

The Influence of Adding Api-Api Mangrove Leaf Extract as an Immunostimulant on Vaname Shrimp Against Vibriosis Disease Caused by *Vibrio parahaemolyticus* Bacteria

Anjani Arum Mahenda¹, Misbakhul Munir² Dian Sari Maisaroh³

^{1,2,3} UIN Sunan Ampel Surabaya, Indonesia

E-mail: anjaniarumm@gmail.com, misbakhul_munir@uinsby.ac.id,
maisaroh.ds@uinsby.ac.id

*Correspondence: anjaniarumm@gmail.com

KEYWORDS

Avicennia Alba,
Immunostimulant,
Vaname Shrimp, *Vibrio*
Parahaemolyticus.

ABSTRACT

Vanname shrimp cultivation is one of the most developed aquacultures in Indonesia and makes it one of the commodities with the highest foreign exchange inputs for the Indonesian state from the fisheries sector. Disease attack is one of the main causes of failure in vaname shrimp culture, especially disease attacks caused by *Vibrio parahaemolyticus* bacteria. Avicennia alba mangrove leaf extract is known to have active compounds that can act as antibacterial. This research was conducted by mixing Avicennia alba mangrove leaf extract with doses of 150 ppm (VA), 250 ppm (VB) and 350 ppm (VC) into commercial feed by re-pelleting. Positive control (KV+) treatment used commercial vitamins added to the feed. The vaname shrimp used was 60 days old with an average initial weight of 8.9 grams. The aquariums used were 15 pieces with a size of 60 x 30 x 40 cm and a volume of 54 liters of water. The white shrimp challenge test with *Vibrio parahaemolyticus* bacteria with a concentration of 10⁶ CfU/ml as much as 0.2 ml. The clinical symptoms of white vaname shrimp include pale white hepatopancreas, blackened tail, grippy swimming legs, necrosis in some parts of the body, necrosis of the tail, blackened and necrotic rectum, broken intestines that are partially brown and starting to turn white, empty intestines, presence of white feces. appears in the aquarium, decreased appetite, as well as not actively swimming. The results showed that Avicennia alba leaf extract had an effect on absolute weight growth (W), growth rate (GR), specific growth rate (SGR), survival rate (SR), total haemocyte count (THC) and phagocytic activity of white shrimp. The dose of 350 ppm VC treatment was the most influential dose as an immunostimulant in this study.

Attribution- ShareAlike 4.0 International (CC BY-SA 4.0)



Introduction

Shrimp farmers in Indonesia often encounter various problems, both internal and external, in their shrimp cultivation efforts. According to (GOAL, 2018), the main problem with the highest percentage of shrimp farming failures is disease. The trend of disease prevention efforts using antibiotics by shrimp farmers can have serious consequences for humans. Antibiotics in shrimp can lead to bacterial resistance that poses health risks to humans when consuming the shrimp. Control of bacterial diseases in shrimp farming is recommended to use environmentally friendly and readily available materials (Muliani, et al., 2003).

The identification of vibrio in shrimp infected with White Feces Disease (WFD) in Indonesia has been reported to occur in Central Java Province, and the species found include *V. parahaemolyticus*, *V. vulnificus*, *V. cholera*, *V. anguillarum* (Jayadi et al., 2016) as mentioned in (Sumini & Kusdarwati, 2020). Immunostimulants are natural substances capable of enhancing various immune cells in shrimp, such as phagocytic cells, lysozymes, respiratory bursts, total white blood cells, and red blood cells (WBC/RBC), as well as hemocytes. Many traditional plants have been proven to prevent and treat some diseases in fish and shrimp. However, the utilization of these cultivated organisms often competes with humans. Hence, there is a need for alternative use of natural materials that do not compete with humans and have the same or even better bioactive compound content than traditional plants that are usually used (Samuria, et al., 2018).

Phytochemical analysis of *Avicennia alba* leaves reveals the presence of active chemicals such as flavonoids, saponins, and tannins. Saponin compounds can damage bacterial cell membranes and kill bacteria (Assani, 1994). The extract of *Avicennia alba* leaves containing these active compounds, when applied through commercial feed regularly, is expected to strengthen the immune system of vaname shrimp itself. This research is needed to test the effect of adding *Avicennia alba* leaf extract at three different doses to commercial feed as an immunostimulant for vaname shrimp against vibriosis, which is caused by *Vibrio parahaemolyticus* bacteria.

The objectives of this study are 4, namely: (a) knowing the effect of adding *Avicennia alba* leaf extract absolute weight, growth rate (GR), specific growth rate (SGR) and survival of vaname shrimp, (b) analyzing the effect of adding *Avicennia alba* leaf extract on the total haemocyte count (THC) of vaname shrimp, (c) analyzing the effect of adding *Avicennia alba* leaf extract against the phagocytosis activity of vaname shrimp before and after the *Vibrio parahaemolyticus* bacterial challenge test, (d) knowing the most influential treatment dose as an immunostimulant of vaname shrimp.

Research Methods

Avicennia alba mangrove leaves are taken from the Wonorejo mangrove conservation area where extract making is carried out at the integration laboratory of UIN Sunan Ampel Surabaya. Vaname shrimp taken from PT. Wirawan Cultivation of Jepara Creations, precisely in the Blebak unit, Rw8, Jambu, Mlonggo District, Jepara Regency, Central Java 59452. In vivo test of vaname shrimp was conducted at the Brackish Water Aquaculture Fisheries Center (BBPBAP) Jepara.

This research lasted for 6 months from August 2020 to January 2021.

Flow

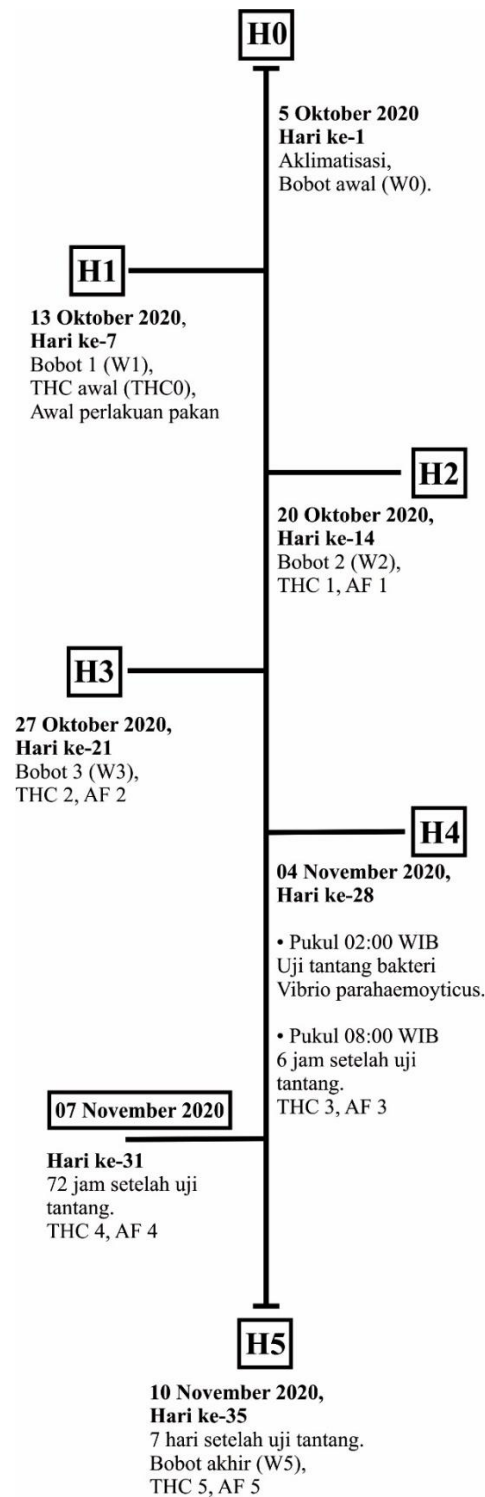


Figure 1. Procedure Flow of THC, AF, and Weights of Vaname Shrimp (*Litopenaeus vannamei*) (Standalone Design, 2020)

Test Animal Preparation

Vaname shrimp are 60 days old with an average initial weight of 8.9 grams. Vaname shrimp are acclimatized for 7 days. Vaname shrimp have been PCR tested and the results are negative for AHPN and WSSV. The number of vaname shrimp used is 10

The Influence of Adding Api-Api Mangrove Leaf Extract as an Immunostimulant on Vaname Shrimp Against Vibriosis Disease Caused by *Vibrio parahaemolyticus* Bacteria

per aquarium. Vaname shrimp weighing is carried out once a week by weighing 10 vaname shrimp at the same time then the weighing results are divided by the number of vaname shrimp, so that vaname shrimp weight data is obtained.

