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The Effect of *Ulva lactuta* Polysaccharide Extract on Total Haemocyte Count and Phagocytic Activity of L. vannamei.

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KEYWORDS
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Abstract This research aimed to evaluate the effect of polysaccharide extract of *Ulva lactuta* on the total haemocytes count (THC) and the phagocytic activities (PA) on *Litopenaeus vannamei*. The concentration of the extract used in this research was 5 ppm and it was applied through the immersion. The haemolymph was collected after the administration in the different periods at hour of 4 and 24. The results showed that the administration of polysaccharide extracts increased THC and PA in *L. vannamei* significantly ($p<0.05$). THC and PA of white-leg shrimp with polysaccharide extract was higher than that without the administration of the extract. The enhancement of THC was found after the administration at hour 4 from 5.00 cells. ml^{-1} to 5.26 x 10^4 cells. ml^{-1}. However, the THC level decreased after 24 hours at the value of 5.19 x 10^4 cells. The increasing pattern was also true for PA of L. vannamei, from 40.44% to 53.67% (hour 4). At the hour of 24, the PA was found decreased to the level and then decreased 45.67 ±1.53. On the contrary, there were no enhancement of THC and PA in the treatment without polysaccharide extract of *Ulva lactuta*.

Introduction

The whiteleg shrimp (*litopenaeus vannamei*) is the biggest farmed species as it has some benefits such as they grow fast, can be farmed in the high density, and have the high market values. However, disease outbreak becomes a primary obstacle in the production the white-leg shrimp (Putri *et al*., 2013). Many efforts were conducted to control the disease of the shrimp by enhancement of the shrimp's immune defence system using the immune stimulant, vitamin and hormone (Johnny *et al*., 2017). Shrimp has the natural body defence system which is non-specific to the pathogen organism. Those were in the form of the physical defence (mechanic), chemistry, cellular, and *humoral*. These natural self-defences were influenced by the genetic factor and environment. Therefore, there will be different levels of natural self-defences depend on the strain, maintenance environment, species, or family (Bellanti, 1989).

Immunostimulants is a chemical compound, medicine or another material which is able to enhance the specific response mechanism and non-specific response mechanism of fish (Anderson, 1992). The administration of immunostimulant is broadly conducted to activate the cell's specific non-immune system such as macrophage in vertebrata and haemocyte in invertebrate (Dugger and Jorry, 1999).

Seaweed is a multicellular alga which immunologically consists of the active substances. The utilization of seaweed is still only limited in the carrageenan product and jelly. The potency of seaweed in the field of disease control is still not well explored and exploited. Some researches showed that seaweeds have potential prospects to be developed of the disease control (Castro *et al*., 2004). Green seaweed is a three major division

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classification of marine macroalgae, which synthesize sulphated polysaccharides. *Ulva lactuta* is a green seaweeds which has sulphated polysaccharide (Tabarsa *et al*., 2012)

The *Ulva* sp has natural bioactive compounds which contain the sulphate polysaccharides (Abd El-Baky et al., 2008). Selvin et al., (2004) found that the active compound in the *Ulva fasciata* could enhance the immunostimulant activity of shrimp. This was indicated by the enhancement of the Total Haemocyte Count (THC) when the *Ulva fasciata* extract was administrated. In addition, Selvin *et al*. (2011) also reported that the extract of the *Ulva fasciata* could increase the survive rate of tiger prawn against the vibrio infection and the THC. Some studies were also revealed that the composition of polysaccharide from the *Ulva's* extract could increase the macrophage cell, phagocytic cell, and *respiratory burst* (RB) of shrimp through the immersion (Castro *et al*., 2006; Tabarsa *et al*., 2012). However, potency of Ulva lactuta polysaccharide have not evaluated yet. Therefore, this study aimed to evaluate the influence of the polysaccharide extract of *Ulva lactuta* on THC and PA of white-leg shrimp (*Litopenaeus vannamei*).

Material and methods

The polysaccharide extract used in this research was the *Ulva lactuta* extract obtained from the coast of Serangan Beach, Bali. *Ulva lactuta* were dried for 5 days, then were grinded and strained with 30 mesh size. The polysaccharide fractionation was performed based on Tabarsa et al. (2018). This research was carried out at the Fish Health and Disease Division, Aquaculture Laboratory (Faculty of Fisheries and Marine Science, University of Brawijaya). The shrimp (average weight of 20 ±0.2 gram) were originated from Karanganyar, Tanjung Pecinan Village, District of Mangaran, Situbondo Regency. White-leg shrimp (10#) were acclimated for two days in the 50 x 30 x 30 cm aquarium.

