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### <sup>1</sup>The Effect of *Avicennia marina* Leaf Extract Addition To the Artificial Feed On the Growth of *Litopenaeus vannamei*

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#### Abstract

Mangrove leaves contain abundant bioactive compounds that can optimize the growth of shrimp. This research examined the effect of adding *Avicennia marina* leaves to feed on the growth and FCR (Feed Conversion Ratio) of Vannamee shrimp and determined the optimal dose. Vannamee shrimp seeds of PL-20 were used in experiments that employed RAL method with 4 treatments and 3 replications. Different doses of *Avicennia marina* leaf extract were used: (A) 125 ppm/500 g feed, (B) 175 ppm/500 g feed, (C) 225 ppm/500 g feed (D). The feed amounted to 3% of the total biomass per treatment every day administered in 5 meals daily for 30 days. The most optimal dose for the growth of shrimp was 225 ppm/500 g (D) with an absolute growth of 7.71 <sup>4</sup>. The statistical analyze showed that the F count is greater than F table which is mean the treatments have had a significant effect on growth. However, the treatment did not affect the survival rate (SR) of shrimp for its value reached 100% in all treatments. The parameters of water quality were temperature of 27.4–30°C, DO 6.3 – 7.9, salinity 13–15 ppt, and pH 7.5 – 7.8.

**Keywords:** *Avicennia marina*, Growth, FCR, *Litopenaeus vannamei*

#### INTRODUCTION

Vannamee shrimp (*Litopenaeus vannamei*) is a favorite commodity, besides it is widely cultivated for having fairly high economic value. In 2020, fishery exports reached 1.26 billion kilograms (kg), with shrimp dominating by 239.28 million kg. In the same year, shrimp production reached 911,200 tons which increased the total marine product export by 18.95% (KKP, 2021). The demand for Vannamee shrimp is high both in the international market and the local market. Its high economic value stimulated massive Vannamee shrimp cultivation (Yustianti *et al.*, 2013).

Vannamee shrimp has better responsiveness to the feed and greater resistance to disease attacks and an unfavorable environment. With these advantages, the cultivation of Vannamee shrimp can be developed. The development of Vannamee shrimp cultivation should be carried out to increase its production to meet the demand of the local market and international market as a mainstay fishery commodity. One of the constraints in aquaculture is the price of feed. The feed should contain adequate protein that will improve the growth rate. Meanwhile, feed costs occupy 60-70% of the total production costs, Nababan *et al.*, (2015). In addition, to make feeding more efficient and effective, the growth rate can be improved by adding *Avicennia marina*

mangrove leaf extract to the feed as a feed additive. Moreover, Handayani (2013) reported that *Avicennia marina* leaves contain 11.04% protein, 69.2% water, 14.91% ash, and 2.21% fat. In addition, some bioactive compounds have been found in *Avicennia marina* leaves including alkaloids, flavonoids, steroids/triterpenoids, and tannins that have antibacterial properties as they can inhibit the growth and development of *Vibrio* sp bacteria (Susanti *et al.*, 2016). *Avicennia marina* can promote growth, boost the immune system and stimulate the feeding response in aquaculture. Flavonoids can act as antimicrobial, antibacterial, antiviral and immunostimulants (Sari *et al.*, 2015). Adding immunostimulants into the feed has been an effective method to improve non-specific defense systems (Irkin and Yigit, 2015).

Research on white mangrove leaf (*Avicennia alba*) has been conducted by Mahenda (2021) to examine the benefits of mangrove leaf extract as an immunostimulant in vanname shrimp against vibriosis from *Vibrio parahaemolyticus* bacteria infection. Arghifari *et al.*, (2019) also examined the contribution of white mangrove leaf powder on the growth of Srikandi tilapia fish was most optimal in 50:50 ratio between artificial feed and white mangrove leaf powder.

Information on the utilization of mangrove leaves is still needed in shrimp farming. For this reason, it is necessary to conduct research on the effect of mangrove leaves on shrimp growth. This research was conducted to examine the effect of adding white mangrove leaf extract into the feed and the optimal dose on the growth of Vanname shrimp.

## MATERIALS AND METHODS

### Location and time of research

This research was conducted from October 04 – November 10, 2022, at the Brackish Water and Marine Laboratory, Faculty of Fisheries, Universitas Pekalongan, Jl. Pantai Dewi, Krapyak Lor, North Pekalongan District, Pekalongan, Central Java.

### Materials

Test animals were the seeds of Vanname shrimp of PL-20 size weighing 0.02 g/each obtained from BBPBAP Jepara. The test containers were 37 cm x 24 cm x 21 cm in size with a volume of 10 liters of water for each treatment. A completely randomized design (CRD) with 4 treatments and 3 replications were conducted with different white mangrove powder concentrations as follows.

