 18 shrimps. Before treatment, shrimps were aclimatated one week in laboratory and they were given commercial weft four times a day of 5% biomass weight.

After acclimatization, each shrimp was injected with extract of *Chaetoceros ceratosporum* on second segment of abdomen ventral with the doses of 10, 50, 100, 500, and 1000 $\mu g.g^{-1}$ of body weight and as the control, it was used phosphate buffer saline (PBS). Therefore, there were 6 treatments for each of them and each was returned for three times.

Observation was carried out on the day of 0, 1, 2, 4, and 6 pascha injection [15] to the response parameter of shrimp immune with indicator of total haemocyte count (THC), differential haemocyte count (DHC), and shrimp sintasan parameter were suitable with life graduated condition (SR).

Measuring of immune response parameter

THC was analyzed by following the method of Van de Braak [22] with taking the hemolim of 50 µL and spet of (1ml #26) from shrimp ventral on the second abdomen and then it was mixed with antikoagulan (10% sodium citspeed, pH 7,2) with the same volume and it was given Trypan blue solution of 100 µl. The quantity of haemocyte was calculated by using haemositometer with the helping of shine microscope.

DHC was analyzed with the method of Grinwald-Giemsa by using the enlarger shine microscope of 1000× [22]. Observation and differentiating inter haemocyte cell based on the existance and inexistance of granular in cell and it was calculated in percentage (%).

Indicator of shrimp graduated life was analyzed in the end of observation by calculating the amount of shrimp which still lived in every experiment place compared with the amount of shrimp in the beginning of research and it was expressed in percentage (%).

RESULTS AND DISCUSSION

Observation result to the shrimp immune response parameter of *L. vannamei* which was injected with the extract of *Chaetoceros ceratosphorum* indicated that there was the increasing of immune response such as

significant increasing was occurred on the quantity of haemocyte (THC) and haemocyte differential (DHC), and it was not influenced the life graduation of shrimp.

Haemocyte total (THC) of *L. vannamei* shrimp after being injected with the extract of *Chaetoceros ceratosphorum* indicated the significant increasing started from the first until sixth day. Injection doses was 10 $\mu g.g^{-1}$ of shrimp body weight significantly produced THC higher than by doses of 50, 100, 500, and 1000 as well as the control treatment of PBS starting from the first until sixth day after injection. Haemocyte total (THC) of *L. vannamei* shrimp with injection doses of 10 $\mu g.g^{-1}$ has increased from the first until fourth day after injection, and starting to decrease on the sixth day and it was higher than the other treatment. It could be said that the highest increasing of THC was reached on the fourth days after injection. However, the giving of extract with the doses of 50 $\mu g.g^{-1}$ still indicated the increasing of THC until the sixth day. The giving of extract with the doses of 100, 500, and 1000 $\mu g.g^{-1}$ indicated that THC was higher but it was not significantly different being compared with control treatment of PBS on the second and fourth day after it was injected by the extract of *Chaetoceros ceratosphorum* (Figure 1).

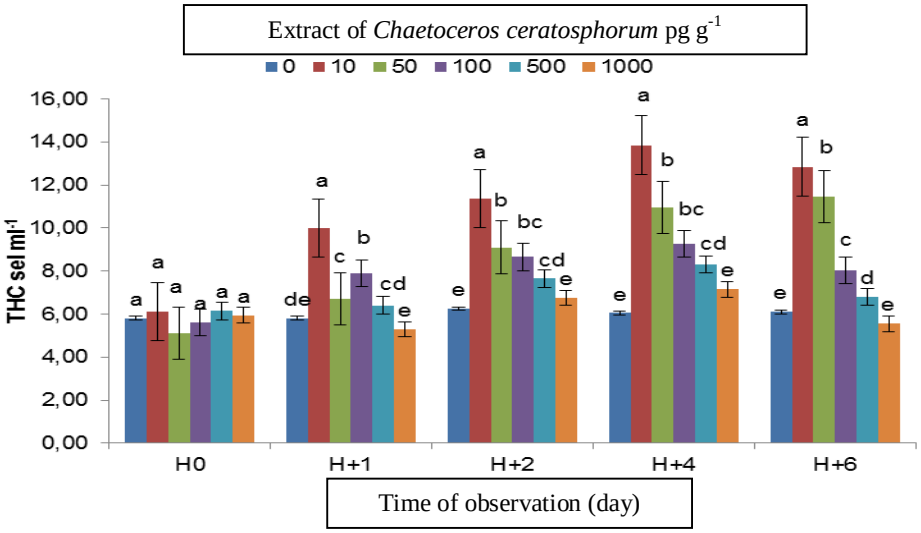

Figure 1 Haemocyte total average (THC) of *L. Vannamei* shrimp before injection and on the day of 1, 2, 4, and 6 after injection with the extract of *Chaetoceros ceratosphorum* on the doses of 0, 10, 50, 100, 500, and 1000 µg.g-1 of body weight