The method used in this research was an experimental method with the Complete Randomized Design with two treatments and 3 replications. The treatments used were treatment A (no polysaccharide extract/control) and treatment B (with 5 ppm polysaccharide extract). Haemolymph was extracted at 0 hour, 4 hours, and 24 hours. Method of THC and PA was performed based on Peraza-Gómez et al. (2014). During this study, the shrimp were commercially fed twice a day at the satiation (Chen and Hou, 2005). The shrimps' haemolymph was conducted at the pleopod at the abdominal segment near the genital hole by using syringe 1 mL (Xian *et al*., 2009). Before haemolymph collection, the syringe was loaded with 0.1 mL of Na-citrate 10% used as an anticoagulant (Vargas-Albores *et al*., 1993). The calculation of the total quantity of haemocyte (THC) was conducted by using a haemocytometer (Abdollahi-Arpanahi *et al*., 2018).

$$\text{Total Haemocyte Count} = \frac{\text{the quantity of calculated cells}}{\text{calculated volume}} \text{ x liquefaction x } 10^4$$

Phagocytic activity was calculated according to Ridlo and Pramesti (2012) by using microscope with 1000 times of magnification.

$$\text{Phagocytic Activity (\%)} = \frac{\text{The quantity of Phagocytic cell}}{\text{The quantity of observed Phagocytic cell}} \text{ x 100\%}$$

The THC and PA data were analysed using the ANOVA test (p=0.05) and were continued by the Duncan test to examine the difference of the treatments.

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Results and Discussion

Total Haemocyte Count (THC)

Table 1. The *Total Haemocyte Count* (THC) of the white-leg shrimps (*L. vannamei*) during study.

Treatment	Log THC (x10^4 cell/ml)		
	hour 0	hour 4	hour 24
A	4.98±0.01[a]	4.99±0.02[a]	5.01±0.01[a]
B	5.00±0.02[a]	5.26±0.01[b]	5.19±0.01[b]

Description: The value with the same *superscript* which is identical in the column showing that there was no significant difference ($p>0.05$).

The non-specific body defence of the white-leg shrimp with administration of immunostimulant of Ulva lactuta was showed by haemolymph profile. The haemolymph profile was including of THC and PA. Haemocyte is a cellular body defence system in shrimp. Haemocyte can extinguish the infection-caused-pathogen through the synthesis and exocytosis of microbicidal protein bioactive molecule (Smith *et al*., 2003; Söderhäll and Cerenius, 1992). In addition, the immune-reactive factors such as *peroxinextin*, peptide anti-bacteria, and *clotting components* are kept in the haemocyte. Therefore, the increase of THC is the measure to depict the ability of some substances in stimulating the shrimps' body defence system (Aguirre-Guzmán et al., 2009).

The THC of whiteleg shrimp's h (*Litopenaeus vannamei*) at hour 4 and 24 after polysaccharide extract of *Ulva lactuta* administration can be seen in Table 1. It can be seen from the Table 1 that there was no significant difference of THC in the treatment with no polysaccharide (A) for every period of haemolymph collection ($p>0.05$). On the other hand, administration of *Ulva lactuta* polysaccharide significantly increased the THC of *L. vannamei* at the hour of 4. However, THC was recorded decrease after 24 hours. Based on the research by Johansson *et al*. (2000), the shrimp's haemocyte held an essential role in the immune response such as *recognition, Phagocytic, melanisation, cytotoxicity* and the communication of the cells. Furthermore, that polysaccharide extract indicated could stimulated the forming of haemocyte cells and released into the shrimp's haemolymph. The result of this research was in line with the research conducted by Selvin *et al*. (2004). They stated that the extract of *Ulva sp* could enhance the total quantity of the shrimps' haemocytes.

A factor that affect the ability to enhance the shrimp's body defence system is an active compound in the material. Later, a material with no active compound could not stimulate the body defence system. The active compound can be act as immunostimulant if they are absorbed by the blood from the digestive system. Furthermore, they are transferred to the place where they can influence the body immune system. If the amount of active substance are lower or higher than required number, they will act as an inhibitor instead of as immunostimulant. Sakai (1999) stated that the immunostimulant ability to increase the immune response and develop the protection to the pathogen infection was influenced by the application dosage. The giving of immune stimulants at the concentration under the minimum value for the immune response would not give an effect to the enhancement of haemocyte's quantity.