Treatment A = No addition of *Avicennia marina* leaf extract to the feed (control)

Treatment B = Adding *Avicennia marina* leaf extract of 125 ppm to 500 gram feed

Treatment C = Adding *Avicennia marina* leaf extract of 175 ppm to 500 gram feed

Treatment D = Adding *Avicennia marina* leaf extract of 225 ppm to 500 gram feed

Those doses were used based on the recommendation of Rusadi *et al.*,(2019) who found the best outcome from the use of 250 ppm added with mangrove leaf extract on the mortality rate of Vanname shrimp.

### Research Procedure

#### Preparation of the Rearing Media

Brackish water was used in the rearing media, which was precipitated for 1 night (Mahenda, 2021). The water is filtered in advance to prevent the presence of harmful particles.

#### The Production of *Avicennia marina* Leaf Extract

According to Mahenda (2021), *Avicennia marina* leaves were dried under direct sunlight before being ground into powder. 200 grams of powder was placed in a macerator to be dissolved in methanol-water with a ratio of 1:5 and set for 3 days with occasional stirring. After that, the concentration process was performed using a rotary evaporator and then hydrolyzed using 2N HCl for 60 minutes at the boiling point temperature of the solvent. The final results were leaf extract with concentrations of 125 ppm, 175 ppm, 225 ppm from mangrove leaf extracts of 0.125 grams, 0.175 grams, and 0.225 grams.

#### Feeding Preparation

The diluted extract was added to the feed through the Spraying method until it was homogeneous. The feed was then administered to the test shrimp with a volume of 3% of the total biomass of the post larva (Rusadi *et al.*, 2019) in 5 feeding times a day at 07.00, 10.00, 13.00, 16.00, and 21.00 WIB (Mahenda, 2021)

#### Test Parameters

Samples for the test were taken every seven days for 30 days to measure the following parameters.

#### Absolute Weight Growth of Vanname Shrimp

The absolute weight growth of Vanname shrimp was calculated using the formula proposed by (Effendi, 1997) as follows.

$$W_m = W_t - W_0$$

Remarks:

W<sub>m</sub> = Individual absolute growth of test animal (gram)

W<sub>0</sub> = Initial shrimp biomass (gram)

W<sub>t</sub> = Final shrimp biomass (gram)

### Survival Rate

The data were then analyzed using a formula proposed by (Effendie, 1997) as follows:

$$SR = \frac{N_t}{N_0} \times 100\%$$

Remarks:

SR : Survival rate (%)

N<sub>t</sub> : Number of living shrimp in the end of the treatment (head)

N<sub>0</sub> : Number of living shrimp before treatment (head)

### Data Analysis

The data of this research was analyzed using the ANOVA (Analysis of Variance) test after the normality and homogeneity of the data had been ensured. The Tukey test would be carried out if the results of the variance test indicated a significant effect on the growth and FCR of Vanname shrimp. The data on the relationship between treatments were put in Microsoft Excel 2013, while the data of water quality were descriptively analyzed.

## RESULTS AND DISCUSSION

### Results

#### The Growth of Vanname Shrimp

The growth of Vanname shrimp (*Litopenaeus vannamei*) during rearing varied and increased along with longer rearing time for all treatments as shown in the following Table 1.

Table 1. Absolute Growth and Specific Growth of Vanname Shrimp

| Treatment | Absolute Growth (gram)    |
|-----------|---------------------------|
| A         | 5.59 ± 0.13 <sup>a</sup>  |
| B         | 6.16 ± 0.09 <sup>ab</sup> |
| C         | 6.68 ± 0.03 <sup>b</sup>  |
| D         | 7.71 ± 0.71 <sup>c</sup>  |

Remarks :

<sup>10</sup> Different letter in one column shows significant difference (P<0.05)

As seen on the Table, the highest absolute growth of Vanname shrimp is in treatment D with 7.71 g, followed by treatment C with 6.68 g, treatment B with 6.16 g, and treatment A with 5.59 g.

<sup>13</sup> The results of the normality of the data on the absolute growth can be seen in Appendix 1. and 2. The data were normally distributed based on  $L_{max} (0.174) < L_{table} 5\% (0.242)$  and  $1\% (0.275)$ . The homogeneity test resulted in  $X^2 (17.205) < X^2 5\% (11.34)$  and  $X^2 1\% (7.81)$ , showing that the data were homogeneous. After that, ANOVA test was performed.

ANOVA test was carried out to determine the effect of adding white mangrove leaf extract to the feed on the absolute growth of Vanname shrimp as presented in Table 2.

