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PHYSIOLOGICAL AND HEMATOLOGICAL RESPONSES OF WHITE SHRIMP (*Litopenaeus vannamei*) FED WITH THE ADDITION MOS PREBIOTICS (*Mannan oligosaccharides*)

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ABSTRACT:-This study aimed to determine the physiological and hematological responses of white shrimp (*Litopenaeus vannamei*) fed Manna Oligosaccharide (MOS). The experimental design in this study was a completely randomized design (RAL) consisting of four treatments with three replicates, namely treatment A feed without the addition of prebiotics (control), B feed with the addition of 1 % prebiotics, C feed with the addition of 1.5% prebiotics, and D feed with the addition of 2% prebiotics. The test animal used in this study was a white shrimp with a weight of 5-7 grams with a stocking density of 1 head/l. The results of this study showed that feeding treatment with the addition of Mannan oligosaccharide prebiotics (MOS) by 1% could increase the Total Haemocyte Count of white shrimp (76.67 x10^6 cells/ml), Differential Hemocyte Count (Granular Cells: 39.36%, Hyaline Cells: 38.62%, and Semi granular Cells 22.02%), and phagocytosis activity (69.95%).

Keywords: *Litopenaeus vannamei*, *Mannan oligosaccharides*, THC, DHC, Phagocytosis Activity

INTRODUCTION

White shrimp (*Litopenaeus vannamei*) is an aquatic organism favored by consumers and has important economic value in the local export market. Currently, white shrimp has been able to show its dominance as one of the mainstay export commodities in the world market. Based on data from the Indonesian Ministry of Maritime Affairs and Fisheries (2022), shrimp production reached 918,550.35 tons, where a decrease of 4% compared to the previous year (2021), which reached 953,177 tons with shrimp production dominated by white shrimp. The success of a cultivation business must be supported by a good environment, such as temperature, salinity, dissolved oxygen, pH, and ammonia levels, and the fulfillment of nutrients that can support the life of white shrimp. However, despite the implementation of a good cultivation system, white shrimp farmers have been unable to overcome disease attacks. Prevention can be achieved by using antibiotics. However, the use of antibiotics in aquaculture has become limited, and indiscriminate use of antibiotics in aquaculture systems has slowly triggered the development of antimicrobial resistance (Jofre *et al*., 2019). Another alternative is to increase the immune response in white shrimp.

When pathogens are present, the immune system is a physiological response mechanism that maintains homeostasis in the body. Nugroho and Firman (2018) claim that antigens activate the body's defensive mechanism against a range of illnesses when infections enter the body. The innate immune system of shrimp is made up of humoral and cellular immunological responses that cooperate to defend against microbial illnesses. Via immune cells like haemocytes, the cellular component of the innate immune response carries out a variety of immunological tasks, such as phagocytosis and apoptosis. Enzymes and non-specific components such as lections, peptides, and antimicrobial peptides (AMPs) kill pathogens in the humoral immune response by either directly destroying them or preventing their growth and spread (Petronio *et al*., 2022).

Nutrients and medications known as immunostimulants can strengthen the body's defences and boost immune system function to combat infections and illnesses (Martinus *et al*., 2019). Immunostimulants such as yeast, carbohydrate complexes, dietary factors, animal extracts, plant extracts, and manufactured medications can be used to boost the immune systems of aquatic animals (Kurniawan *et al*., 2018). Prebiotics are substances that the host is unable to digest, such as *mannan oligosaccharides* (MOS), but they have the ability to promote the development and activity of good bacteria in the gastrointestinal system (Dawood *et al*., 2017). According to Mohan *et al*. (2018), adding MOS to white shrimp feed results in a decrease in *Vibrio* spp. and an increase in *Lactobacillus* spp. Shrimps' improved microbial balance supports their immunity and digestive function. White shrimp growth and health can also be enhanced by MOS. Fish and crustaceans raised in aquaculture have demonstrated the beneficial effects of MOS (Nawaz *et al*., 2020).

White shrimp (*Litopenaeus vannamei*) have a robust immune system, and haemocytes are essential for the body's defence against infections. Shrimp immune system improvement is facilitated by phagocyte cells from haemocytes that are more active. According to Eleftherianos *et al*. (2021) haemocytes are involved in the phagocytosis, encapsulation, and melanization of invasive pathogens. According to Shangshang *et al*. (2021), the addition of MOS may improve the growth, fortify the immune systems, and increase the capacity of *L. vannamei* plants to withstand hyposalin stress. The ideal MOS prebiotic dosage, however, has yet to be discovered by researchers in order to improve the haematological and physiological responses of white shrimp and thereby boost their resilience to illness and stress.