Container Preparation

There are 15 aquariums measuring 60 x 30 x 40 cm with a water volume of 54 liters, of which 3/4 of the volume is filled with seawater and 1/4 is filled with fresh water to produce water salinity of 30 ppt. The aquarium is labeled with treatment codes KV (+), KV (-), VA, VB, and VC with three iterations so that the test code is generated as follows:

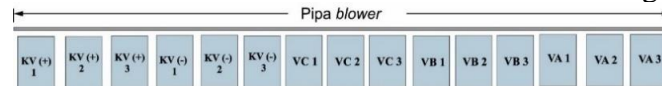


Figure 2. Illustration of Vaname Shrimp Rearing Aquarium (*Litopenaeus vannamei*) with Blower Pipe Position (Standalone Design, 2020)

Information:

KV (+): Positive Control, feeding treatment with the addition of commercial vitamins

KV (-) : Negative Control, feeding treatment without any addition

VA : Treatment of adding *Avicennia alba* mangrove leaf extract to feed at a dose of 150 ppm.

VB : Treatment of adding *Avicennia alba* mangrove leaf extract to feed at a dose of 250 ppm.

VC : Treatment of adding *Avicennia alba* mangrove leaf extract to feed at a dose of 350 ppm.

Aeration hoses, aeration taps, aeration stones, ballast, aquariums and seawater are sterilized by soaking using $\text{Ca}(\text{ClO})_2$ which is chlorine and left overnight and then rinsed. Prepare 1 fiber bath with a capacity of 1 ton for sterile seawater supply and given $\text{Ca}(\text{ClO})_2$ left to evaporate for 5 days (Samuria, et al., 2018). Preparing 2 fiber tubs for acclimatization of vaname shrimp.

Creation of Bacterial Suspension

The *Vibrio parahaemolyticus* bacteria used in this research were obtained from the laboratory collection of MKHA, Balai Besar Perikanan Budidaya Air Payau (BBPBAP) Jepara. A single bacterial colony of *Vibrio parahaemolyticus* was taken and suspended in sterile 0.9% NaCl solution, then homogenized using a vortex mixer (Hasbiah, et al., 2015). The next step was determining the bacterial count spectrophotometrically ($\lambda = 580 \text{ nm}$, transmittance 25%) (Hasbiah, et al., 2015; Rauf, et al., 2016), resulting in a bacterial density of 106 CFU/ml.

Preparation of *Avicennia alba* Mangrove Leaf Extract

Avicennia alba mangrove leaves were washed with water, then cut into small pieces using scissors and dried indirectly without direct exposure to sunlight for 7 days. Afterward, they were oven-dried at 40°C for 24 hours (Datu, 2017). The dried leaves were ground using a blender and sieved to obtain *Avicennia alba* leaf simplisia (Datu, 2017). The next process involved cold extraction using the maceration method with methanol as the solvent. A total of 200 grams of leaf powder was soaked (macerated) in methanol at a ratio of 1:5 (Saptiani, et al., 2018) for 3 x 24 hours (Fitri, et al., 2018).

The *Avicennia alba* leaf maceration results were filtered using filter paper, and the filtrate was collected in glass containers. The filtration results underwent a hot extraction process, with the filtrate evaporated using a rotary evaporator at 50°C with a speed of 200 rpm to separate the solvent from the *Avicennia alba* leaf extract (Lantah, et al., 2017). The *Avicennia alba* leaf extract in paste form was stored in petri dishes and kept in a low-temperature refrigerator to preserve its bioactive content (Datu, 2017).

Feed Supplementation

Avicennia alba mangrove leaf extract paste was weighed for three different doses: 150 ppm, 250 ppm, and 350 ppm. Commercial pellet feed produced by PT. Evergreen Indonesia with feed code 922-3M was used in this research. Three kilograms of the feed were ground using a blender and sieved. Then, 1 kg of each was mixed with *Avicennia alba* extract according to the respective doses. Afterward, squid fishmeal attractant was gradually added to the feed to mask its odor, following a sequence of 1 tablespoon, 1 ½ tablespoons, and 2 tablespoons for each treatment. Additionally, each treatment received a proglucagon solution as a feed binder. Once thoroughly mixed, the feed was reshaped and dried without direct exposure to sunlight. A positive control treatment was included by adding commercial vitamins to the pellet feed at a rate of 0.2 grams per 1 kg of feed and left overnight.

Feeding

Vannamei shrimp were fed five times a day. The feeding rate was 3% of the total shrimp biomass per day for each aquarium (Rusadi, et al., 2019; Putri, et al., 2013). The feeding times were adjusted according to the vannamei shrimp feeding schedule in the ponds, which were at 07:00 AM, 10:00 AM, 01:00 PM, 04:00 PM, and 09:00 PM.

Total Hemocyte Count (THC) Calculation

The method for calculating the total hemocytes in shrimp was based on (Liu & Chen, 2004). It began with the preparation of a 10% EDTA anticoagulant solution, where 0.1 grams of sodium citrate was dissolved in 1 ml of aquabides and homogenized with a vortex mixer. Hemocyte samples from vannamei shrimp in each aquarium, measuring 0.1 ml, were collected using a 1 ml syringe that had been coated with the 10% EDTA anticoagulant solution. Hemocytes were then taken in an amount of 10 µl using a micropipette and counted using a hemocytometer under a microscope with a magnification of 200 – 400 times.

Preparation of Phagocytosis Activity

Phagocytosis activity treatment using *Vibrio parahaemolyticus* bacteria on vannamei shrimp was conducted through two different methods: direct and indirect. The indirect method involved the incubation of 20 µl of shrimp hemocytes, taken simultaneously with the THC observation samples, with 10 µl of *Vibrio parahaemolyticus* bacteria in a microplate for 30 minutes (Rusadi, et al., 2019). The preparations were soaked in alcohol for 10 minutes to remove surface lipids (Putri, 2012).

After drying the preparations with tissue, 10 µl of the incubation result containing shrimp hemocytes and *Vibrio parahaemolyticus* bacteria was placed on a glass slide and flattened using a cover glass at a 45° angle. The preparations were air-dried, fixed with 95% alcohol for 15 minutes, and then repositioned at a 45° angle until completely dry. Giemsa stain was applied to the preparations to cover the entire surface using a dropper

The Influence of Adding Api-Api Mangrove Leaf Extract as an Immunostimulant on Vaname Shrimp Against Vibriosis Disease Caused by *Vibrio parahaemolyticus* Bacteria

and left for 30 minutes, then repositioned until dry. The preparations were observed under a microscope with an immersion oil lens at a magnification of 400 - 1000 times.

