



Effects of the *Sargassum* Sp Enrichment Time on Post-Larvae White Shrimp (*Litopenaeus vannamei*)

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Abstract

Sargassum sp has been applied in aquaculture and has shown promising results; however, the application in shrimp larvae through artemia bio-encapsulation and the length of Artemia enrichment is still unknown. This study aimed to evaluate the effect of *Sargassum* sp and its duration of enrichment on *Artemia* sp on the survival, growth, and immunity of white-leg shrimp (*Litopenaeus vannamei*) post-larvae. This study was an experimental study using a completely randomized design (CRD). The treatment was the duration of Artemia enrichment in *Sargassum* sp crude extract fed to vaname shrimp post larvae, namely: an enrichment time of 30 minutes (A), an enrichment time of 60 minutes (B), an enrichment time of 90 minutes (C), and without *Sargassum* sp extract (control) (D). The test animals were 1,440 PL1 stage white-leg shrimp (*L. vannamei*), stocked into 12 jars, each containing 2 L of water with a density of 60 shrimp/L (120 shrimp/jar). Daily feeding was carried out twice with *Artemia* sp and 4 times with flour feed, reared for 20 days. An environmental stress test was then carried out at a pH of 5 on the 20th day and the shrimp were observed 24 hours after the stress test. Observation parameters consisted of larvae survival, growth, and immunity. The results showed that the bio-enrichment of *Artemia* sp with *Sargassum* sp extract could improve the growth performance, survival, and hematology of white-leg shrimp after being reared for 20 days. Similarly, the environmental stress testing at a pH of 5 showed that the shrimp larvae had high survival rates and hematology, especially in the treatment with an enrichment duration of 90 minutes. Therefore, it was concluded that the bio-enrichment of *Artemia* sp with *Sargassum* sp extract could improve the survival rate, growth, and immunity profile of white-leg shrimp post larvae.

Keywords: Bio-encapsulation; Crude Extract; Hematology; Enrichment Time; White-Leg Shrimp.

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1. Introduction

Fish production is one of the food production sectors with the highest growth globally with a production of 82,095 thousand tons in 2018 [1]. White-leg shrimp, *Litopenaeus vannamei*, is one of the most important aquaculture products in the last decade, being better than other types of shrimp and types of crabs and lobsters for consumption [2] due to various cultivation supporting factors, including the simplicity of cultivation activities, cultivation area size, and tolerance to the environment, especially salinity (0.5–45 ppt) and temperature (15–28 °C), and is in very high demand in various countries. Seaweed is a source of bioactive compounds such as alkaloids, flavonoids, polysaccharides, fatty acids, polyphenols, *et cetera*, with antioxidant, antimicrobial, anticancer, and immune-enhancing properties that make seaweed have more potential as a functional food [3-5]. Among these various sources, seaweed is considered a good source of polysaccharides such as alginate, carrageenan, fucoidan, galactan, ulvan [6]. These polysaccharides are biological macromolecules consisting of large units of monosaccharides and their derivatives which have potential biological activity [7-9]. These polysaccharides have been used in the aquaculture industry and have shown increasing use in recent years, shown by an increase in high growth performance and disease resistance [10-15].

The problem with materials such as *Sargassum* sp polysaccharides on larvae is that it is difficult to apply orally because the larvae feed is still in the form of flour; therefore, this study tested it through the application through artemia bioencapsulation. *Artemia nauplii* play an important role in the aquaculture industry at the global level due to the presence of essential nutrients such as protein, essential amino acid, and fatty acid. In addition to the complete nutritional content, *Artemia salina* is also often used as animal bio-encapsulation of several important ingredients such as probiotics, prebiotics, fatty acids, and various other ingredients to improve the profile of high-quality fish and shrimp larvae such as the survival rate, growth rate, and high resistance against various diseases [16-18].

Several studies have shown that *Sargassum* sp has been applied at the research level and demonstrated good results; however, the application in shrimp larvae through artemia bioencapsulation and the length of artemia enrichment is still unknown. Therefore, this study aimed to evaluate the effectiveness of *Sargassum* sp extract and the duration of its enrichment on *Artemia* sp on the survival, growth, and immunity profile of white-leg shrimp (*L. vannamei*).

2. Materials and Method

2.1. Animals and Research Containers

The post larvae were reared in 2.5-liter jars cleaned using fresh water and detergent by scrubbing all parts of the jars using a sponge, then rinsed with fresh water and dried. The water for rearing the larvae was treated by filtering and sedimented in a holding tank. The water was then put into the jars and filtered using a filter bag and then the aeration was installed. The test animals used in this study were 1,440 post-larvae-1 (PL-1) stage white-leg shrimp (*L. vannamei*), spawned from a shrimp hatchery in Barru Regency. Before stocking into the rearing media, acclimatization was carried out in a temporary holding container for 15-20 minutes, then the bag of fries was opened and water was slowly added then the bag was tilted so that the shrimp fries could leave on its own

accord.