RESEARCH METHODS

Research Design

This study used an experimental method based on a completely randomized design (RAL) with three treatment repeats. Each treatment was performed as follows:

A = Feeding without the addition of MOS prebiotics (Control)

B = Feeding with the addition of 1 % MOS prebiotics

C = Feeding with the addition of 1.5 % MOS prebiotics

D = Feeding with the addition of 2% MOS prebiotics

Test Feed Preparation

We employed *mannan oligosaccharides* (MOS)-containing prebiotic Bio-MOS to screen for *Saccharomyces cerevisiae*. Prior to usage, the prebiotics underwent microbial isolation. Using a digital scale, we measured the prebiotics in accordance with the treatment dosage. A commercial artificial feed containing 30% protein was utilized. We used the pellet-making process for commercial feed enrichment. MOS is added artificial feed at concentrations of 1%, 1.5%, and 2%, based on the treatment. Fresh water was added to the fake feed as a binder, and it was then swirled until it was all mixed in. Using a pellet molding tool, we formed the dough-forming feed, let it to air-dry for a full day, and then stored it.

Research Procedure

Twelve 35-liter basin containers were used in this investigation. Then, we sanitized and cleaned the instruments and containers. Additionally, we added salinized seawater at 29 ppt to the container, which had already undergone treatment and had a 15-liter capacity. Next, in order to provide the white shrimp with oxygen, we added an aeration pipe.

Preparation of Test Animals

The test animal was a white shrimp, or *Litopenaeus vannamei*. The shrimp, which weighed between five and seven grams each, were acquired from the BPBAP Takalar hatchery. Before stocking the white shrimp, we let them acclimate for half an hour in a fiber pond. This helped them become used to their new environments.

Test Animal Maintenance

White shrimp were maintained in a rearing container that had been set up with a stocking density of one shrimp/liter. Before feeding them, we killed the shrimp for a full day. Every day, we fed the mixed feed containing BIO-MOS into containers three times a day at 06.00, 12.00, and 18.00 WITA. We kept the shrimp alive for a period of fifteen days.

Research Parameters

a. **Total Haemocyte Count**

The total number of hemocytes in the hemolymph of white shrimp is known as the total haemocyte count, or THC. THC sampling was done at the start and finish of the investigation. Using a haemocytometer, we determined the THC after obtaining the hemolymph sample. Kurniawan *et al*. (2018) also provided a method for figuring out the THC.

b. Differential Haemocyte Count

The proportion of distinct haemocyte types is measured by the differential haemocyte count (DHC) method applied to white shrimp hemolymph. DHC sampling was done at the start and finish of the investigation. Under a 400 magnification microscope, we divided haemocyte cells into three cell types: granular, semi-granular, and hyaline cells. This allowed us to complete the DHC calculation. We counted 100 different types of haemocytes and used the algorithm (Tampangallo *et al*., 2012) to determine the percentage of each type of cell.

c. Phagocytosis Activity

The process by which some cells, including phagocytes, take up and eliminate foreign particles that enter the shrimp's body is known as phagocytosis activity. At the start and finish of the investigation, we measured the phagocytic activity. We used the following formula to calculate the microscopic observations (Suleman *et al*., 2019).

RESULTS AND DISCUSSION

Total Haemocyte Count

The haemocyte count has increased, which is significant because haemocytes are essential to shrimp immunity. The primary immune cells that fight infections and pathogens are haemocytes. In the hemolymph, a variety of protein types interact with immunological and antimicrobial peptides to provide synergistic defence against infection (Kumar *et al*., 2023). Shrimp's capacity to react to and combat the threat of disease improves along with their haemocyte count. The study's findings are shown in Figure 1, which indicates that the prebiotic *mannan-oligosaccharide* (MOS) significantly affected the total haemocytes (total haemocyte count/THC) in the diet of white shrimp (*Litopenaeus vannamei*). Treatment B with the addition of 1% MOS showed the highest increase in THC (76.66 x10^6 cells/ml) followed by treatment C (43.83 x10^6 cells/ml), D (35.67 x10^6 cells/ml), and the lowest treatment A (31.67 x10^6 cells/ml).

