

The Performance Of Pacific White Shrimp Infected By *Vibrio harveyi* After Mangrove Leaf Extract Supplementation

Linayati Linayati^{1*}, Maghfiroh², Adhitya Wisnu Prasetyo³, M. Zulkham Yahya⁴

¹Department of Aquaculture, Faculty of Fisheries, Pekalongan University

²Department of Batik Technology, Faculty of Engineering, Pekalongan University

³Bachelor Degree Programme of Aquaculture, Faculty of Fisheries, Pekalongan University

⁴Post Graduate Programme of Water Resource Management, Faculty of Fisheries and Marine Science, Diponegoro University

*Corresponding author : linayatiunikal@gmail.com

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Abstract

This research investigated the advantages and determined the optimal dosage of mangrove leaf supplementation to enhance growth and disease resistance in shrimp. PL-20 Pacific White Shrimp were given different doses of mangrove leaf extract added to their feed: 0 ppm/500 g (A), 225 ppm/500 g (B), 250 ppm/500 g (C), and 275 ppm/ 500 g (D), over a 30-day period. This experimental research was conducted using a completely randomized design with four treatments and three replications each. Several parameters were observed: growth, feed conversion ratio (FCR), energy protein productivity (EPP), and subsequent exposure to *Vibrio harveyi* to assess survival rate (SR). The obtained data underwent ANOVA analysis, which results demonstrated significant influence in shrimp growth, with the most notable improvement observed in treatment D. Treatment D also exhibited the highest SR value following the challenge test. Throughout the study, water quality parameters remained within the suitable range for fostering shrimp growth

Keywords: shrimp, temperature, *Vibrio* sp., water quality.

INTRODUCTION

Pacific White Shrimp are extensively farmed in Indonesia due to their relatively easy cultivation process and competitive market value on a global scale (Ariadi et al, 2019). Statistical data reveal that out of the total fish production weighing 1,260,000,000 kg, a substantial portion of 239,280,000 kg comprises the shrimp commodity (Ariadi and Mujtahidah, 2022). The demand for Pacific White Shrimp transcends international boundaries, extending to robust local markets as well. As a result,

this trend has spurred a rapid expansion of Pacific White Shrimp farming activities throughout Indonesia (Irani et al, 2023).

Pacific White Shrimp cultivators encounter a range of challenges, prominently including shrimp mortality due to diseases, sluggish growth rates, and the notably steep cost of feed. The elevated expense of feed production, accounting for 60-70% of the overall costs, significantly undermines the sustainability of the shrimp farming process (Quiroz-Guzman et al, 2023).

Furthermore, the industry contends with another pressing issue – the widespread mortality of shrimp brought about by viral infections and assaults from *Vibrio* sp. bacteria (Xin et al, 2020).

An approach to address the aforementioned issues involves incorporating extracts of natural compounds sourced from plants into the feed. An example of such a compound is derived from mangrove leaves. These leaves harbor active compounds that can enhance growth, improve feed efficiency, and bolster the shrimp's immune system against diseases. The coastal regions are abundant in mangrove leaves, yet their optimal utilization remains largely untapped.

Research findings indicate that *Avecnia marina* mangrove leaves encompass the antibacterial component *Vibrio harveyi*, as highlighted by Anjani, (2021). Furthermore, the *R. apiculata* species displays a notable resistance to *Edwardsiella tarda* bacterial infections, as documented by Ardiantami et al, (2015). *Rhizopora apiculata* mangrove leaves supplementation into the feed yielded a positive outcome, leading to a growth enhancement of 0.35 g/day in Pacific White Shrimp, as demonstrated by Linayati et al in 2022.

