Journal of Fish Health
Volume 5, Issue 1, February 2025
https://doi.org/10.29303/jfh.v5i1.6358

Efficiency of Using Kappa-Carrageenan as an Immunostimulant Agent in the Non-Specific Immune System of Vannamei (*Litopenaeus vannamei*) Infected with AHPND (Acute Hepatopancreatic Necrosis Disease)

Fariq Azhar[1*], Andre Rachmat Scabra[1], Nuri Muahiddah[1], Rangga Idris Affandi[1]

[1]Aquaculture Study Program, Department of Fisheries and Marine Science, Faculty of Agriculture, University of Mataram, Mataram, Indonesia
Jl. Pendidikan No. 37 Mataram, NTB

Correspondence:
fariqazhar@unram.ac.id

Received:
January 9th, 2025
Accepted:
February 14th, 2025

Keywords:
AHPND, *Kappaphycus alvarezii*, Kappa-carrageenan, Vannamei, *Vibrio parahaemolyticus*

ABSTRACT

Vibrio parahaemolyticus is one of the bacteria that can attack whiteleg shrimp and cause Acute Hepatopancreatic Necrosis Disease (AHPND) or Early Mortality Syndrome (EMS) in shrimp. One alternative that can be used is the use of seaweed-based immunostimulants, namely k-carrageenan. The purpose of this study was to evaluate the effectiveness of k-carrageenan mixed in feed with different doses on increasing the immune system of whiteleg shrimp infected with AHPND. This study 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 + *Vibrio parahaemolyticus* Infection; P2 (Negative Control): Commercial Feed + 0.9% NaCl Infection; P3: Commercial Feed + Kappa-carrageenan 8 g / kg + *Vibrio parahaemolyticus* Infection; P4: Commercial Feed + Kappa-carrageenan 10 g/kg + *Vibrio parahaemolyticus* Infection; P5: Commercial Feed + Kappa-carrageenan 12 g/kg + *Vibrio parahaemolyticus* Infection. The results of this study indicate that the addition of 12 g/kg of k-carrageenan to the feed affects the survival rate and immune system of whiteleg shrimp infected with *Vibrio parahaemolyticus*.

INTRODUCTION

Disease is a major obstacle that needs attention besides feed. This is because disease attacks are generally sudden and can cause shrimp death. Praja (2018), revealed that AHPND (Acute Hepatopancreatic Necrosis Disease) or also known as Early Mortality Syndrome (EMS) is one of the diseases that attacks shrimp caused by *Vibrio parahaemolyticus*. Indonesia as a tropical country, has vast waters with abundant seaweed and has even been intensively cultivated. Among these seaweeds, the most widely cultivated is *Kappaphycus alvarezii* (Metungun, 2012). *Kappaphycus alvarezii* is widely cultivated in the NTB area and is a livelihood for fishermen when they are not at sea (Cokrowati, 2021). Seaweed of the *Kappaphycus alvarezii* type produces primary metabolites of hydrocolloid compounds called carrageenan. Carrageenan produced from *Kappaphycus alvarezii* type seaweed is the kappa-

carrageenan type (Yuniati, 2011). Sulfate polysaccharides in carrageenan have beneficial bioactive compounds as anticoagulants, antivirals, antioxidants, anticancer, and immune modulation activation (Wijesekara et al., 2011). Polysaccharides can stimulate the non-specific immune system of shrimp in this case is phagocytosis activity. The application of kappa-carrageenan against *Vibrio parahaemolyticus* bacterial attacks has never been done, so research is needed to determine the effectiveness of kappa-carrageenan mixed in feed with different doses.

METHODS

Time and Place

This research was conducted in March-August 2023 at the Fish Health Laboratory, Department of Fisheries and Marine Sciences, Faculty of Agriculture, University of Mataram.

Ethical Approval

All experimental procedures and animal husbandry were handled in accordance with animal welfare under national accreditation no. SNI 7311:2009 of the Republic of Indonesia.

K-carrageenan & Feed Preparation

Kappa-carrageenan flour mixed with commercial shrimp feed with 40% protein content was weighed in doses (8, 10, and 12 g/kg feed), then dissolved in a little bit of water, then coated with egg white and air-dried at room temperature. Shrimp were fed as much as 5-7% of the biomass weight of each container.