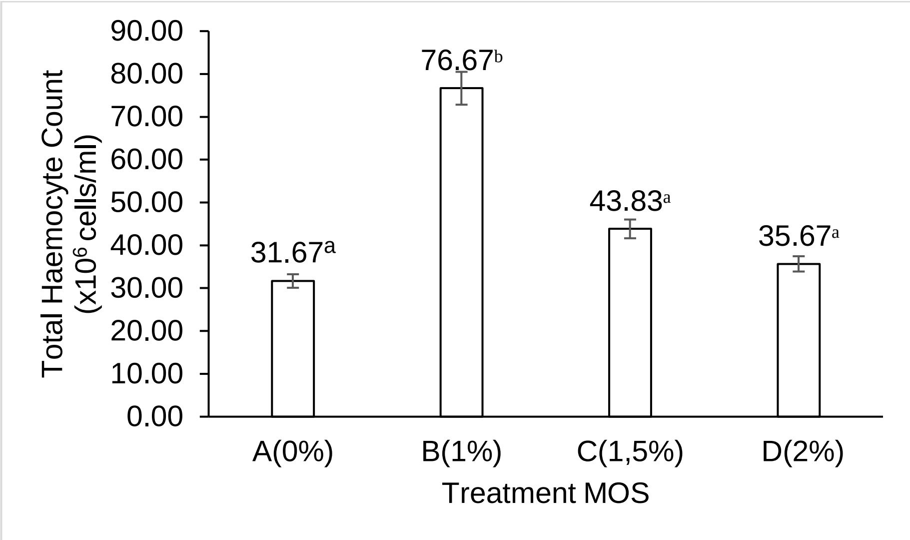

Figure 1. Total hemocyte count (THC) *L. vannamei* during maintenance (different letters on the histogram show significantly different results (P<0.05)

Prebiotics known as *manan oglycosaccharides* (MOS) are made of brief mannose chains that are extracted from the *Saccharomyces cerevisiae* yeast's cell wall. The 1% addition of MOS to this meal suggests that MOS can boost the amount of haemocytes in the immune system of white shrimp. This could be as a result of MOS boosting the micro biota in the shrimp's intestine, which boosts immunity. MOS can be used as a substrate by beneficial bacteria in the white shrimp's digestive system to preserve the microbial balance in the gut. This substrate is indigestible to white shrimp's digestive enzymes, but it can be used by bacteria non the shrimp's digestive system. Beneficial bacteria in the gut can ferment MOS, but host digestive enzymes are unable to break it down (Shang *et al*., 2018).

The same study's results were achieved by Widanarni *et al*. (2018), who discovered that the THC value of the MOS prebiotic treatment in white shrimp larvae was higher than when prebiotics were not present. Mameloco and Traifalgar (2020) also found that improved THC values were obtained with prebiotic supplementation containing 0.2% MOS and β-glucan. As to Hoseinifar *et al*. (2018), the gut microbiota is modulated by MOS prebiotics, hence potentially enhancing the growth performance and overall health of shrimp. *Lactobacillus* and *Bifid bacterium* are two beneficial bacteria that can be obtained with MOS prebiotic supplementation (Wang *et al*., 2018). Numerous beneficial effects on gut health, such as the synthesis of short-chain fatty acids and the fortification of the intestinal barrier, are attributed to these bacteria. Because gut infections must compete with one another for resources like food and space, their numbers tend to decrease as the population of beneficial bacteria increases. Better immune system performance, illness resistance, and gastrointestinal health are all aided by this for white shrimp.

Furthermore, a number of antimicrobial substances, including organic acids (lactic and acetic acids), hydrogen peroxide, reuterin, and bacteriocin, can be produced by probiotic bacteria, including lactic acid bacteria (Hasan *et al*., 2020). Because they can reduce the pH of the surrounding environment and generate an environment that is not favorable to the growth of pathogenic bacteria, bacteriocins function as antibacterial substances. Shrimp that have stronger immune systems are better able to withstand environmental stressors and use their energy more effectively for growth rather than illness prevention. Xue *et al*. (2022) report that MOS supplementation can enhance the activity and capacity of antioxidant enzymes in white shrimp. Shrimp may experience oxidative stress due to a variety of physiological and environmental reasons, which this boost in antioxidant capacity may assist them overcome.