2.2. Experimental design

This study was an experimental study using a completely randomized design (CRD). The treatment was the duration of *Artemia* sp enrichment in *Sargassum* sp crude extract for feeding the white-leg shrimp PL. The length of time of immersion of *Artemia* nauplii in *Sargassum* sp extract in each treatment consisted of 30 minutes of enrichment (A), 60 minutes of enrichment, 90 minutes of enrichment (C), and without *Sargassum* sp extract (the control).

2.3. Sargassum Collection and Extraction

Sargassum sp was washed with seawater to separate it from foreign materials such as epiphytes, sand particles, gravel, and shells. Then it was washed thoroughly with tap water, followed by distilled water, smeared on blotting paper, and spread on thick paper to air dry at room temperature (25 °C) overnight. The air-dried seaweed was then aired in the sun to dry and then made into a powder by flouring with a blender and sieved with a number 16 mesh sieve. The flour extraction was conducted using the maceration method [19] by placing it in a glass jar and adding 96% ethanol solvent at a (1:4) ratio until it was submerged while stirring occasionally. It was allowed to stand for 24 hours, the dregs formed were separated, and the filtered product was called macerate I. The dregs were macerated again twice by repeating the initial step and then allowed to stand for 24 hours. The macerates were called macerate II and macerate III. All the macerates were collected and concentrated using a rotary evaporator until a thick extract was obtained. The extract was stored in a bottle whose weight was known and then the yield of the extract was calculated. Then the extract was put in a fridge at 4°C until it was used for *Artemia* sp enrichment to feed white-leg shrimp larvae.

2.4. Bioencapsulation of Artemia sp

The *Artemia* sp used in this study is a product of Mackay Marine. The *artemia* nauplii enrichment with *Sargassum* sp extract with enrichment (soaking) durations was carried out according to the instructions in Nieves-Soto and colleagues [20]. Enrichment was carried out by culturing *Artemia* sp in a transparent jar with a volume of 1 L with a density of 500 shrimp/L, while the dose of *Sargassum* sp extract was 300 ppm. Three durations of *Artemia* sp enrichment were evaluated, namely 30, 60, and 90 minutes, and one *Artemia* sp group was unenriched (as a control). Each treatment was carried out with three replications.

2.5. Feeding Trial and Sampling

The study was begun by stocking PL1 stadium white-leg shrimp larvae into 12 jars filled with 2 L of water at a density of 60 individuals/L (120 individuals/jar). The shrimp were fed 6 times per day; the encapsulated *Artemia* feed was given twice a day and the commercial feed (Lansy Shrimp MPL) at a dose of 5% of the weight of shrimp biomass was given at a frequency of 4 times a day. The water quality was monitored every morning and afternoon. The parameters measured were temperature, salinity, pH, and dissolved oxygen. The temperature was measured using a thermometer with an accuracy of 0.5 °C, salinity was measured using a hand-refractometer,

pH was measured using a pH meter, and dissolved oxygen was measured with a DO meter. Water in PL1-4 was changed by 30-40%, PL 5-8 was changed by 40-50%, PL 9-12 was changed by 50-80%, and PL13-16 was changed by 60-90%. The maintenance medium water was maintained using constant aeration at an oxygen content of 5.2-5.9 ppm, pH 8.0-8.4, salinity 28-30 ppt, and water temperature 29-31 °C.

Observations of immunity, growth, and survival were conducted on day 20 (at the end of the study). A sampling of shrimp immunity was done by taking 10 fries at random from each treatment unit, then were tested for Total Hemocytes Count (THC), Phagocytic Activity (PA), and Differential Hemocytes Count (DHC). The Survival Rate (SR) was determined by counting the number of fries that lived up to day 20, while growth observation was conducted by weighing 10 fries per experimental unit.

2.6. pH Challenge

After 20 days of rearing, a stress test was conducted at a low pH of 5. The decrease began from pH 8.0-8.4 during maintenance to pH 5 using HCl. The stress test was conducted by pouring 1 liter of pH 5 water into glass jars and then placing 20 shrimp fries in each jar. Observations on the pH stress test were carried out for 24 hours by counting the number of surviving larvae to determine the SR of the shrimp larvae.

2.7. Immunological Parameters

The test parameters observed in this study consisted of THC, DHC, PA, and SR, observed on day 20 (at the end of the study) and day 21 (post-stress test). The THC was calculated based on the Campa-Cordova and colleagues [21] formula, the DHC based on the Martin dan Graves [22] formula, the Phagocytic activity based on the Kim and Austin [23] formula, the SR and the growth rate based on Lugert and colleagues [24].

2.8. Statistical analysis

The research parameter data which included the immune response (THC, DHC, PA) and survival and growth rates were statistically analyzed using SPSS version 22. Parameters indicating significance were continued with Duncan's follow-up test.