Vibrio parahaemolyticus Infection Procedure

The type of bacteria used was *Vibrio parahaemolyticus* obtained from the collection of the Fish Health Laboratory, Department of Fisheries and Marine Sciences, University of Mataram. The bacterial isolate was re-cultured and purified to obtain younger and more virulent bacteria. then re-characterization and Total Plate Count (TPC) were carried out. The *Vibrio parahaemolyticus* isolate used was cultured in 25 ml of liquid SWC media for 18 hours in a water shaker at 29^{o}C. Furthermore, a dilution stage was carried out with a bacterial density of 10^{6} cfu/ml (Oktaviana, 2014). The process of injecting *Vibrio parahaemolyticus* bacteria was carried out intramuscularly into the shrimp body on the 3rd walking leg as much as 0.1 ml/tail, except for the negative control which was infected with 0.9% NaCl as much as 1 ml because it was related to its role in the study as a comparison with other post-infection treatment conditions. Furthermore, the shrimp were reared again for 10 days to determine the Survival Rate of the shrimp.

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 doses of kappa-carrageenan to the feed. Each treatment was carried out with 3 repetitions so that 15 experimental units were formed as follows:

P1 : Commercial Feed + *Vibrio parahaemolyticus* Infection (Positive Control)
P2 : Commercial Feed + NaCl 0.9% Infection (Negative Control)
P3 : Commercial Feed + Kappa-carrageenan 8 g/kg + *Vibrio parahaemolyticus* Infection
P4 : Commercial Feed + Kappa-carrageenan 10 g/kg + *Vibrio parahaemolyticus* Infection
P5 : Commercial Feed + Kappa-carrageenan 12 g/kg + *Vibrio parahaemolyticus* Infection

Survival Rate (SR)

The survival rate can be calculated using Azhar (2014), as follows:

$$SR = \frac{Nt}{No} \text{ x } 100\%$$

Information:
SR : Survival rate (%)
Nt : Number of shrimps alive at the end of maintenance (shrimp)
No : Number of shrimps alive at the beginning of maintenance (shrimp)

Hemolymph Sampling

Hemolymph sampling for each treatment was carried out after 10 days of maintenance using a 1 ml syringe. The syringe was filled with 0.6 ml of anticoagulant. Then hemolymph samples were taken from 3 shrimps with each hemolymph taken from each shrimp as much as 0.1 ml from the 3rd walking leg and placed in a microtube (Hidayatullah, 2019).

Total Haemocyte Count (THC)

The hemolymph that had been taken was used to measure immune response parameters. Calculation of the Total Haemocyte Count (THC) levels was carried out by dripping the hemolymph sample that had been taken on a hemacytometer then covered with a cover glass. Furthermore, the hemolymph sample was observed using a microscope and the number of cells was counted using a hemacytometer. Hemocytes were observed at a magnification of 400 times and calculated using the following formula (Oktaviana, 2014):

$$\text{THC} = \text{Average amount cell count x } \frac{1}{\text{Haemocytometer volume}} \text{ x FP}$$

Information:
FP : Dilution factor

Differential Haemocyte Count (DHC)

The slide was soaked in methanol for 5 minutes and dried at room temperature. The slide was then dripped with 1 drop of hemolymph and spread evenly over the entire slide using another slide and dried. The slide was then re-fixed by immersion in methanol for 10–15 minutes. After drying, the slide was soaked in Giemsa solution for 10–15 minutes and air-dried. The slide was rinsed with distilled water and dried, then observed under a microscope. Differential Haemocyte Count (DHC) was calculated using the following formula (Muharrama, 2020):

$$\text{DHC (hyaline) (\%)} = \frac{\Sigma \text{ hyaline cells}}{\Sigma \text{ hemocytes observed}} \times 100$$

Phagocytic Activity (PA)

Phagocytic activity measurement was carried out by mixing 100 µL of hemolymph sample with 25 µL of *Streptococcus aureus* bacteria in a microplate and incubating for 20 minutes. The incubation results were dripped onto a glass slide that had been soaked in methanol beforehand and were evenly smeared. The smear preparation was air-dried and soaked in methanol for 10–15 minutes. Furthermore, Giemsa staining was carried out for 10–15 minutes. The preparation was then observed under a microscope at a magnification of 400 times. Phagocytic activity is based on calculating the percentage of cells that perform phagocytosis (Hidayatullah, 2019). The calculation of the PA value is as follows:

$$\text{Phagocytic Activity} = \frac{\sum \text{phagocytic cells}}{\sum \text{whole hemocyte cells}} \text{ x } 100\%$$

