Application of kappa-carrageenan as immunostimulant agent in non-specific defense system of vannamei shrimp

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Abstract. The burden of disease due to the Infectious Myo Necrosis Virus (IMNV) which mostly attacks shrimp cultures, is faced by shrimp farmers in Indonesia, especially in West Nusa Tenggara. Meanwhile, using ineffective antibiotics to overcome diseases is no longer considered as an acceptable strategy. The use of immunostimulants can be an alternative to treat the IMNV disease. The kappa-carrageenan is a natural ingredient made from seaweed, which contains polysaccharides composed of polyanionic galactose sulfate units produced from *Kappaphycus alvarezii* seaweed. This study aimed to evaluate the effectiveness of kappa-carrageenan mixed in different doses of feed in increasing the immune response of white vannamei shrimp infected with IMNV. This research was conducted for 60 days with an experimental method in the form of a Completely Randomized Design (CRD), consisting of 5 treatments and 3 replications, namely, P1 (positive control): commercial feed + IMNV infection; P2 (negative control): commercial feed + NaCl placebo (0.9%); P3: 15 g kg^{-1} k-carrageenan + commercial feed + IMNV infection; P4: 20 g kg^{-1} k-carrageenan + commercial feed + IMNV infection; P5: 25 g kg^{-1} k-carrageenan + commercial feed + IMNV infection. The research parameters observed in this study included the vannamei shrimp survival rate and immune response. This study showed that adding k-carrageenan 20 g kg^{-1} to the feed affected the survival rate and immune system of vannamei shrimp infected with IMNV.
Key Words: antibacterial, k-carrageenan, *Litopenaues vannamei*, IMNV, immune system.

Introduction. The disease that has most frequently attacked white shrimp in recent years is the Infectious Myonecrosis Virus (IMNV) or better known as myovirus. Melo et al (2014) stated that IMNV first appeared and was reported in 2002, in Brazil. Meanwhile, in Indonesia, the first case of IMNV was found in Situbondo, East Java, in 2006 (Taukhid & Nur'aini 2009). The IMNV samples of field isolates from Situbondo and Lampung showed that both had genome similarities up to 99% with the Brazilian isolates (Widowati et al 2012). Besides and the clinical symptoms were also similar (Prasad et al 2017).

Clinical symptoms of IMNV are tissue damage and the presence of white color in skeletal muscles, red color at the base of the tail or the last segment of the shrimp's abdomen, and causes the infected shrimp to become weak (Sukenda & Sari 2011). Furthermore, vannamei shrimp (*Litopenaeus vannamei*) will die gradually at the bottom of the pond. The disease can spread widely if there is no early monitoring. Naim et al (2014) suggested that IMNV transmission can occur horizontally due to cannibalism and through water, while the vertical transmission is thought to occur from broodstock to fry. IMNV has several distinctive and specific characteristics. The virus will be lethal when there is a sudden change in water quality parameters. Jha et al (2021) stated that shrimp muscle's distinctive white and red colors first appeared in the second and third segments, then in the fifth and sixth segments, with the most active metabolic rate. IMNV infection prevents the oxygen supply, which causes tissue death and turning white and red.

One of the efforts to prevent IMNV attacks on shrimp farming that do not impact consumer health and the environment is by administering immunostimulants from natural, environmentally friendly ingredients. Immunostimulants are the safest alternative to improve the function of the immune system by using natural ingredients that can induce the appropriate immune response (Sivasankar et al 2015). One of the natural ingredients that can be used as an immunostimulant compound that is safe and environmentally friendly is carrageenan from seaweed.

As a tropical country, Indonesia has vast waters with abundant seaweed and is even intensively cultivated. Among these seaweeds, the most widely cultivated is *Kappaphycus alvarezii*. Cheng et al (2008) stated that Kappa k-carrageenan was extracted from *Eucheuma cottonni*. Carrageenan is a family of linear sulfated polysaccharides extracted from red seaweed and is available in powder form and various other forms (Parmar et al 2012). Sulfate polysaccharides in carrageenan have beneficial bioactive compounds with anticoagulant, antiviral, antioxidant, anticancer, and immune-modulating activation properties (Wijesekara et al 2011). Studies reported its ability to increase the phagocytic activity of *Cyprinus carpio* carp and also its resistance to *Edwardsiella tarda,* and *Aeromonas hydrophila*, via intraperitoneal injection (Murthy 2017). In addition, carrageenan or sodium alginate has been reported to enhance the innate immune response of grouper *Epinephelus coioides* and its resistance to *Vibrio alginolyticus*, through injection techniques (Cheng et al 2007).

