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THE EFFECT OF IMMUNOSTIMULANTS FROM NATURAL INGREDIENTS ON VANAMEI SHRIMP (*LITOPENAEUS VANNAMEI*) IN INCREASING NON-SPECIFIC IMMUNITY TO FIGHT DISEASE

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ABSTRACT

Vanamei shrimp Litopeneus *vannamei* is a commodity with high economic value. Shrimp is Indonesia's main export commodity. The high value of sales and production of vanamei shrimp is constrained by the many disease attacks in the vanamei shrimp farming industry. An alternative solution that has been continuously researched for several years to combat shrimp disease is using immunostimulants from natural ingredients. Using natural ingredients for immunostimulants can increase the non-specific immunity of vanamei shrimp to ward off disease and be environmentally friendly. Immunostimulants can be administered orally, by immersion, or by injection. Immunostimulants from natural ingredients such as *spirulina platensis*, polysaccharides from seaweed such as carrageenan, sodium alginate, and fucoidan, extracts from red seaweed, as well as the use of polysaccharides from the bacterium *Nodulisporium* sp. KT29, *Trichoderma* sp. able to increase the non-specific immune system in *L. vannamei shrimp.* These immunostimulants increase non-specific immunity, such as total hemocyte count, phagocytosis activity, phenoloxidase, phagocytic, respiratory burst, superoxide dismutase, and total plasma protein. Immunostimulants from these natural ingredients can also fight infectious disease attacks.

INTRODUCTION

The vannamei shrimp *Litopeneus vannamei* is a commodity with high economic value and is Indonesia's main export commodity. The export value of this commodity reaches USD 1 billion or 40.1% of the total export value (DITJEN PDSPKP, 2021). Vannamei shrimp originated from Hawaii and Florida and were later introduced to Indonesia. They are widely cultivated because they have several advantages, including fast growth and low feed conversion value, and are very suitable for cultivation in ponds with high stocking densities (Lestari et al., 2018).

Even though the export value of vanamei shrimp is high, it still has problems in the form of many disease attacks on the vanamei shrimp farming industry. Disease caused by *Vibrio* *harveyi* resulted in the death of around 80-100% vannamei shrimp (Widanarni et al., 2012). Vibriosis is a shrimp disease problem not only in Indonesia but also a global problem (Cao et al., 2015). Another disease that attacks the shrimp farming industry is *infectious myonecrosis* (IMN) which caused losses of up to 300 billion in Indonesia in 2009 (KKP, 2010). IMN disease causes the death of 40-70% of vanamei shrimp (OIE, 2009). In addition to diseases caused by bacteria, there are also diseases caused by viruses, namely the *White Spot Syndrome Virus* (WSSV), *Yellow Head Virus* (YHV), and *Taura Syndrome Virus* (TSV), which are considered the most dangerous pathogens in paneid shrimp because they cause losses. Large ones (Loh et al., 1997; Wang et al., 2007).

Many vannamei shrimp cultivators cope with disease attacks by using antibiotics (Defoirdt et al., 2011). Apart from antibiotics, some use vaccines or chemotherapy to treat diseases in vanamei shrimp but are considered ineffective (OIE, 2009). However, the use of this antibiotic raises a new problem, namely the resistance of the bacteria that attack vanamei shrimp to antibiotics, which threatens the shrimp's health and the health of the shrimp consumers.

The vanamei shrimp *L. vannamei* has a very primitive immune system compared to fish and other vertebrates (Kwang, 1996). The crustacean shrimp group only has innate or non-specific immune defense abilities and does not produce specific antibodies (Anderson, 1992). This non-specific immune system helps shrimp to fight disease. This non-specific defense can only be stimulated daily, so the stimulation should be done periodically and continuously, either by oral or immersion which is a treatment with little risk.

An alternative solution that has been continuously researched for several years to tackle vanamei shrimp disease is the use of immunostimulants from natural ingredients (Febriani, 2013). According to Sakai (1999), immunostimulants can increase non-specific immunity in shrimp. Improving the non-specific immune system is positively correlated with increasing the health of shrimp to fight disease. Using natural ingredients for immunostimulants can increase shrimp's non-specific immunity to ward off disease and be environmentally friendly (Zahra et al., 2017). Giving immunostimulants from natural ingredients to vanamei shrimp is expected to solve disease problems in vanamei shrimp by increasing non-specific shrimp immunity and being environmentally friendly.