Water Quality

Water quality measurements were based on parameters such as salinity, pH, dissolved oxygen levels, and temperature. Dissolved oxygen levels in the aquariums were measured using a Dissolved Oxygen meter, while salinity was measured using a refractometer. Temperature was measured using a digital dip thermometer, and pH was measured using a pH meter.

DATA ANALYSIS

Absolute Weight Growth (W) of Vannamei Shrimp The absolute individual weight growth of vannamei shrimp was calculated using the formula (Effendie, 1979):

$$W = W_t - W_0 \quad (1)$$

Where:

W = Growth in absolute individual weight of test animals (g)

W_0 = Shrimp weight at the beginning of the study (g)

W_t = Shrimp weight at the end of the study (g)

Growth Rate (GR) of Vaname Shrimp

Observation of growth rate is carried out by weighing vaname shrimp using digital scales. The data used were shrimp weight data at the beginning of the study and at the end of the study. The data is analyzed using formulas:

$$GR = \frac{W_t - W_0}{t} \quad (2)$$

Information:

GR : Growth rate

t : Duration of observation

W_t : Final weight of shrimp

W_0 : Shrimp starting weight

Specific Growth Rate (SGR) of Vaname Shrimp

The SGR data of vaname shrimp is obtained from the results of multiplying the growth rate (GR) by 100%, so that the percentage of SGR of vaname shrimp is obtained. The formula used is the formula proposed by (Fajar, et al.,2014) in (Nurhijrah, 2019), as follows:

$$GR = \frac{W_t - W_0}{t} \times 100\% \quad (3)$$

Where:

SGR : Daily growth rate (%)

W_t : The average weight of vaname shrimp at the end of the study

W_0 : The average weight of vaname shrimp at the beginning of the study

t : Duration of maintenance (days)

Kelulushidupan (Survival Rate / SR) Udang Vaname

Survival observations were carried out directly by recording the number of shrimp at the beginning of the study and at the end of the study (Nuhman, 2009). The data on the number of shrimp is then processed using the formula (Effendie, 1979):

$$SR = \frac{N_t}{N_0} \times 100\% \quad (4)$$

Information:

SR : Survival rate (%)

N_t : Number of live shrimp at the end of the study

N_0 : Number of live shrimp at the start of the study

Perhitungan Total Haemocyte Count (THC) Udang Vaname

The calculation results of total shrimp hemocytes or THC are then entered into the formula (Brock & Madigan, 1991) in (Tampangallo & Susianingsih, 2011), the following:

$$THC = N \times 25 \times 50 \times 10^3 \text{ sel/ml} \quad (5)$$

Information:

THC : The number of vaname shrimp hemocytes counted in the large haemocytometer box

N : Average hemocytes found

Phagocytosis activity of vaname shrimp

The results of the calculation of phagocytosis cells in the form of both active and inactive cells are then entered into the Anderson and Siwicki (1995) in (Putri, et al., 2013), formula is below:

$$\%AF = \frac{\text{Jumlah sel fagositosis}}{\text{Jumlah sel diamati}} \times 100\% \quad (6)$$

Results and Discussions

Water Quality Control During Maintenance

Water quality measurement is carried out once a week, while the water quality parameters measured include; (i) Dissolved oxygen content (D.O), (ii) Salinity, (iii) Temperature, and (iv) pH. Measurement of dissolved oxygen levels using a D.O meter, while temperature measurement using a digital dip thermometer. Salinity measurement using a refractometer and pH measurement using a pH meter. The results of water quality measurements during the study can be seen in Table 1 below.

Table 1. Water Quality Measurement

Parameter	Code				
	KV (+)	KV (-)	VA	VB	VC
Dissolved oxygen levels (Mg/L)	5,88 – 6,33	5,73 – 6,31	5,85 – 6,21	5,91 – 6,33	5,88 – 6,37
Temperature (°C)	28,0 – 28,6	28,1 – 28,6	28,1 – 28,8	28,1 – 28,8	28,1 – 28,8
Salinitas (PPT)	30				

pH	8,21 – 8,51	8,35 – 8,58	8,20 – 8,55	8,20 – 8,36	8,35 – 8,45
----	-------------	-------------	-------------	-------------	-------------

According to Liao and Muarai (1986) as cited in (Sahrijanna & Sahabuddin, 2014), the optimal temperature range for the survival of shrimp is 20 - 30°C. Therefore, the aquarium temperature during the study is still within the optimum range. The recommended dissolved oxygen level for shrimp maintenance is >5 mg/L (Boyd and Tucker, 1998) as cited in (Adiyana, et al., 2017), and in this study, the dissolved oxygen level ranged from a minimum of 5.88 to a maximum of 6.37, which is considered optimal. The dissolved oxygen level was higher during the acclimatization treatment in two fiber tanks, measuring 8.87 and 8.85, respectively, because each tank used 3 and 4 aeration hoses. This higher oxygen level was necessary as the fiber tanks accommodated a large number of vannamei shrimp after transportation from the ponds. Additionally, the high shrimp density required more aeration, and the aeration setup also considered the size of the fiber tank in relation to the shrimp size and stocking density.

According to Budiardi (2008) as cited in (Adiyana, et al., 2017), the optimum pH for shrimp is 7.5 - 8.5. The pH measurements in the study ranged from 8.23±0.06 to 8.48±0.05, indicating that the pH levels in this study are still within the optimum range for vannamei shrimp growth. The optimum salinity range for vannamei shrimp is between 15 ppt - 25 ppt (Boyd, 1989) as cited in (Adiyana, et al., 2017). However, according to (Li, et al., 2007) as cited in (Adiyana, et al., 2017), an increase in salinity up to 30 - 45 ppt does not affect the growth of vannamei shrimp. This suggests that vannamei shrimp have a relatively wide tolerance for salinity levels.

The preliminary results of this study indicate that vannamei shrimp raised in the aquarium cannot tolerate excessive aeration, as it leads to high dissolved oxygen levels, with dissolved oxygen levels above 7 mg/L considered too high for vannamei shrimp to tolerate. The optimal range for dissolved oxygen is between 5 mg/L and >7 mg/L. According to (Sumeru & Anna, 1992), excessive dissolved oxygen can lead to Gas Bubble Disease, which can cause shrimp mortality, as observed in the preliminary experiments of this study. In conclusion, the efforts undertaken aim to minimize shrimp mortality during maintenance due to external factors, ultimately obtaining survival data for vannamei shrimp influenced by the treatment variables in this study.

Absolute Weight Growth (W), Growth Rate (GR) and Specific Growth Rate (SGR) of Vaname Shrimp

The maintenance of vaname shrimp (*Litopenaeus vannamei*) was carried out when the shrimp was 60 days old, the maintenance time during the study was 35 days, so that the age of vaname shrimp at the end of the study was 95 days. The weight of vaname shrimp is weighed once a week precisely for 5 weeks, so that data is obtained 5 times the weight of shrimp. Absolute weight growth (Wm), Growth rate (GR) and Specific growth (SGR) calculated by the formula can be seen in Table 2.

