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EFFECTIVENESS OF CAPPA-CARRAGEENAN TO INCREASE TO RESISTANCE OF FRESHWATER CRAYFISH *Cherax quadricarinatus* TO *Aeromonas hydrophila* INFECTION

(Efektivitas Pemberian Kappa-Karaginan Untuk Meningkatkan Resistensi Lobster Air Tawar Cherax quadricarinatus terhadap Infeksi Bakteri Aeromonas hydrophila)

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ABSTRACT

Crayfish Cherax quadricarinatus is one of the fishery commodities that has business prospects not only as an ornamental fish commodity but also as a consumption commodity. However, as an aquatic animal, it is inseparable from disease attack. One of the disease attacks caused by bacteria, so one of the efforts made to increase the resistance of crayfish by giving kappa carrageenan is to increase the resistance of the crayfish. The purpose of this study was to determine the level of resistance of lobster to Aeromas hydrophila bacterial infection by giving kappa carrageenan with different frequencies which is effective for increasing lobster survival after challenge tests with Aeromnas hydrophila bacteria. The study was conducted using a completely randomized design (RAL) with five treatments and three replications, namely control (without injection of kappa carrageenan), 1 time, 2 times, 4 times, and 6 times of injection kappa carrageenan. During maintenance, kappa carrageenan was given at a dose of 5 µg/g of oyster by dissolving 2.5 mg in 1 ml of Phosphate Buffer Saline (PBS) and then injected into lobster as much as 20µl/head. on day 30, challenged with A. hydrophila bacteria. The results showed that giving kappa-carrageenan 2, 4, and 6 times showed the same results (P>0.05) on the absolute growth of crayfish. The survival rate of crayfish given kappa-carrageenan showed that the frequency of giving 4 times showed the highest survival rate. Water quality during the study consisting of oxygen 6.25 mg/l, carbodioxide 3.68 mg/l, pH 6.8, ammonia 0.17 mg/l, and temperature 26.9 °C showed that it was still in the range that was feasible as a maintenance media.

Keywords: *A. hydrophila bacteria, kappa-carrageenan, crayfish C. quadricarinatus, survival rate,*

ABSTRAK

Lobster air tawar Cherax quadrinatus merupakan salah satu komoditas perikanan yang memiliki prospek bisnis tidak hanya sebagai komoditas ikan hias tetapi juga sebagai komoditas yang bisa dikonsumsi. Namun demikian, sebagai hewan akuatik, lobster air tawar tidak terlepas dari serangan penyakit. Serangan penyakit salah satunya disebabkan oleh bakteri, sehingga salah satu Upaya yang dilakukan adalah meningkatkan daya tubuh lobster air tawar dengan pemberian kappa karaginan. Tujuan dari penelitian ini adalah untuk mengetahui tingkat resistensi lobster terhadap infeksi bakteri *Aeromnas hydrophila* dengan pemberian kappa karaginan dengan frekuensi yang berbeda untuk meningkatkan tingkat kelangsungan hidup lobster setelah uji tantang dengan bakteri *Aeromonas hydrophila*. Penelitian menggunakan Rancangan Acak Lengkap (RAL) dengan 5 perlakuan 3 ulangan yaitu control, 1 kali, 2 kali, 4 kali dan 6 kali injeksi kappa karaginan. Selama masa pemeliharaan, kappa karaginan diberikan dengan dosis 5 µg/g dengan cara melarutkan kedalam 1 ml *Phospate Buffer Saline* (PBS)

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kemudian diinjeksikan ke dalam tubuh lobster sebanyak 20 µg/ekor dan pada hari ke 30, ditantang dengan bakteri *Aeromonas hydrophila.* Hasil penelitian menunjukkan bahwa tingkat kelangsungan hidup lobster air tawar yang diberi kappa karaginan menunjukkan bahwa frekuensi pemberian 4 kali memberikan tingkat kelangsungan hidup yang tertinggi. Selama masa penelitian kualitas yang terdiri oksigen 6.25 mg/l, karbodioksida 3.68 mg/l, pH 6.8, amoniak 0.17 ml/l dan suhu 26.9 ^{o}C menunjukkan masih pada kisaran yang layak sebagai media pemeliharaan.

