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The Potential of *Melastoma malabathricum* Leaf Extract, Chitosan, and Probiotics in Enhancing the Growth and Survival of Giant Tiger Prawn (*Penaeus monodon*)

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Abstract

The quality of post-larvae is critical to the prosperous cultivation of *Penaeus monodon*. This study aims to evaluate the effect of a combination of *Melastoma malabathricum* leaf extract, chitosan, and probiotics on the growth and survival of *P. monodon*., The post larvae of *P. monodon* were treated with various combinations of *M. malabathricum* leaf extract (50 ppm), chitosan (100 ppm), and probiotics (5 ppm) over 30 days. Growth performance was evaluated through weight gain, absolute length, specific growth rate (SGR), and survival rate. The results showed that the PCH treatment (100 ppm chitosan) yielded the highest growth, with an absolute weight gain of 0.168 g and an absolute length of 3.03 cm. The combination of *M. malabathricum* leaf extract, probiotics, and chitosan resulted in the highest survival rate of 67.5%. Water quality parameters remained within regulatory standards throughout the study. These findings corroborate the significant potential of chitosan and *M. malabathricum* as bioactive growth enhancers in *P. monodon* cultivation.

INTRODUCTION

The giant tiger prawn (*Penaeus monodon*) is one of the *Penaeus* species currently cultivated commercially in several countries and has a sizable international market (FAO, 2023). According to the Ministry of Marine Affairs and Fisheries, shrimp farming is the largest industry in Indonesia, and the government is constantly trying to develop it. In 2024, the Ministry of Marine Affairs and Fisheries successfully boosted the production of five leading export commodities: shrimp, crab, seaweed, lobster, and tilapia. The shrimp production increased to 1.13 million tons, compared to 2023, it is about 941 thousand tons. According to the FAO data from 2023,

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Indonesia ranks among the world's largest shrimp producers, standing as the fourth-largest shrimp exporter globally with a 6.6% share of total global shrimp exports in 2022. Tarakan City has an area of 657.33 km^2 and enormous potential for fishery resources. The giant tiger prawn is a fishery commodity favoured by pond farmers for cultivation due to its high market price.

The success of the shrimp farming industry is strongly influenced by the production of high-quality post-larvae (PL) through the hatchery phase (Truong *et al*., 2023). Hatchery can produce larger and healthier postlarval shrimp and adjust the PL conditions to the rearing pond conditions. Seeding also increases feeding efficiency, provides better-controlled conditions than in ponds, and improves biosecurity and facility utilization. Therefore, quality maintenance during the seeding phase is the most critical aspect of the success of the shrimp-rearing phase (De la Peña *et al*., 2023).

Common problems faced in shrimp hatcheries are slow growth due to delays in the molting process and diseases caused by fungi and parasites. The importance of moulting for shrimp growth is reviewed, as well as physiological cycles, induction triggers, behavioural changes, and composition. The success of molting also depends on pond water quality, health, and nutritional status (Lemos and Weissman, 2020). In addition to the molting process, diseases also impact shrimp farming. Flegel (2012) stated that around 60% of disease losses in shrimp aquaculture are caused by viral pathogens and 20% by bacterial pathogens.

At present, the hatchery industry relies on drugs to overcome these problems. However, the obstacles mentioned above are still often found in seed maintenance. Some preventive efforts to overcome these conditions include the use of herbs as immunostimulants that has been conducted by several researchers by adding turmeric flour to fish feed (Awaludin *et al*., 2023) and *M. malabathricum* extract to accelerate shrimp growth through the moulting process (Rahayu, 2022). The use of natural compounds, such as plant extracts, chitosan, and probiotics, has gained significant attention in aquaculture due to their potential to enhance the growth, immunity, and survival of aquatic species. *Melastoma malabathricum*, a plant known for its medicinal properties, has been studied for its role in improving reproductive performance and growth in crustaceans.

