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Non-specific immune response of Pacific white shrimp *Litopenaeus vannamei* by supplementation of sodium alginate of *Sargassum* collected from Lampung Indonesia

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Abstract. This study aims to determine the effectiveness of supplementation of sodium (Na) alginate *Sargassum* sp. from Lampung waters to enhance the non-specific immune response and the resistance of Pacific white shrimp (*Litopenaeus vannamei*) against white spot disease. This study used a completely randomized design with 3 treatments, namely feeding without alginate supplementation (A) or control, dietary Na alginate *Sargassum* supplementation at a dose of 2.0 (B), and 4.0 g kg^{-1} feed (C), each with four replications, for 14 days. Hemolymph sampling was performed on days 0, 7 and 14 to observe the parameter of shrimp hematology included *total haemocyte count* (THC), *phagocytocyte activity* (PA), *phagocytic index* (PI), and total plasma protein (TPP). The hepatopancreas histology profile and water quality were observed at the end of treatment. Data were analyzed by Anova at 95% of confidence interval and continued with Duncan test. Result showedthat the supplementation of Na alginat from *Sargassum* sp. significantly enhance several shrimp immune response namely THC, PA, and TPP. Meanwhile, the histology of the hepatopancreas of both shrimp treatment and control showed not significantly effect on tissue damage. The water quality was still in normal condition during the treatment. The results of this study indicate that the application of *Sargassum* alginate supplementation at a dose of 2gr / kg of feed is the best treatment to enhance the immune response of Pacific white shrimp.

1. Introduction

Lately, Pacific white shrimp or vannamei shrimp farming is having serious problems with the emergence of various diseases both caused by long-attacking pathogens such as WSSV, TSV, IMNV, as well as new-found types of diseases such as EMS (AHPND), CMNV, DIV-1 and other types of diseases [1]. One of the efforts in controlling the disease is to increase the shrimp resistance to the disease by adding immunostimulants.

Alginate, polysaccharides in brown algae, proved effective in improving immune response as in fish [2], shrimp [3], [4], and human [5]. Application of alginate administration in aquaculture can be done by supplement through feed, soaking in maintenance media, or by injection [6]. On the other hand, bioactivity of polysaccharides can be influenced by several factors such as the type of brown algae species, extraction methods, and the geographical location of the source of brown algae obtained [7]. So far, in Indonesia, the use of alginate to improve the immune response in Pacific white shrimp has been done before by using sodium, acid, and calcium alginate from *Sargassum* originating from

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the waters of the south coast of Java [3], [4] and calcium alginate from Lampung waters [8]. There has been no study of the use of *Sargassum* sodium alginate from Lampung waters to trigger the immune response of Pacific white shrimp. This study aims to determine the effectiveness of *Sargassum* sodium alginate supplementation from Lampung waters to trigger the immune response of Pacific white shrimp.

2. Material and method

Sargassum sp. was collected from two locations namely Sebalang Beach, Tarahan, South Lampung, Lampung and Biha Beach, Pesisir Barat, Lampung (Figure 1) in January – March 2020. *Sargassum* was washed with fresh water, stored at room temperature until dry, and cut into *Sargassum* powder.