Table 2. Growth Results of Vaname Shrimp (*Litopenaeus vannamei*)

Code	KV (+)	KV (-)	VA	VB	VC
Starting Wei	H 0	8.90 ± 0.00			

ght											
Day	H	10,70	±	11,80	±	11,67	±	12,50	±	12,07	±
-7	1	1,14		1,68		0,49		0,30		0,23	
Day	H	13,65	±	14,36	±	14,25	±	15,03	±	15,04	±
-14	2	0,95		0,75		0,88		0,90		1,71	
Day	H	16,12	±	16,49	±	17,12	±	19,40	±	19,97	±
-21	3	1,44		1,91		1,81		0,42		1,35	
Day	H	18,14	±	18,38	±	18,22	±	20,86	±	22,14	±
-28	4	1,09		1,79		1,07		0,86		1,94	
Day	H	20,87	±	18,66	±	20,59	±	22,34	±	23,32	±
-35	5	2,33		0,62		1,30		1,26		0,94	
Average Absolute Weight Growth / W (gr)		11,97 2,33 ^{ab}	±	9,76 0,62 ^b	±	11,69 1,30 ^{ab}	±	13,44 1,26 ^a	±	14,42 0,94 ^a	±
Average GR Growth Rate (gr/day)		0,34 0,07 ^{ab}	±	0,28 0,02 ^b	±	0,33 0,04 ^{ab}	±	0,38 0,04 ^{ab}	±	0,41 0,03 ^a	±
Average Specific Growth Rate / SGR (%)		34,19% 0,07	±	27,89% 0,02	±	33,39% 0,04	±	38,39% 0,04	±	41,20% 0,03	±

Description: Superscript letters behind different numbers show a noticeable difference ($P < 0.05$) at a 95% confidence interval

H0 data is the initial research data, while H1, H2 and H3 data are weight data after the treatment of adding mangrove leaf extract, positive control with vitamins and negative control without treatment. H0, H1, H2 and H3 data are vaname shrimp weight data before the challenge test treatment, while H4 and H5 data are vaname shrimp weight data after the challenge test. The absolute weight growth of vaname shrimp before the challenge test treatment was in H3, namely KV (+) of 16.12 ± 1.44 grams, **KV (-)** of 16.49 ± 1.91 grams, VA of **17.12 ± 1.81** grams. VB of 19.40 ± 0.42 grams and VC of **19.97 ± 1.35** grams. The absolute weight growth of vaname shrimp after the challenge test precisely at the end of maintenance on H5 was **KV(+)** of **20.87 ± 2.33 grams**, **KV(-)** 18.66 ± 0.62 grams, **VA** 20.59 ± 1.30 grams, **VB** of 20.59 ± 1.30 grams and VC of **23.32 ± 0.94** grams.

The results of One way Anova analysis showed that there was an effect of treatment on the growth of absolute weight of vaname shrimp both before and after the *Vibrio parahaemolyticus* bacterial challenge test. The results of the tukey test showed that VB and VC treatment most affected the absolute weight growth of vaname shrimp. The results of One way Anova analysis showed that there was an effect of treatment on the growth rate (SGR) of vaname shrimp both before and after the challenge test. The results of the tukey test showed that VC treatment had the most effect on the growth rate of vaname shrimp. Specific growth rate (SGR) is a percentage of growth rate (GR), where

The Influence of Adding Api-Api Mangrove Leaf Extract as an Immunostimulant on Vaname Shrimp Against Vibriosis Disease Caused by *Vibrio parahaemolyticus* Bacteria

the highest specific growth rate is in VC treatment of $41.20\% \pm 0.03$ gr/day, VB of $38.39\% \pm 0.04$ gr/day, KV (+) of $33.39\% \pm 0.04$ gr/day, VA of $27.89\% \pm 0.02$ gr/day and KV (-) $27.89\% \pm 0.02$ gr/day .

The Calculation of Total Haemocyte Count (THC) Vaname shrimp

The calculation of total haemocyte count (THC) which is the total number of hemocyte cells in shrimp was carried out as many as 6 sampling (see Figure 4.4-1). H0 sampling of shrimp hemocytes is carried out at the end of acclimatization, namely on the 7th day before the application of treatment to the entire aquarium. H1 hemocyte sampling was carried out a week after the application of treatment, namely in week 2, while H2 hemocyte sampling was carried out in week 3, exactly 13 hours before the challenge test was carried out. H3 hemocyte sampling was carried out at 6 hours after challenge test treatment with *Vibrio parahaemolyticus* bacteria. H4 hemocyte sampling was carried out at 72 hours after the challenge test, and H5 hemocyte sampling was carried out at week 4, namely at 168 hours after the challenge test. The calculation of total hemocytes of vaname shrimp can be seen in Table 3.

Table 3. Total Haemocyte Count (THC) Vaname Shrimp (*Litopenaeus vannamei*)

THC x 10 ⁶ sel/ml	KV (+)	KV (-)	VA	VB	VC
H0	17.53 ± 8,21	13,71 ± 3,07	17,56 ± 6,05	23,03 ± 4,77	21,63 ± 6,02
H1	22,32 ± 6,79	16,68 ± 0,66	23,49 ± 7,78	36,84 ± 1,14	23,23 ± 6,51
H2	24,82 ± 7,76	26,35 ± 5,01	25,89 ± 10,69	29,58 ± 3,79	36,35 ± 0,33
H3	23,82 ± 6,40	15,24 ± 6,35	31,09 ± 1,60	23,76 ± 9,01	35,51 ± 0,96
H4	12,07 ± 5,08	4,73 ± 1,80	19,83 ± 8,21	23,16 ± 2,84	27,48 ± 3,25
H5	7,18 ± 2,79	3,41 ± 0,93	26,31 ± 5,14	24,17 ± 0,24	28,32 ± 4,48

The last total hemocytes of vaname shrimp (see Table 4.4-1) before the challenge test treatment on H2 were highest in VC treatment of $36.35 \pm 0.33 \times 10^6$ cells/ml while the lowest in KV(+) aquariums of $24.82 \pm 7.76 \times 10^6$ cells/ml. The total hemocytes of vaname shrimp which ranks 2nd are in VB treatment of $29.58 \pm 3.79 \times 10^6$ cells / ml, third place in KV (-) treatment of $26.35 \pm 5.01 \times 10^6$ cells / ml and the last 4th place in VA treatment of $25.89 \pm 10.69 \times 10^6$ cells / ml. The observation of H2 negative control treatment with the code KV(-) has increased significantly, indicating the development of good adaptation in vaname shrimp. All treatments improved except for the VB treatment which had a slight decrease in the number of hemocytes.

The calculation of total vaname shrimp hemocytes at H5 carried out at the end of maintenance, namely 168 hours after the challenge test with *Vibrio parahaemolyticus*, showed the following results: VA treatment of $26.31 \pm 5.14 \times 10^6$ cells / ml, VB treatment of $24.17 \pm 0.24 \times 10^6$ cells / ml, VC treatment of $28.32 \pm 4.48 \times 10^6$ cells / ml, KV (+) treatment of $7.18 \pm 2.79 \times 10^6$ cells / ml, and KV(-) treatment of $3.41 \pm 0.93 \times 10^6$ cells/ml.

The results of the One way Anova test showed that there was a significant effect on the treatment of total vaname shrimp hemocytes both before and after the *Vibrio parahaemolyticus* bacterial challenge test. The results of the tukey test concluded that the treatment of VA, VB, and VC has the ability to stimulate the immune system of vaname

shrimp in terms of its effect on the total number of vaname shrimp hemocytes. However, between the treatment of VA, VB and VC the results of the analysis did not have a noticeable difference in effect. While the significant effect of total hemocytes of vaname shrimp occurs in H5.

Observation of phagocytosis activity of vaname shrimp

Making preparations for the observation of phagocytosis activity is by counting the number of active cells and inactive cells. Hemocyte cells that actively phagocytosis in *Vibrio parahaemolyticus* bacteria are included in the active cell count, while cells that do not react to *Vibrio parahaemolyticus* bacteria are included in the inactive cell count (see Figure 3). Observation of phagocytosis activity between active cells and inactive cells can be seen in the picture.



(a)



(b)

Figure 3. observation of phagocytosis activity; (a) Active Granular Cells, (b) Non Active Granular Cells

(Author's Documentation, 2020)

H0 sampling of blood collection is carried out at the end of acclimatization on the 7th day before the application of treatment to the entire aquarium. H1 hemocyte sampling was carried out a week after the application of treatment, namely in week 2, while H2 hemocyte sampling was carried out in week 3, exactly 13 hours before the challenge test was carried out. H3 hemocyte sampling was carried out at 6 hours after challenge test treatment with *Vibrio parahaemolyticus* bacteria. H4 hemocyte sampling was carried out at 72 hours after the challenge test, and H5 hemocyte sampling was carried out at week 4, namely at 168 hours after the challenge test.