This research has been carried out previously by Febriani et al (2014), by administering kappa-carrageenan at a dose of 15 g kg^{-1} of feed, producing a high immune response. However, the optimal dose of kappa-carrageenan that vannamei shrimp infected with IMNV can tolerate needs to be determined. Therefore, it was necessary to research the effect of different doses of kappa-carrageenan supplementation on the immune system of IMNV-infected vannamei shrimp.

Material and Method

Description of the study sites. Maintenance, treatment, and analysis of vannamei shrimp immune parameters were performed at the Fish Health Laboratory, Department of Fisheries and Marine Sciences, Faculty of Agriculture, University of Mataram (Agreement number: 9289/UN18/HK/2021), West Nusa Tenggara, Indonesia.

Material. *L. vannamei* PL-10 specimens, imported from the hatchery of PT Talenta Mandiri Sumbawa, were fed with commercial pellets of the Deus Ruby brand mixed with (κ)-carrageenan flour extracted from *K. alvarezii* in the Sekotong Tissue Culture Laboratory, West Lombok. The virus used was Infectious Myo Necrosis Virus (IMNV) obtained from infected live shrimp and it was administered orally. *Staphylococcus aureus* NCTC 8532 [IAM 12544; R. Hugh 2605] for phagocytosis activity. The used disinfectant material consisted of Ca-hypochlorite (chlorine) and 70% alcohol. Other chemicals used to measure the immune parameters are: methanol, Giemsa dye, NaCl, and anti-coagulant (Trisodium citrate 1.765 g and Sodium chloride 3.978 g)., EDTA 0.744 g, Aquades (200 mL), trypticase soy agar (TSA) medium (Microbiology GranuCult, Merck Germany) and thiosulfate citrate bile salt sucrose medium (TCBS; Microbiology; Merck; Germany).

Ethical approval. All animal experimentation and rearing were handled according to the animal welfare procedures, under the national accreditation no. SNI 7311:2009 of the Republic of Indonesia.

Kappa-carrageenan and feed preparation. Kappa-carrageenan flour, mixed with commercial shrimp feed with a protein content of 40%, was first weighed based on predetermined doses (15, 20 and 25 g kg^{-1} feed). Each kappa-carrageenan portion that has been weighed was dissolved in a small quantity of water, then mixed evenly with the commercial feed and air-dried at room temperature. After the feed was dry, it was coated with egg white and air-dried again at room temperature. The ready-to-eat feed was put in a plastic container and stored in the refrigerator until it was ready to be used. Shrimp are fed at 5-7% of their biomass weight per container. Feeding is done 4 times daily, namely at 07.00, 12.00, 17.00 and 22.00 WITA, with doses of 30, 20, 20 and 30%.

Research procedure. The research method used was an experimental method using a completely randomized design (CRD) with 5 treatments, consisting of 2 treatments as

controls and 3 treatments with the addition of different carrageenan doses in the feed, as follows:
P 1 : Commercial feed + IMNV infection (positive control)
P 2 : Commercial feed + 0.9% NaCl placebo (Negative control)
P 3 : 15 g Commercial feed + Kappa-carrageenan + IMNV infection
P 4 : 20 g Commercial feed + Kappa-carrageenan + IMNV infection
P 5 : 25 g Commercial feed + Kappa-carrageenan + IMNV infection
Each treatment was carried out with 3 replications to form 15 experimental units.

IMNV infection procedure. IMNV infection refers to the study of Febriani (2014), with an oral application. The IMNV used are from shrimp that have been infected and showed clinical signs of red muscle in the last segment. First, the prawns were cleaned from the head, carapace, and tail so that the muscle or flesh of the shrimp is obtained as a whole, then crushed and homogenized. After that, it was weighed according to the needs, and the infected shrimp muscles were given to the experimental shrimp specimens as feed, three times a day, at 10% of their biomass, then the shrimp were reared for 10 days.

Data analysis. All data were tabulated in Microsoft Excel 365, and one-way ANOVA followed by Duncan's Multiple Distance Test (DMRT) (P=0.05) were performed with the SPSS v. 22.0 software (SPSS Inc., USA).