3. Materials and Method

3.1. Survival Rate

Based on a study carried out with the length of rearing of white-leg shrimp larvae for 20 days at the PL1-PL20 stage, the application of *Sargassum* sp could increase the survival rate (Fig. 1) of white-leg shrimp larvae, differing based on the *Artemia* sp immersion treatment duration. The statistical analysis showed that the different immersion durations in *Sargassum* sp extract on *Artemia* sp affected the larvae survival rate ($P < 0.05$). Duncan's follow-up test showed that immersion for 90 minutes increased survival the most ($P < 0.05$) (79.20%) and was the best time among the lengths of time tested compared to 30 minutes (50.85%) and 60 minutes (55.80%), and the control (52.23%).

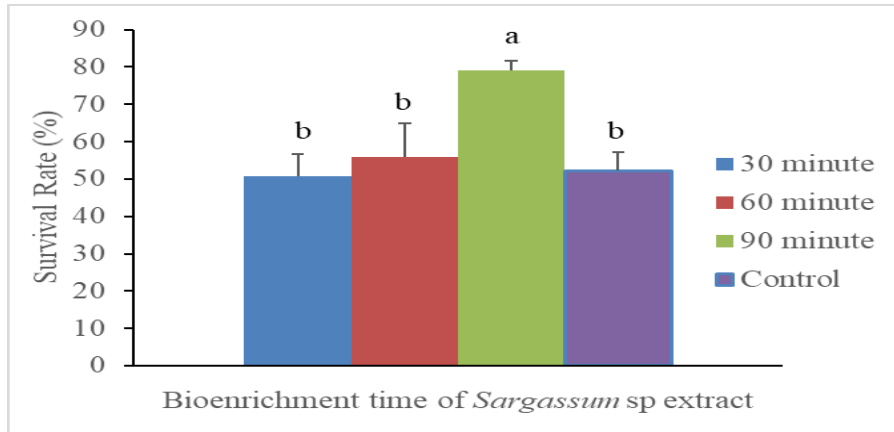


Figure 1: The survival rate of white-leg shrimp (*Litopenaeus vannamei*) larvae during 20 days of rearing (PL1-PL20) fed *Artemia* sp enriched with *Sargassum* sp crude extract with different enrichment durations. Data are presented as mean \pm standard deviation. Means with different superscript letter were significantly different ($P < 0.05$) as determined by Duncan’s test.

3.2. Growth

The growth rate of the white-leg shrimp larvae increased during the 20 days of rearing (Fig 2). The absolute weight growth of white-leg shrimp larvae was influenced by the presence of *Sargassum* sp extract in the *Artemia* sp natural feed. The results of statistical analysis showed that the application of *Sargassum* sp through *Artemia* sp significantly increased absolute growth ($P < 0.05$). Duncan’s follow-up test showed that the treatment with 90 minutes of enrichment was the best enrichment duration (0.0251 mg) compared to 30 and 60 minutes of enrichment (0.0148 and 0.0152 mg respectively) while the lowest was in the control (0.0049 mg) ($P < 0.05$).

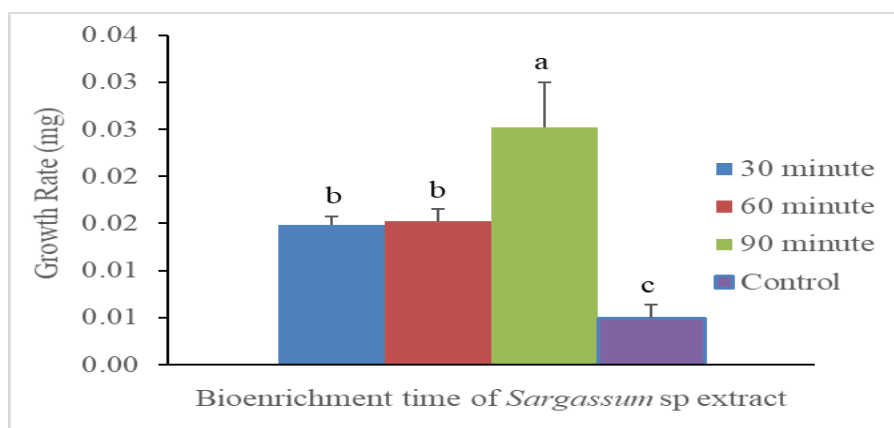


Figure 2: The growth rate of white-leg shrimp (*Litopenaeus vannamei*) larvae during 20 days of rearing (PL1-PL20) fed *Artemia* sp enriched with *Sargassum* sp crude extract with different enrichment groups. Data are presented as mean \pm standard deviation. Means with different superscript letter were significantly different ($P < 0.05$) as determined by Duncan’s test.

3.3. Shrimp Hematology

The white-leg shrimp hematology observed in this study consisted of THC (Fig. 3), PA (Fig. 4), and DHC (Fig. 5) parameters. Overall, the three observed immunity parameters were influenced by the enrichment of *Sargassum* sp. The statistical test showed that the enrichment of *Sargassum* sp extract of *Artemia* sp with different immersion durations had a significant effect on the THC, PA, and DHC of shrimp larvae ($P < 0.05$). Duncan's follow-up test showed that treatment with an enrichment period of 90 minutes could increase the THC (8.0×10^6 cell/mL) and PA (38.4%) the most and they were significantly better than the other treatments ($P < 0.05$), followed by treatment with an enrichment duration of 60 minutes and 30 minutes, and the lowest in the control (5.1×10^6 cell/mL and 22.1% respectively).