The Influence of Adding Api-Api Mangrove Leaf Extract as an Immunostimulant on Vaname Shrimp Against Vibriosis Disease Caused by *Vibrio parahaemolyticus* Bacteria

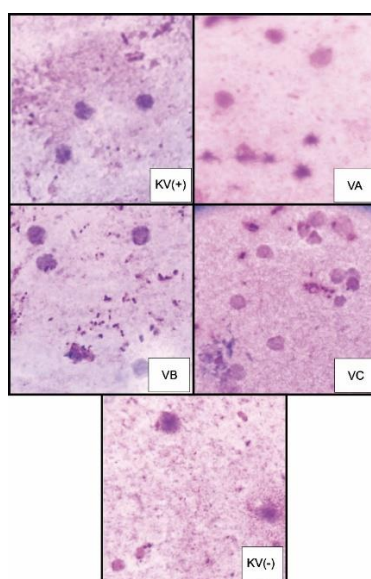


Figure 4. Observation of Vaname Shrimp Phagocytosis Activity Preparations (Author's Documentation, 2020)

The finished preparation is then observed under a microscope with magnification of 400x – 1000x (see Figure 4). The calculation of total hemocytes of vaname shrimp can be seen in Table 4.

Table 4. Phagocytosis Activity Percentage of Vaname Shrimp

Pengamatan	KV (+)	KV (-)	VA	VB	VC
H0	78,03% ± 0,02	76,96% ± 0,03	77,28% ± 0,03	76,84% ± 0,02	77,45% ± 0,04
H1	81,96% ± 0,02	81,34% ± 0,02	82,89% ± 0,03	80,95% ± 0,02	82,80% ± 0,02
H2	80,79% ± 0,02	83,08% ± 0,02	83,98% ± 0,01	82,55% ± 0,03	84,12% ± 0,01
H3	88,01% ± 0,02	87,19% ± 0,02	89,62% ± 0,02	90,79% ± 0,01	91,43% ± 0,02
H4	88,39% ± 0,02	79,96% ± 0,05	86,64% ± 0,04	90,60% ± 0,03	91,67% ± 0,01
H5	82,96% ± 0,05	82,52% ± 0,05	84,02% ± 0,05	83,13% ± 0,05	87,96% ± 0,02

The last percentage of phagocytosis activity (see Table 4) before the challenge test, namely in H2 included KV(+) of 80.79% ± 0.02, VA of 83.98% ± 0.01, VB of 82.55% ± 0.03, VC of 84.12% ± 0.01, and KV(-) of 83.08% ± 0.02. The results of the percentage of phagocytosis activity at the end of maintenance, namely 168 hours after the test against *Vibrio parahaemolyticus* bacteria, were shown in H5 including KV(+) of 82.96% ± 0.05, VA of 84.02% ± 0.05, VB of 83.13% ± 0.05, VC of 87.96% ± 0.02, and KV(-) of 82.52% ± 0.05.

The results of One way Anova analysis showed that the treatment had an effect on the phagocytosis activity of vaname shrimp, but the effect of phagocytosis activity treatment before and after the challenge test did not have a significant effect. The results of the tukey test showed that VC treatment had the most influence on the phagocytosis activity of vaname shrimp. The peak of influence of phagocytosis activity occurs in H3 and H4.

Analysis

Immunostimulants are chemical compounds, drugs, or other substances capable of enhancing the specific and nonspecific immune responses of fish (Putri, et al., 2013; Mastan, 2015). The administration of immunostimulants in feed can be used to boost the body's defense mechanisms against diseases, in addition to providing a balanced nutrient composition in the diet. Immunostimulants directly affect the immune system cells, stimulating them to become more active (Ekawati, et al., 2012). Immunostimulants are natural substances capable of enhancing various immune cells, such as phagocytes, lysozymes, respiration burst, total white blood cells, red blood cells (WBC/RBC), and hemocytes in shrimp. Many traditional plants have been shown to prevent and treat several diseases in fish and shrimp. However, the use of these cultivated plant organisms often competes with humans. Therefore, there is a need for alternative use of natural substances that do not compete with humans and contain bioactive compounds that are equal to or even better than those of traditionally used plants (Samuria, et al., 2018).

The shrimp's immune system relies heavily on nonspecific defense processes as a defense against infection (Lee & Shiau, 2004). Shrimp's first line of defense against diseases is carried out by hemocytes through phagocytosis, encapsulation, and nodule formation. The main immune defense mechanism in shrimp is the cellular immune defense by hemocytes, where the proliferation and increase in the total number of hemocytes are assumed to be a form of cellular immune response in shrimp (Van de Braak, 2002) as cited in (Putri, 2012).

The total haemocyte count (THC) variable in shrimp indicates the health condition of the shrimp. When the total number of hemocytes is within the normal range, the shrimp is considered healthy (Manoppo, et al., 2011). Phagocytosis activity shows the percentage of active hemocytes performing phagocytosis against pathogens (Manoppo et al., 2011). During the maintenance period, it is evident that the treatments influenced the increase in vannamei shrimp hemocyte counts and the increase in phagocytosis percentage.

In conclusion, the increased total hemocyte count in vannamei shrimp, along with the increased phagocytosis activity percentage, indicates that hemocytes not only increased in number but also improved in their phagocytic abilities. Hemocytes are known to be crucial factors in nonspecific cellular defense systems. To confirm that hemocytes are cellular immune defense components, their ability to perform phagocytosis can be seen as an increase during infection. In the presence of infection, nonspecific cellular defense systems are stimulated, which is expected to counteract diseases (Fontaine and Lighter, 1974) as cited in (Putri, 2012).

In Tables 3 and 4, there are cases where the total hemocyte count decreases after the challenge test, indicating a possible reduction in shrimp hemocytes due to the need to combat bacterial infections. However, phagocytosis activity remains high because it is part of the body's defense against infection. This is consistent with the statement by (Effendy & Akbar, 2004) that when a pathogen attack occurs in shrimp, hemocytes play multiple roles, including degranulation, cytotoxicity, and lysis of the material. Therefore, the number of circulating hemocytes may appear to decrease. There are also cases where the total hemocyte count increases but phagocytosis activity decreases. This is because hemocytes have multiple roles in the body's defense system, including wound healing through cellular clumping and carrying and releasing prophenoloxidase system (pro-PO) (Manoppo et al., 2011).

The extract of *Avicennia alba* mangrove leaves, when introduced into the shrimp's body, stimulates hemocytes as part of the nonspecific immune system in shrimp to undergo degranulation and release various proteins, including binding molecules (β -

Glucan-binding protein / β G-BP), Lipopolysaccharide-binding protein / PG-BP), coagulation factors (transglutaminase), prophenoloxidase-related factors (prophenoloxidase activating enzyme, prophenoloxidase, peroxinectin), protein inhibitors (α 2 macroglobulin), and antimicrobial substances (penaedin, lectin) (Manoppo et al., 2011). Some of these secreted proteins play a role in immune responses, such as phagocytosis, encapsulation, melanization, coagulation, prophenoloxidase enzyme activity, opsonization, activation of antimicrobials, and other humoral and cellular processes (Johansson & Soederhall, 1989; Van De Braak, 2000).