Results. The survival rate of the vannamei shrimp after 60 days of rearing with commercial feed on various treatments and added k-carrageenan ranged from 58-84% (Figure 1).

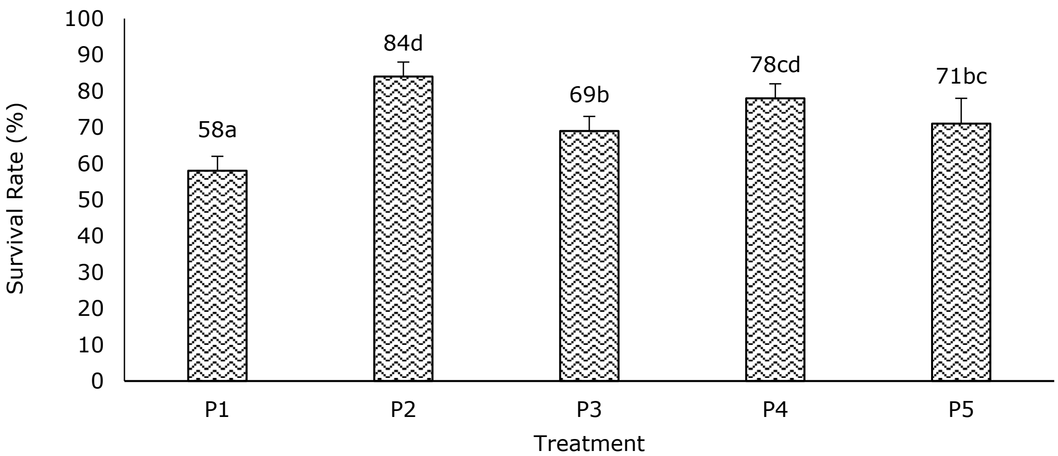

Figure 1. Survival rate: P 1 (Positive control); P2 (Negative control); P3 (15 g kg^{-1} k-carrageenan); P4 (20 g kg^{-1} k-carrageenan); P5 (25 g kg^{-1} k-carrageenan).

The results of the analysis of variance (ANOVA) showed that the addition of k-carrageenan to feed had a significant effect ($P<0.05$) on the survival rate of vannamei shrimp infected with IMNV. The results were significantly different, namely: P1 (positive control) 58%, P2 (negative control) 84%, P3 69%, P4 78% and P5 71%. P2 (negative control) showed the highest survival rate, which was not significantly different from P4, but significantly different from P1, P3 and P5. Meanwhile, P3 showed significantly different results from P4 but not significantly different from P5.

Total haemocyte count. The mean total haemocyte count of shrimp after 60 days of rearing with commercial pellet feed in various treatments and added k-carrageenan ranged from 15.14×106 cells mL^{-1} to 24.04×106 cells mL^{-1} (Figure 2).