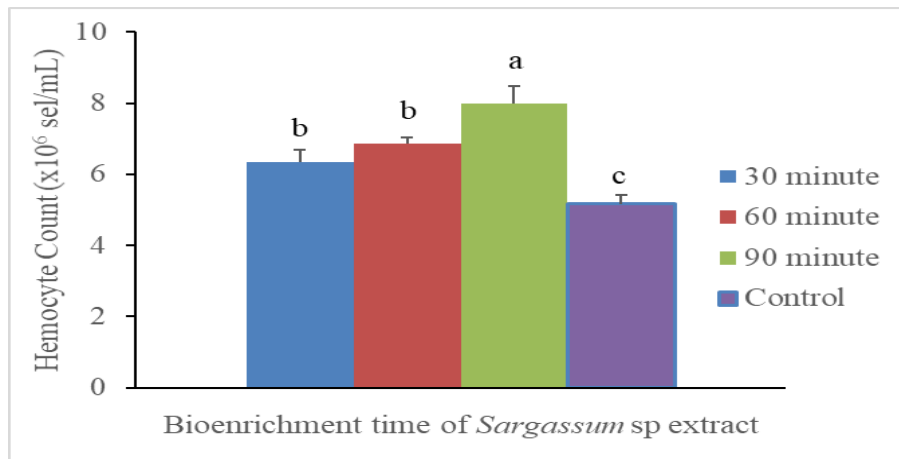


Figure 3: The Haemocyte Count of white-leg shrimp (*Litopenaeus vannamei*) larvae during 20 days of rearing (PL1-PL20) fed *Artemia* sp enriched with *Sargassum* sp crude extract with different enrichment durations. Data are presented as mean \pm standard deviation. Means with different superscript letter were significantly different ($P < 0.05$) as determined by Duncan's test.

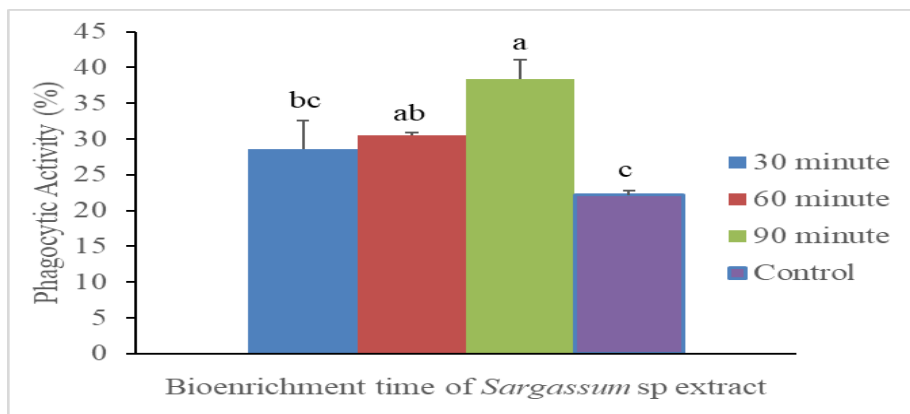


Figure 4: The Phagocytic Activity of white-leg shrimp (*Litopenaeus vannamei*) larvae during 20 days of rearing (PL1-PL20) fed *Artemia* sp enriched with *Sargassum* sp crude extract with different enrichment durations. Data are presented as mean \pm standard deviation. Means with different superscript letter were significantly different ($P < 0.05$) as determined by Duncan's test.