Kata kunci: *A. hydrophila bacteria* , kappa-carrageenan, kelangsungan hidup, lobster

INTRODUCTION

Cherax quadricarinatus is one of type of freshwater crayfish that has prospects for development. This is supported by Indonesia's favorable climate and geographical condition, evolving farming techniques, and genuine customer market with lucrative business opportunities for processed products. As aquatic animals, lobsters cannot avoid disease. Diseases in crayfish are caused by three factors: poor physical condition, unfavorable environment, and the presence of pathogens or other disease-carrying animals (Lukito and Prayugo, 2009). Disease attacks in lobsters often occur during the nursery period. One of the diseases that often attacks young crayfish is MAS (*Motile Aeromonas Septicemia*). This disease causes crayfish to move passively, and their legs and claws fall off. To avoid this, efforts must be made to increase the resistance of crayfish to this disease. One possible attempt is to use seaweed, which is now widely used not only for food purposes but also for industrial purposes. Seaweed, also known as macroalgae, is commonly known and used by coastal communities for food and traditional medicine. Currently, with the development of increasingly advanced technology, seaweed is used to meet the diverse needs of modern industry by using the chemical content of seaweed in the form of kappa carrageenan as a mixture. Suitable for many types of products, for example in the pharmaceutical industry, cosmetics, food and others.

Algae have antidiabetic activity (Abirami & Kowsalya, 2013), antibacterial (Vijayabaskar & Shiyamala, 2011), anticancer (Moussavou et al., 2014) and antioxidant activity (Kreckhoff et al., 2019). *Kappaphycus alvarezii* (*K. alvarezii*) can inhibit the growth of *Bacillus cereus* (Hutabarat et al., 2017), has bacteriostatic properties against *Streptococcus* mutans and has a diameter of 10.55 mm to inhibit the growth of *Staphylococcus aureus* and *Salmonella typhimurium* 8.75 mm, bacteriostatic antibiotic against *Staphylococcus yellow* and *Escherichia coli* (Soelama et al., 2015). *K. alvarezii* contains alkaloid compounds, flavonoids and saponins (Hudaifah, 2020), alkaloids, flavonoids, phenolic hydroquinone and tannins (Safia, 2020) . Differences in the levels of bioactive compounds produced in the alga *K. alvarezii* are believed to be related to the solvent used in the compound selection process (Tatiya et al., 2011). Plant extracts contain different types of phenolic compounds with different solubilities in different solvents. Additionally, the environment can also influence the levels of bioactive compounds in plants (Safia, 2020).

Based on this basis, a study was conducted title Effectiveness of using cappa-

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carageenan from *K. alvarezii* algae to increase the resistance of crayfish *C. quadricarinatus* to infection with *A. hydrophila* bacteria. This research aims to increase the survival rate and the right frequency for giving the kappa-carrageenan. This research is expected to benefit farmers by using natural compounds from seaweed to improve the immune systems of farmed aquatic animals.

MATERIAL AND METHODS

The research was conducted from October to December 2022 at the Fish Health Laboratory and Wet Laboratory at Politeknik Negeri Pangkajene dan Kepualuan. The research method used in the research plan is the laboratory experimental method. This study was designed using a completely randomized design (RAL). The treatment tested was the frequency of kappa-carrageenan administration during 30 days of rearing with 5 treatments and 3 replicates each. The treatments tested were:

A = control (without giving injection of cappa-carrageenan)
A1 = 1 time injection
A2 = 2 times injection
A3 = 4 times injection
A4 = 6 time injection

Research Parameter

The parameters observed in the research activities include :

1. **Absolute Growth**
   Growth was measured at the beginning and end of the study by measuring the weight of the lobster with the formula: Everhart et al (1975) in Effendie (1997), namely: H = Wt - Wo where H = Absolute growth, Wt = Total weight of the test lobster at the end of the experiment and Wo = total weight of the test lobster at the end of the experiment
2. **Survival Rate**
   The lobster survival rate was observed on the first day of the challenge test until the end of the study which was calculated using the Effendi 2002 formula as follows SR = Nt/No x 100%, where SR = Survival rate (%), Nt = number of live lobsters at the end of the study (tails), No = number of live lobsters at the beginning of the study (tails).