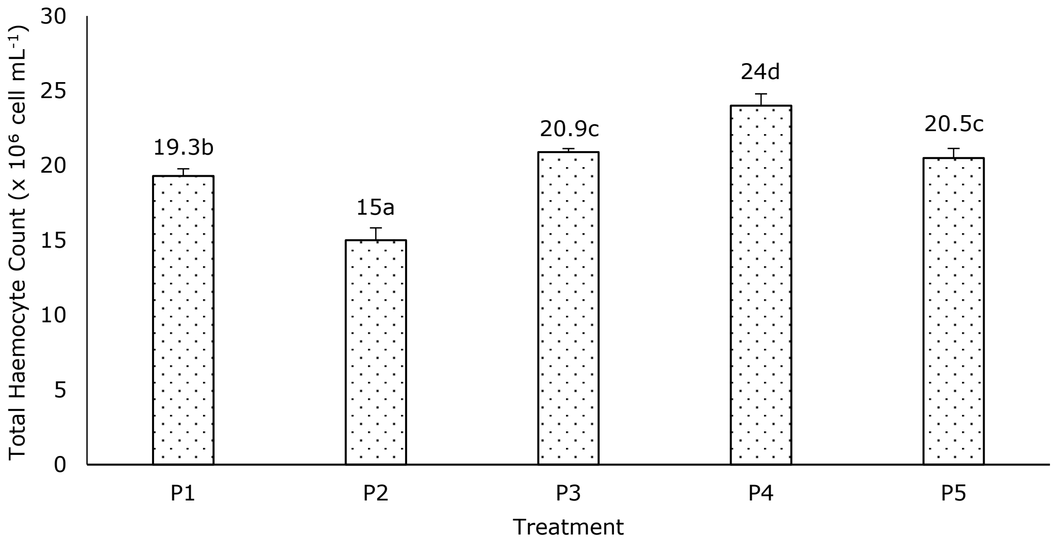

Figure 2. Total haemocyte count: P 1 (Positive control); P2 (Negative control); P3 (15 g kg^{-1} k-carrageenan); P4 (20 g kg^{-1} k-carrageenan); P5 (25 g kg^{-1} k-carrageenan).

The results of the analysis of variance (ANOVA) showed that the addition of k-carrageenan to feed had a significant effect ($P<0.05$) on the total haemocyte count of vannamei shrimp infected with IMNV. In P2 (negative control), the results differed significantly from all treatments. The highest total number of hemocytes was observed for P4. The results obtained for P1 (positive control) were 19.3×106 cells mL^{-1}, while in P2 (negative control) the value was lower, namely 15×106 cells mL^{-1}. All treatments showed higher total haemocyte count values (20.9× 106 and 20.5×106 cells mL^{-1}, in P3 and P5, respectively), but in P4 it was the highest 24×106 cells mL^{-1}.

Differential haemocyte count. The mean differential haemocyte count of the vannamei shrimp after 60 days ofrearing on various treatments with added k-carrageenan mixed with commercial feed and (Figure 3).

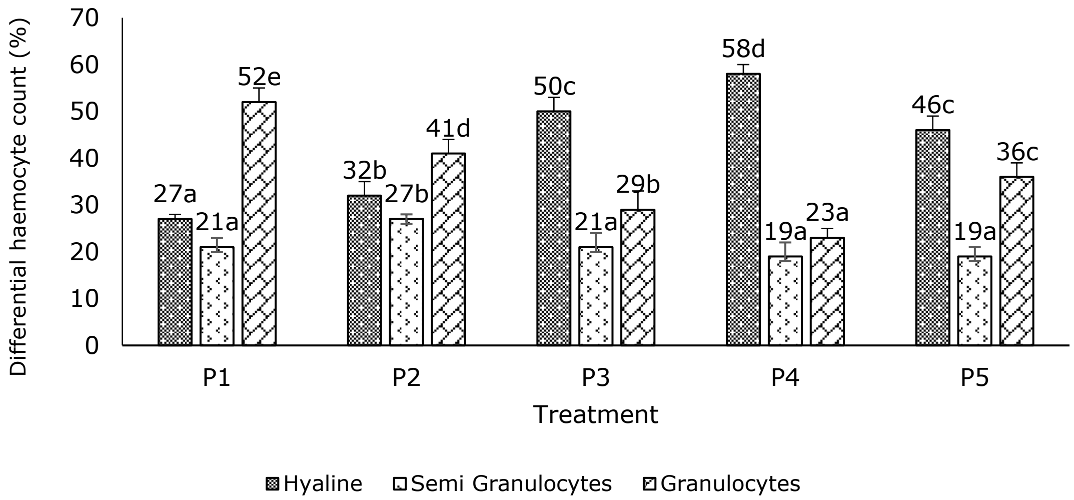

Figure 3. Differential hemocyte count: P 1 (Positive control); P2 (Negative control); P3 (15 g kg^{-1} k-carrageenan); P4 (20 g kg^{-1} k-carrageenan); P5 (25 g kg^{-1} k-carrageenan).

The results of the analysis of variance (ANOVA) showed that the addition of k-carrageenan to feed had a significant effect ($P<0.05$) on the differential haemocyte count of vannamei shrimp infected with IMNV. Hemocytes in shrimp consist of hyaline cells, semi-granulocytes, and granulocytes. The hyaline cells count significantly differred between P1 (positive control) 27% and P2 (negative control) 32% versus P3 50%, P4 58% and P5

46%. The semi-granulocytes count for P1 (positive control) 21% was significantly different from P2 (negative control) 27% and P3 21% but not significantly different from P4 19% and P5 19%. Meanwhile, the granulocyte count showed significantly different results between P1 (positive control) 52% and P2 (negative control) 41%, P3 29%, P4 23% and P5 36%.