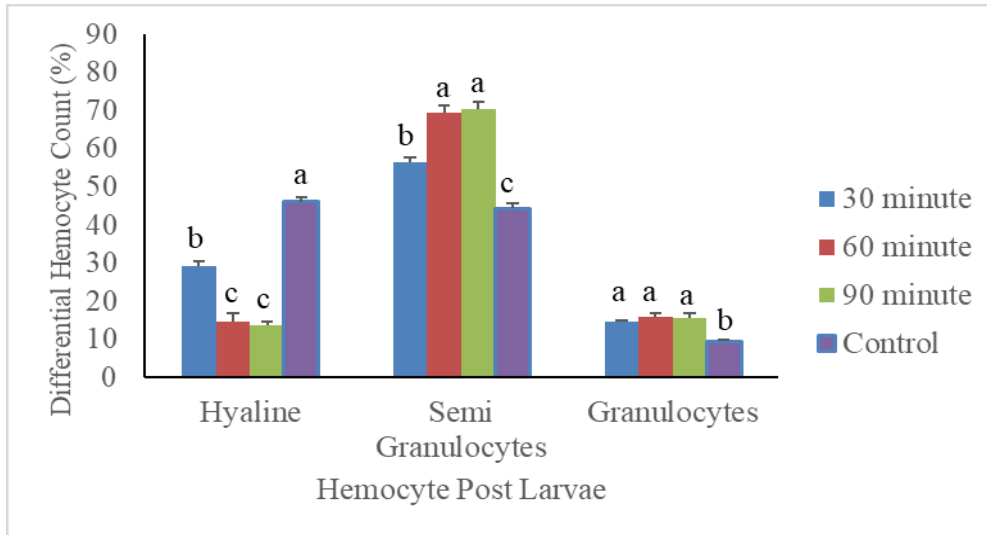


Figure 5: The Differential Hemocyte Count of white-leg shrimp (*Litopenaeus vannamei*) larvae during 20 days of rearing (PL1-PL20) fed *Artemia* sp enriched with *Sargassum* sp crude extract with different enrichment durations. Data are presented as mean \pm standard deviation. Means with different superscript letter were significantly different ($P < 0.05$) as determined by Duncan’s test.

The research results on the parameters of the DHC white-leg shrimp larvae showed differences between hyaline, semi-granular, and granular. The ANOVA test showed that feeding *Artemia* sp enriched with *Sargassum* sp extract for 20 days had a significant effect on the hemocyte types in the shrimp PL. Duncan’s follow-up test showed that shrimp PL fed *Artemia* sp enriched with *Sargassum* sp for 90 minutes and 60 minutes demonstrated the highest increase in semi-granulocytes (masing-masing 70.58% and 69.46%) and granulocytes (15.70% and 15.89% respectively) compared to enrichment for 30 and the control ($P < 0.05$). Different results were found for hyaline, where the PL fed *Artemia* sp enriched with *Sargassum* sp for 60 and 90 minutes were lower than those fed *Artemia* sp with 30 minutes enrichment and the control ($P < 0.05$).

3.4. SR Post Stress-Test

Environmental stress test on the white-leg shrimp PL by lowering the pH of the rearing water (pH 5) was carried out at the end of the 20-day rearing period. Observation of larval survival was conducted for 24 hours post-stress test (Fig. 6). The results showed that the survival rate of the larvae after the stress test was different for each treatment, where the larvae fed *Artemia* sp feed containing *Sargassum* sp could shield themselves against low pH rearing water, so they had a higher survival rate compared to the control. The results of statistical analysis showed that the administration of *Sargassum* sp could increase the survival rate of shrimp larvae post-pH stress test. Duncan’s follow-up test showed that *Artemia* sp feed with an enrichment period of 90 minutes could better maintain larval survival after the stress test (90.83%) compared to all other treatments, and the lowest was in the control ($P < 0.05$) (48.52%).

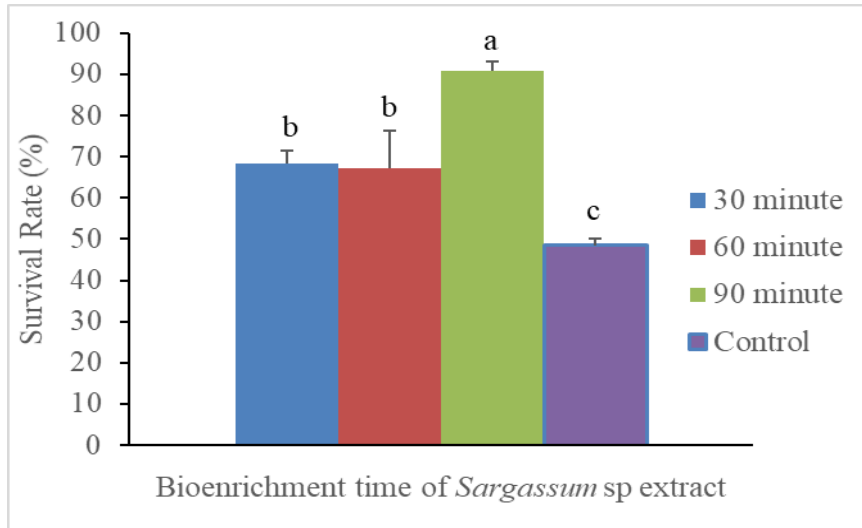


Figure 6: The survival rate of white-leg shrimp (*Litopenaeus vannamei*) larvae was 24 hours after the pH 5 stress test. Data are presented as mean \pm standard deviation. Means with different superscript letter were significantly different ($P < 0.05$) as determined by Duncan's test.

3.5. Haematology Post pH Stress Test

Hematology after the stress test with a low pH (pH 5) aquatic environment showed changes in THC (Fig. 7), PA (Fig. 8), and DHC (Fig. 9) parameters. Statistically, the hematology of PL post-pH-stress-test was influenced by the *Sargassum* sp enrichment of the *Artemia* sp natural feed ($P < 0.05$). Duncan's follow-up test showed that enrichment using *Sargassum* sp extract for 90 minutes resulted in the parameters of THC (7.92%) and PA (38.40%) which were better than those of the other treatments and the control (3.05% and 22.19 % respectively) ($P < 0.05$).