Data Analysis

The resulting data were analyzed using analysis of variance. if the analysis proves that the treatment has a significant effect, it is continued with the Least Significant Difference Test (BNT) to determine the best treatment (Gasperz, 1991). Water quality was analyzed descriptively according to standard criteria for lobster growth and survival.

Research producer

1. Preparation
   The aquarium container was cleaned with fresh water mixed with 10 ppm chlorine to sterilize the container. with chlorine with a concentration of 10 ppm to sterilize the container. After soaking with chlorine for one day, the aquarium is then washed using sunlight soap and rinsed with fresh water. Aquariums that have been washed clean are then dried for about two days. After the drying process was complete, the water was filled into the container to a height of 15-20 cm, aerated and sheltered. Crayfish are put into the aquarium and maintained for approximately one week to acclimatize.
2. Stock provision of cappa-carrageenan
   The dosage of kappa-carrageenan used was 5 μg/g of lobster. Preparation of kappa-carrageenan stock is done by

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dissolving 2.5 mg in 1 ml Phosphate buffer saline (PBS). The stock solution is then injected into crayfish as much as 20µl/head on the cephalothorax on the second segment after the carapax. This dose is given to each treatment with a different frequency.

3. Growth Sampling
   Sampling of absolute weight growth was done at the beginning and day 30 of rearing. Sampling is done by weighing crayfish using analytical scales.
4. Cappa-carrageenan Injection
   The study was conducted during 30 days of rearing with the giving of kappa carrageenan as much as 20ul/head with different frequencies. Kappa-carrageenan was injected into each crayfish in the cephalothorax at the second segment after the carapax.
5. Provision of challenge test material
   The challenge test material was *A, hydrophila* bacteria. Virulence enhancement was carried out on *A. hydrophila* bacteria by infecting healthy shrimp. Furthermore, the bacteria were isolated in the hepatopancreas and cultured on TSA (Tipticase Soy Agar) media and incubated for 24 hours at room temperature, single bacterial colonies that grow, were isolated on TSA media with quadrant technique and then incubated for 24 hours. Bacterial colonies that grow separately and have different morphologies are isolated on tilted TSA media to obtain pure cultures at room temperature for 24 hours. Determination of bacterial virulence that killed shrimp by 50% was done by conducting LD-50 (Lethal Dose 50%) test by infecting healthy shrimp. The concentration of *A. hydrophila* bacteria at 10 cfu/ml is considered lethal in crayfish (Suryati, 2011). *A. hydrophila* bacteria isolated from the oblique media was taken one ose. Furthermore, *A. hydrophla* bacteria were inoculated into an Erlemeyer containing NB (Sodium Broth) media, and incubated for 24 hours in a water bath shaker at 28°C as a pure culture stock. The pure stock culture was centrifuged at 3000 rpm for 15 minutes at 4°C. The supernatant was removed and the bacterial pellets were resuspended in PBS (Phosphate-Buffered Saline) solution as bacterial suspense stock for the challenge test.
6. Challenge Test
   The challenge test was conducted on day 30 of rearing by infecting freshwater with *A. hydrophila* bacteria. Observations on the survival rate of lobsters were made from day 1 to day 7 after the challenge test.
7. Water Quality Measurement
   Water quality measurements consisted of oxygen, pH, temperature, and ammonia which were carried out at the beginning and end of the study.

RESULTS AND DISCUSSION

Absolute Growth

The results of the analysis of variance, the absolute growth of crayfish during 30 days of maintenance showed that all treatments were not significantly different were $F_{Count} < F_{Table}$ or P>0.05 (Tabel 1).