Phagocycic Activity

The shrimps' body defence system also can be known from the phagocytic activity (PA) (Söderhäll and Cerenius, 1992; Johansson *et al*.,

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2000). PA is the non-specific response immune cell's ability in phagocytising the disease agents which entered the shrimps' body (Ridlo and Pramesti, 2012). The function of haemocyte as a cellular self-body defence can be seen from its ability to phagocyte the microbe or foreign materials. By increasing the phagocyte activity, the cellular non-specific self defence system can prevent the disease outbreak (Putri *et al*., 2013). The Phagocytic activities on the white-leg shrimps are presented in the Figure 1.

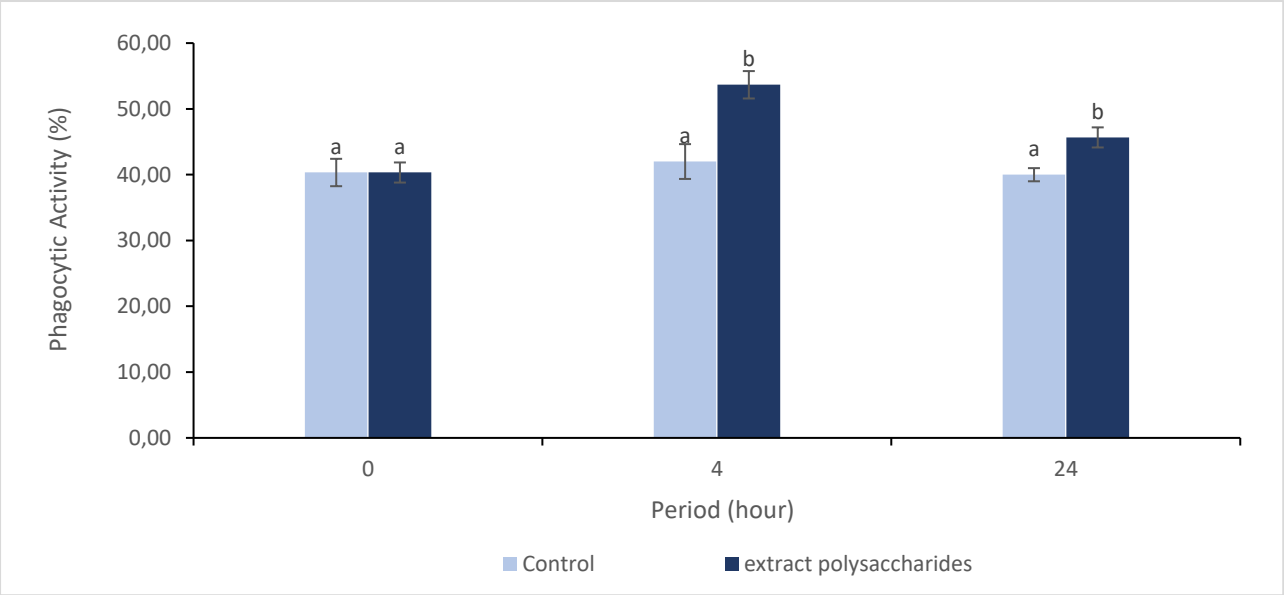

Figure 1. Phagocytic Activities of L. vannamei after Ulva lactuta polysaccride administration.

The phagocytic activity showed that the treatment without polysaccharide did not affect in all periods of time ($p>0.05$). After 4 hours of ulva lactuta polysaccride administration, the PA significantly increased (53.67±2.08%) ($p<0.05$). Yet, it decreased again after 24 hours of administration (45.67±1.53%).

Based on the research results, the addition of polysaccharide extract of *Ulva lactuta* could enhance the phagocytic activity of the whiteleg shrimp (*L.vannamei*). It due to the content of polysaccharide compounds. Chojnacka *et al*. (2012) reported total the amount of polysaccharide from seaweed was 76% (dry basis). In addition, they noted that the polysaccharide from algae were galactan, fucoidan, laminarin and alginate (include sodium alginate). Sodium alginate could increase the phagocytic activity of L. vannamei (Cheng *et al*. (2005). Gannam and Schrock (1999) reported that immunostimulant is a substance which stimulates or enhances the immune system by interacting directly with the cells which activates the immune system. In addition, the mechanism of immunostimulant in stimulating the body immune system was by increasing the activities of Phagocytic cells (Yin *et al*., 2006). The phagocytic activity is an essential way in controlling and devastating the foreign material. The defence process through the phagocytic was divided into some processes, i.e. chemotactic, recognition, and internalization (Bachère *et al*., 1995).

Conclusion

Based on the result in this study, it can be concluded that the addition of polysaccharide extract with the dosage 5 ppm gave significant effect ($p<0.05$) on THC (5.26±0.01 X10^4 cell/ml) and phagocytic activity (53.67±2.08 %) of L. vannamei.

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