Although not as well as treatment B, treatments C (prebiotic 1.5%) and D (prebiotic 2%) also shown an increase in THC. According to this research, the best MOS dosage should be taken into account in order to have the most immunostimulant impact. Dawood *et al*. (2018) state that optimum immunological response and growth of aquatic organisms depend on the proper dosage of prebiotics. A dose of MOS that is too high might not have any further benefits or possibly work against you, while a dose that is too low might not have enough of an impact.

Differential Haemocyte Count

The haemocyte differentiation profile of white shrimp (*Litopenaeus vannamei*) was significantly impacted by the addition of *manan-oligosaccharide* (MOS) prebiotics to their diet. The study's findings are displayed in Table 1, which highlights a shift in the proportion of hemocyte cells a crucial part of the shrimp's defensive mechanism against infections.

Table 1. Differential Haemocyte Count (DHC) *L. vannamei*

Treatment	**Differential Haemocyte Count (%)**		
	Granular	**Hyaline**	**Semi Granular**
A (control)	36.28 ± 0.494^{a}	33.07 ± 1.993^{a}	30.65 ± 1.801^{b}
B (Pre 1%)	39.36 ± 1.957^{a}	38.61 ± 1.226^{a}	22.01 ± 1.539^{a}
C (Pre 1,5%)	38.18 ± 2.432^{a}	34.83 ± 3.253^{a}	26.98 ± 5.033^{ab}
D (Pre 2%)	37.35 ± 2.383^{a}	33.20 ± 1.553^{a}	29.44 ± 2.503^{ab}

Description : different superscript letters between columns indicate significantly different results (P<0.05)

Based on the results of the research in Table 1. Granular cells showed no noticeable difference in each treatment. But the highest granular cells were obtained at treatment B (39.36%). Granular cells are crucial to the immune system of shrimp, especially when it comes to phagocytosis and pathogen encapsulation (Xing and Zhang, 2023). The granular cell count has increased, suggesting that the shrimp's defence mechanism is getting stronger. Because they contain a variety of immunological proteins and antimicrobial enzymes, granular cells are essential to the functioning of the shrimp immune system. Phagocytosis increases with the amount of granular cells. Himzanah *et al*. (2023) Shrimps use their granular cells to activate the prophenoloxidase (proPO) system in response to infections or other stimuli. The phenoloxidase (PO) system is responsible for initiating many immunological responses. According to Ardiansyah *et al*. (2023), proteins like PO are released when the proPO system is engaged. These proteins aid in the direct destruction of microorganisms as well as melanisation, coagulation, and opsonisation.

Hyalin cells in Table 1. also showed no significant difference between treatments, and the highest DHC was obtained in treatment B (38.62%). The rise in hyaline cells indicates that adding MOS may improve shrimp's ability to phagocyte, which may strengthen their resistance to illness. Hyaline cells are crucial for controlling the immune response because they are involved in phagocytosis and the synthesis of cytokines. Pathogens or foreign particles that enter the shrimp body are engulfed and destroyed via the phagocytosis process of hyaline cells (Jannah *et al*., 2018). Granular cells actively combat infections or threats from pathogens, as indicated by lower levels in granular cells compared to hyaline cells. The MOS prebiotic is administered An immunostimulant given to white shrimp enhances the release of cytokines and immunological mediators from immune cells in their intestines, which in turn increases the formation of haemocyte cells to combat pathogens that enter their bodies. Alvanou *et al*. (2023) report that crayfish feed supplemented with MOS prebiotics has beneficial effects on the fish's immune system against bacterial exposure. The first line of defence of the shrimp against pathogen invasion can be strengthened by an increase in the quantity of hyaline cells.

The results of the study on semi-granular cells (Table 1.) showed that there was a significant difference between treatment A (control) and treatment B (MOS 1%). In addition, there was a decrease in the number of semi-granular cells in the treatment with the addition of MOS prebiotics. However, there was no significant difference observed between the treatments for semi-granular cells. The decrease in semi-granular cells may possibly be attributed to a shift in cell development towards granular cells and hyaline, which are more active in the immunological response of white shrimp. Semi-granular cells are involved in the maturation of hyaline cells, according to Wangi *et al*. (2021). The hyaline cell is the first to respond to a pathogen attack, preventing the development of semi-granular cells. Consequently, there are fewer semi-granular cells in the haemocytes. MOS prebiotics are added to white shrimp diet, which not only boosts the quantity of specific immune cells but also intricately alters how those cells interact with one another. Shrimp's immunity is strengthened as a result, increasing their resistance to infections and enhancing the coordination of their immune response.