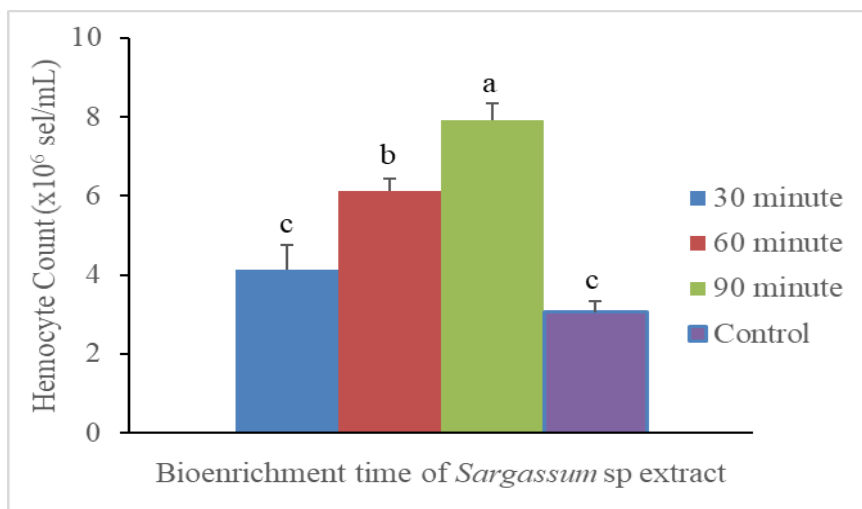


Figure 7: The Hemocyte Count of white-leg shrimp (*Litopenaeus vannamei*) larvae 24 hours after the pH 5 stress test. Data are presented as mean \pm standard deviation. Means with different superscript letter were significantly different ($P < 0.05$) as determined by Duncan's test.

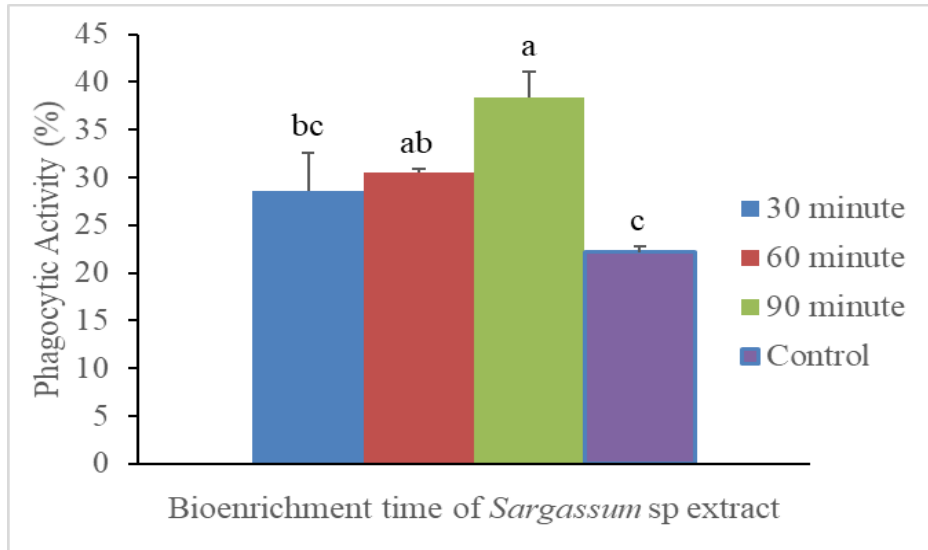


Figure 8: The Phagocytic Activity of white-leg shrimp (*Litopenaeus vannamei*) larvae 24 hours after the pH 5 stress test. Data are presented as mean ± standard deviation. Means with different superscript letter were significantly different ($P < 0.05$) as determined by Duncan’s test.

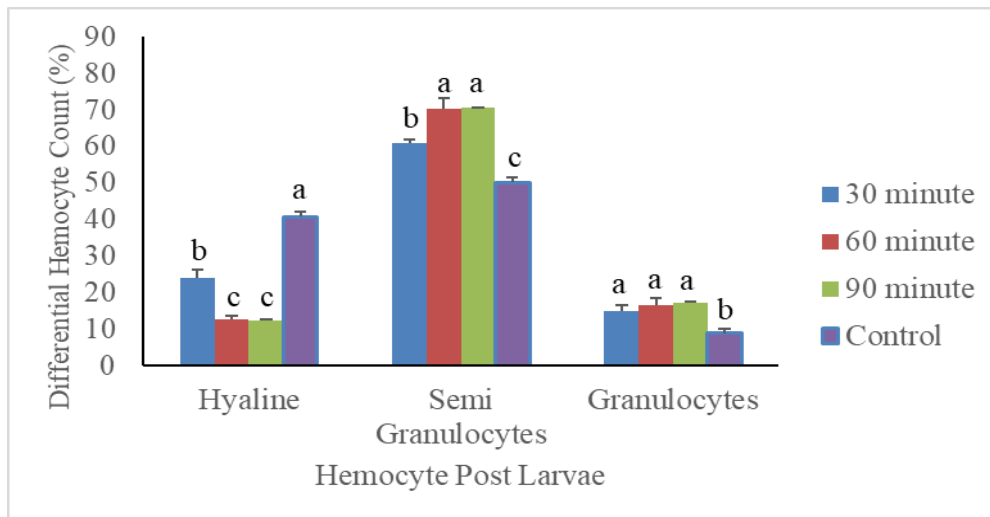


Figure 9: The Differential Hemocyte Count of white-leg shrimp (*Litopenaeus vannamei*) larvae 24 hours after the pH 5 stress test. Data are presented as mean ± standard deviation. Means with different superscript letter were significantly different ($P < 0.05$) as determined by Duncan’s test.