DISCUSSION

The use of natural ingredients as immunostimulants to treat disease in *L. vannamei vanamei shrimp* has been widely studied lately. Using natural ingredients as immunostimulants are considered because they are environmentally friendly and can potentially increase non-specific immunity. Natural ingredients that are widely used come from the seaweed group (Muahiddah & Sulystyaningsih, 2021). Many groups of seaweed are used as immunostimulants from red seaweed, namely gracilaria, brown seaweed, sargassum, and laminarin. Apart from seaweed, there are also natural ingredients from herbal ingredients, namely black cumin and garlic, as immunostimulants in improving health conditions, quality of life of fish, growth and increasing immunity and enabling fish to cope with stress caused by artificial infections (Ahmed et al., 2008).

Seaweed cell walls are rich in polysaccharides which can be helpful as immunostimulants, anticoagulants, antioxidants, antibacterial, antiviral, anti-cancer, and immune-modulating activation (Wijesekara et al., 2011). Red, green, and brown seaweeds are a source of immunostimulants because they contain bioactive that produce secondary metabolites with a broad spectrum of activity and can enhance shrimp non-specific immunity

(Febriani et al., 2013). Polysaccharides from seaweed often used are carrageenan (red seaweed), fucoidan, and sodium alginate (brown seaweed). In addition to polysaccharides from seaweed, polysaccharides from bacterial walls such as *Nodulisporium* sp. KT29 and *Trichoderma* sp.

Immunostimulants can be administered orally, by immersion, or by injection. Research on determining the dose and frequency is still being carried out to determine the most effective way to use immunostimulants. The use of immunostimulants in vannamei shrimp (*L. vannamei*) in the following references:

Immunostimulants	**Method**	**Results**	**Resistant to disease**	**Reference**
Kappa-Carageenan	Oral	Total Haemocyte Count (THC) Phagocytosis Phenoloxidase Survival Rate (SR)	Infectious myonecrosis (IMN)	Febriani et al., 2013
Extract *Gracilaria verrucosa*	Oral	Total Haemocyte Count (THC) Respiratory burst (RB) Phenoloxidase Survival Rate (SR)	White spot syndrome virus (WSSV)	Zahra et al., 2017
Fucoidan	Oral	Total hemocyte count (THC) Phagocytosis (AF) activity Phagocytosis index (IF) Superoxide dismutase (SOD) activity Phenol oxidase (PO) activity Total plasma protein (TPP)	*White spot syndrome virus* (WSSV)	Setiawan, 2019
Sodium Alginate	Oral	Total hemocyte count (THC) Phagocytosis (AF) activity Phagocytosis index (IF)	-	Surur, 2018
Spirulina platensis	Oral	Total hemocyte count (THC) Differential total hemocytes (DHC) Phagocytosis (AF) activity	*Vibrio harveyi*	Ghowina, 2018
Nodulisporium sp. KT29	Oral	Respiratory burst (RB) Phenoloxidase Survival Rate (SR)	*Vibrio harveyi*	Wahjuningrum et al., 2020
Trichoderma sp.	Injection	Total hemocyte count (THC) Survival Rate (SR) Phagocytic index Phenoloxidase activity	*Vibrio harveyi*	Pebrianto, 2009

Polysaccharides from seaweed or bacterial walls can stimulate non-specific immunity, such as phagocytic activity, and respiratory burst through interaction from the receptor surface (Siregar et al., 2012). Polysaccharides increase the total hemocyte count. The total hemocyte count is a non-specific indicator of both humoral and cellular defenses. Change in total hemocytes is an indicator not only of stress but of the health status of the shrimp. In addition, hemocytes are involved in synthesizing and releasing essential molecules such as α-2-macroglobulin (α2M), agglutinins, and antibacterial peptides as a reaction of the body's defense in shrimp (Rodriguez & Moullac, 2000). Total hemocytes are closely related to proPO stimulation to induce phenoloxidase activity. Phenoloxidase activity indicates the ability of vannamei shrimp to recognize foreign bodies that enter the body (Garcia-Carreno et al., 2008) and the activity of the shrimp's body defense (Costa et al., 2009).