Phagocytic activity. The phagocytic activity of vannamei shrimp, after being reared for 60 days under various treatments with commercial feed mixed with k-carrageenan, ranged between 39 and 81% (Figure 4).

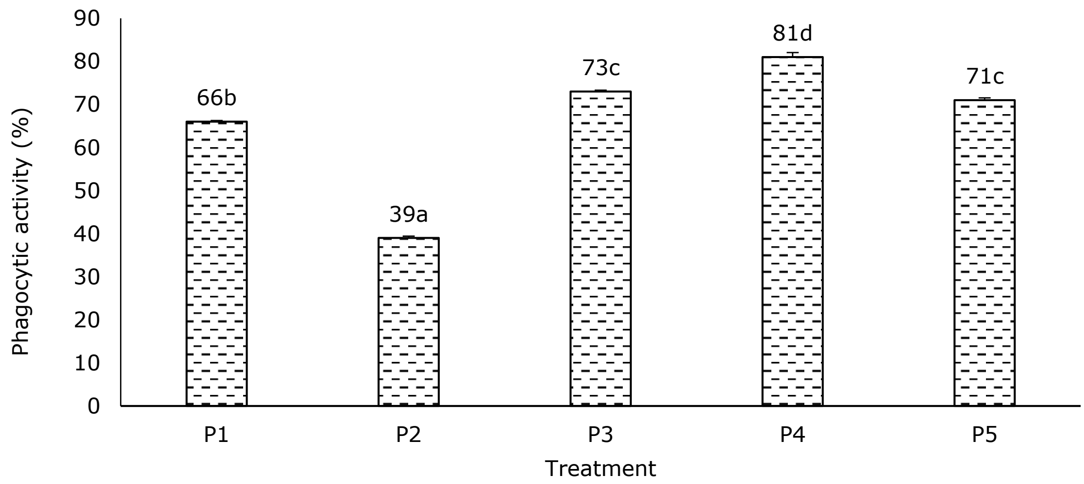

Figure 4. Phagocytic activity: P 1 (Positive control); P2 (Negative control); P3 (15 g kg^{-1} k-carrageenan); P4 (20 g kg^{-1} k-carrageenan); P5 (25 g kg^{-1} k-carrageenan).

The results of the analysis of variance (ANOVA) showed that the addition of k-carrageenan to the feed had a significant effect ($P<0.05$) on the phagocytic activity of vannamei shrimp infected with IMNV. In P1 (positive control), the results differed significantly from all treatments. In P1 (positive control), the value is 66%, while in P2 (negative control) it has a lower value, of 39%. In added k-carrageenan treatments, the phagocytic activity's pattern ressembled to the other fitness indicators: P3 73% and P5 71%, with the highest value in P4 81%.

Total bacteria count. The total gut bacteria, measured as the optical density (nm), of the vannamei shrimp after 60 days of rearing with commercial feed on various treatments, without and with added k-carrageenan, ranged between 2.22-3.8 nm (Figure 5).

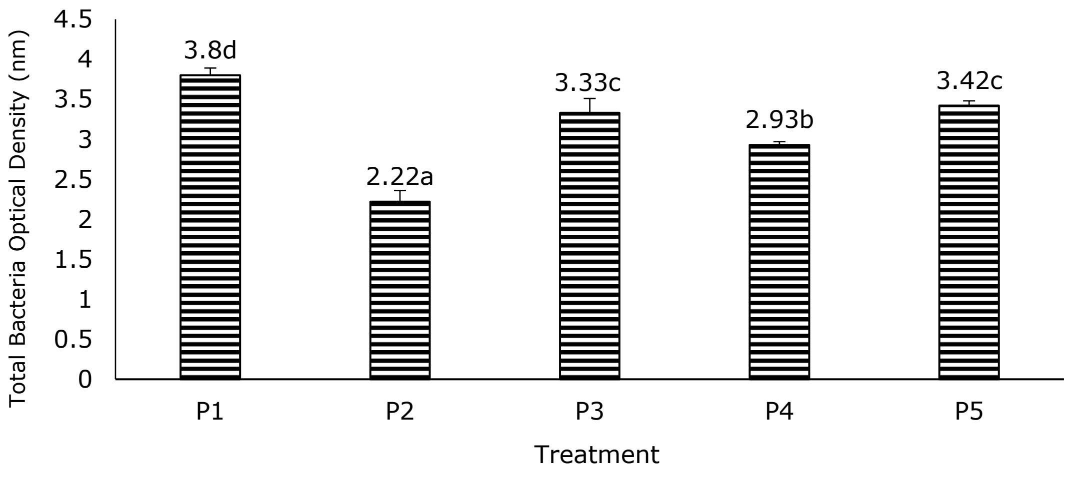

Figure 5. Total intestinal bacteria: P 1 (Positive control); P2 (Negative control); P3 (15 g kg^{-1} k-carrageenan); P4 (20 g kg^{-1} k-carrageenan); P5 (25 g kg^{-1} k-carrageenan).