The types of hemocytes after the stress test with a low pH (pH 5) aquatic environment in general did not change much in dynamic patterns compared to before the stress test. The results of the ANOVA test showed that the enrichment of *Artemia* sp with *Sargassum* sp extract had a significant effect on the types of hemocytes 24 hours after the stress test. Duncan’s follow-up test showed that the semi-granulocyte and granulocyte hemocytes in the *Sargassum* sp enrichment treatment were higher than the control ($P < 0.05$), while hyaline in the *Sargassum* sp enrichment was lower than that of the control ($P < 0.05$).

4. Discussion

This research has provided information about the effectiveness of *Sargassum* sp extract on the growth performance, survival, and hematology of whiteleg shrimp larvae, information about the effectiveness of *Artemia* sp bioenrichment using *Sargassum* sp extract, and information about the length of time for *Sargassum* sp extract enrichment on *Artemia* sp. The results, which are presented in Fig. 1 and Fig. 2, show that bio-enrichment with *Sargassum* sp extract can increase the survival and absolute growth of white-leg shrimp larvae during the 20 days of rearing. Similarly, larval hematology indicated by the total hemocyte count (Fig. 3) and phagocytic activity (Fig. 4) parameters showed an increase in shrimp larvae fed *Sargassum* sp extract, especially in those fed with a 90-minute enrichment time. These results differed from the control which showed a slight increase in all test parameters.

The results of environmental stress testing with a water pH of 5 showed that shrimp larvae fed *Artemia* sp containing *Sargassum* sp could survive in extreme environments with a high survival rate (90.83%) (Fig. 6), especially in the treatment with an enrichment duration of 90 minutes, at odds with the control that had a low survival rate (48.52%). The high survival rate in the treatment with *Sargassum* sp enrichment, especially in the treatment with an enrichment period of 90 minutes, is due to the increase in larval hematological parameters (Fig. 3,4,5), enabling the larvae to adapt to an unsuitable (extreme) environment during the stress test. The high survival rate post-stress-test was also influenced by the larvae's high immunity, which was indicated by high hematology compared to the other treatments during the 20-day rearing period (Fig 7,8,9). The high results in all the test parameters were in line with the duration of enrichment as the treatment indicated that the components of *Sargassum* sp were well absorbed by the *Artemia* sp and subsequently consumed by shrimp larvae.

In this study, the growth of shrimp larvae given *Sargassum* sp, especially at 90 minutes of enrichment, was higher than that of larvae not given *Sargassum* sp. This effect is due to the content of low molecular weight polysaccharides and oligosaccharides that are potential prebiotics [25-28]. This will improve the larval gut health status and increase antioxidant and immunostimulation functions [29,30]. Tapia-Paniagua and colleagues [31] stated that the nature of food in aquatic animals affects the composition of the gut microbiota which play a key role in modulating the immune system and resistance to pathogen invasion [31,32], although, in this study, the composition of the bacteria in the gut was not studied. Furthermore, Shi and colleagues [30] stated that *Sargassum* sp can reduce the effects of stress on organisms, indirectly affecting the growth of shrimp.

In terms of immunity, this study also demonstrated high larval immunity as indicated by higher total hemocytes and phagocytic activity and subsequently shown as a high survival rate during both rearing and post-stress-testing. This is because *Sargassum* sp extract which contains tannins, alginate, carrageenan, fucoidan, galactan, ulvan, and some other active ingredients can stimulate non-specific immunity which is an immunostimulant in nature [33].

Artemia sp is commonly used as feed for the larvae and juveniles of fish, cephalopods, and crustaceans. *Artemia* sp has the advantage that it can be produced in large quantities in a short time [20]. In this study, encapsulation of *Artemia* sp was carried out and proved effective in transferring *Sargassum* sp to shrimp larvae. In a similar

study, *Artemia* sp was used for transferring some supplemental ingredients [34]. The enrichment time of *Artemia* sp studied in this study showed that an enrichment duration of 90 minutes improved the performances tested in general and was better than the other enrichment durations. Nieves-Soto and colleagues [20], who studied the enrichment period of *Artemia franciscana*, demonstrated that enrichment for 6 hours resulted in the highest DHA content, while the highest EPA and ARA content was obtained at 3 hours of enrichment.