Hyaline cell percentage of *L. Vannamei* shrimp on the day of 1, 2, 4, and 6 after being injected with the extract of *Chaetoceros ceratosphorum* with the dosis of 10 $\mu g.g^{-1}$ was significantly higher than with the doses of 50, 100, 500, and 1000 $\mu g.g^{-1}$ as well as the control treatment of PBS. Semi granular cell of *L. vannamei* setelah shrimp was injected with 10 $\mu g.g^{-1}$ of *Chaetoceros ceratosphorum* extract, significantly indicated that the increasing was higher than with the doses of 50, 100, 500, and 1000 $\mu g.g^{-1}$ as well as the control treatment of PBS. Granular cell of *L. vannamei* shrimp on the day of 2, 4, and 6 after being injected with the doses of 10, 50, 100, 500, and 1000 $\mu g.g^{-1}$ with the extract of *Chaetoceros ceratosphorum* significantly indicated the decreasing being compared with control treatment of PBS. The Granular cell increasing of *L. vannamei* shrimp was occurred on the first day after injection with the doses of 500 and 1000 $\mu g.g^{-1}$ by the extract of *Chaetoceros ceratosphorum* (Figure 2.)

During the research, life continuity of *L. Vannamei* shrimp did not significantly change. Life continuity of *L. Vannamei* shrimp in the end of research was in the average of 89%.

