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SUPPLEMENTATION OF *GRACILARIA*. SP. AND *SARGASSUM*, SP. INTO THE FEED AS AN IMMUNO-STIMULANT AGENT FOR NON-SPESIFIC DEFENCE OF WHITE SHRIMP (*L. VANNAMEI*)

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ABSTRACT

This study aims to clarify the effect of seaweed (*Gracilaria* sp. and *Sargassum* sp.) extract as immune-stimulant agent for non-specific defense system of white shrimp (*L. vannamei*). The study conducted with the initial body weight of white shrimp ±15 g. The dried seaweed was extracted by boiling in water for 2 hours then supplemented into the shrimp feed pellet with 10g/kg pellet. Hematological parameters of Total Haemocyte Count (THC) and Phagocytosist Activity (PA) was then tested at 5, 10, and 15 days period after initial treatment. The result showed that supplementation of seaweed extract was increased the number of haematocyte. It was recorded that the highest of haematocyte in day 10 from White shrimp fed by supplemented Gracilaria, Sp. with the number of haematocyte 0.879 ± 0.382 x 107 cell/L and in day 15 fed by pellet contained *Sargassum* sp. with the number of haematocyte 1.248 ± 0.192x107 cell/L. The Extract of *Sargassum* sp. gave the most significant effect with total phagocytosist activity of 97.305 ± 0.554 at day 10 without any significant different with *Gracilaria*, sp.

INTRODUCTION

Disease is one of the limiting factors in the cultivation of vannamei shrimp (*Litopennaeus vannamei*). The high mortality rate of cultured shrimp is thought to be caused by viral or bacterial pathogenic infections. Stated that the common pathogenic bacteria that attack in aquaculture are Vibrio alginolyticus, V. flufialis, V. vulfinicus, and V. ordalii[14]. Epidemics that mostly attack shrimp culture are White Spot Syndrome Virus (WSSV), Taura Syndrome Virus (TSV) and Yellow Head Virus (YHV)[19]. shrimp body defense, namely by using immunostimulants, vitamins and hormones[10]. Shrimp has a natural resistance that is non-specific against pathogenic organisms in the form of physical (mechanical), chemical, cellular and humoral defenses. This natural resistance is influenced by genetic and environmental factors, so there are different levels depending on the strain, rearing environment, species and family[2].

The shrimp immune system depends on non-specific defense processes as a defense against infection[13]. The first defense against disease in shrimp is carried out by haemocytes through phagocytosis, encapsulation and nodule formation. Phagocytic activity can be increased by activating the prophenol oxidase (Pro-PO) system located in semigranular and granular haemocytes[18]. Immunostimulation is usually done by giving microbial components such as -glucan and lipopolysaccharide (LPS) or bacterial cells that have been killed[18]. The weakness of this immunostimulant is that the price is relatively expensive, so it is necessary to find alternative sources of immunostimulants that are cheap and easy to handle, one of which is from seaweed. Seaweed is a multicellular algae that contains immunologically active substances. The use of seaweed so far is still limited to carrageenan and agar products. The potential of seaweed in the field of disease control is still not much explored and exploited. Several studies have shown that seaweed has prospects that are still open for development in the field of disease control. Seaweed extract has been known to have antitumor activity, increase macrophage chemotaxis activity, stimulate oxygen radical secretion activity and phagocytosis in peritoneal and splenic murine macrophages[4]. Secondary metabolites of Halimeda macroloba have antifungal bioactive compounds[22]. Seaweed *Ulva* sp., *Dendrilla* sp., *Spirulina* sp., *Enteromorpha* sp., *Dictyota* sp., and *Porphira* sp. has been shown to increase the immunostimulant activity of shrimp[4,18]. Polysaccharides from red algae (carrageenan) can increase macrophage phagocytic activity and are able to fight bacterial infections after intraperitoneal injection in Cyprinus carpio fish[4]. Polysaccharides are known to be essential components for all organisms and have various vital biological functions including antitumor, anti-inflammatory, anticoagulant, anticomplementary, immunological and antiviral properties. Test the effectiveness of seaweed application as an immunostimulant for the non-specific defense system of shrimp L. vannamei can be done by observing the non-specific immune system based on its hematological picture, namely by counting the number of haemocytes and phagocytic activity. This study aimed to analyze the ability of seaweed extract as an immunostimulant agent for non-specific defense systems in commercial vannamei shrimp (*Litopennaeus vannamei*).