The increased hemocytes in larvae will play an important role in the body's defense system through recognition, phagocytosis, melanization, cytotoxicity, and communication between cells [35] through the process of synthesis and release of α -2-macroglobulin (α 2M) molecules, agglutinins, and antibacterial peptides as a crustacean defense reaction [36]. Pourmzaffar and colleagues [37] and Jahromi and colleagues [32] stated that hemocytes play a role in nodulation, elimination of foreign materials, exoskeleton hardening, and encapsulation. Because of the role of hemocytes in crustacean cellular immunity, THC is often an indicator to evaluate the immune status of shrimp, influenced by materials, the animal's life stage, route of administration, duration of treatment, and environmental conditions of rearing. In this study, the application of *Sargassum* sp, especially in the bioencapsulation duration of 90 minutes, showed the highest increase in THC, while the control was the lowest [38,32].

The results which showed better immunity compared to controls, both at the beginning of larval rearing, after being reared for 20 days and 24 hours after the stress test indicated that *Sargassum* sp extract had an immunomodulatory effect. This information was indicated by total hemocytes and higher phagocytic activity compared to the control. This causes the larvae to have a higher survival during rearing and even more so during stress testing. The presence of the immunomodulatory effect caused the larvae to be in optimal conditions so that growth was also better than the control, and the highest was in the treatment with 90 minutes of enrichment. According to Schleder and colleagues [39] and Jahromi and colleagues [32], the benefits of feed containing seaweed depend on the type of algae, dose, and route of administration.

The THC and DHC indicate the immune status. Differential hemocyte count indicates that the shrimp are in homeostasis or stress due to infection. If the animal is infected, the total hemocyte will be lower than that in homeostatic conditions because it plays a role in destroying foreign objects [40]. In this study, there was an increase in THC during rearing in the shrimp given *Sargassum* compared to the control shrimp; however, when the stress test was conducted, there was a decrease in THC but it was still high compared to the control [41]. Low pH stress testing in this study contributed to the decrease in THC [42,43].

In addition to total hemocytes, there was an increase in phagocytic activity before and after the stress test. One of the cellular immune responses in shrimp in response to foreign bodies is the phagocytosis mechanism. In several studies, the increase in phagocytic activity in shrimp occurs after challenge testing as a shrimp defense mechanism [44, 45].

During phagocytosis, bacterial particles are recognized by receptors on the cell surface, then engulfed by cells that rearrange the cytoskeleton to form phagosomes. In the first formation, the phagosomes undergo maturation by cleavage and fusion with lysosomes and become mature phagolysosomes. Bacteria in phagolysosomes will

be destroyed by low pH conditions, hydrolysis, and radicals [46].

Phagocytosis is a process performed by shrimp hemocytes that play a role in destroying foreign particles, including pathogens that enter the body [47].

The hemocytes play a role in the encapsulation process to ingest and subsequently destroy the foreign particles [48,49,50]. According to Zhang and colleagues [51], shrimp hemocytes consist of 3 types: hyaline comprising 5-15%, granulocytes 10-20%, and semi-granulocytes approximately 75% of hemocytes. Hyaline has no direct phagocytic activity, while granulocytes play a role in the phagocytosis process and store the prophenoloxidase (proPO) enzyme. The greatest role is held by the semi-granular in phagocytosis, encapsulation, and clotting activities [52].

In this study, enrichment with *Sargassum* sp resulted in a higher percentage of semi-granular and granular hemocytes than the control, indicating that shrimp larvae already had a stronger immune response that will control foreign materials entering the body such as pathogens or extreme environments.

Seaweeds, including *Sargassum* sp, contain many bioactive ingredients such as alkaloids, flavonoids, polysaccharides, fatty acids, polyphenols, and others, with antioxidant, antimicrobial, anticancer, and immune-enhancing properties that give seaweeds more potential as a functional food. [53]. Seaweeds such as *Sargassum* sp are a source of polysaccharides, including alginate, carrageenan, fucoidan, galactan, and ulvan [35], which are macromolecules that have biological activity [8, 9].

Several studies on the use of seaweed in shrimp, including by Yudiati and colleagues [14,15,53]. The results of this study showed a positive effect of the active ingredients in seaweed, including an increased survival rate, improved growth performance, and decreased FCR. Po-Sang and colleagues [54] investigated the effect of *Sargassum* sp as an immunomodulator on *L. vannamei* through the feed for four weeks, demonstrating an increase in immunity and growth performance in white-leg shrimp, increasing growth, hematology, hepatopancreas histology, and intestinal microbiota of *Litopenaeus vannamei* and ultimately inhibiting the development of *Vibrio* spp. in the intestine [32].

5. Conclusion

Feeding *Artemia* sp enriched with *Sargassum* sp extract to white-leg shrimp PL can improve the growth performance, survival rate, and hematology of white-leg shrimp larvae after being reared for 20 days. Similarly, the results of environmental stress testing with a pH of 5 showed that the shrimp PL had a high survival rate and hematology, especially in the treatment with an enrichment period of 90 minutes.

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