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Table 1. Analysis variance of absolute growth

Uniformity Source	df	Sum of squares	Mean square	F_{Count}	F_{table} (0.05-0.01)
Treatment	4	0.163	0.041	0.17^{tn}	3.28-5.99
Random	10	2.337	0.234		
Total	14	2.500			

Remark: Numbers followed by tn letters are not significantly different at the 5% BNT test level.

The insignificant addition of absolute weight between each treatment given kappa-carrageenan is thought to be due to stress in crayfish. Stress occurs as a result of the injection process that is too frequent. According to Aodoys (2009), injection in fish generally will experience stress due to the injection treatment. will experience stress due to the injection treatment given. So that the handling afterward needs to be prepared so that the fish becomes normal again. Jasmanindar (2009) also explained that stress occurs in shrimp due to frequent injections, thus affecting the addition of absolute weight at the end of the treatment. thus affecting the addition of absolute weight at the end of treatment which is the highest absolute weight gain in the control treatment.

Survival Rate

The survival rate of lobsters that have been challenged with *A. hydrophila* bacteria shows significantly different results where $F_{Count} > F_{Table}$ or $P < 0.05$, by giving an injection of kappa-carrageenan with the sample 2 times, 4 times and 6 times injection compared to the control, except by giving 1 time injection is not significantly different ($P>0.05$) (Tabel 2).

Table 2. Analysis variance of survival rate

Uniformity Source	df	Sum of squares	Mean square	F_{Count}	F_{Table} (0.05-0.01)	
Treatment	4	1960	490	12.25**	3.48	5.99
Random	10	400	40			
Total	14	2360				

Remarks: ** significantly different at the 5% BNT test level.

The results showed that giving kappa carrageenan with a frequency of 4 times and 6 times gave a high survival rate of 80% and 76.67% compared to the treatment with a frequency of giving twice 66.67%, once 56.67% and control 50%. The frequency of 4 times 80% and 6 times 76.67% showed differences ($p>0.05$), but significantly different ($p<0.05$) with 2 times 66.67% and very real at 1 time injection 56.67% and 50% control. 6 times injection was not significantly different ($P>0.05$) with 2 times, but very significantly different ($P<0.05$) with 1 time injection and control. 6 times Injection was not significantly different ($P>0.05$) with 2 times, but very significantly different ($P<0.05$) with 1-time injection and control. Giving 2 times was not significantly different ($P>0.05$) from 1 time and significantly different from the control. At the same time, the control and 1-time injection showed results that were not significantly different ($p>0.05$) (Table 3).

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Table 3. Significant difference test for the best treatment

Treatment	Average	BNT 5%=√2(40):3)x(t0.05(10)=2.228)=11.49				
		4 times	6 times	2 times	1 time	control
4 times	80.00					
6 times	76.67	3.33				
2 times	66.67	13.33*	10.00 tn			
1 time	56.67	23.33**	20.00 **	10.00 tn		
control	50.00	30.00**	26.67**	16.67*	6.67tn	

Remarks: ** (Very significantly), * (significantly), tn (not significantly)

The survival rate of crayfish during 30 days of rearing, after being challenged with *A. hydrophila* bacteria showed that giving of kappa-carrageenan 4 times and 6 times gave the highest survival rate compared to the control. By giving of 4 and 6 times injections did not show a significant difference. However, 4 times injections showed higher survival (80%). (80%). It is suspected that the injection of kappa-carrageenan with a frequency of 4 times is considered to be able to provide opportunities for host cells to recognize microorganisms that enter the lobster body.

This is following the opinion of Van de Braak (2002) that the most important thing is the immunity system in crustaceans. immunity system in crustaceans is the recognition of microorganisms that enter the shrimp body mediated by hemocytes and plasma proteins. Several recognition proteins have been described and are known as pattern recognition proteins (PRPs). These PPRs recognize carbohydrate groups from components of the microorganism cell walls, such as lipopolysaccharides (LPS) or peptidoglycan (PG) from bacteria, B-1,3-glucan derived from fungi, and peptidoglycan (PG) from bacteria (Vargas-Albores et al, 1996). This is also reinforced by Suryati (2010), who explained that the provision of kappa-carrageenan was able to stimulate and increase resistance in fish to infection with *A. hydrophila* bacteria.