Bacterial Population Calculation in the Intestine

The calculation of the number of bacteria in the intestine was carried out at the end of the treatment (day 60). The calculation of the number of bacteria in the shrimp intestine consists of the Total Viable Bacterial Count (TBC) and the Total Presumptive Vibrio Count

(TVC). The intestine was taken as much as 0.1 g collected from 3-5 shrimps and then homogenized in 0.9 ml of Phosphate Buffer Saline (PBS) solution. Bacterial counting used the spread plate counting method, using Sea Water Complete (SWC) media for TBC calculations and specific TCBS Thiosulfate Citrate Bile Salt Sucrose (TCBS) media for TVC calculations (Oktaviana, 2014).

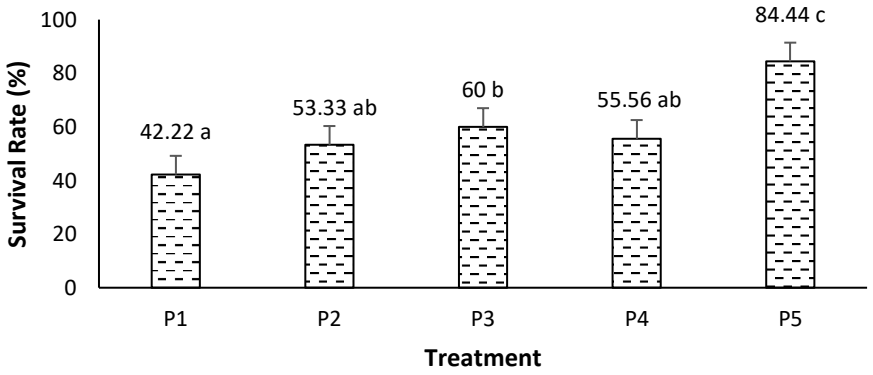
Data Analysis

Data from the research results will be analyzed using Analysis of Variance (ANNOVA) with SPSS at a significance level of 5% to determine the effect of the treatment in the study. If the data shows a real effect, then further analysis is carried out with Duncan's advanced test.

RESULTS

Survival Rate

The survival rate of whiteleg shrimp after 60 days of maintenance with commercial feed in various treatments and added k-carrageenan ranged from 42.22-84.44% (Figure 1).

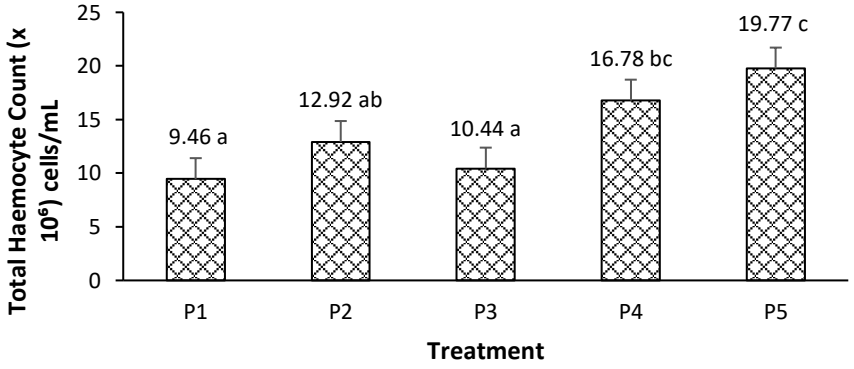


Description: P1 (Positive Control); P2 (Negative Control); P3 (8 g/kg k-carrageenan); P4 (10 g/kg k-carrageenan); (12 g/kg k-carrageenan).

Figure 1. Survival Rate

Total Haemocyte Count

The total haemocyte count of shrimp after 60 days of maintenance with commercial pellet feed in various treatments and added k-carrageenan ranged from 9.46 × 10^6 – 19.77 × 10^6 cells/ml (Figure 2).