The results of the analysis of variance (ANOVA) showed that the addition of k-carrageenan to the feed had a significant effect (P<0.05) on the total gut bacteria of white vannamei shrimp. The results of P1 (positive control) 2.8 nm were significantly different from all treatments. However, although between P3 3.33 nm and P5 3.42 nm there was no significant difference, the total gut bacteria count in the mentioned treatments was significantly different from P2 2.22 nm and P4 2.93 nm. In P2 (negative control), a physiological solution of NaCl was injected with a dose of 0.1 mL, compared with other treatments which were infected with IMNV, which demonstrated that shrimp mortality was indeed affected by IMNV infection.

Total vibro bacteria count. The total vibrio in the vannamei shrimp gut, measured as the optical density (nm), after being reared for 60 days with commercial feed given through various treatments with added k-carrageenan, was 2.12-3.72 nm (Figure 6).

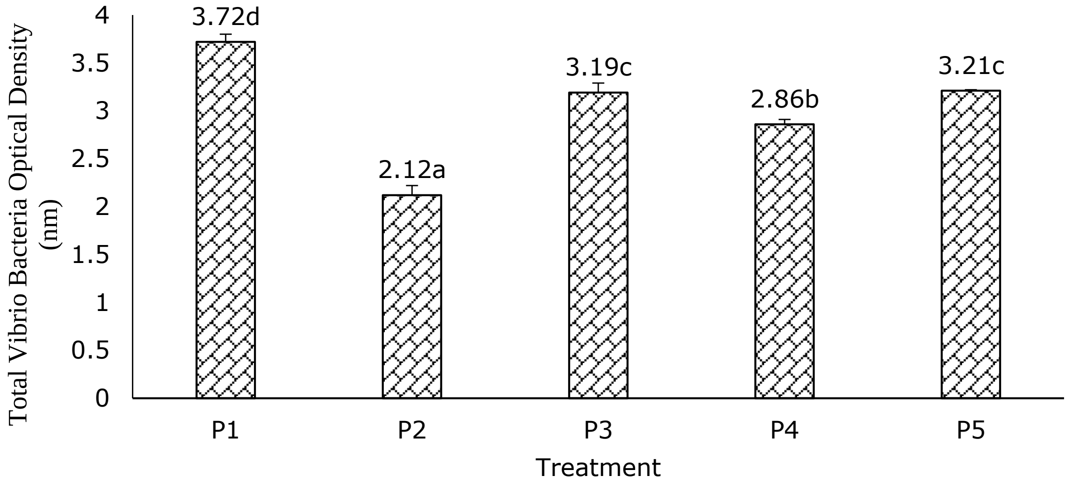

Figure 6. Total vibrio bacteria: P 1 (Positive control); P2 (Negative control); P3 (15 g kg^{-1} k-carrageenan); P4 (20 g kg^{-1} k-carrageenan); P5 (25 g kg^{-1} k-carrageenan).

The results of the analysis of variance (ANOVA) showed that the addition of k-carrageenan to the feed had a significant effect (P<0.05) on the total vibrio in the vannamei shrimp intestine. For P1 (positive control) 3.72 nm, the results are significantly different from all treatments: P2 (negative control) 2.12 nm, P3 3.19 nm, P4 2.86 nm and P5 3.21 nm.

Discussion. The low survival rate at P5, with the administration of 25 g kg^{-1} of k-carrageenan, indicated that the administration of 20 g kg^{-1} k-carrageenan was the optimum dose that white vannamei shrimp could tolerate. Consequently, it appears that an increase in total hemocytes after adding k-carrageenan provides protection for white vannamei shrimp after infection with IMNV. The mechanism of k-carrageenan in enhancing the immune system in shrimp is still being studied. Cheng et al (2008) stated that phagocytic activity and respiratory burst increased in vannamei shrimp treated with carrageenan, indicating the role of carrageenan receptors on hemocytes.