Additional research outcomes underscore the nutritional significance of *Avecnia marina* mangrove leaves, which contain protein, fat, minerals, as well as active compounds like flavonoids, tannins, and alkaloids. These compounds play a pivotal role in stimulating growth and reinforcing the shrimp's immune system against disease threats, as indicated by Ardiantami et al in both 2015. Ananthavalli and Karpagam in 2017 emphasize that mangroves serve as a reservoir of carbohydrates, amino acids, and minerals, and they also harbor diverse active constituents such as flavonoids, terpenoids, and vitamins, including vitamins B and C.

Based on the information gleaned from the aforementioned data, the primary objective of this study was to ascertain the advantages and identify the most effective dosage of mangrove leaves as a natural component to enhance growth and bolster the disease resistance of shrimp. This standpoint gains further credence from the outcomes of the author's investigation, which reveal that the utilization of *Avecnia marina* leaves at a dosage of 225 mg/L did not yield optimal growth rates for Pacific White Shrimp, as

reported by Linayati et al in 2023. The composition of other compounds within mangrove leaves comprises 11.04% protein, 69.2% water content, 14.91% ash content, and 2.21% fat content (Hinokidani et al, 2022).

MATERIAL AND METHODS

This research was conducted at the Brackish and Sea Water Laboratory, Faculty of Fisheries, Universitas Pekalongan.

Materials

Test animal was PL-20 Pacific White Shrimp fry, maintained at a density of 1 fish per liter. Shrimp were put in containers with dimensions of 37 cm x 24 cm x 21 cm. The provided feed contained 30-33% protein content, as established by Junaidi et al. in 2018, Supono in 2017, and Anjani et al. in 2021. All equipment were pre-sterilized before treatments were given. The arrangement of the containers was adapted from Heriyanto (1996) as follows.

C3	D2	D3	C2	B2	D1
A2	C1	A1	A3	B1	B3

Figure 1. Layout of Experiment Arrangement

Remarks :

A : No supplementation

B : 225 ppm of *A. marina* leaf extract /500 g feed

C : 250 ppm of *A. marina* leaf extract /500 g feed

D : 275 ppm of *A. marina* leaf extract/500 g feed

1,2,3 : Replications

Method

This experimental research was performed using a completely randomized design (CRD). Four treatments were conducted with three replication each to gain novel insights while corroborating findings from earlier studies, as emphasized by Heriyanto, (1996). Feed doses were determined based on the recommendation from prior researchers, especially for the dose of *Avecenia marina* leaf extract for Pacific White Shrimp feed which was set at 225 ppm per 500 g of feed, as proposed by Linayati et al. in 2023.

Container Preparation

Brackish water with a salinity level of 28-30 ppt from water sources nearby the research location was used as a test medium (Hinokidani et al, 2022).

The Extraction of *Avecenia marina* Mangrove

The preparation of liquid solid *Avecenia marina* mangrove leaf extract as conducted as follows (Hinokidani et al, 2022):

1. Prior to the extraction, *Avecenia marina* mangrove leaves were sun-dried until completely dry.
2. 200 grams of *Avecenia marina* mangrove leaf powder was placed into a mortar, lined with cotton, and a methanol-water solvent (at a ratio of 1:5) was added.
3. The mortar was allowed to stand for 3 days with occasional stirring.
4. The concentration process was carried out using a rotary evaporator, followed by hydrolysis using 2N HCl for 60 minutes at the boiling point temperature.

Test Animal Preparation

There were 120 Pacific White Shrimp of PL-20 fry with an average weight of 0.02 each obtained from BBPBAP Jepara used in this study. The fry underwent a preparatory period involving a 7-day adaptation and acclimatization to the containers with 10 liters of water volume.

Rearing and Sampling

The rearing extended over a 30-day period, during which shrimp sampling and biomass sampling were carried out at seven-day intervals. In each instance, a total of 10 individual shrimp were taken out of each test container for measurement. The weight of the sampled

shrimp was assessed using a digital scale accurate to 0.01 grams.