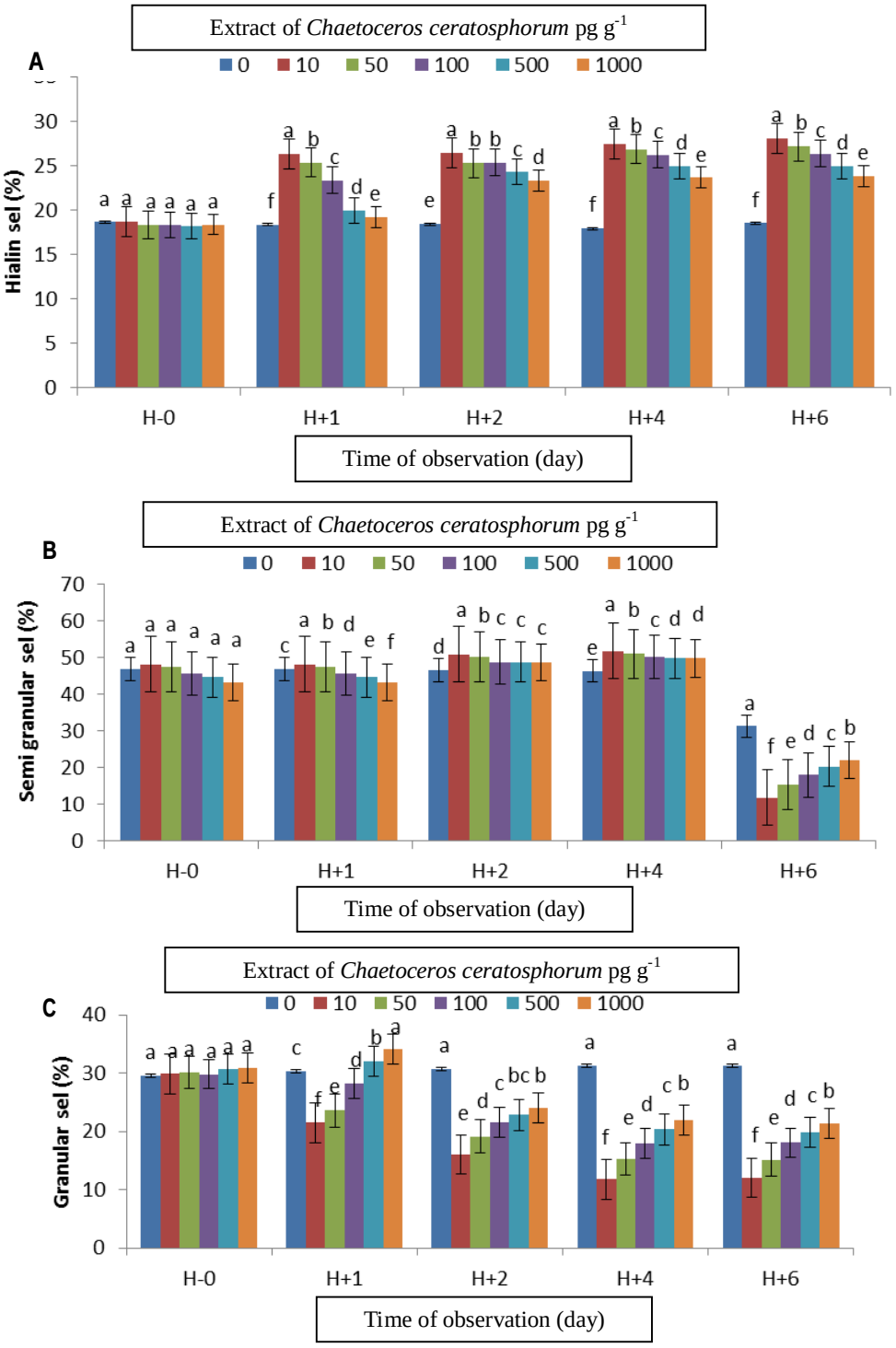

Figure 2 hialin cell (A), semigranular cell (B), and granular cell (C) of *L. vannamei* shrimp before injection and on the day of 1, 2, 4, 6 after injection with the extract of *Chaetoceros ceratosphorum* with the doses of 0, 10, 50, 100, 500, and 1000 µg.g^{-1} of body weight

Haemocyte is a cell that is on the hemolim. This cell has an important function on shrimp immune response to infection [5]. Haemocyte is as the main component of defence system on invertebrata that is circulated in hemolim and has a function as security which is ready to response the pathogen attack or wound network [23]. Total of haemocyte can influence foster-mother for reacting against stranger material and any responses to infection [8].

Result of the research indicated that injection by the extract of *Chaetoceros ceratosphorum* can increase the immune response of *L. vannamei* shrimp. Extract of hot water from some alga species has been reported to be able to increase immune of fish and shrimp to pathogen infection. The giving of hot water extract on some species of red or brown alga was reported to be able to increase resistance of *Cyprinus carpio* and *Seriola quinqueradiata* to *Edwardsiella tarda* and *Streptococcus* sp [24].; *L. vannamei* which was soaked or injected with the extract of hot water. *G. tenuistipitata* [15] as well as *S. duplicatum* [15] could increase the immune and resistance to infection. *Vibrio alginolyticus. L. vannamei* which was soaked in sea water that was content of hot water extract. *G. tenuistipitata* can accelerate the immune recovery of *L. vannamei* shrimp after being injected by *Vibrio alginolyticus* [25]..

Hot water extract of *G. tenuistipitata* was content 30% of sugar with the main component was gelatos [26] that were as the differentiation of polysaccharide. Compound of polysaccharide or the differentiation can be produced of extraction process by using water [15][14], H Cl [26] etc. Storreth *et.al*.[28][29] have proved the available structure of β-D-(1,3)-glycan on *Chaetoceros mulleri*, while in 2006, Storseth *et al*. [28][29] obtained the structure of β-D-(1,3; 1,6)-glycan on *Chaetoceros debilis.*

However as being known, some researchers as above has found that polysaccharide compound as the extraction result with water and other dissolver significantly can increase the shrimp immune response and resistance to pathogen. The giving of sodium alginate from brown alga extract of *Macrocystis pyrifera* and *Lessonia nigrescens* can increase the resistance of *L. vannamei* to infection of *Vibrio alginolyticus* [31][32]. Extract of Chaetoceros *mulleri* can increase the life graduation and growth of Cod fish larva [12]. *Chaetoceros calcitrans* was more of fat acid of 20:5(n-3) and 20:4(n-6) and had positive influence to total of haemocyte count (THC), percentage of granulocyte, phagocytosis speed, and oxidative activity on shell haemocyte species of *Crassotrea gigas* and *Ruditapes phillipinarum* compared to the use of *Isochrysis* sp. and *Tetraselmis suecica* [17].