Hemocytes play an essential role in the immune system of shrimp. First, hemocytes can remove foreign particles or disease in the hemocoel through phagocytosis, encapsulation, and nodular aggregation mechanisms (Soderhall & Cerenius, 1992). Both hemocytes in shrimp play a role in wound healing through cellular aggregation and initiation of the coagulation process through the release of factors required for plasma gelation (Johansson & Soderhall, 1989; Omori et al., 1989; Vargas-Albores et al., 1998), and the release of prophenoloxidase proPO system (Johansson and Soderhall, 1989 Hernandez-Lopez et al., 1996).

Melanization is essential to the shrimp's immune defense system against pathogens. Phenoloxidase is an enzyme that plays a role in the process of melanization. This enzyme is produced through the proPO (prophenoloxidase) system, which is activated in the presence of an immunostimulant. ProPO plays an essential role in introducing foreign bodies, including phagocytosis, melanization, production of cytotoxic reactants, particle encapsulation, and formation of nodules and capsules (Amparyup et al., 2013). Activation of the proPO system induces the production of melanin, a brown pigment responsible for various processes, such as activation of foreign particles, protecting their distribution in the host's body, and repairing cuticle damage.

Phagocytosis is the most common response in cell protection. Through phagocytosis, the cellular immune response in shrimp responds to foreign bodies. Advantages in phagocytosis activity in shrimp also occur in several post-challenge test studies as a shrimp defense mechanism (Jasmanidar, 2009; Febriani et al., 2013). During phagocytosis, particles or microorganisms enter the cell and form digestive vacuoles called phagosomes. The mechanism of particle removal by phagocytic cells, called the respiratory burst, involves the release of degradative enzymes into the phagosome (an oxygen-dependent killing mechanism) and the production of ROI (reactive oxygen intermediates) (Rodriguez & Moullac, 2000). An increase in the respiratory burst is correlated with an increase in phagocytosis activity and vice versa (Jasmanindar, 2009; Sirirustananun et al., 2011). During phagocytosis, viral particles are recognized by receptors on the cell surface and are engulfed by cells that re-prepare the cytoskeleton for phagosome formation. In the first stage, phagosomes undergo maturation through cleavage and fusion with lysosomes and become mature α phage-lysosomes. Viruses in phage-lysosomes will be destroyed by low pH conditions, hydrolysis processes, and radicals (Xu et al., 2014).

Wongprasert et al. (2013) stated that sulfated galactan could stimulate the shrimp immune system, possibly mediated by the interaction between sulfated galactan and receptors on the hemocyte surface. The existence of a relationship between galactan sulfate and these receptors will activate the signaling process of increased hemocyte proliferation and stimulation of immune system activity (Zahra et al., 2017).

Li and Xiang (2013) stated that shrimp receptors, namely pattern recognition receptors (PRRs), play an essential role in the shrimp immune system and consist of lipopolysaccharide, -1,3-glucan binding protein (LGBP), and toll receptors. Pathogen recognition by PRRs is triggered by activation via the serine protease pathway and finally cleaved to proPO to produce phenoloxidase. Galactan sulfate in *Gracilaria verrucosa extract* containing 1,3-glucan interacts with LGBP in the hemocyte membrane, which activates the phenoloxidase enzyme. Other receptors that play an essential role in the shrimp immune system are toll-like receptors (TLRs).

CONCLUSION

Immunostimulants from natural ingredients such as *spirulina platensis*, polysaccharides from seaweed such as carrageenan, sodium alginate, and fucoidan, extracts from red seaweed, as well as the use of polysaccharides from the bacterium *Nodulisporium* sp. KT29, *Trichoderma* sp. able to increase the non-specific immune system in *Litopeneus vannamei shrimp.* These immunostimulants increase non-specific immunity, such as total hemocyte count, phagocytosis activity, phenoloxidase, phagocytic, respiratory burst, superoxide dismutase, and total plasma protein. Immunostimulants from these natural ingredients can also fight infectious myonecrosis (IMN), *White spot syndrome virus* (WSSV), and *Vibrio harveyi bacteria* by increasing the survival rate of vannamei shrimp that are not given immunostimulants.

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