For instance, Awaludin and Ridwan (2016) demonstrated that the ethanol extract of *M. malabathricum* significantly improved the growth and survival rate of giant tiger prawn larvae, attributed to the presence of lanosterol, a precursor for steroid hormones essential for growth and reproduction. Similarly, Alam *et al*. (2019) found that *M. malabathricum* leaf extract stimulated spawning in blue swimming crabs (*Portunus pelagicus*), suggesting its potential as a natural inducer for gonadal maturation in crustaceans. Furthermore, Awaludin and Ridwan (2016) reported that *M. malabathricum* extract increased progesterone levels and accelerated ovarian maturation in white shrimp (*Litopenaeus vannamei*), highlighting its role in enhancing reproductive performance. These findings collectively support the potential of *M. malabathricum* as a natural growth promoter and reproductive enhancer in aquaculture.

Research by Martang (2020) and Hartinah (2020) reported that enrichment of artemia using *M. malabathricum* extract affected the growth and immunity of the shrimp larvae. Martang (2020) reported that *P. monodon* post-larvae given artemia enriched with *M. malabathricum* extract at a concentration of 100 ppm had a survival rate of 100% and immunity to *Vibrio harveyi* bacteria compared to the control treatment. In addition, Hartinah (2020) also found that *P. monodon* post-larvae fed *Artemia* saline-treated with enrichment using *M. malabathricum* extract had a significantly better growth and survival rate than the treatment without extract.

The use of *M. malabathricum* in aquaculture activities has become more intensive in recent years (Awaludin and Ridwan, 2016; Farizah *et al*., 2017; Alam *et*

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al., 2019). The use of herbs can increase fisheries productivity by playing role as a tonic, an agent in improving the fish growth rate, as an antibacterial, and an agent in improving reproductive performance (Velmurugan and Citarasu, 2010; Maulianawati and Suharni, 2022) and moulting performance (Maulianawati *et al*., 2020; Iromo *et al*., 2023). Additionally, the integration of chitosan and probiotics has been shown to improve immune responses and disease resistance in shrimp, further emphasizing the synergistic potential of combining these natural compounds for sustainable aquaculture practices.

The selection of chitosan in this study was based on several things, including the fact that chitosan is biodegradable, meaning it is easily biodegradable and does not contain toxins. Chitosan contains the enzyme lysozyme and amino polysaccharide molecules that can inhibit microbial growth. This ability is because chitosan has positively charged polycations that can inhibit mold and bacteria (Meng *et al.*, 2023). As immunomodulators, chitosan and nano chitosan have been shown to increase resistance to bacterial infections, improve growth performance, modulate haematological and biochemical parameters, and reduce mortality in fish and crustacean species at different chitosan concentrations (Adebisi *et al*., 2023).

Udo *et al*. (2018) stated that chitosan supplementation proved to significantly increase daily weight gain, survival, and the flesh quality of *C. gariepinus* fish seeds, while chitosan nanoparticle supplementation significantly improved water quality, daily weight gain, feed utilization, survival, and body composition. Probiotics have been widely utilized in fish farming activities. Probiotics are microorganisms that can modify the composition of bacteria in the digestive tract of aquatic animals, the water, and the sediment and can be used as feed supplements that can improve host health and act as biocontrol agents. Chin *et al*. (2024) added that probiotics can improve water quality, increase immune and nutritional responses, and eliminate pathogenic bacteria. Probiotics are also known to inhibit the growth of pathogenic bacteria such as *V. parahaemolyticus* and to prevent *Aeromonas hydrophila* infections (Maulianawati *et al*., 2018). Commercial probiotics are known to have many benefits in shrimp farming and to provide a significant influence in increasing shrimp productivity, improving the farming environment, and increasing immune responses and FCR (Jamal *et al*., 2019). In this study, the probiotics contained several types of bacteria: *Bacillus megaterium*, *Bacillus polymyxa*, *Lactobacillus plantarum*, *Nitrobacter winogradskyi*, and *Nitrosomonas europaea*. Therefore, using *M. malabathricum* extract and adding chitosan and probiotics in this study is expected to increase the growth and survival of the giant tiger prawn.

METHODOLOGY

Ethical Approval

There are no animals harmed or improperly treated during this research. The test animals in this study were treated properly according to the optimal environment and there was no addition of harmful bacteria or toxic material.

Place and Time

This study was carried out between January and February of 2022 at the Mini Hatchery, part of the Faculty of Fisheries and Marine Sciences at Borneo Tarakan University. Water quality analysis was performed at the Water Quality Laboratory within the same faculty.