The Challenge Test

The selected organisms underwent a challenge involving the introduction of *Vibrio harveyi* bacteria, administered at a concentration of 10^6 bacteria per milliliter. The *V. harveyi* bacteria were put into the container based on the principle of the formula $V_1N_1 = V_2N_2$. Subsequently, the shrimp that had been exposed to the bacterial infection were cultivated alongside feed supplemented with mangrove leaf extract. The survival of these shrimp was meticulously monitored over a period of 7 days.

Parameters to Observe

1. Pacific White Shrimp Weight Growth

The absolute weight growth of the shrimp was calculated using the following formula (Effendi, 1997):

$$Wm = Wt - W_0$$

Remarks :

Wm : Absolute weight growth of individual test animal (grams)

W0 : Shrimp biomass before treatment (grams)

Wt : Shrimp biomass after treatment (gram)

2. Feed Conversion Ratio (FCR)

The Feed Conversion Ratio (FCR) is a numerical representation denoting the relationship between the quantity of feed dispensed and the weight of the biomass

generated. It is computed utilizing the formula outlined in the works of Latife et al. in 2015 and Effendi in 1997 as follows.

$$FCR = \frac{F}{(Bt+Bm)-Bo}$$

Remarks :

FCR : Feed Conversion Ratio
 F : Amount of feed consumed (gr)
 Bt : Absolute biomass after rearing (gr)
 Bm : Absolute biomass during rearing (gr)
 Bo : Absolute biomass before rearing (gr)

3. Feed Utilization Efficiency (FUE)

Feed utilization efficiency pertains to the proportion, expressed as a percentage, between the produced body weight and the amount of feed administered during the maintenance phase. This efficiency was determined through the equation as established by Effendi (1997).

$$FUE = \frac{Wt - Wo}{F} \times 100\%$$

Remarks :

FUE : Feed Utilization Efficiency (%)
 Wt : Biomass after the treatment (gr)
 Wo : Biomass before the treatment (gr)
 F : Amount of feed consumed during the treatment (gr)

4. Clinical Symptoms

The assessment of clinical manifestations was conducted by observing any irregularities that manifested in the macro anatomy of the

examined shrimp throughout the treatments.

5. Survival Rate of Pacific White Shrimp

The evaluation of shrimp survival was conducted by calculating the number of the shrimp before and after the treatments. Data were then analyzed using the formula proposed by Effendi in 1997.

$$SR = \frac{Nt}{No} \times 100\%$$

Remarks:

SR : Survival rate (%)
 Nt : Number of shrimp alive after treatment
 No : Number of shrimp alive before treatment

6. Water Quality Measurement

The water quality was measured based on several key parameters; temperature, dissolved oxygen (DO) levels, pH acidity, salinity, and ammonia concentration. The measurement was conducted at regular intervals as recommended by Effendi, (1997).

Data Collection Method

Primary data acquired from the research outcomes and secondary data obtained from supplementary sources such as literature reviews and other corroborating references were regarded as recommended by Hasan in 2002. The data then underwent a series of statistical

assessments, including tests to check the data normality and homogeneity, ANOVA analysis, and the Tukey test on SPSS version 16 software.

RESULT AND DISCUSSIONS

The highest Absolute Biomass value was observed in treatment D, while the lowest is found in treatment A (See Table 1). The outcomes of the ANOVA test demonstrate a substantial impact of the treatment on growth, with an F count exceeding the critical F value. The Tukey test was performed to discern

inter-treatment differences, revealing that treatment D exhibited a significant distinction from treatment C. On the contrary, treatment B did not display a significant difference when compared to treatment E. These observations were consistent across the EPP and FCR values. Regarding survival rates, treatments A and B had 5 deaths each, while the highest count of surviving shrimp following the *Vibrio* bacteria challenge was 10 individuals.