MATERIAL AND METHODS

Gracilaria sp. seaweed samples. and Sargassum sp. taken from the waters of the Thousand Islands. The seaweed samples were cleaned and then dried by airing and not exposed to direct sunlight. As the test animal was L. vannamei shrimp with a weight of ± 15 grams. Seaweed that has been air-dried, cut into small pieces (± 0.5 cm). A total of 500 grams of seaweed is boiled until boiling with 2 liters of distilled water for 2 hours, then filtered. The dregs are boiled again, then filtered and the filtrate is combined with the results of the first extraction. The extracted solution is evaporated using a rotary evaporator until dry. The seaweed extract obtained was added to commercial shrimp feed (composition: 35% crude protein, 11% water and 3%). At first the pellet was crushed by grinding, then 10 g/kg of seaweed extract was added, mixed until homogeneous and made pellets used for testing. L. vannamei shrimp were acclimatized in a tank equipped with an aeration system and water circulation for 15 days and ad libitum feeding of commercial pellets (until they were full), each aquarium contained 15 shrimp[16]. Feeding during the treatment was carried out 4 times a day, namely morning (05.30), afternoon (11.30), afternoon (17.30), and evening (23.00) as much as 5% of the shrimp body weight[7,16,8].

Hemolymph was taken at intervals of 5 days for 15 days, then placed in a sterile microtube and stored in a cool box. The total number of haemocytes (THC) was calculated using a haemocytometer using[3].

$$\text{Haemosite count} = \frac{Cell\ count}{Volume} Dilution\ x\ 10^6$$

Phagocytic activity was determined according to the procedure of[9]. Bacterial cells of V. alginoliticus were mixed with formalin solution until all the suspension precipitate was submerged for 24 hours, then centrifuged at 1000 rpm for 3 minutes and the filtrate was separated. A total of 250 L of haemocytes was mixed with 500 L of bacterial suspension that had been killed, then incubated at room temperature for 60 minutes. Phagocytosis activity was observed using a microscope with 1000 times magnification.

$$\text{Phagocytic Activity (100\%)} = \frac{Number\ \ of\ Active\ phagocytes}{Numbe\ r\ of\ cells\ observed}\ x\ 100\%$$

Results and Discussion

The non-specific defense system of L. vannamei shrimp against the application of immunostimulants from seaweed is shown by the appearance of the hematocytes, namely the total number of haemocytes and phagocytic activity. Hemocytes are a form of cellular defense of the body. Hemocytes are able to kill infectious agents through the synthesis and exocytosis of microbicidal protein bioactive molecules[19]. Immunoreactive factors such as peroxinextin, antibacterial peptides and clotting components are stored in haemocytes, so an increase in the number of haemocytes is a measure of the ability of a substance to stimulate the shrimp's immune system.

The results showed that the number of haemocytes and phagocytic activity varied, depending on the type of seaweed used. Giving seaweed extract tends to increase the total number of haemocytes, especially on the 10th and 15th days. The highest total number of haemocytes was achieved by Sargassum sp. on day 15 (1,248 x 107 cells/L ± 0.192) (Table 1). On the 10th day, Gracilaria sp. showed the highest value compared to other types of seaweed. This indicated that the administration of seaweed extract was able to stimulate an increase in the total number of L. vannamei shrimp haemocytes. According to, shrimp hematocytes play an important role in the immune response including through recognition, phagocytosis, melanization, cytotoxicity and communication between cells[11].

The results of the study above are in accordance with the research of which states that the extract of Ulva sp. and Dendrilla sp. can increase the total number of shrimp haemocytes up to 0.672 x 107 cells/L and 0.620 x 107 cells/L, respectively[18]. Similar studies have shown that the immune response of shrimp can be enhanced by the application of glucans[5] and peptidoglycans[21]. The results of this study are in accordance with[20], which states that Halimeda sp. able to increase the THC value by 96.242% on day -12.