Research Materials

The study utilized a range of equipment and materials to ensure accurate and reliable results. The equipment included aluminium foil, spatula, analytical scales (Mettler Toledo, Swiss), aquariums, aeration equipment, aerators, styrofoam, bottles, measuring cups, fish scoops, pipettes, thermometer (Gea S-006, China), pH meter (Lutrin pH-208, Taiwan), DO meter (Lutron 5510, Taiwan), hand held-refractometer

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(Atago, Japan). The materials used in the study were equally diverse, including *M. malabathricum* extract, giant tiger prawn larvae (PL-10) from the same broodstock, probiotics (Paraqua, Indonesia), Chitosan, and seawater.

Research Design

The experimental design of this research used a randomized block design consisting of five treatments and four replications. The treatments consisted of treatment without extract and the addition of probiotics and chitosan (PK), *M. malabathricum* extract at a concentration of 50 ppm (PKM), chitosan (PCH) at a concentration of 100 ppm, combination of 50 ppm *M. malabathricum* extract and 5 ppm probiotic (PKP), combination of 50 ppm *M. malabathricum* extract and 100 ppm chitosan (PKK).

Work Procedure

Preparation of Tank and Shrimp

The culture tanks used for larvae rearing were 16 aquariums, with a volume of 30 liters each, which were thoroughly cleaned and sterilized before the use. Aeration equipment was installed in the tanks and labelled according to the treatment tested. The test animals were 240 giant tiger prawn larvae obtained from a hatchery in Tarakan City, all of which had entered the post-larvae (PL) 10 phase. Each aquarium contained 20 giant tiger prawn larvae. Before acclimatizing the shrimp larvae, the initial sampling was conducted to measure the length and weight of the shrimp.

Shrimp Stocking and Management

The *M. malabathricum* extract was administered by dissolving the extract in an aquarium filled with 5 liters seawater (the final concentration of the extract was 50 ppm). Next, the shrimp were transferred to a tank (20 shrimp/container) and dipped for 16 hours. After 16 hours, seawater was added (last volume of 10 liters), and Chitosan (the final concentration was 100 ppm) and probiotics (the final concentration was five ppm) were added according to the research design. Probiotics were administered every three days. The larvae were maintained for 30 days and fed *Artemia salina* (20 ind/PL) three times a day namely at 06.00 a.m, 02.00 p.m, and 09.00 p.m. Ockstan (2010) stated that the standard for feeding shrimp at the post-larval stage was around 10-20 *A. salina* per shrimp. The shrimp larvae were fed three times a day in the morning, afternoon, and evening.

Study Parameters

The parameters investigated included absolute length and absolute weight growth, survival rate, specific growth rate, average daily weight, and water quality listed in Table 1.

Table 1. Parameters Measured in Shrimp Larvae Study.

Study Parameter	Formula	Reference
Absolute Length	$Lm = Lt - Lo$	
Absolute Weight	$Wm = Wt - Wm$	
Survival Rate	$SR(\%) = \frac{Nt}{No} \times 100$	
Specific growth rate (SGR)	$SGR = \frac{(\ln W_t - \ln W_0)}{t} \times 100$	Landsman *et al.* (2019)
Average Daily Gain (ADG)	$ADG = \frac{Wt - Wo}{t} \times 100$	
Water quality	Including temperature, pH, salinity, dissolved oxygen (DO), and ammonia	

Data Analysis

The research data obtained were analyzed statistically using Analysis of

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Variance (ANOVA) P <0.05 to determine the effect of *M. malabathricum* extract, chitosan, and probiotics on the growth and survival of shrimp larvae. If the result was significantly different, the further test was carried out by using the Duncan test. This statistical analysis used SPSS 26.

RESULTS AND DISCUSSIONS

Table 2 shows the growth trend of giant tiger prawns receiving different treatments during maintenance. The growth parameters included absolute weight, absolute length, SGR, and ADG. The PCH treatment demonstrated the best results (100 ppm chitosan) with an absolute weight of 0.168 g and an absolute length of 3.63 cm. The lowest results were in the control treatment at an absolute weight of 0.062 g and an absolute length of 1.93 cm. The highest survival rate was found in the combination of extract and probiotics and the combination of extract and chitosan at 67.5%. The lowest survival rate was found in the control treatment at 48.75%. There was no significant difference in the survival rate of giant tiger prawns during maintenance. Based on the ANOVA (P<0.05), there was a significant difference between the treatments on the absolute weight, total length, SGR, and ADG parameters. The PCH treatment (100 ppm chitosan) was the best based on Duncan's follow-up test.