Table 1. Shrimp absolute growth (gr)

Replicate	Treatment				Total
	A	B	C	D	
1	14,76	15,50	17,26	18,51	
2	14,54	15,87	16,70	18,67	
3	14,79	14,90	16,84	18,12	
Total	44,09	46,27	50,80	55,30	196,46
Average	14,69±0,13	15,417±0,48	16,933±0,29	18,437±0,28	

Absolute Growth

The inclusion of *A.marina* leaf extract treatment during the rearing led to enhanced growth in comparison to the control treatment (A), while treatment D exhibited the highest growth among all treatments. This is suspected to be influenced by the composition of *A.marina* leaves, which potentially improves the nutritional quality of the shrimp feed. *A.marina* leaves are recognized for containing crucial amino

acids (Al Husnain et al, 2023), which play a vital role as building blocks of proteins during the fish growth process. These amino acids serve as energy sources for metabolic activities and aid in cellular repair.

According to Cao et al, (2012), the presence of amino acids in the diet contributes to heightened protein retention, a factor that supports optimal growth by fostering a higher protein content in the feed. This notion aligns

with the perspective put forth by Ariadi et al, (2021), that increased protein consumption and digestion lead to greater nutrient utilization for growth. Furthermore, protein is a pivotal element in mending and sustaining shrimp body tissues, as emphasized by Cao et al, (2012). Protein also plays a crucial role in the molting process of shrimp, which serves as a growth indicator (Herawati & Hutabarat, 2015).

The content of secondary metabolism such as flavonoids and tannins in *A. marina* leaves also helps to increase shrimp growth while maintaining shrimp health. The several phenolic compounds such as flavonoids and tannins are antioxidants which can improve shrimp health so as to optimize the absorption of nutrients in the feed consumed (Ramamoorthy et al, 2023). Flavonoids help growth by improving the nutritional value of feed digestibility such as crude fiber and protein (Ariadi et al, 2022). The presence of flavonoids also serves to protect the body from the threat of free radicals that can damage cells and reduce the immune system of shrimp. This is in line with Hatami et al., (2020) that flavonoids stimulate tissue repair and the formation of new metabolic cells from free radical

damage. These free radicals can come from the environment or from the rest of the metabolic processes in the body. Furthermore, alkaloids also have the potential to increase growth through a protective mechanism as antioxidants Bussabong et al., (2021) stated that alkaloids are also potential growth promoters.

The secondary metabolites present in *A. marina* leaves, including flavonoids and tannins, contribute to the enhancement of shrimp growth while concurrently supporting their overall health. Fitri & Usman, (2021), pointed out that certain phenolic compounds, such as flavonoids and tannins, possess antioxidant properties that have a positive impact on shrimp well-being, thereby facilitating the optimized absorption of nutrients from the consumed feed. Flavonoids play a pivotal role in promoting growth by augmenting the digestibility of key nutrients in the feed, such as crude fiber and protein, as highlighted (Hayat et al, 2020). Flavonoids also strongly contribute in safeguarding the shrimp's body against the detrimental effects of free radicals, which have the potential to inflict cellular damage and compromise the immune system of the shrimp. This

assertion is aligns with the findings of Hatami et al. (2020), who demonstrated that flavonoids actively stimulate tissue repair and the generation of fresh metabolic cells in response to the damage caused by free radicals. These free radicals may originate from both external environmental sources and internal metabolic processes within the body. Moreover, alkaloids also possess the capacity to foster growth through their antioxidant properties, thereby functioning as protective agents. Bussabong et al. (2021) emphasized that alkaloids, in addition to their antioxidant role, exhibit promise as potential promoters of growth.

Despite the addition of *A.marina* leaves in Treatments B and C, the administered dosage was suboptimal in meeting the nutritional requirements of the shrimp, resulting in a diminished manifestation of the beneficial effects attributed to *A.marina* leaves on the growth performance of Pacific White Shrimp. In Treatment A which exhibited

the lowest growth outcome, *A.marina* leaves were unable to exert a discernible influence on the enhancement of Pacific White Shrimp growth.