According to (Johansson & Soederhall, 1989), immunization can stimulate hemocytes to release the proPO system (communication system) and protein pro-binding TGKpa (signal molecules) that are responsible for communication and activation of cells to perform phagocytosis and encapsulation in crustacean species. The administration of immunostimulants to shrimp, also known as vaccination, is considered to have no side effects and is highly effective in organisms that do not have memory cells in their immune systems. This stimulation or maximization of the nonspecific immune response in shrimp is achieved (Kwang, 1996) as cited in (Darwanti, et al., 2016).

Clinical Symptoms

The challenge test in this study using *Vibrio parahaemolyticus* bacteria employed a 1ml syringe with a bacterial concentration of 10^6 CFU/ml, administered at a dosage of 0.2 ml per vannamei shrimp (see Figure 4.7-1). The challenge test was conducted during the third week of vannamei shrimp cultivation. The challenge was carried out in the early morning, precisely at 02:00 AM, to allow for sampling and observation six hours later, precisely at 08:00 AM. Clinical symptom observations were performed three times: 6 hours after injection, 3 times 24 hours after injection, and 7 times 24 hours after injection.

These clinical symptoms can be observed in Figure 4.7-2. Clinical symptoms of vannamei shrimp infected with *Vibrio parahaemolyticus* bacteria include necrosis of muscle tissue, characterized by a reddish body color and black spots on the swimmerets. Additionally, the intestines become empty (Jannah et al., 2018). According to (Rahmanto, et al., 2014), clinical symptoms include a pale body, reddened legs and tails, wrinkled and reddish-brown to blackish tails, soft carapace, empty intestines, and the appearance of black spots on the shrimp's body and necrosis (Jannah, et al., 2018). Clinical symptoms of white feces disease are characterized by changes in the color of the hepatopancreas to pale and white feces floating on the surface of the pond (Somboon et al., 2016) as cited in (Sumini & Kusdarwati, 2020), as well as a change in the color of the intestines to white (Rajendran et al., 2016) as cited in (Sumini & Kusdarwati, 2020). Clinical symptoms based on morphology are indicated by a soft and loose carapace that does not adhere to the body. Additionally, healthy vannamei shrimp exhibit a clear and transparent body color, while external symptoms include a dark body color followed by body and tail melanization (Sumini & Kusdarwati, 2020).

Following the challenge test with *Vibrio parahaemolyticus* bacteria in vannamei shrimp, the water in the aquarium becomes dirtier more quickly, necessitating more frequent cleaning. This is due to a decrease in the shrimp's appetite as a result of the bacterial infection's influence, leading to leftover feed accumulating at the bottom of the aquarium and causing the water to become dirty. The hepatopancreas is a primary organ responsible for the absorption of feed, transportation, secretion of digestive enzymes, and the deposition of lipids, glycogen, and some other minerals. Disruptions in the

hepatopancreas can disturb the nutrition in the vannamei shrimp's body, resulting in stunted shrimp growth. This condition leads to a decrease in the Average Daily Gain (ADG) and, consequently, an increase in the Feed Conversion Ratio (FCR) due to the accumulation of feed provided during cultivation that is not digested by the vannamei shrimp.

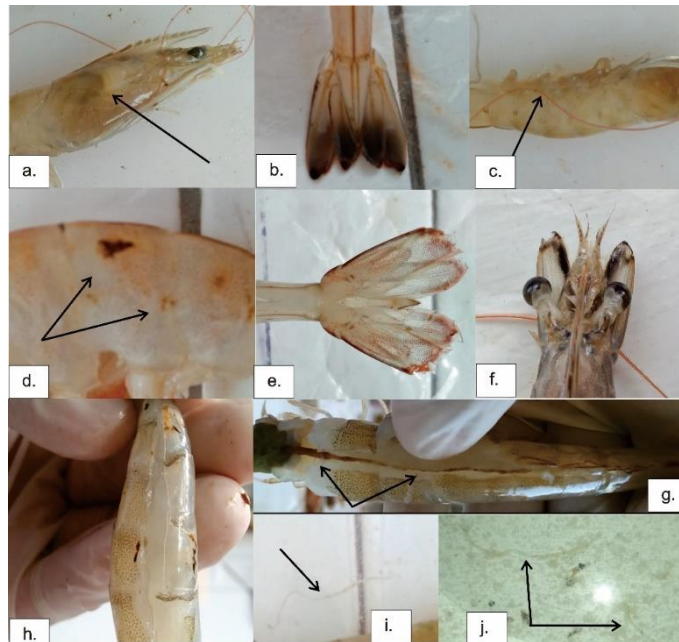


Figure 5. Clinical Symptoms of Vaname Shrimp After Challenge Test with *Vibrio parahaemolyticus* Bacteria
(Author's Documentation, 2020)

The clinical symptoms shown in figure 4.7-2 include (a) Hepatopancreas is pale white, (b) Blackened tail, (c) Gripped swimming legs, (d) Necrosis on some parts of the body, (e) Necrosis on the tail, (f) Blackened rectum and necrosis, (g) Dropped-pustus intestine is partially brown and begins to be white, (h) Empty intestine, (i) & (j) The presence of white feces appears in the aquarium.

Observation 6 hours after injection has not shown noticeable symptoms. Observation of clinical symptoms 24 hours after the challenge test was shown from the behavior of vaname shrimp that approached aeration, some vaname shrimp are still actively swimming while other shrimp only stay at the bottom of the aquarium or at the bottom of the corner of the aquarium. Observation 48 hours after the test challenge vaname shrimp the amount of feed residue began to increase at the bottom of the aquarium, showing a decrease in appetite of vaname shrimp, vaname shrimp also became less active swimming. Vaname shrimp can be seen still actively swimming and eating as usual.

Necrosis is the death of cells or tissues that cause cells and tissues to become abnormal or no longer intact where this necrosis can be caused by bacterial infections, injuries to body parts, stress, and toxic waters (Zahrah, et al., 2016). Observation 3 x 24 hours after injection began to show symptoms of bacterial infection *Vibrio parahaemolyticus*, these symptoms were accompanied by the death of vaname shrimp in some aquariums. Death that occurs after injection with *Vibrio parahaemolyticus* bacteria can be seen in Figure 3.

The Influence of Adding Api-Api Mangrove Leaf Extract as an Immunostimulant on Vaname Shrimp Against Vibriosis Disease Caused by *Vibrio parahaemolyticus* Bacteria



Figure 6 Observation of Vaname Shrimp That Died 72 Hours After *Vibrio parahaemolyticus* Bacteria Challenge Test
(Author's Documentation, 2020)

Observations 3 days after the challenge test in Figure 4.7-3 showed the death of vaname shrimp in several aquariums including VA1 treatment there was 1 dead shrimp, VA2 no one died, VA3 there were 2 dead shrimp, VB1 no one died, VB3 there were 2 dead shrimp, VB3 there was 1 dead shrimp, VC1 no one died, VC2 had 1 dead, and VC3 no one died. Positive control or K(+) treated with feed with the addition of commercial vitamins showed the death of vaname shrimp, namely at KV(+)₁ there were 2 dead shrimp, KV(+)₂ there were 3 dead shrimp, and K(+)₃ there were 3 dead. Negative control or KV(-) that was not given any treatment showed the death of vaname shrimp, namely in KV(-) there were 4 dead shrimp, in K(-)₂ there were 3 dead, and in KV(-)₃ there were 4 dead.

Symptoms caused by observation 72 hours after injection of *Vibrio parahaemolyticus* bacteria in vaname shrimp (see Figure 3) include necrosis, blanched shrimp body, reddened swimming legs and rostum and gripis, reddish tail and gripis, vaname shrimp have decreased appetite characterized by a lot of feed residue at the bottom of the aquarium, inactive shrimp swim more often stay at the bottom of the aquarium, The shrimp looks limp. The majority of vaname shrimp found dead show signs of decay as seen from the color of the shrimp that has turned red.