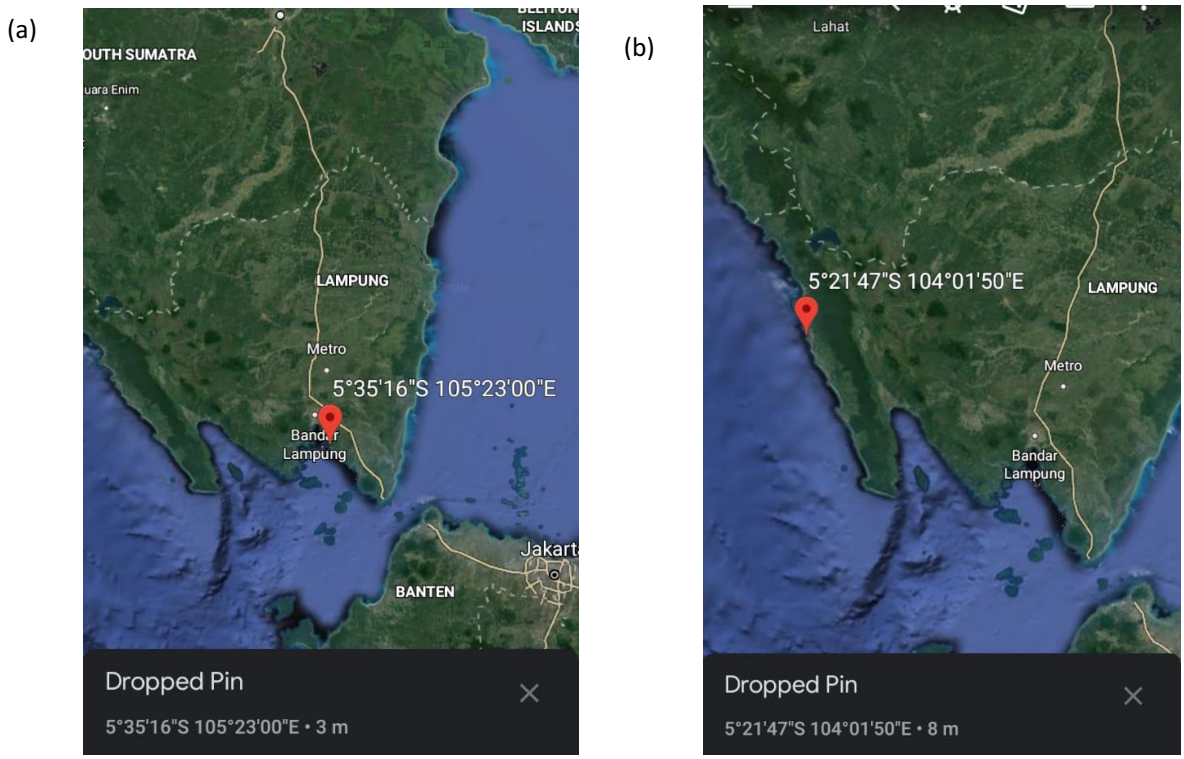
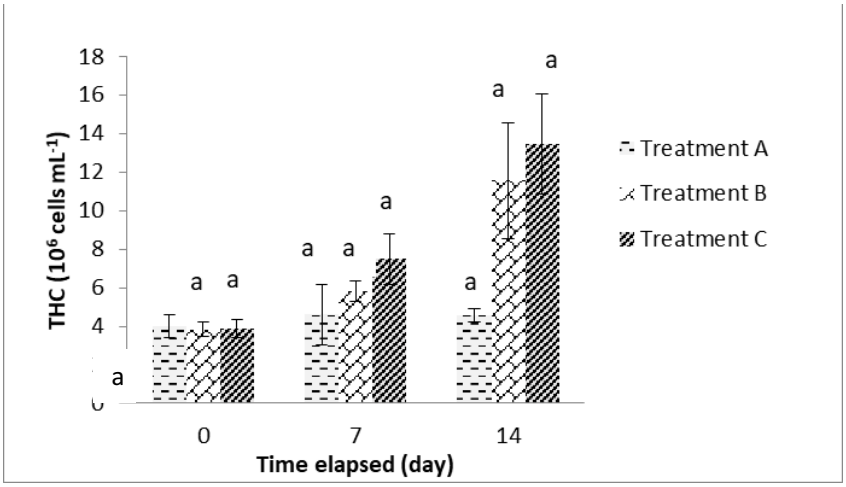


Figure 1. Map of the Sargassum collecting site at Sebalang Beach (a) and Biha Beach (b), in Province of Lampung, Indonesia (Google Earth, 2021)