Feed Conversion Ratio (FCR)

An effective feed ratio is denoted by a low FCR (*Feed Conversion Ratio*) value. Conversely, a high FCR value suggests inadequate utilization of the provided feed by aquatic organisms, as highlighted by Ariadi et al. (2022). Within the spectrum of FCR values, falling between 1.27 and 1.71 indicates favorable feed efficiency for shrimp, as outlined (Carmen et al, 2023). The findings from this investigation underscore that the incorporation of *A.marina* leaf extract into the feed prompted an enhanced utilization of the feed by the shrimp, surpassing the feed utilization of shrimp fed without the extract. Particularly noteworthy, Treatment D emerged as the most effective, yielding the lowest FCR value among all other treatments.

Table 2. *Feed Conversion Ratio (FCR)*

Replicate	Treatment			
	A	B	C	D
1	1,701	1,620	1,519	1,465
2	1,726	1,582	1,608	1,452
3	1,697	1,685	1,557	1,496
Average	1,709±0,01	1,628±0,05	1,561±0,04	1,470±0,02

The active compounds present in *A.marina* leaves exert a notable influence on the FCR (Feed Conversion Ratio) value. This impact is attributed to the flavonoid and tannin compounds, which contribute to enhancing digestive conditions, thereby facilitating optimal utilization of the feed. Flavonoids, acting as prebiotics within the digestive tract, foster a symbiotic relationship with probiotics (Linayati et al., 2022). Elevated levels of beneficial bacteria (Probiotics) stimulate the activity of proteolytic enzymes responsible for the breakdown of proteins into simpler amino acids, thus promoting their efficient absorption within an acidic pH environment in the intestines (Ariadi et al, 2023). The heightened functionality of these enzymes is also accompanied by an escalation in overall metabolic activity (Dawood et al., 2019). Furthermore, the enhanced performance of these enzymes correlates with improved digestibility of the consumed feed, leading to effective utilization (Soeprapto et al, 2023).

Efficiency of Protein Utilization

The Efficiency of Protein Utilization (EPP) value obtained in this study demonstrated a favorable result, with the highest score observed in

treatment D, reaching 80%. An EPP value is deemed satisfactory when it falls within a range exceeding 50% or even approaching 100%, as highlighted by Linayati et al. (2023). The attainment of an EPP value of up to 80% could be attributed to the presence of mangrove extract within the feed, which serves to enhance feed quality, thereby promoting greater feeding efficiency. As per Linayati et al. (2022), a commendable EPP value signifies superior feed quality, leading to enhanced digestibility and heightened efficiency in feed utilization. Notably, the inclusion of flavonoids in the feed stimulates the proliferation of *Lactobacillus*, a factor that significantly contributes to augmenting feed efficiency.

Arief, (2013), underscores the significance of *Lactobacillus* in creating an acidic environment, crucial for optimizing the functionality of endogenous enzymes within the feed. This environment facilitates the enhanced absorption of nutrients, further amplifying feed efficiency. Furthermore, the enrichment of the feed with essential amino acids stemming from the incorporation of mangrove extract further bolsters feed utilization. According to Linayati et al. (2022), an

elevated protein content within the feed maximizes its utility, while posit that an accurate assessment of feed quality can

be deduced from its protein content (Ariadi and Puspitasari, 2021).

Table 3. Efficiency of Protein Utilization

Replicate	Treatment			
	A	B	C	D
1	58,78	61,72	65,82	68,25
2	57,90	63,20	62,16	68,84
3	58,90	59,33	64,22	66,81
Average	58,50±0,54	1,628±1,9	64,05±1,8	67,98±1,04

The control treatment (A) had the lowest because there was no function of the bioactive compounds in *A.marina* leaves which could have helped improve feed quality and shrimp digestibility for most optimal use of the feed.