Phagocytosis Activity

A cellular defence mechanism called phagocytosis eliminates foreign objects that enter the body of a white shrimp (Takwin *et al*., 2022). The findings demonstrated that phagocytosis activity was positively impacted by the addition of *mananoligosaccharides*, or MOS prebiotics, to the diet of white shrimp (*Litopenaeus vannamei*).

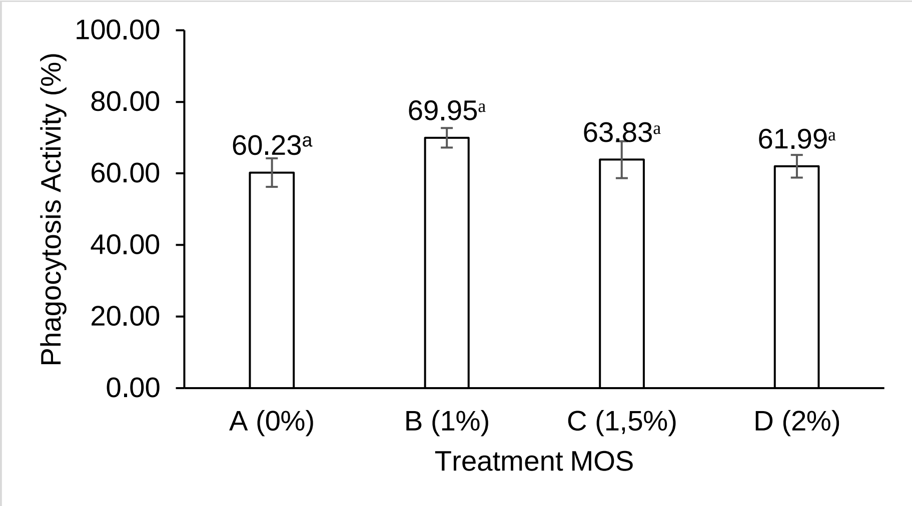

Figure 2. Phagocytosis activity of *L. vannamei* during rearing (the same letters on the histogram show no significant difference in results (P>0.05)

The immune system is being stimulated by the rise in phagocytic activity in MOS fed shrimp. Higher phagocytic activity suggests that shrimp are more resilient to possible illnesses. According to Hoseinifar *et al*., (2018), MOS can bind to phagocyte mannose receptors, which initiates a series of internal signals that activate immune cells. Increased synthesis of lytic enzymes and reactive oxygen species (ROS) is the outcome of this activation, and both are crucial for phagocytosis. The phagocytic activity of white shrimp treated with MOS prebiotics was higher than that of the control treatment, as reported by Widanarni *et al*. (2018).

Mannan oligosaccharides (MOS), short-chain prebiotics of mannose, are made by the yeast *Saccharomyces cerevisiae* from its cell wall. Shrimp's intestinal microbiota can be modulated by *mannan oligosaccharides* (MOS), which act as prebiotics to stimulate the establishment of beneficial micro biota in the digestive tract. This can enhance the health and immunity of shrimp. Ringo *et al*. (2018) claim that MOS can boost the number of good bacteria that are known to have immunomodulatory effects, like *Lactobacillus* and *Bifid bacterium*. In the course of the immunological response, these bacteria produce peptidoglycans, organic acids, bacteriocins, exopolysaccharides, and enzymes that are crucial in shielding shrimp cells from oxidative damage. This is consistent with study by Noman *et al*. (2024), who demonstrated that through immune system modulation and enhancement of the shrimp gut microbiota, prebiotic supplementation can increase the growth and survival rates, health status, and resistance to disease in shrimp.

CONCLUSION

Our conclusion, based on the study's findings, was that feeding white shrimp with 1% extra Mannan oligosaccharide prebiotic (MOS) as treatment B helped strengthen their immune system, as shown by the shrimps' THC (76.67 x 10^6 cells/ml), DHC (granular cells: 39.36%, hyaline cells: 38.62%, and semigranular cells: 22.02%), and phagocytosis activity (69.95%).

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