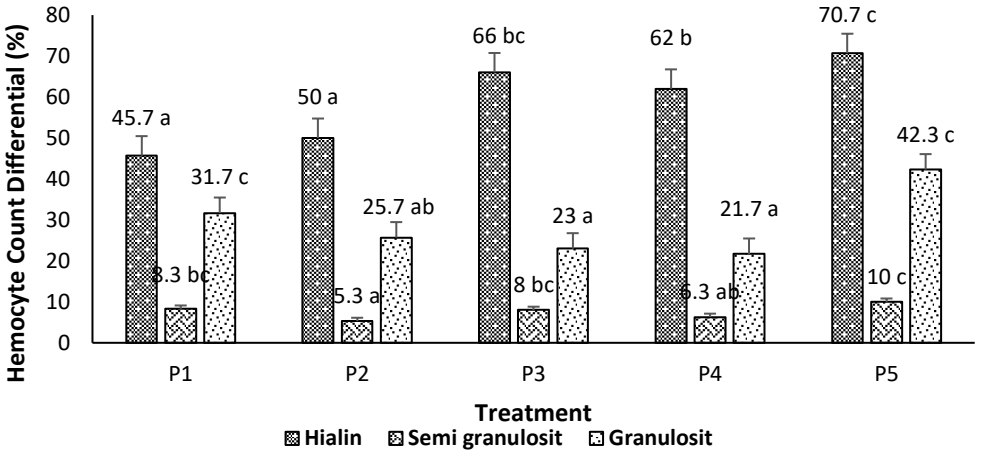


Description: P1 (Positive Control); P2 (Negative Control); P3 (8 g/kg k-carrageenan); P4 (10 g/kg k-carrageenan); (12 g/kg k-carrageenan).

Figure 2. Total Haemocyte Count

Differential Haemocyte Count

Differential Haemocyte Count of whiteleg shrimp after 60 days of maintenance with commercial feed in various treatments and added k-carrageenan (Figure 3).

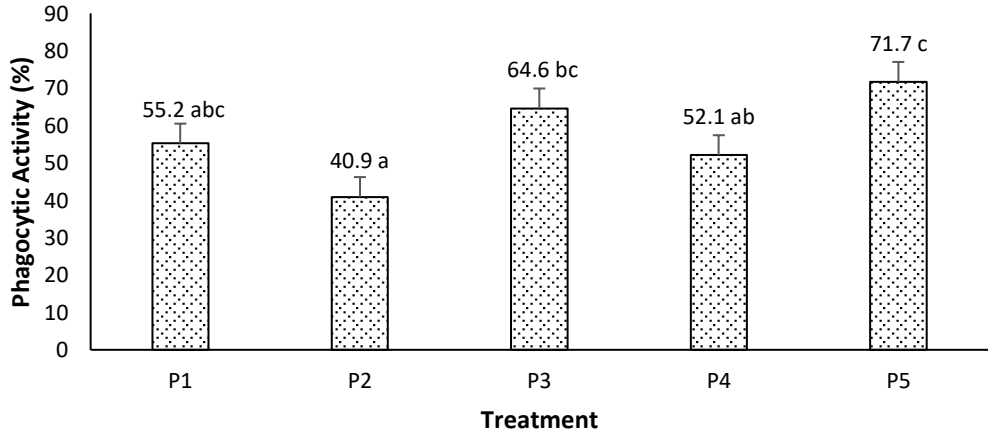


Description: P1 (Positive Control); P2 (Negative Control); P3 (8 g/kg k-carrageenan); P4 (10 g/kg k-carrageenan); (12 g/kg k-carrageenan).

Figure 3. Differential Haemocyte Count

Phagocytic Activity

The phagocytic activity of whiteleg shrimp after being maintained for 60 days with commercial feed in various treatments and added k-carrageenan was 40.9-71.7% (Figure 4).