Increased body immunity and wound healing in infection are ways for shrimp to respond to pathogens (Wachid et al 2022). In this study, the survival rate of vannamei shrimp infected with IMNV and treated with k-carrageenan showed better results than for the specimens infected with IMNV and not treeated with k-carrageenan. This shows that the administration of immunostimulants in the form of k-carrageenan can be applied to maintain the survival rate of white vannamei shrimp.

Shrimp rely solely on the innate immune system, which may have some form of adaptive immunity in defense against pathogens. Pope et al (2011) revealed that in the shrimp immune system, hemocytes are the most involved in cellular immune reactions such as phagocytosis, nodule formation, and encapsulation, as well as humoral immunity reactions such as the prophenoloxidase (proPO) cascade and the release of antimicrobial peptides and lysozyme for self-protection. In this study, adding k-carrageenan to the vannamei shrimp feed significantly increased the hemocyte value after IMNV infection. Smith et al (2003) revealed that hemocytes perform inflammatory reactions such as phagocytosis, agglutination, production of reactive oxygen metabolites, and release of microbicidal proteins.

Hemocyte cell differentiation consists of hyaline cells, semi-granulocytes, and granulocytes that have their respective functions in hemocyte circulation. The percentage of hyaline cells in this study reached 27-58% of the total hemocytes. Hyaline cells treated with k-carrageenan in this study showed better results than those not treated with k-carrageenan. Smith et al (2003) stated that hyaline cells significantly increase phagocytic cells against pathogens attack. Semigranulocyte cells are described as cells that have several small granules whose function is recognizing and responding to foreign molecules or pathogens that enter the shrimp body. At the same time, granular cells are described as cells with many granules whose function is storing and releasing the proPO system and as cytotoxicity together with semigranular cells (Smith et al 2003). The proPO system produces phenoloxidase, and the presence of immunostimulants will activate the phenoloxidase activity.

Increased phagocytic activity may indicate that adding k-carrageenan to feed can improve the shrimp's immune system. The entry of pathogens into the shrimp’s body will be recognized by plasma and these will be bound. There is a response in granular and semigranular cells by releasing an activated proPO system, and hyaline cells will stimulate phagocytosis and encapsulation by semigranular cells. In the process of phagocytosis, respiratory bursts (RBs) occur via activation of NADPH-oxidase, which in turn causes the release of superoxide anions and other reactive oxygen species (ROS) (Bogdan et al 2000). Several parameters, such as hemocyte count, phenol oxidase activity, respiratory burst, lysozyme activity, and phagocytosis, were considered for shrimp health evaluation.

From the results of research conducted by Zhu et al (2017), it was found that oxidized k-carrageenan can damage bacterial cell walls and cytoplasmic membranes, which effectively suppresses bacterial growth. The treatment with the addition of k-carrageenan showed better results or could suppress the growth of vibrio bacteria in the shrimp intestines compared to the treatment without the addition of k-carrageenan in the feed. In addition, the results of Junior's study found that the antibacterial activity of N-alkyl-kappa-carrageenan derivatives, compared with the N-alkyl-(1-deoxylactitol-1-yl)-amines using the microdilution test, showed higher antibacterial activity.

Conclusions. Based on the results of this study, the addition of 20 g kg^{-1} of k-carrageenan had a significant effect on improving the immune system of the vannamei shrimp infected with IMNV, with the following results: survival rate 78%, total haemocyte count 24.04×106 cells mL^{-1}, differential haemocyte count 58%, semi-granulocytes 19%, granulocytes 23%, phagocytic activity 81%, total bacteria count 2.93 nm and total Vibro bacteria count 2.86 nm.

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Conflict of interest. The authors declare no conflict of interest.

References

Bogdan C., Röllinghoff M., Diefenbach A., 2000 Reactive oxygen and reactive nitrogen intermediates in innate and specific immunity. Current Opinion in Immunology 12(1):64–76.

Cheng A. C., Chen Y. Y., Chen J. C., 2008 Dietary administration of sodium alginate and κ-carrageenan enhances the innate immune response of brown-marbled grouper epinephelus fuscoguttatus and its resistance against vibrio alginolyticus. Veterinary Immunology and Immunopathology 121(3–4):206–215.