Table 2. The Results of ANOVA Test on Absolute Growth

| JK        | DB | JK    | KT   | F COUNT | F TABLE |       |
|-----------|----|-------|------|---------|---------|-------|
|           |    |       |      |         | 5%      | 1%    |
| Treatment | 3  | 7.293 | 2.43 | 18.5075 | 4.0662  | 7.591 |
| Galat     | 8  | 1.051 | 0.13 |         |         |       |
| Total     | 11 | 8.344 |      |         |         |       |

Remarks :

\*\* : Strong Significant Difference

Table 3 shows the presence of strong significant influences of the treatments on the growth of the Vanname shrimp. The Tukey test was then performed to examine the growth with the following results.

Table 3. The Results of Tukey Test on Absolute Growth

| Treatment | 9 | Subset for alpha = 0.05 |        |        |
|-----------|---|-------------------------|--------|--------|
|           |   | 1                       | 2      | 3      |
| A         | 3 | 5.5900                  |        |        |
| B         | 3 | 6.1567                  | 6.1567 |        |
| C         | 3 |                         | 6.6833 |        |
| D         | 3 |                         |        | 7.7067 |
| Sig.      |   | 0.294                   | 0.348  | 1.000  |

### Survival Rate (SR)

Table 4. Survival Rate

| Treatment | No | Nt | SR (%) |
|-----------|----|----|--------|
| A         | 10 | 10 | 100    |
| B         | 10 | 10 | 100    |
| C         | 10 | 10 | 100    |
| D         | 10 | 10 | 100    |

Remarks:

<sup>5</sup>SR : Survival rate (%)

Nt : Number of living shrimp in the end of the treatment (head)

No : Number of living shrimp before treatment (head)

<sup>2</sup>The addition of *Avicennia marina* leaf extract to the feed did not affect the Vanname shrimp's survival. <sup>2</sup>Treatments A, B, C, and D resulted in a 100% survival rate, which means that there were no deaths in the study.

### Water Quality

Water quality parameters observed in this research were temperature, DO, salinity, and pH level as presented in Table 5.

Table 5. Parameters of Water Quality in this Research

| Parameter        | Outcome   | Reference (Supono, 2017) |
|------------------|-----------|--------------------------|
| Temperature (°C) | 27.4 – 30 | 26 – 33                  |
| DO (ppm)         | 6.3 – 7.9 | >4                       |
| Salinity (ppt)   | 13 – 15   | 10 – 30                  |
| pH               | 7.5 – 7.8 | 7.5 – 8.5                |

## Discussion

### The Growth of Vanname Shrimp

Treatment D and treatment A showed distinct results, while other treatments showed average results. Therefore, the treatment variable only had a significant effect in treatment D. The addition of *Avicennia marina* leaf extract to the feed was found significant as shown by significant increases in the absolute weight growth. Treatment A obtained the lowest yield of 5.59 grams, because the feed provided did not contain feed additives such as alkaloids, flavonoids, steroids/triterpenoids, and tannins, making the shrimp unable to digest the feed optimally. In addition, *Avicennia marina* mangrove leaf extract in the feed provided additional nutrients to support the growth of Vanname shrimp. Several bioactive compounds in *Avicennia marina* include alkaloids, flavonoids, steroids/triterpenoids, and tannins (Susanti *et al.*, 2016) and essential oil (Guo *et al.*, 2008). *Avicennia marina* leaf extract is a natural feed additive that can be used as an antioxidant. Wulansari *et al.*, (2020) stated that the addition of *Avicennia marina* leaf extract as a feed additive in Vanname shrimp feed was able to improve the absolute weight growth. Treatment B obtained an absolute growth of 6.16 grams and treatment C obtained 6.68 grams. The low growth values in treatments B and C were due to alkaloids, flavonoids, steroids/triterpenoids, and tannin deficiency in the feed. That compound has an effect on increasing appetite that can be affected to growth. According to Muhlisah (1999), mentioned that drugs containing flavonoids, saponin, and essential oils can be used to increase appetite. Moreover, flavonoids could stimulate the presence of *Lactobacillus*, which is an important bacterial for the digestive process (Boubakeur, 2015).

Lack of flavonoids made the digestive process fail to run properly. The feed will be processed through the digestion process, while the nutritional elements and nutrients will be absorbed by the body to build tissues and grow (Karimah & Samidjan., 2018).

The high growth of Vannamee shrimp in treatment D is shown by the yield of 7.71 grams which could be affected by the addition of *Avicennia marina* leaf extract. Handayani (2013) found that *Avicennia marina* leaves contained 11.04% protein, 69.2% water, 14.91 ash, and 2.21% fat. The protein content in *Avicennia* leaves can help meet the protein needs for the growth of Vannamee shrimp. *Avicennia marina* mangroves contain essential amino acids, such as glutamine and alanine (Shinpei *et al.*,2013). It is affected into greater levels of amino acids in the feed that can be absorbed and available for use by the body, resulting the growth. Essential oils are able to improve the process of digesting food inside the body and have a sedative effect to maintain calm in the condition of fish. Furthermore, Linayati *et al.*,(2021) also explained that essential oil in *Kaempferia galanga* could help the digestive system in milkfish (*Chanos chanos*).