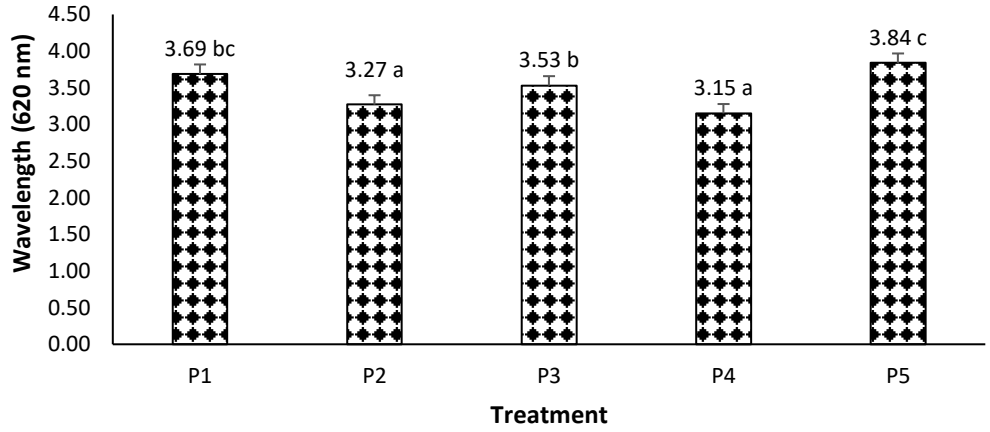


Description: P1 (Positive Control); P2 (Negative Control); P3 (8 g/kg k-carrageenan); P4 (10 g/kg k-carrageenan); (12 g/kg k-carrageenan).

Figure 4. Phagocytic Activity

Total Bacteria Count

Total bacteria in the intestines of vaname shrimp after maintenance for 60 days with commercial feed in various treatments and added k-carrageenan was 3.15-3.84 nm (Figure 5).

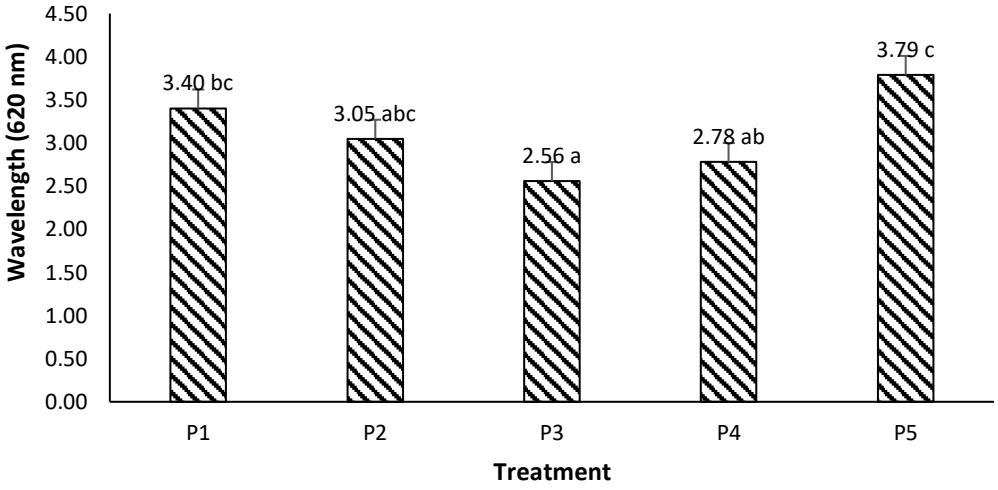


Description: P1 (Positive Control); P2 (Negative Control); P3 (8 g/kg k-carrageenan); P4 (10 g/kg k-carrageenan); (12 g/kg k-carrageenan).

Figure 5. Total Bacteria Count

Total Vibrio Count

Total vibrio in the intestines of vaname shrimp after being maintained for 60 days with commercial feed in various treatments and added k-carrageenan was 2.56-3.79 nm (Figure 6).



Description: P1 (Positive Control); P2 (Negative Control); P3 (8 g/kg k-carrageenan); P4 (10 g/kg k-carrageenan); (12 g/kg k-carrageenan).

Figure 6. Total Vibrio Count

DISCUSSION

Survival Rate

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 survival rate of whiteleg shrimp infected with *Vibrio parahaemolyticus*. In P1 (positive control) showed results that were not significantly different, namely 42.22% with P2 (negative control) of 53.33%, and P4 of 55.56% but significantly different from P3 of 60%, and P5 84.44%. P5 showed the highest value at the survival rate level and showed significantly different results with all treatments.

In this study, the survival rate of whiteleg shrimp infected with *Vibrio parahaemolyticus* and given kappa-carrageenan treatment showed better results compared to the treatment

without kappa-carrageenan. This shows that the administration of immunostimulants in the form of kappa-carrageenan can be applied to maintain the survival rate of whiteleg shrimp. According to Widigdo (2013), the survival rate of vaname shrimp after being infected with bacteria with results >70% is still categorized as good.

Total Haemocyte Count

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 Haemocyte Count of whiteleg shrimp infected with *Vibrio parahaemolyticus*. In P1 (positive control) showed significantly different results from P4 and P5. The highest total number of haemocytes was in P5. The results obtained in P1 (positive control) were 9.46×10^6 cells/ml, in P2 (negative control) namely 12.92×10^6 cells/ml, and P3 which had a Total Haemocyte Count of 10.44×10^6 cells/ml, then the highest Total Haemocyte Count in P5 namely, 19.77×10^6 cells/ml, and followed by P4 of 16.78×10^6 cells/ml.