Abnormal or excessive frequency of administration of kappa-carrageenan is thought to suppress the level of immunity in lobsters. Excessive is thought to be able to suppress the level of immunity in lobsters so that it does not have a significant effect on the level of lobster survival rate as in the administration of kappa-carrageenan as much as 6 times. Based on the opinion of Robertsen et al (1990) Jasmanindar (2009) explains that the frequency gives more response or more often stimulates the resistance system of vaname shrimp so that optimal protection is obtained. However, too frequent or excessive administration will indirectly affect the survival rate of lobsters caused by hemocyte cells that are full of extract particle charges due to too frequent stimulation.

Water Quality

Table 3. Water Quality

Parameters	Range
Oxygen	6.25 mg/l
Carbodioxide	3.68 mg/l
pH	6.8
Ammonia	0.17 mg/l
Temperature	26.90 C

Remarks: Primer Data

Water quality is one of the supporting factors to ensure the survival of lobsters.

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During maintenance, water quality is still in the optimal range which can be seen in Table 3.

Water quality data on oxygen parameters during the study were in the range of 6.25 mg/l and suitable for use as a maintenance medium. Lobsters are known to be tolerant of very low dissolved oxygen content. To grow and develop properly they need dissolved oxygen levels of more than 4 mg/l (Taufiq et all, 2016). Temperature conditions in the maintenance media are one thing that needs to be considered because they affect the metabolic process and oxygen solubility of the media. During the study, the media temperature ranged from 26.9 ºC, which shows the optimal range for lobster rearing. The degree of acidity (pH) during the study was 6.8. According to Setiawan (2006), the ideal pH for crayfish growth is 6-8 and this indicates that the maintenance media is still in a feasible range. Ammonia is the result of the extraction and decomposition of organic matter such as crayfish feces and excessive feed residue in the container. Ammonia levels according to the Sukabumi Freshwater Aquaculture Center water quality standards are no more than 1 mg/l. The results of ammonia measurements during maintenance were 0.17 mg/l and this shows that it is still in the normal range. The carbon dioxide level during the study was 3.68 mg/l, indicating an optimal range. Kurniawan and Hartono 2006 explained that a good carbon dioxide content for lobsters is less than 10 mg/l.

CONCLUSION

Based on the results of the research it can be concluded that cappa-carageen can increase the survival rate of the lobster and by given 4 times injections of cappa-carrageenan will be effective in increasing the resistance of the lobster than 2 times, 4 times dan 6 times injections.

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REFERENCES

Abirami, R. G., & Kowsalya, S. (2013). Antidiabetic activity of ulva fasciata and its impact on carbohydrate metabol-ism enzymes in alloxan induced diabetic rats. *International Journal of Research in Phytochemistry and Pharmacology*, 3(3), 136–141

Aodoys, (2009). *Teknik Penyuntikan Ikan*. http://aodoys.blogspot.com/2009/08/teknik-penyuntikan-pada -ikan.html. diakses pada Februari 2012.

Effendie. (1997). *Biologi Perikanan*. Yayasan Pustaka Nusatama: Yogyakarta. 163 ha

Effendi, (2002). *Biologi Perikanan*. Yayasan Pustaka Nusantara, Yogyakarta.

Gaspersz, V. (1991). *Metode Perancangan Percobaan*. CV.ARMICO. Bandung

Hakim, D. M., Tjahjaningsih, W., Sudarno, Abdilla, A.A. (2016). Pengaruh ekstrak alga merah (Kappaphycus alvarezii) terhadap jumlah total bakteri dan nilai organoleptik ikan Kembung (Rastrelliger sp.). *Jurnal Ilmiah Perikanan dan Kelautan*.

Hudaifah, I. (2020). Komponen bioaktif dari euchema cottonii, ulva lactuca, halimeda opuntia, dan padina australis. *Jurnal Lemuru*, 2(2), 63–70.