Cheng A., Tu C., Chen Y., Nan F., Chen J., 2007 The immunostimulatory effects of sodium

alginate and iota-carrageenan on orange-spotted grouper epinephelus coicoides and its resistance against vibrio alginolyticus. Fish & Shellfish Immunology 22(3):197–205.
Febriani D., Sukenda, Nuryati S., 2014 Kappa-carrageenan as immunostimulant to control Infectious Myonecrosis (IMN) disease in white shrimp *Litopenaeus vannamei*. Journal of Indonesian Aquaculture 12(1):70.
Jha R. K., Babikian H., Srisombat S., 2021 Managing Infectious Myonecrosis Virus (IMNV) in vannamei shrimp culture: learning by doing. International Journal of Fisheries and Aquatic Studies 9(1):385–391.
Melo M. V. C., Guedes M. I. F., Malaga S. R., Alameida L. M. D., Moreira M. D. F., Oliveira T. R., 2014 Molecular characterization of Infectious Myonecrosis Virus (IMNV) isolated from the shrimp *Litopenaeus vannamei* farmed in ceará state, Brazil. Latin American Journal of Aquatic 42(3):649-652.
Murthy H. S., 2017 Effect of dietary administration of carrageenan on growth, survival and feed utilization of common carp, *Cyprinus carpio*. Journal of Aquaculture & Marine Biology 5(1).
Naim S., Brown J. K., Nibert M. L., 2014 Genetic diversification of penaeid shrimp infectious myonecrosis virus between Indonesia and Brazil. Virus Research 189:97–105.
Parmar P. V., Murthy H. S., Tejpal C. S., Naveenkumar B. T., 2012 Effect of brewers yeast on immune response of giant freshwater prawn, macrobrachium rosenbergii, and its resistance to white muscle disease. Aquaculture International 20(5):951-964.
Pope E. C., Powell A., Roberts E. C., Shields R. J., Wardle R., Rowley A. F., 2011 Enhanced cellular immunity in shrimp (*Litopenaeus vannamei*) after vaccination. Plos One 6(6):E20960.
Prasad K. P., Shyam K., Banu H., Jeena K., Krishnan R., 2017 Infectious Myonecrosis Virus (IMNV) an alarming viral pathogen to penaeid shrimps. Aquaculture 477:99–105.
Sivasankar P., Santhiya A. V. A., Kanaga V., 2015 Review on plants and herbal extracts against viral diseases in aquaculture. Journal of Medicinal Plants Studies 3(2):75-79.
Smith V. J., Brown J. H., Hauton C., 2003 Immunostimulation in crustaceans: does it really protect against infection fish & shellfish. Immunology 15(1):71–90.
Sukenda N., Sari I. R., 2011 Administration of meniran phyllanthus niruri for the prevention of IMMUNV infection (Infectious Myonecrosis Virus) in *Litopenaeus vannamei* shrimp. Journal of Indonesian Aquaculture 10(2):192–202.
Taukhid, Nur'aini Y. L., 2009 Infectious Myonecrosis Virus (IMNV) in pasific white shrimp (*Litopenaeus vannamei*) in Indonesia. The Israeli Journal of Aquaculture-Bamidgeh 6(1):255–262.
Wachid B. A. A., Setyowati D. N., Azhar F., 2022 Effectiveness of meniran leaf extract (*Phyllanthus niruri* L.) as immunostimulant in vannamei shrimp (*Litopenaeus vannamei*) against vibriosis disease. Journal of Aquaculture and Fish Health 11(2):182–192.
Widowati Z., Firma F., Rustian D., Silaban M. T. W., Riatmo F., Astuti S. T., Wuryastuti H., 2012 The development and application of reverse transcription loop-mediated isothermal amplification for the diagnosis of infectious myonecrosis virus in the white shrimp (*Litopenaeus vannamei*). Jurnal Sain Veteriner 30(2).
Wijesekara I., Pangestuti R., Kim S. K., 2011 Biological activities and potential health benefits of sulfated polysaccharides derived from marine algae. Carbohydrate Polymers 84(1):14–21.
Zhu M., Ge L., Lyu Y., Zi Y., Li X., Li D., Mu C., 2017 Preparation, characterization and antibacterial activity of oxidized κ-carrageenan. Carbohydrate Polymers 174:1051–1058.

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