Haemocytes are one of the most important components in the cellular defense system which is non-specific. Hemocytes play a role in the process of phagocytosis, encapsulation, degranulation, and nodular aggregation of pathogens and foreign particles as well as the production and release of prophenoloxidase (Sahoo et al., 2008). Based on the research that has been done, the administration of kappa-carrageenan can increase the total hemocytes. It is proven that P5 showed higher results compared to P1 which was only given commercial feed. This indicates that the high value of hemocytes in P5 after the *Vibrio parahaemolyticus* challenge test is suspected to be due to the immune response reaction in the shrimp body in responding to foreign particles that enter because it is influenced by the administration of kappa-carrageenan.

Differential Haemocyte Count

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 Differential Haemocyte Count of whiteleg shrimp infected with *Vibrio parahaemolyticus*. Haemocytes in shrimp consist of hyaline, semi-granulocytes and granulocytes. The hyaline value showed significantly different results between P1 (positive control) which was 45.7% followed by P2 (negative control) of 50% and P3 66%, P4 62% and P5 70.7%. The semi-granulocyte value in P1 (positive control) of 8.3% showed significantly different results with P2 (negative control) of 5.3%, P3 of 8% but not significantly different with P4 of 6.3% and P5 of 10%. Meanwhile, the granulocyte value showed significantly different results between P1 (positive control) of 31.7% with P2 (negative control) of 25.7%, P3 of 23%, P4 of 21.7% and P5 of 42.3%.

Kurniawan (2018), reported that the percentage of hyaline in normal vaname shrimp consists of 60% -93% of total hemocytes. From this statement, it shows that hyaline cells in the test shrimp are still in the normal range. The decrease in the percentage of hyaline cells in this study is not a negative effect of the administration of kappa-carrageenan but is an implication of an increase in granular cells. The difference in the high value of hyaline cells between the control and the treatment given kappa-carrageenan indicates that there is an effort to resist the test shrimp against *Vibrio parahaemolyticus* which has been previously infected into the shrimp's body. Darwantin (2016), stated that the shrimp body's defense mechanism does not have immunoglobulin like other fish, immunoglobulin in shrimp is replaced by Prophenoloxidase Activating Enzyme (PPA) which is located in the shrimp's granular hemocyte cells. In this study, PPA was activated by immunostimulants that entered the shrimp's body, which could induce hyaline cells to increase their activity in the phagocytosis process.

The low total semi-granulocytes in the treatment added with kappa-carrageenan was due to the low production of semi-granulocyte cells which are the maturation of hyaline cells. Munaeni et al. (2014) stated that semi-granulocyte cells are characterized by the presence of granules in the cytoplasm. These cells are capable of carrying out the encapsulation process and play a small role in the phagocytosis process. Encapsulation is a defense reaction against particles in large numbers and cannot be phagocytosed by hemocyte cells.

Putri (2018), reported that the percentage of granulocyte cells in normal shrimp ranges from 17-40% of the total hemocytes. This shows that the range of granulocyte cells in this study is still within the normal range. Ekawati (2012), stated that the function of granulocyte cells is more focused on the process of producing phenoloxidase enzymes which have an important role in the non-specific defense system.

Phagocytic Activity

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 whiteleg shrimp infected with *Vibrio parahaemolyticus*. In P1 (positive control) showed significantly different results with all treatments. The highest total number of hemocytes was in P5. The value of P1 (positive control) was 55.2% while in P2 (negative control) it had a lower value of 40.9%. In contrast to P3 which had a value of 64.6% cells/ml, then the highest phagocytic activity value was in P4, namely 52.1%, and P5 was 71.7%.

Phagocytosis is a non-specific cellular defense mechanism that can generally protect against pathogen attacks (Jasmanindar, 2009). Based on the research that has been done, after giving kappa-carrageenan P5 produced a higher phagocytic activity value compared to P1 without giving kappa-carrageenan. According to Febriani et al. (2013), phagocytic activity is influenced by total hemocytes, increasing total hemocytes will increase the ability of cells to phagocytize. The high value of phagocytic activity in the treatment given kappa-carrageenan after the challenge test is caused by the high value of hemocytes after the challenge test. According to Rodriguez & Moullac (2000), a high value of phagocytic activity illustrates that the organism has the ability to produce higher phagocytic cells, so that when exposed to pathogens, phagocytic cells are ready to perform phagocytosis.