The extraction of sodium alginate was carried out with reference to previous study (3). Sodium alginate was then coated in shrimp feed using Progol (Surabaya, Indonesia) binder in accordance with the treatment doses of 0 g kg^{-1} feed (treatment A or control), 2 g kg^{-1} feed (treatment B), dan 4 g kg^{-1} feed (treatment C) [8]. Feed that had been coated with sodium alginate was then given to shrimp (±15 g) which was maintained in containers (40 L) with a density of 10 shrimp per-container. Feeding ratio as much as 3% was given into shrimp with the frequency of administration 4 times a day. Hemolymph was individually withdrawn on days 0, 7, and 14 to evaluate the parameters of shrimp immune response including total hemocyte count (THC), phagocytic activity, phagocytic index, and total plasma protein (TPP) by the method as described in previous study [9]. The immune response parameter data was then analyzed with analysis of variance (ANOVA) and continued with Duncan's test at a 95% confidence level. At the end of the study, observation on the profile of shrimp hepatopancreas tissues was performance indivually from each treatment. Water quality of culture media was maintained to always be within the optimum range for Pacific white shrimp farming.

3. Result

The results of total hemocyte observations showed that *Sargassum* sodium alginate supplementation from Lampung waters significantly (P<0,05) was able to increase the amount of Pacific white shrimp hemocyte on the 7th and 14th days of treatment. The highest increase in THC occurred in the treatment of administration of 4 g kg^{-1} on the 14th day with the amount of hemocyte reaching 13.4 x 10^6 cells mL^{-1} (Figure 2).



Description: The value is the average of the 3 replication ± standard deviation. The difference in notation in each treatment showed a statistically significant difference from the Duncan Test at a confidence level of 95%

Figure 2. Total hemocyte count of Pacific white shrimp

The supplementation of *Sargassum* sodium alginate from Lampung waters significantly (P<0.05) was also effective in increasing phagocytic activity of shrimp that fed with 4 g kg^{-1} sodium alginate coated-feed (Figure 3.). Increased activity phagocytosis of shrimp occurred on the day-7 as well as on day-14 of treatment on the treatment reached 91% and 96%, respectively. However, the increase in phagocytosis activity was not followed by the phagocytosis index which showed no significant difference with the control. The phagocytic index value of shrimp is in the range of 1.4 to 2.0 from start to finish treatment (Figure 4).

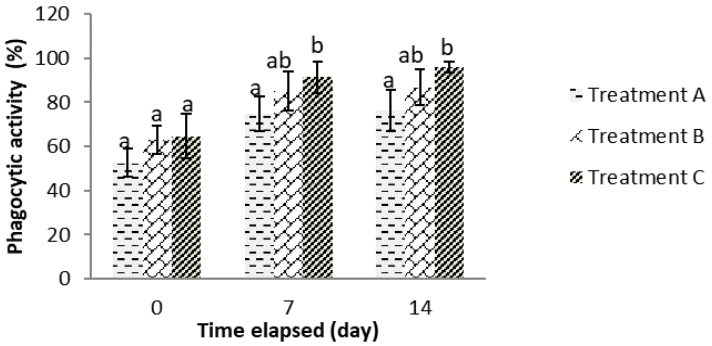


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Figure 3. Phagocytic activity of Pacific white shrimp

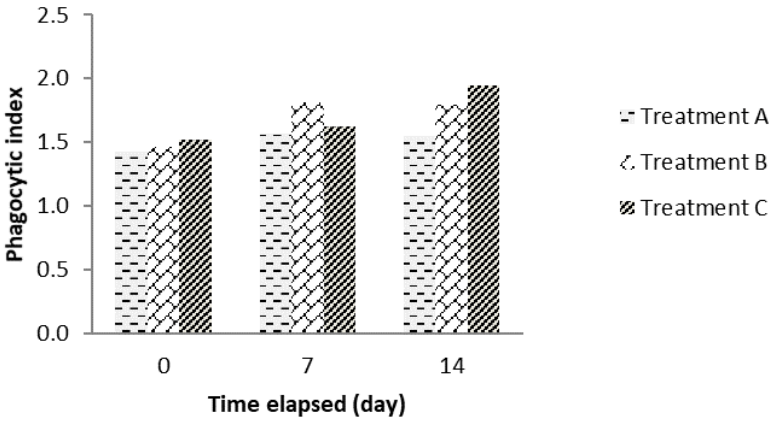

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Figure 4. Phagocytic index of Pacific white shrimp