Table 1. The Effect of Giving Seaweed Extract on the Total Hemocyte Count (THC) of Shrimp L. Vannamei

Seaweed	Total Hemocyte Count (x 10^7 cells/L)		
	5th day	10th day	15th day
Control	0.583 ± 0.122a	0.602 ± 0.149a	0.673 ± 0.299a
Gracilaria sp.	0.632 ± 0.033a	0.879 ± 0.382b	0.701 ± 0.282a
Sargassum sp.	0.611± 0.241a	0.698 ± 0.292a	1.248 ± 0.192b

Note: Values followed by the same letter are not significantly different at the 95% confidence level (p> 5%)

Table 2. Effect of Seaweed Extract on Phagocytic Activity of Shrimp L. vannamei

Seaweed	Phagocytic Activity (%)		
	5th day	10th day	15th day
Control	73.601 ± 0.123a	70.342 ± 1.239a	72.334 ± 0.443a
Gracilaria sp.	80.571 ± 0.342b	92.123± 0.586b	88.342 ± 1.332b
Sargassum sp.	82.134 ± 0.321b	97.305 ± 0.554b	91.332 ± 0.965b

Note: Values followed by the same letter are not significantly different at the 95% confidence level (p > 5%)

Several factors that affect the ability of an ingredient to increase the shrimp's defense system include the active compounds contained in the extract that do not have activity to stimulate the body's defense system, the active compounds are in a form that cannot be absorbed from the digestive system. The active compound will show its activity if it can reach its target location, which means it must be absorbed by the blood from the digestive tract to be subsequently carried/transferred to a place where the substance will give its activity effect or the amount of the active compound is smaller than the minimum amount required to elicit an immunostimulant effect. or even vice versa the dose is too high so that it does not give effect or behave as an inhibitor. Stated that the ability of immunostimulants to enhance the immune response and develop protection against pathogenic infections is influenced by the dose of application[17]. Administration of immunostimulants at concentrations below the minimum value for the occurrence of an immune response will not have an effect on increasing the number of haemocytes.

Parameters of the shrimp defense system can also be known from phagocytic activity, namely the ability of non-specific immune response cells to phagocytose disease agents that enter the body. The results showed that the administration of seaweed extract was able to increase the phagocytic activity of L. vannamei shrimp, especially on the 10th day (See Table 2). This is because both types of seaweed contain alginate polysaccharide compounds. According to, sodium alginate can increase the phagocytic activity of shrimp[6]. In addition, stated that the seaweed extract of Halimeda sp. can increase phagocytic activity on day 12 by 76.78%[20].

Phagocytic activity is one of the most important ways to control and destroy foreign particles. The defense process through phagocytosis is divided into several processes, namely: chemotaxis, recognition, and internalization [1]. According to, haemocytes perform inflammatory-type reactions such as phagocytosis,

haemocyte clumping, production of oxygen-reactive metabolites and release of microbicidal proteins[19].
Based on the results of this study, feeding with seaweed extract supplementation to increase the number of haemocytes of L. vannamei shrimp can be given up to the 15th day, while to increase the phagocytic activity it is given up to the 10th day. The best results of hemocyte count and phagocytic activity were achieved by Sargassum sp. on day 15 and on day 10, respectively.

Conclusion

Gracilaria sp., and Sargassum sp. seaweed extract supplementation. at a dose of 10 g/kg feed was able to increase the total number of haemocytes and phagocytic activity of L. vannamei shrimp for at least 10 to 15 days. Of these two types of seaweed, Sargassum, sp. provide the best benefits as an immunostimulant in vannamei shrimp.

References

[1] Bachere, E., E. Mialhe, D. Noel, V. Boulo, A. Morvan, & J. Rodriquez, 1995, Knowledge and Research in Marine Mollusk and Crustacean Immunology. *Aquaculture* 132: 17-32.

[2] Bellanti, J. A. 1989. Immunology III, Gadjah Mada University Press. Yogyakarta

[3] Campa-Courdova, A.I., N.Y. Hernaundez-Saavedra, R. De Phillipis, & F. Ascentio, 2002, Generation of Superoxide Anion and SOD Activity in Haemocytes and Muscle of American White Shrimp (*Litopenaeus vannamei*) as a Response to *beta* -glucan and Respiratory Burst Activity of Turbot Phagocytes, *Aquaculture* 229: 67–78

[4] Castro, R. I. Zarrab, & J. Lamas. 2004, Water-soluble Seaweed Extracts Modulate the *Pantoea agglomerans* lipopolysaccharide (LPS). *Fish Shellfish Immunol*, 10:555–558.

[5] Chang C-F, H-Y Chen, M-S Su, & I-C Liao. 2000, Immunomodulation by Dietary *beta* 1,3 glucan in the Brooders of the Black Tiger Shrimp, *Penaeus monodon*. *Fish Shellfish Immunol,* 10:505–14.