Hutabarat, M. A. A., Sari, N. I., & Leksono, T. (2013). *The Antibacterial Effectiveness Of Seaweed (Eucheuma Cottonii) Extract On Bacillus Cereus And Pseudomonas Aeruginosa*. Riau University.

Jasmanindar (2009). *Pengggunaan Ekstrak Gracilaria verrucosa untuk*

DOI: 10.32663/ja.v21i2.4205

Meningkatkan Sistem Ketahanan Udang Vaname Litopnenaeus Vannamei. Bogor. Institut Pertanian Bogor.

Kreckhoff, R. L., Ngangi, E. L. A., Undap, S. L., & Kusen, D. J. (2019). Crude extracts of Kappaphycus alvarezii algae cultivated in several seaweed production centers in North Sulawesi, Indonesia as an immunostimulant. *Aquaculture, Aquarium, Conservation & Legislation*, 12(2), 678–686

Kurniawan dan Hartono. (2007). *Pembesaran Lobster Air Tawar Secara Cepat*. Jakarta: Penebar Swadaya.

Lukito A dan Prayogo. (2007). *Panduan Lengkap Lobster Air Tawar*. Penerbar Swadaya: Jakarta.

Moussavou, G., Kwak, D. H., Obiang-Obonou, B. W., Ogandaga Maranguy, C. A., Dinzouna Boutamba, S.-D., Lee, D. H., Manvoudou, P, O. G., Ko, K., Seo, J. I., & Choo, Y. K. (2014). Anticancer effects of different seaweeds on human colon and breast cancers. *Marine Drugs*, 12(9), 4898–4911.

Safia, W. (2020). Kandungan nutrisi dan bioaktif rumput laut (*Euchema cottonii*) dengan metode rakit gantung pada kedalaman berbeda. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 23(2), 261–271

Setiawan. (2006). *Teknik Pembenihan dan Cara Cepat Pembesaran Lobster Air Tawar*. Jakarta: Agromedia Pustaka.

Siregar, A.F., Sabdono, A., Pringgenies, D. (2012). Potensi antibakteri ekstrak rumput laut terhadap bakteri penyakit kulit *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, dan *Micrococcus luteus*. *Journal Of Marine Research*, 1(2), 152-160.

Soelama, H. J. J., Kepel, B. J., & Siagian, K. V. (2015). Uji minimum inhibitory concentration (MIC) ekstrak rumput laut (*Eucheuma cottonii*) sebagai antibakteri terhadap *Streptococcus mutans*. *E-GiGi*, 3(2).

Suriyati (2011). *Pemberian Kappa-karaginan Kappaphycus alvarezii Untuk Meningkatkan Respon Imun No Spesifik dn Resistensi Lobsyer Air Tawar Cherax qudircarinatus terhadap Penyakit Mas (Motile Aeromonad septicemia)*. Pangkep Politeknik Negeri Pangkep.

Tatiya, A. U., Tapadiya, G. G., Kotecha, S., & Surana, S. J. (2011). *Effect of Solvents on Total Phenolics, Antioxidant and Antimicrobial Properties of Bridelia retusa Spreng. stem bark*

Taufiq, M., Dewi, K.M.C., Handono., Rosidi I. (2016). Pengaruh pemberian berbagai jenis pakan terhadap pertumbuhan lobter air tawar *Cherax quadricarinatus*. *Educational and Human Development Journal*, 01(01).

Van de Braak, K. (2002). *Haemocytic Defence in Black Tiger Shrimp (Penaeus monodon)*. Thesis. The Wageningen University, Netherland. 186 pp.

Vargas-Albores F, Jimenes-Vega f, Soderhall K (1996). A plasma protein Isolated from brown shrimp (*Penaeus californiemsis* which enhances the activation of prophenoloxidase system by β-1,3-glucan. *Develop Comp Immunol* 20.299-306

Vijayabaskar, P., & Shiyamala, V. (2011). Antibacterial activities of brown marine algae (*Sargassum wightii* and *Turbinaria ornata*) from the gulf of mannar biosphere reserve. *Advances in Biological Research*, 5(2), 99–102