The total plasma protein of shrimp fed with sodium alginate supplement on dose 2 g kg^{-1} feed increased significantly on the 7th day of treatment up to 257.94 mg mL^{-1} or more than three multiplied from control (67.78 mg mL^{-1}). However on the 14th day there was no significant difference in total plasma proteins between control and treatment, although all three group of treatment simultaneous increased (Figure 5).

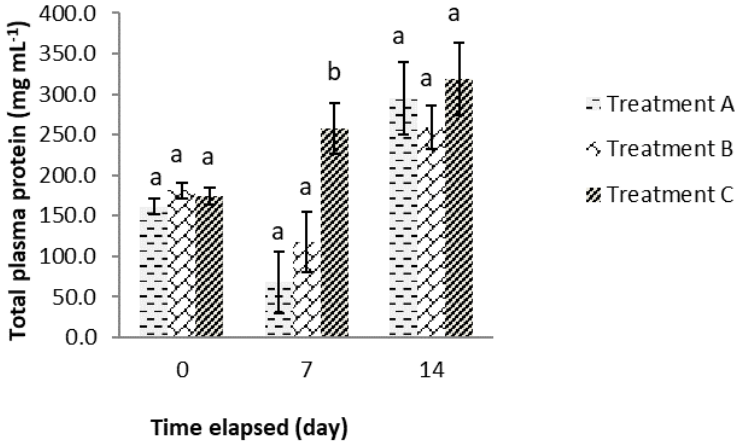

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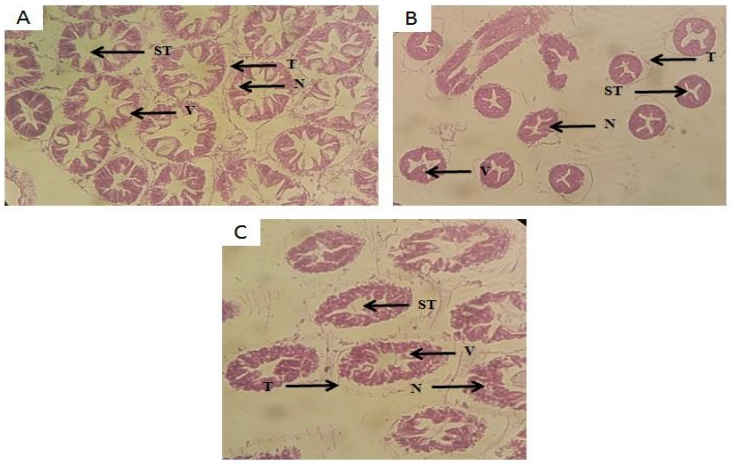
Figure 5. Total plasma protein of Pacific white shrimp

The histological profile of the shrimp hepatopancreas organ at the end of the treatment showed almost the same structure between control and treatment, although on hepatopancreas shrimp control had a more compact profile of its cells than in shrimp treatment. All hepatopancreas tissue of shrimp both control and treatment underwent vacuolasi and necrosis although in small amounts. (Figure 6).