[6] Cheng W., CH Liu, CH Tsai, & JC Chen, 2005, Molecular Cloning and Characterisation of a Pattern Recognition Molecule, lipopolysaccharide- and b-1,3-glucan binding protein (LGBP) from the White Shrimp *Litopenaeus vannamei*, *Fish & Shellfish Immunology,* 18 :297- 310

[7] Esteban MA, Cuesta A, Ortuno J, & Meseguer J. 2001. Immunomodulatory Effects of Dietary Intake of Chitin in Gilthead Seabream (*Sparus aurata* L.) Innate Immune Response. *Fish & Shellfish Immunology,* 11:303–15.

[8] Haliman, R. W. 2005. Udang Vannamei, Penebar Swadaya. Jakarta

[9] Isnansetyo, A, 2007, Petunjuk Praktikum Hematologi Ikan (Evaluasi Pertahanan Non Spesifik Ikan), Pelatihan Hematologi Ikan, Laboratorium Hama dan Penyakit Ikan, Universitas Gadjah Mada, Yogyakarta.

[10] Johny, F. Roza, D. K. Mahardika. Zafran & A. Prijono. 2005. Penggunaan Immunostimulan Untuk Meningkatkan Kekebalan Nonspesifik Benih Ikan kerapu Lumpur, *Epinephelus coiodes* Terhadap infeksi Virus irido. *Jurnal Penelitian Perikanan Indonesia,* XI(5):75-83

[11] Johansson M W, P. Keyser, K. Sritunyalucksana, & K Soderhall, 2000. Crustacean Haemocytes and Haematopoisis, *Aquaculture* 191 : 45-52

[12] Lee, M. H. and S. Y Shiau,. 2002. Dietary Vitamin C and Its Derivatives Effect Immune Responses in Grass Shrimp equirements of Juvenile Grass Shrimp, *P. monodon* . *Fish & Shellfish Immunology.* 12:119 - 129

[13] Lee, M. H. & S. Y Shiau 2004. Vitamin E Requirements of Juvenile Grass Shrimp, *P. monodon* and Effects on Nonspecific Immune Responses, *Fish & Shellfish Immunology.* 16:475 — 485

[14] Nitimulyo, K. H., Isnansetyo, A. Triyanto, I. Istiqomah, & M. Murdjani. 2005. Isolasi, Identifikasi, dan Karakterisasi *Vibrio spp.* Patogen Penyebab Vibriosis pada Kerapu, Balai Budidaya Air Payau, Situbondo. *Jurnal Perikanan* VII (2):80 — 94

[15] Rachmaniar. 1993. Antimicrobial Activity of Crude Extract and its Fractions of Seaweed. ODI. 26: 1 - 11

[16] Rodryguez, A. Cuesta, A. Esteban, M. A. & Meseguer, J. 2003. The Effect of Dietary Administration of The Fungus *Mucor circnneloides* on Nonspecific Immune Responses of Gilthead Seabream, *Fish & Shellfish Immunology.* (16):241 – 249

[17] Sakai, M. 1999. Current Research Status of Fish Immunostimulants. *Aquaculture* 172 : 63 - 92

[18] Selvin J., A.J. Huxleya, & A.P. Lipton, 2004, Immunomodulatory Potential of Marine Secondary Metabolites Against Bacterial Diseases of Shrimp, *Aquaculture* 230: 241–248

[19] Smith V J., J H. Brown, & C. Hauton , 2003, Immunostimulation in Crustaceans: Does it Really Protect Against Infection. *Fish & Shellfish Immunology* 15 : 71–90

[20] Subagiyo. 2009. Uji Pemanfaatan Rumput Laut *Halimeda* sp. Sebagai Sumber Makanan Fungsional Untuk Memodulasi Sistem pertahanan Non Specifik Pada Udang Putih (*Litopenaeus vannamei)*. *Ilmu Kelautan* 14 (3): 195–199.

[21] Takahashi Y, M Kondo, T Itami, T Honda, H Inagawa, & T Nishizawa, 2000, Enhancement of Disease Resistance Against Penaeid Acute Viraemia and Induction of Virus-Inactivating in Haemolymph of Kuruma Shrimp, *Penaeus japonicus*, by Administration of Sulphated Polysaccharide, *Fish & Shellfish Immunology* 12: 353–366

[22] Widiastuti, H.N. 2003. Uji aktivitas metabolit sekunder dari rumput laut *Halimeda macroloba* sebagai senyawa bioaktif antijamur *Candida albicans* dan *Torula histolutica. Ilmu Kelautan,* 8(2): 114-118

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