In this research, *L. vannamei* that was injected by the extract of *Chaetoceros ceratosphorum* with the 10 $\mu g.g^{-1}$ doses of shrimp body indicated the highest increasing of immune response than the doses of 50, 100, 500, and 1000 $\mu g.g^{-1}$ as well as the control of PBS on the first until sixth day after being injected (Figure 1 and 2). The giving of *G. tunuistipitata* extract [14] or *Gelidium amansii* [32] on the shrimp of *L. vannamei* gave the increasing of THC on the first day. The increasing of THC was caused by lectine molecule that was as part of shrimp humoral resistance which was functioned for carrying out the introduction to stranger thing (non self recognition) which was entered to shrimp body [33].

The increasing of THC was beginning from the first day after being injected, but after the fourth day there was occurred the decreasing of THC, except for the doses of 50 $\mu g.g^{-1}$, which still indicated the increasing of THC on the sixth day. The same case was occurred on the research of Hou *et.al* [14] such as THC, activity of phenol oxidase, and respiratory burst of shrimp which was given the extract of *G. tenuistipitata* with the dosis of 4 and 6 $\mu g.g^{-1}$ has increased on the first day and has higher resistance after sixth day of treatment. Hemocyanine would increase on the pascha larva of *L. vannamei* which was given weft that was content of β-glycan [34]. The injection of stranger particle like alginate, *karaginan* or hot water extract of sea grass polysaccharide significantly increased THC, activity of phenol oxidase, and respiratory burst of *L. vannamei* white shrimp [32].

The increasing of THC on this research was suitable with the increasing of hyaline and semi granular cell. Haemocyte was playing an important function on the shrimp immune response included introduction, phagocytosis, melanises, cytotoxicity, and communication inter cell [8]. THC was increasing and it would increase the ability for fasgosite because there were produced many haemocyte cells for carrying out this function such as hyaline and semi granular cell. The highest increasing of hyaline and semi granular cell was obtained on the 10 $\mu g.g^{-1}$ doses of body weight. The increasing of hyaline cell was as the response of peroxinectin appearance which was produced from the degranulation process of semi granular and granular cells[35]. The increasing of hyaline cell gave big contribution to the increasing of THC. The increasing of hyaline cell generally was related to the increasing of resistance to pathogen [36].

Semi granular cell was able to response polysaccaharide from bacteria wall or β-glycan. This cell can carry out the process of encapsulasion and had a little function in phagocytosis process [8]. On the first until sixth day after being injected by the extract of *Chaetoceros ceratosphorum* with 10 $\mu g.g^{-1}$ doses of body weight, semi granular cell significantly increase than the treatment of 50, 100, 500, and 1000 $\mu g.g^{-1}$ as well as the control.. If the extract of *Chaetoceros ceratosphorum* could increase the response of shrimp immune, it meant that the extract of *Chaetoceros ceratosphorum* could increase the synthesis of hemocyanine, whereas β-glycan and the other polysaccharide. The increasing of synthesis to hemocyanine directly would increase haemocyte included hyaline cell and semi granular. The quantity of haemocyte larva which was given the weft of β-glucan would increase until the maximal level every 2 to 3 days [37]. In this research, the giving extract of *Chaetoceros ceratosphorum* with the doses of 10 $\mu g.g^{-1}$ indicated the increasing of shrimp haemocyte until fourth day, it was higher than the dosis of 50, 100, 500, and 1000 $\mu g.g^{-1}$.

The increasing of haemocyte on the shrimp of *L. vannamei* by giving the extract of *Chaetoceros ceratosphorum* was not influence the life graduation. It indicated that the extract of *Chaetoceros ceratosphorum*

with high dosis could still be tolerated by shrimp body and did not influence its life. Therefore, extract of *Chaetoceros ceratosphorum* could be said that there did not have toksik characteristic for shrimp.

CONCLUSION

This research indicated that *L. vannamei* shrimp which has been injected by the extract of *Chaetoceros ceratosphorum* with 10 $\mu g.g^{-1}$ doses of body weight indicated the increasing of immune response through the increasing of haemocyte quantity (THC), differential haemocyte (DHC), and it was not dangerous for life.

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