Table 1. The giant tiger prawn growth data during the maintenance period with different treatments.

Treatment Code	Absolute weight (gr)	Absolute length (cm)	SGR	ADG (%)	SR (%)
PK	0.062 ± 0.016^{a}	1.30 ± 0.61^{a}	10.8 ± 1.17^{a}	0.002 ± 0.005^{a}	48.75 ± 4.79^{a}
PKM	0.107 ± 0.001^{ab}	1.73 ± 0.32^{b}	12.9 ± 0.68^{a}	0.004 ± 0.000^{a}	62.50 ± 2.89^{a}
PCH	0.168 ± 0.040^{d}	1.90 ± 1.63^{c}	14.4 ± 0.27^{b}	0.006 ± 0.001^{b}	62.50 ± 2.89^{a}
PKP	0.111 ± 0.002^{bc}	1.30 ± 0.22^{a}	13.1 ± 0.70^{a}	0.004 ± 0.001^{a}	67.50 ± 2.89^{a}
PKK	0.112 ± 0.001^{bc}	3.03 ± 1.32^{c}	13.1 ± 0.67^{a}	0.004 ± 0.000^{a}	67.50 ± 2.89^{a}

Description : PK (Control), PKM (Addition of 50 ppm *M. malabathricum* Extract), PCH (Addition of 100 ppm chitosan), PKP (Addition of 50 ppm *M. malabathricum* Extract and 5 ppm Probiotic), P5 (Addition of 50 ppm *M. malabathricum* Extract and 100 ppm Chitosan). n = 4. Different lowercase letters indicate significant differences between treatments. P<0.05).

This study evaluated the effect of combining *M. malabathricum* extract with chitosan and probiotics on the growth of giant tiger prawn fries. Chitosan, a natural polysaccharide derived from chitin, has been widely recognized for its immunostimulatory properties and potential to enhance growth performance in aquatic organisms, including tiger shrimp (Mohan *et al*., 2023). The mechanism by which chitosan increases weight and length growth in tiger shrimp can be attributed to its ability to enhance nutrient absorption, improve gut health, stimulate the immune system, and improve growth efficiency. Chitosan enhances intestinal lining permeability, facilitating the absorption of nutrients such as proteins, lipids, and carbohydrates, which directly contributes to increased weight gain and growth in shrimp.

Studies have demonstrated that chitosan supplementation in shrimp diets leads to higher feed conversion efficiency, as more nutrients are utilized for growth rather than being excreted (Niu *et al*., 2013). Additionally, chitosan acts as a prebiotic, promoting the growth of beneficial gut bacteria while inhibiting pathogenic microorganisms. A healthy gastrointestinal microbiota is essential for efficient digestion and nutrient assimilation. By maintaining a balanced gut environment, chitosan ensures that shrimp can derive maximum nutritional value from their feed, resulting in improved growth rates (Chen *et al*., 2022; Liang *et al*., 2020).

Chitosan is also known to activate the innate immune system of shrimp by stimulating the production of immune-related enzymes and molecules such as lysozyme, phenoloxidase, and superoxide dismutase. These enzymes play crucial roles in defending against pathogens and reducing stress-induced mortality. A stronger immune system allows shrimp to allocate more energy toward growth rather than to combat diseases, resulting in increased weight and length (Cheng *et al.*, 2021). Furthermore, chitosan possesses antioxidant properties that help to mitigate oxidative stress, which can otherwise impair growth and development in shrimp. By reducing oxidative damage to cells and tissues, chitosan supports healthier growth and development, leading to improved weight and length metrics (Niu *et al.*, 2013). Research has shown that chitosan supplementation can significantly improve shrimp growth performance. For example, studies have reported weight gain increases of 10-20% and length growth improvements of 5-15% in shrimp fed chitosan-supplemented diets compared to control groups. These improvements are attributed to the combined effects of enhanced nutrient absorption, gut health, and immune stimulation.