Descryption: V: Vacuolation; N: necrosis; T: Tubules; ST: Tubule ducts
Figure 6. Histopathology profile of hepatopancreas of Pacific White shrimp

4. Discussion

Sodium alginate is proven to be potentially one of the immunostimulants in shrimp vannamei that effectively protects shrimp from viral and bacterial diseases. Previous research has shown the effectiveness of sodium alginate in protecting against disease such as WSSV [4] and vibriosis [10]. Protection of sodium alginate in shrimp by triggering several non-immune response such as THC, phagocytic activity, phagocytic index, phenol oxidase (PO) activity, superoxide dismutase (SOD) activity, and TPP, as well as by up-regulating the immune-related genes such as LGBP, Toll, proPO, and lectin [3]. In this study, supplementation of *Sargassum* sodium alginate from Lampung waters was able to trigger an increase in THC, phagocytosis activity, and total plasma proteins and did not damage the hepatopancreas tissue of Pacific white shrimp.

The effectiveness of alginate administration in improving the nonspecific immunity of shrimp can be known by observation of hematological profiles. The total hemosite of shrimp supplemented with *Sargassum* sodium alginate showed a significant increase (Picture 2). Hemosite on the body of crustaceans is very important in maintaining resistance to pathogens. If the total hemosite is high, it can increase the ability of the blood to phagocytosis. The occurrence of increased THC due to lectin molecules that are part of the humoral defense of shrimp serves to introduce foreign bodies (non self recognition) that enters the body of shrimp [11]. Total increase in shrimp hemosite may also increase granular cells which can stimulate the activation of Prophenoloxidase (ProPO) to produce phenoloxidase (PO) activity, to defend shrimp from pathogenic attacks. Oral administration of acid and sodium alginate may increase the activity of the proPO system that stimulates hemosite and phagocytosis [3].

Shrimp that fed *Sargassum* sodium alginate 2 gr kg^{-1} feed showed a significant increase (P<0.05) in shrimp phagocytic activity compared to control, both on the 7th to 14th day of treatment (Figure 3). Administration of *Sargassum siliquossum* sodium alginate 2 gr kg^{-1} feed has been able to increase in phagocytcic activity of Pacific white shrimp [3]. The activity of phagocytosis

supported by a higher total amount of hemosite will increase the activity of phagocytosis to control foreign materials entering the body [12]. However, the increased activity of phagocytosis in this study was not accompanied by an index of phagocytosis that showed no significant difference compared to control (Figure 4).

Total plasma protein (TPP) of shrimp administered administered *Sargassum* sodium alginate at 2 gr kg^{-1} increase significantly to reach 258 mg mL^{-1} on 7 days of treatment (P<0.05) (Figure 5). The value is much greater than previous studies showing that the administration of *Sargassum siliquossum* sodium alginat at same dose reached 9 mg mL^{-1} on 10 days of treatment [3]. The value of shrimp TPP given sodium alginate is almost the same as the value of shrimp TPP given calcium alginate from the same *Sargassum* species and the location of lampung waters [8]. Plasma proteins have an important role in the immune system of the crustaceans. Plasma proteins are a major part of hemolymph plasma consisting of a very complex mixture of simple proteins and conjugated proteins such as glycoprotein and various forms of lipoprotein [13].

Supplementation of *Sargassum* sodium alginate from Lampung waters in addition to effectively improving the immune response of Pacific white shrimp, also proven safe to use in shrimp cultivation. This is demonstrated by the histological profile of the hepatopancreas organ shrimp treatment with the shrimp control group showing almost the same profile.. In general, the cells in the shrimp hepatopancreatic tissue of control group showed more solid and tightness than the cells in shrimp treatment. In addition, there was little damage such as vacuolation and necrosis that is likely caused by the activity of ezim alkaline phosphatase (ALP) and acid phosphatase (ACP) are considered as important regulative enzymes in animals metabolic process including alginate [6].

5. Conclusions

Suplementation of *Sargassum* sodium alginate from the Lampung waters, Indonesia is able to trigger Pacific white shrimp immune response indicated by an increase in total hemocyte, phagsitosis activity and total plasma proteins. Supplementation *Sargassum* sodium alginate from Lampung waters is also proven safe to be administered to shrimp by referring to the histological profile of hepatopancreas. This study complements previous studies that prove the potential of alginate as an immunostimulant in the cultivation of shrimp vannamei.

Acknowledgements

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