Total Bacteria Count

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 intestinal bacteria of whiteleg shrimp. In P1 (positive control) which was 3.69 nm showed a significantly different result from P2 which was 3.27 nm. P3 which was 3.53 nm showed a significant difference with P4 which was 3.15 nm and P5 which was 3.84 nm. From the results of research conducted by Zhu (2017), it was found that oxidized k-carrageenan can damage bacterial cell walls and cytoplasmic membranes which effectively suppress bacterial growth.

Total Vibrio Count

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 intestines of whiteleg shrimp. In P1 (positive control) which is 3.40 nm showed results that were not significantly different from P2 (negative control) which is 3.05 nm, P4 which is 2.78 nm, and P5 3.79 nm, but significantly different from P3 which is 2.56 nm. This shows that the treatment with the addition of k-carrageenan shows better results or can suppress the growth of vibrio bacteria in the shrimp intestines compared to the treatment without the addition of k-carrageenan to the feed. From the results of Junior (2021), it was stated that the antibacterial

activity of N-alkyl-kappa-carrageenan derivatives was compared with N-alkyl-(1-deoxylactitol-1-yl)-amine using a microdilution test, which showed higher antibacterial activity.

CONCLUSION

Based on the results of this study, the addition of 12 g/kg k-carrageenan had a significant effect on increasing the immune system of whiteleg shrimp infected with *Vibrio parahaemolyticus*, with the SR results obtained being 84.44%, THC being 19.77 × 10^6 cells/ml, DHC with a hyaline percentage of 70.7%, semi-granulocytes being 10%, and granulocytes being 42.3%, PA being 71.7%, TBC being 3.84 nm and TVC being 3.79 nm.

ACKNOWLEDGEMENT

The author would like to thank all parties who have helped in the implementation of this research so that it can be carried out properly. In particular, the author would also like to thank the Institute for Research and Community Service, University of Mataram for the grant program so that this research can be carried out.

REFERENCES

Akbar S, Kurnia B, Istiqomah. (2001). Technology Cultivation Seaweed (*Kappaphicus alvarezi*i). Department Marine Affairs and Fisheries, Directorate General Fishery Cultivation, Marine Cultivation Center. Lampung.

Azhar, F. (2014). The Influence Giving Probiotics and Prebiotics to Juvenile Grouper Performance Duck (*Comileptes altivelis*). Bulletin Udayana Veterinary, 6 (1). 1-9. Retrieved from https://ojs.unud.ac.id/index.php/buletinvet/article/view/8877

Azhar, F. (2018). Application Combined Biofloc with Probiotics For Prevention *Vibrio parahaemolyticus* Infection in Livestock Shrimp Vaname (*Litopenaeus vannamei*). Journal of Aquaculture Science, 3(1): 128 – 137.

Central Bureau of Statistics. (2017). Exports Shrimp by Main Destination Country 2000-2015. https://www.bps.go.id/LinkCatatanSt atis /view/id/ 1015 [April 21, 2017].

Castro R, Piazzon MC, Zarra I, Leiro J, Noya M, Lamas J. (2006). Stimulation of Turbot Phagocytes by *Ulva rigida* C. Agardh Polysaccharides. Aquaculture 254: 9 – 20.

Chandrakala, N., Menaka, R. (2017). Vibriosis Detection and Pathology. International Journal of Current Innovation Research, 3: 622-625.

Cokrowati. N., Aluh. N., Zainal. A., Edy. S., Hardawiansyah., Erwansyah. (2021). Development Cultivation Seaweed *Kappaphycus alvarezii* in Waters Buer District, Sumbawa Regency. Journal Master of Science Education Community Service, 4 (2). 168-172. http://dx.doi.org/10.31258/jipas.9.2.p.94-98

Directorate General Processing and Marketing of Fisheries Products KKP. (2010). Recapitulation rejection RAS case 2005-2009. Ministry of Marine Affairs and Fisheries.