Chitosan's immunostimulant properties have been quantified in various studies, with shrimp fed chitosan-supplemented diets showing a 20-30% increase in lysozyme activity, which enhances bacterial defense, and a 15-25% increase in phenol oxidase activity, which improves the shrimp's ability to encapsulate and neutralize pathogens. Additionally, chitosan supplementation has been associated with a 10-20% reduction in mortality rates when shrimp are challenged with pathogenic bacteria such as *Vibrio* species (Mohan *et al.*, 2023).

The mechanism by which chitosan enhances the weight and length growth of tiger shrimp involves a combination of improved nutrient absorption, gut health modulation, immune system stimulation, and antioxidant activity. Collectively, these effects contribute to improved growth performance and survival rates, making chitosan a valuable additive in shrimp aquaculture. The quantifiable improvements in growth and immune response highlight the potential of shrimp asle and effective growth promoter in shrimp farming. Our results showed that the treatment with chitosan (PCH) provided the best results regarding absolute weight, absolute length, and Specific Growth Rate (SGR) compared to other treatments. The PCH treatment (100 ppm chitosan) resulted in an absolute weight of 0.168 ± 0.040 g and an absolute length of 3.63 ± 1.63 cm, which was significantly higher than other treatments. This result showed that Chitosan can increase giant tiger prawns' weight and length growth. Chitosan is known to have immunostimulant properties that can increase the immune response of organisms (Kamilya and Khan, 2020), ultimately leading to better growth.

The PKK treatment (50 ppm *M. malabathricum* and 100 ppm chitosan), combining *M. malabathricum* and chitosan extracts, also showed promising results. However, they did not exceed the PCH treatment in weight and length growth. These results differed from those obtained from the studies conducted by Hartinah (2020). In this study, the *M. malabathricum* extract was directly applied to the maintenance media; unlike the previous studies, the extract was given through an enrichment process on natural feed given to shrimp fry. These different results are suspected due to the complex interaction between active ingredients affecting the bioavailability and overall effectiveness (Imam *et al.*, 2021).

The PCH treatment (100 ppm chitosan) also had the highest SGR value (18.9 ± 0.27). This result indicates that adding chitosan to the maintenance media can significantly increase the daily growth rate of giant tiger prawns. The nature of chitosan as a nutrient binder is thought to contribute to the increasing feed conversion efficiency, directly affecting the SGR (Zaki *et al.*, 2015). In PKP (50 ppm *M.*

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malabathricum and 5 ppm probiotic) and PKK (50 ppm *M. malabathricum* and 100 ppm chitosan) treatments, the results were not significantly different in the specific growth rate (SGR) of shrimp fry. However, the value was higher compared to the control treatment. These results indicated that giving chitosan solely to shrimp maintenance media is more effective in increasing the growth rate than administering chitosan combined with *M. malabathricum* extract.

The interaction of a combination of bioactive compounds can generally be classified into three types: reacting synergistically, responding antagonistically, and working additively. Synergistic is when combining two or more chemical compounds work together to produce a greater effect than the single effect of the compounds individually. Using a combination of compounds can benefit the targeted biological system or produce the desired harmful effects. On the other hand, antagonism is a phenomenon when a combination of compounds produces an overall effect that is less than the additive effect of the individual compounds (Bulusu *et al*., 2016).

The highest ADG was also found in the PCH treatment, directly proportional to the SGR results and weight and length growth. The result confirms that chitosan has a robust positive effect on the daily growth of giant tiger prawns (Cheng *et al*., 2021). The Survival Rate did not demonstrate significant differences between treatments; all the treatments had relatively stable and high survival rates, with SR values ranging from 48.75 ± 4.79% to 67.50 ± 2.89%. The survival rate in the control treatment had the lowest value compared to the other treatments. The result showed that combining ingredients did not harm shrimp survival (Alam *et al*., 2019; Toledo *et al*., 2019).

Compared to the other treatments, treatment with the addition of chitosan (PCH) was shown to provide the most effective results in increasing the growth and performance of giant tiger prawns. The positive effects of chitosan on crucial growth parameters such as weight, length, and daily growth rate indicate the great potential of chitosan as a bioactive agent in shrimp cultivation. The use of chitosan in high concentrations is more strongly recommended compared to a combination of other ingredients, especially in terms of optimizing growth. The current study's results align with Rochana *et al*. (2019), who used chitosan as a feed additive and demonstrated significant results on the weight growth of giant tiger prawns, percentage of weight growth, and SGR compared to the control treatment. Similar results were also demonstrated by Ashraf *et al*. (2022), where giant tiger prawns fed chitosan experienced increased growth and SGR compared to those without chitosan-supplemented feed. In addition, the FCR value also showed that the use of chitosan could increase feed efficiency and thus increased shrimp growth. Based on the results of the ANOVA ($P<0.05$), the best treatment in this study was using chitosan to increase the growth of the giant tiger prawn.