Flavonoid compounds have antioxidant properties that can boost the immunity of Vannamee shrimp (Handayani, 2013). The antioxidant mechanism of flavonoids works by directly capturing free radicals, preventing cell regeneration, and indirectly increasing the antioxidant activity of cellular antioxidant enzymes (Nuraeni & Sulistijowati, 2021). Saponins, tannins, and alkaloids have the same function as an antioxidant, in an amount that is not excessive could help improve the growth performance of the shrimp so as to increase productivity. Junaedi *et al.*, (2020) explained that adding 2 % of *Rhizopora apiculata* containing flavonoids, tannin, saponin, and alkaloids has to provide a positive effect on the growth of shrimp. Furthermore, Linayati *et al.*, (2022) also mentioned that the addition of other natural ingredients such as aloe vera can also increase the immune system of shrimp, especially phagocytosis activity and also its growth.

## <sup>2</sup> Survival Rate (SR)

<sup>2</sup> The addition of *Avicennia marina* leaf extract to the feed did not affect the Vannamee shrimp's survival. Treatments A, B, C, and D resulted in a 100% survival rate, which means that there were no mortality in the study. Internal and external factors affect the low survival rate (Aliyas *et al.*, 2016). Internal factors are genetics, while external factors are the quality of water and feed. Internal factors include the agility of the shrimp seeds in moving, the completeness of organs such as intestines, and hepato-pancreas (stomach) filled with feed, and the uniform size of the seeds. According to (SNI 7549:2009), healthy seeds can move agilely against the water current and they have complete organs. External factors include the commercial feed that contains 30% protein and is added with *Avicennia marina* mangrove leaf extract. The amount of protein needed for growth is 30% (SNI 7549:2009).

In addition, the health condition of shrimp would be a valuable factor to improve their growth and survival rate of shrimp. Although the results showed no effect on survival rate, but the addition of mangrove leaves could not be ignored. Mangrove leaves play an important role in improving the survival rate due to their ability to improve shrimp health. *Avicennia marina* leaf extract contains flavonoid compounds that can act as antimicrobial, antibacterial, antiviral, and immunostimulants (Sari *et al.*, 2015). The mechanism of flavonoids as antibacterial can be divided into three: the inhibition of nucleic acid synthesis, the inhibition of cell membrane function, and the inhibition of energy metabolism (Rijayanti, 2014). Alkaloids are antibacterial, but they work by destruct the peptidoglycan component in bacterial cells, resulting in the formation of imperfect cell wall layers, thereby causing the bacterial cell to die (Mufti *et al.*, 2022). Tannins also have antimicrobial properties as they have the ability to inactivate microbial cell adhesion on the cell surface (Ngajow *et al.*, 2013).

The combination of immunostimulants and feed has been found effective in increasing the non-specific defense systems (Irkin & Yigit, 2015). The water quality parameters during this research were within optimal range as the water quality was checked and maintained every seven days. The high survival rate of Vannamee shrimp is affected by the optimal water quality, healthy seed, and the selection of good feed. Likewise, Rakhmanda *et al.*, (2020) also found water quality as one of the factors affecting the survival rate of Vannamee shrimp.

## Water Quality

The temperature ranges between 27.4 – 30°C (optimal) based on the recommended water temperature for Vannamee shrimp culture of 26 – 33 °C (Supono, 2017). A temperature that exceeds the optimal limit can cause faster metabolism which increases the need for oxygen. The dissolved oxygen was obtained in the range of 6.3 – 7.9 ppm (optimal). The optimal DO value for shrimp cultivation is greater than 4 ppm (Supono, 2017). pH level is defined as the negative logarithm of the sensitivity of hydrogen ions released in a liquid. The pH level of the rearing media in this research ranged from 7.5 to 7.8 (optimal). According to Supono (2017) that the optimal pH range for shrimp growth is 7–8.5, and shrimp can tolerate a pH level between 6.5–9. The results of salinity value ranged from 13–15 ppt (not optimal). The optimal salinity for shrimp growth should be between 10 – 30 ppt (Supono, 2017). Some factors affect water salinity, including the number of river estuaries around the pond location, rainfall, and seasons (rainy or dry).

## Conclusion

The addition of *Avicennia marina* mangrove leaf extract to the feed showed a strong significant effect on the growth of Vannamee shrimp. The best dose of *Avicennia marina* mangrove leaf extract was found in treatment D (225 ppm / 500 grams of feed) which resulted in a growth of 7.69 gr.

## Suggestion

Regarding the results of this research, future researchers are encouraged to analyze the effect of *Avicennia marina* mangrove leaf as an additional ingredient in Vannamee shrimp artificial feed.

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