Febriani, D., Sukenda., & Nuryati S. (2014). Kappa-carrageenan as Immunostimulant to Control Infectious Myonecrosis (IMN) Disease in White Shrimp *Litopenaeus vannamei*. Journal Indonesian Aquaculture, 12 (1), 70-78. https://doi.org/10.19027/jai.12.70-78

Fernando, E. (2016). The Influence Variation Dosage and Frequency Giving Probiotics in Feed Against Growth as Well as Mortality (*Litopenaeus vannamei*). Thesis. Surabaya: Biology Study Program, Faculty of Science and Technology, Airlangga University.

Firdaus, IA, Reni, A. (2019). Attack Level Ectoparasites in Shrimp Vannamei (*Paneaus Vannamei*) in Aquaculture Land Polyculture Sidoarjo. Journal Bio Lantern ,8 (2): 127-135.

Hidayat, RP (2017). Evaluation Administration of Crude Protein Zoothamnium penei on Growth Rate, Immune Response and Survival Shrimp Vaname (*Litopenaeus Vannamei*) in Ponds. Journal Biosciences, 19 (2), 111-132. http://dx.doi.org/10.20473/jbp.v19i2.2017.111-126

Hidayatullah, M. (2019). Immune Response and Activity Enzyme Digestion Shrimp Vaname (*Litopenaeus Vannamei*) in Ponds During Outbreaks White Feces Disease. [Thesis]. Bogor: Institute Bogor Agriculture.

Jasmanindar, Y., Sukenda, Alimuddin, Junior, MZ, Utomo, NBP (2018). The Chemical Composition of Gracilaria verrucosa Extract and its Utilization on Survival and Growth Litopenaeus vannamei, Omni-Aquatica. 14(3): 1-9.

Metungun, J. (2012). Administration of Kappa-Carrageenan Orally on Dumbo Catfish *Clarias* Sp. For Increase Non-Specific Immune Response and Resistance to *Aeromonas hydrophi*la. Thesis. Institut Agriculture Bogor: Bogor.

Muharrama, ARW (2020). Growth, Gene Expression and Response Immunity Shrimp Vaname Given Probiotics Bacillus Np5 and Honey Prebiotics and Infection *Vibrio Parahaemolyticus*. [Thesis]. Bogor: Institute Bogor Agriculture.

Naim S, Brown JK, Nibert ML. (2014). Genetic diversification of Penaeid shrimp infectious myonecrosis virus between Indonesia and Brazil. Virus Research 189: 97-105

Nardianto, B. (2019). Feasibility Study and Development Strategy Cultivation Shrimp Vannamei (*Litopenaeus Vannamei*) in Ponds Plastic in Kaur Regency, Bengkulu Province (Case Study at PT Xyz). Thesis. Bandar Lampung: University of Lampung.

Oktaviana, A., Widanarni., Munti, Y. (2014). The Use of Synbiotics to Prevent IMNV and *Vibrio harveyi* Co-Infection in *Litopenaeus vannamei*. Journal of Biosciences, 21 (3). 127-134. http://dx.doi.org/10.4308/hjb.21.3.127

Sahoo PK, DAS A, Mohanty S, Mohanty BR, Pillai BR, Mohanty J. (2008). Short Communication: Dietary β-1-3-glucan Improves the Immunity and Disease Resistance of Freshwater Prawn *Macrobrachium rosenbergii* Challenged with *Aeromonas hydrophila*. Aquaculture Research. 39(2): 1574-1578.

Tobing, S. P. L. (2019). Growth and Survival Shrimp Vaname *Litopenaeus vannamei* (Boone, 1931) at 5 Ppt Salinity with Different Density. Thesis. Bandar Lampung: University of Lampung.

Wijesekara I, Pangestuti R, Kim SK. (2011). Biological Activities and Potential Health Benefits of Sulfated Polysaccharides Derived from Marine Algae. Carbohydrate Polymers. 84 (4): 14-21.

Yuniati, E. (2011). Characteristics Physicochemical Carrageenan and Histology Sea Grass *Kappaphycus alvarezii* from Area Origin Seeds and Different Harvest Age. [Thesis]. School Postgraduate Institute Bogor Agriculture, Bogor.

Zahra, A., Sukenda, Dinamella Wahjuningrum, & Dendi Hidayatullah. (2019). Feed Contains *Gracilaria verrucosa* Seaweed Extract to Improve Survival Rate of *Litopenaeus vannamei* White Shrimp Infected by WSSV (White Spot Syndrome Virus). Intek Aquaculture, 3(1), 128-138. https://doi.org/10.31629/intek.v3i1.1262