In our study, chitosan was applied to the water. When chitosan is added directly to water, it acts as a water conditioner by binding to suspended particles, organic matter, and pathogens, thereby reducing the microbial load in the culture environment (Younes and Rinaudo, 2015). Additionally, chitosan, known for its immunostimulant and growth-promoting properties, may exert effects through interaction with the shrimp's gills and possibly exoskeleton surface, particularly when applied in nano or soluble form. While gills are the potential site for absorption, further studies are required to confirm the extent of chitosan uptake via these routes (Andriani *et al*., 2023). This dual mechanism enhances the overall health and growth performance of shrimp. However, the growth-enhancing effects of water-based chitosan are generally less pronounced compared to dietary supplementation, as it primarily influences the external environment and immune system rather than directly improves nutrient absorption or gut health.

The effectiveness of chitosan in water depends on some factors such as concentration, water quality, and exposure time. While low concentrations may have minimal effects, higher concentrations can cause stress or toxicity, and prolonged exposure is often necessary to achieve significant immune and growth benefits. Chitosan's ability to improve water quality, such as reducing ammonia and nitrite levels, indirectly supports shrimp growth and health, though its direct growth-promoting effects remain limited compared to dietary application (Ahmed *et al*., 2020).

Combining *M. malabathricum* extract with probiotics or chitosan did not show significantly different results from the treatment of chitosan and *M. malabathricum* separately; however, it was still better than the control treatment. Previous studies have shown that *M. malabathricum* extract can increase the growth of giant tiger prawns because it accelerates the moulting process. However, in this study, the results were not better than those of the treatment with chitosan. Further research is necessary to examine the duration of dipping and concentration that can affect the absorption process of secondary metabolite compounds in *M. malabathricum* extract into the body of giant tiger prawns.

The water quality parameters which were measured included temperature, salinity, DO, pH, and ammonia (Table 2). The results of the quality parameter measurements carried out every week during the study showed that they were still in accordance with the quality standards set by the Minister of Marine Affairs and Fisheries Regulation No. 75 of 2016 concerning Guidelines for the Rearing of Giant Tiger Prawn and Vannamei Shrimp (*Litopenaeus vannamei*).

Table 3. Water quality data during the maintenance period.

No.	Parameter	Concentration Range	Quality Standard
1.	Temperature (°C)	27-30	
2.	Salinity (Ppt)	13-15	
3.	pH	6.5-7	MMAF Decree No. 75 of 2016
4.	DO (mg/L)	5.1-7.2	
5.	Ammonia	0.111-0.171	

Water quality conditions are the determining factor in giant tiger prawn (*P. monodon*) cultivation. Right water quality conditions are suitable for survival, directly and indirectly, affecting the growth of giant tiger prawns. They can increase the survival rate of giant tiger prawns during maintenance. The data of water quality parameters during maintenance showed that the water condition was appropriate for maintaining the giant tiger prawn. There were no differences in water quality conditions in the different treatments, and all were within the range according to the quality standards stipulated in the MMAF Decree 75 of 2016. Therefore, in this study, water quality was not a limiting factor in maintaining giant tiger prawns with different treatments.

CONCLUSION

Chitosan, *M. malabathricum*, or a combination of *M. malabathricum* extract with chitosan and probiotics significantly influenced the growth and survival of giant tiger prawn (*P. monodon*). Supplementation with chitosan is the best treatment compared to other treatments. Further studies are required to add additional data, such as amino acid profiles, fatty acids, and histological tests, to obtain data that supports the initial hypothesis better.

CONFLICT OF INTEREST

There is no conflict of interest in this manuscript between all authors upon writing and publishing this manuscript.

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AUTHOR CONTRIBUTION

Diana Maulianawati led the research by writing and analyzing the results. Rusmiati and Ainun Hikmah contributed to the preparation and data collection in the field. Nuril Farizah played a role in data analysis and focused on writing scientific article.

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