According to (Marbun, et al., 2019), *Vibrio parahaemolyticus* bacteria are indeed able to attack shrimp quickly so that they can cause death in 1-3 days after infection. In this study, it was proven that there had been a death of vaname shrimp on the 3rd day after the challenge test. However, there are still vaname shrimp that survive with mild to chronic symptoms of vibriosis that can cause vaname shrimp to secrete white feces which is a sign of infection in shrimp hepatopancreas. The presence of white feces is found in 5 aquariums out of 15 aquariums, namely KV(-)₂, KV(-)₃, KV(+)₂, VA1, and VA2.

Table 5 Classification of Final Clinical Symptoms of Vaname Shrimp Rearing Based on Criteria

Code	Looping	Symptom			Total
		Light	Medium	Chronic	
KV (+)	1	-	4	1	5
	2	1	3	2	6

	3	1	2	2	5
KV (-)	1	-	2	2	4
	2	-	-	1	1
	3	-	-	3	3
VA	1	-	3	4	7
	2	1	1	3	5
	3	-	3	2	5
VB	1	-	5	2	7
	2	1	1	4	6
	3	2	3	2	7
VC	1	3	4	2	9
	2	-	5	3	8
	3	1	5	1	7
Total		10	41	34	85

Vaname shrimp at the end of the study in Table 3 showed diverse clinical symptoms so that researchers made criteria for clinical symptoms of white fecal disease in vaname shrimp.

Description of the criteria for classifying clinical symptoms of vaname shrimp at the end of maintenance:

Mild Symptoms: Necrosis is characterized by reddish body discoloration and black patches on the swimming legs, pale body, reddened legs and tail, thinned tail and brownish red or blackish color, swimming close to aeration, good appetite, still actively swimming (Rahmanto, et al., 2014; Jannah, et al., 2018), intestines are still full (see Figure 7).



Figure 7 Mild Symptoms of Vaname Shrimp After *Vibrio parahaemolyticus* Bacteria Challenge Test

Moderate Symptoms: Symptoms are characterized by hepatopancreas begins to yellowish-brown in color, dotted intestines, carapace that begins to soften, decreased appetite, staying at the bottom of the aquarium, slow movement (Marbun, et al., 2019; Helda, et al., 2018).

The Influence of Adding Api-Api Mangrove Leaf Extract as an Immunostimulant on Vaname Shrimp Against Vibriosis Disease Caused by *Vibrio parahaemolyticus* Bacteria



Figure 8 Moderate Symptoms of Vaname Shrimp After *Vibrio parahaemolyticus* Bacteria Challenge Test

Chronic Symptoms: Clinical symptoms are characterized by a soft carapace, hepatopancreas that is already pale white, the body becomes pale white, the intestine is completely empty or the intestine is white, begins to secrete white feces, until death occurs (Marbun, et al., 2019; Sumini & Kusdarwati, 2020).



Figure 9 Chronic Symptoms of Vaname Shrimp After *Vibrio parahaemolyticus* Bacteria Challenge Test

Table 5 shows that all vaname shrimp at the end of the study showed symptoms of *Vibrio parahaemolyticus* bacterial infection. KV(+) treatment contained 2 vaname shrimp with mild symptoms, 9 with moderate symptoms and 5 with chronic symptoms. KV(-) treatment did not have mild symptoms of vaname shrimp, 2 moderately symptomatic vaname shrimp and 6 chronically symptomatic shrimp. VA treatment contained 1 vaname shrimp with mild symptoms, 7 animals with moderate symptoms and 9 with chronic symptoms. VB treatment contained 3 mildly symptomatic vaname shrimp, 9 moderately symptomatic shrimp and 8 chronically symptomatic shrimp. VC treatment contained 4 vaname shrimp with mild symptoms, 14 with moderate symptoms, and 6 with chronic symptoms. Of the total 85 vaname shrimp that are still alive, 10 of them experienced mild symptoms, 41 experienced moderate symptoms and 34 developed chronic symptoms.

Conclusion

This study is a study using *Avicennia alba* mangrove leaf extract as an immunostimulant that is shown into commercial feed vaname shrimp against vibriosis caused by *Vibrio parahaemolyticus* bacteria. There are 3 doses of mangrove leaf extract used, namely, VA treatment of 150 ppm, VB of 250 ppm and VC of 350 ppm. The conclusions of this study are as follows:

Mangrove leaf extract affects the growth of absolute weight (W), growth rate (GR), specific growth rate (SGR), and survival (SR) of vaname shrimp, where the most influential on absolute weight is the dose treatment of VC and VB, the most influential on GR and SGR is VC, and all dose treatments of VA, VB and VC are very influential on the survival (SR) of vaname shrimp. Mangrove leaf extract affects the Total Haemocyte Count (THC) of vaname shrimp before and after the *Vibrio parahaemolyticus* bacterial challenge test. The treatment of adding mangrove leaf extract doses of VA, VB and VC has the most effect on the THC of vaname shrimp.

Mangrove leaf extract affects the phagocytosis activity of vaname shrimp before and after the bacterial challenge test. The treatment of adding mangrove leaf extract with VC dose is the most influential on the phagocytosis activity of vaname shrimp. The most effective dose of mangrove leaf extract as an immunostimulant in this study was VC treatment with a dose of 350 ppm because it affected the total haemocyte count (THC) as well as phagocytosis activity of vaname shrimp.

References

- Adiyana, K., Anandasari, R. V., Wahyuni, T. & Thesiana, L., 2017. Kondisi Kualitas Air dan Respon Pertumbuhan Pada Pemeliharaan Udang Vaname (*Litopenaeus vannamei*) Menggunakan Sumber Energi Surya. Jakarta, Badan Penelitian dan Pengembangan kelautan dan Perikanan.
- Assani, S., 1994. Mikrobiologi Kedokteran. Jakarta: Fakultas Kedokteran Universitas Indonesia.
- Darwanti, K., Sidik, R. & Mahasri, G., 2016. Efisiensi Penggunaan Immunostimulan Dalam Pakan Terhadap Laju Pertumbuhan, Respon Imun Dan Kelulushidupan Udang Vanamei (*Litopenaeus vannamei*). Jurnal Biosains Pascasarjana, Volume 18, pp. 1-17.
- Datu, S. S., 2017. Skrining Antibakteri Ekstrak Sargassum sp. Terhadap Bakteri *Vibrio parahaemolyticus* Dan *Vibrio harveyi*, Makassar: UNIVERSITAS HASANUDDIN.
- Effendie, M. I., 1979. Metode Biologi Perikanan. Jakarta: Gramedia Pustaka Utama.
- Effendy, S. R. A. & Akbar, T., 2004. Peningkatan Hemosit Benur Udang Windu (*Penaeus monodon* Fabricus) Pasca Perendaman Ekstrak Ragi Roti (*Saccharomyces cerevisiae*) Pada Konsentrasi Yang Berbeda. Jurnal Sains dan Teknologi, 14(2), pp. 46-53.
- Ekawati, A., Nursyam, H., Widjayanto, E. & Marsoedi, M., 2012. Diatomae *Chaetoceros ceratosporum* dalam Formula Pakan Meningkatkan Respon Imun Seluler Udang Windu (*Penaeus monodon* Fab.). The Journal of Experimental Life Science, 2(1), pp. 20-28.
- Fitri, Z. M., K. & Mubarak, A. S., 2018. Daya Antibakteri Ekstrak Daun Api-Api (*Avicennia alba*) terhadap *Vibrio harveyi* Penyebab Vibriosis secara Invitro. Jurnal Ilmiah Perikanan dan Kelautan, 10(2), pp. 131-136.
- GOAL, 2018. Goal Aquaculture Alliance: World Shrimp Aquaculture (Including Rosenbergii) by Species. s.l.:s.n.
- Hasbiah, Dwyana, Z. & Haedar, N., 2015. Potensi Beberapa Isolat Probiotik Sebagai Antibakteri Terhadap Pertumbuhan *Vibrio* sp.. Universitas Hasanuddin, pp. 1-9.
- Helda, Y., Harpeni, E. & Supono, S., 2018. Aplikasi Ekstrak Daun Ketapang (*Terminalia catappa* L.) Terhadap Udang Vaname (*Litopenaeus vannamei*) Yang Terinfeksi

The Influence of Adding Api-Api Mangrove Leaf Extract as an Immunostimulant on Vaname Shrimp Against Vibriosis Disease Caused by *Vibrio parahaemolyticus* Bacteria

- Penyakit White Feces Disease (WFD). *Jurnal Sains Teknologi Akuakultur*, 2(2), pp. 7-15.
- Jannah, M., Junaidi, M., Setyowati, D. N. & Azhar, F., 2018. Pengaruh Pemberian *Lactobacillus* sp. Dengan Dosis Yang Berbeda Terhadap Sistem Imun Udang Vaname (*Litopenaeus vannamei*) yang Diinfeksi Bakteri *Vibrio parahaemolyticus*. *Jurnal Kelautan*, 11(2), pp. 140-150.
- Johansson, M. W. & Soederhall, K., 1989. Cellular Immunity In Crustaceans And The proPO System. *Parasitology Today*, 5(6), pp. 171-176.
- Lantah, P. L., Montolalu, L. A. & Reo, A. R., 2017. Kandungan Fitokimia Dan Aktivitas Antioksidan Ekstrak Methanol Rumpu Laut *Kappaphycus alvarezii*. *Jurnal Media Teknologi Hasil Perikanan*, 5(3), pp. 167-173.
- Lee, M. H. & Shiau, S. Y., 2004. Vitamin E Requirements of Juvenile Grass Shrimp, *P. monodon* and Effects on Nonspecific Immune Responses. *Fish & Shellfish Immunology*, Issue 16, pp. 475-485.
- Liu, C.-H. & Chen, J.-C., 2004. Effect of Ammonia on the Immune Response of White Shrimp *Litopenaeus vannamei* And Its Susceptibility to *Vibrio alginolyticus*. *Fish & Shellfish Immunology*, Volume 16, pp. 321-334.
- Manoppo, H. et al., 2011. Peningkatan Respon Imun Non-Spesifik, Resistensi, dan Pertumbuhan Udang vaname (*Litopenaeus vannamei*) Melalui Pemberian Pakan Nukleotida. *Jurnal Akuakultur Indonesia*, I(10), pp. 1-7.
- Marbun, J., Harpeni, . E. & Wardyanto, W., 2019. Penanganan penyakit white feces pada udang vaname *Litopenaeus vannamei* menggunakan aplikasi pakan yang dicampur ekstrak lengkuas merah *Alpinia purpurata* k. schum. *Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan*, 8(2), pp. 76-86.
- Mastan, S., 2015. Use of Immunostimulants in Aquaculture Disease Management. *International Journal of Fisheries and Aquatic Studies*, 2(4), pp. 277-28.
- Muliani, B. R., T. & Atmomarsono, M., 2003. Penyebaran dan Prevalensi White Spot Syndrome Virus (WSSV) pada Budidaya Udang Windu (*Penaeus monodon*). *Jurnal Riset Aquaculture*, 2(2), pp. 231-241.
- Nuhman, 2009. Pengaruh Prosentase Pemberian Pakan Terhadap Kelangsungan Hidup dan Laju Pertumbuhan Udang Vanname (*Litopenaeus vannamei*). *Jurnal Ilmiah Perikanan dan Kelautan*, 1(2), pp. 193-197.
- Nurhijrah, S., 2019. Kinerja Pertumbuhan Udang Vaname (*Litopenaeus vannamei*) yang Diberi Pakan dengan Penambahan Mannan oligosakarida (MOS), Makasar: Universitas Muhammadiyah Makasar.
- Putri, F. M., 2012. Pengaruh Penambahan *Spirulina* sp. dalam Pakan Buatan Terhadap Jumlah Total Hemosit dan Aktivitas Fagositosis Udang Vaname (*Litopenaeus vannamei*), Semarang: Universitas Diponegoro.
- Putri, F. M., S. & S., 2013. Pengaruh Penambahan *Spirulina* sp. dalam Pakan Buatan Terhadap Jumlah Total Hemosit dan Aktivitas Fagositosis Udang Vaname (*Litopenaeus vannamei*). *Journal of Aquaculture Management and Technology*, 2(1), pp. 102-112.
- Rahmanto, S., Sarjito, D. & Chimawati, 2014. Karakterisasi dan Uji Postulat Koch Bakteri Genus *Vibrio* yang berasal dari Media Kultur Massal Mikroalga. *Journal of Aquaculture Management and Technology*, 3(4), pp. 230-237.
- Rauf, A., Haeria & Anas, D. D., 2016. Efek Imunostimulan Fraksi Daun Katuk (*Sauropus androgynus* L. Merr.) Terhadap Aktivitas Dan Kapasitas Fagositosis Makrofag Pada Mencit Jantan (*Mus Musculus*). *Jurnal FIK UINAM*, 4(1), pp. 9-15.

- Rusadi, D., Wardiyanto & Diantari, R., 2019. Treatment Of Vibriosis Disease (*Vibrio harveyi*) In Vaname Shrimp (*Litopenaeus vannamei*, Boone 1931) Using *Avicennia alba* Leaves Extract. *Rekayasa dan Teknologi Budidaya Perairan*, 8(1), pp. 909-916.
- Sahrijanna, A. & Sahabuddin, 2014. Kajian Kualitas Air Pada Budidaya Udang Vaname (*Litopenaeus vannamei*) dengan Sistem Pergiliran Pakan di Tambak Intensif. *Prosiding Forum Inovasi Teknologi Akuakultur*, pp. 313-320.
- Samuria, S. A., Nur, I. & Hamzah, M., 2018. Pengaruh Ekstrak Daun Mangrove (*Avicennia marina*) terhadap Ketahanan Tubuh Udang Vaname (*Litopenaeus vannamei*). *Jurnal Sains dan Inovasi Perikanan*, 2(2), pp. 49-54.
- Saptiani, G., Asikin, A. N., Ardhani, F. & Hardi, E. H., 2018. Tanaman Bakau Api-api Putih (*Avicennia marina*) Berpotensi Menghambat Mikrob Patogen dan Melindungi Post Larva Udang Windu. *Jurnal Veteriner*, 19(1), pp. 45-54.
- Sasongko, J., 2016. Pencapaian Produksi Udang 2016 Proyeksi Produksi 2017. *Shrimp Club Jawa Barat dan Banten*, pp. 1-8.
- Sumeru, S. U. & Anna, S., 1992. *Pakan Udang Windu (Penaeus monodon)*. Yogyakarta: Kanisius.
- Sumini, S. & Kusdarwati, R., 2020. The Discovery of *Vibrio harveyi* on *Litopenaeus vannamei* Infected White Feces Disease in Situbondo, East Java. *Jurnal Perikanan Universitas Gadjah Mada*, 22(1), pp. 9-18.
- Tampangallo, B. R. & Susianingsih, E., 2011. Total Sel Hemosit Udang Windu (*Penaeus monodon*) Yang Dipapar dengan Bakteri *Vibrio harveyi*. Maros, Balai Riset Perikanan Budidaya Air Payau Maros Sulawesi Selatan, pp. 595-599.
- Van De Braak, K., 2000. *Haemocytic Defence in Black Tiger Shrimp (Penaeus monodon)*, Germany: Van Wareningen Universiteit.