Shrimp Survival Rate

Following a 7-day observation subsequent to the *Vibrio harveyi* bacterial challenge test, the highest survival rate was evident within treatment D. This notable outcome suggests that the inclusion of *A.marina* leaf extract in the shrimp feed potentially bolstered the shrimp's immune defense against *V. harveyi* bacterial attacks. The

bioactive constituents present in *A.marina* leaves, particularly alkaloids, are presumed to contribute significantly to this defense mechanism. Alkaloids are attributed with shielding the shrimp's body against *V. harveyi* bacteria. This defense mechanism arises from the ability of alkaloids to disrupt the formation of the bacterial cell wall layer, leading to bacterial demise (Aulya, 2012). Moreover, alkaloids operate by inhibiting the synthesis of bacterial cell peptidoglycan (Znee et al, 2023) and curtailing both nucleic acid synthesis and bacterial cell division (Schulz et al, 2022).

Tabel 4. Survival Rate of shrimp after *Vibrio harveyii* infection

Replicate	Treatment			
	A	B	C	D
1	7	4	2	1
2	6	3	1	0
3	6	4	4	2
Growth	19	11	7	3
Nt	11	19	23	27
Survival Rate (%)	36	63	76	90

Flavonoids, in addition to their antioxidant properties, also serve as antibacterial agents due to their lipophilic nature (Ariadi et al, 2019). The antibacterial role of flavonoids plays a crucial part in safeguarding shrimp body cells against pathogenic assaults. Flavonoids exhibit antibacterial effects through several mechanisms, including hindering bacterial synthesis processes essential for their survival. Additionally, flavonoids disrupt the integrity of the bacterial plasma membrane system, inducing an imbalance between intracellular and extracellular components, ultimately leading to bacterial demise. As noted by Linayati et al. (2023), flavonoids impede nucleic acid synthesis, curtail the functioning of the cytoplasmic membrane, and interfere with bacterial energy metabolism. Furthermore, flavonoids form complex compounds that interact with extracellular proteins, dismantling the structural integrity of bacterial cells' core tissue and causing the release of intracellular compounds within bacterial cells (Han et al, 2023). This substantiates that the presence of flavonoids can effectively suppress pathogens like *Vibrio harveyi* upon their entry into the shrimp's body.

Moreover, the tannin compound within the mangrove extract also contributes to impeding the entry of *Vibrio harveyi* bacteria into the shrimp's body. Tannins exhibit notable antibacterial properties, demonstrating considerable toxicity against bacteria. This toxicity effect of tannins operates by degrading the bacterial cell wall, as documented by Linayati et al. (2022), and constricting the bacterial cell nucleus membrane, leading to disruption in cellular permeability and bacterial function, eventually culminating in bacterial demise (Ardiantami et al., 2015). The presence of tannins introduces a formidable barrier for *V. harveyi* bacteria, obstructing their ability to infect shrimp and thus fostering an elevated survival rate, as observed in treatment D.

The control treatment exhibits a lower stress response (SR) level compared to the other treatments. This difference can be attributed to the lack of active compounds present in *A.marina* leaves, which typically offer protective and immune-enhancing effects for shrimp, aiding them in effectively countering attacks from pathogens.

Water Quality

The temperature observations conducted on Pacific White Shrimp culture media over a 30-day period demonstrated a consistent range of 28 to 30°C which is considered favorable for shrimp growth. Assert that the ideal temperature range for achieving optimal growth in shrimp is between 26-32°C (Ariadi et al, 2021). Similarly, the salinity level maintained during rearing ranged from 20 to 25 ppt, who suggest that both shrimp and fish can thrive optimally within the range of 15-25 ppt salinity (Ariadi et al, 2020). The dissolved oxygen (DO) content, crucial for aquatic life, measured between 4 to 5 mg/L. Likewise, Ariadi et al. (2021) specify that the most advantageous oxygen concentration for successful shrimp cultivation lies between 3-6 mg/L. The pH of the Pacific White Shrimp culture medium during the rearing period exhibited slight variations, ranging from 7 to 7.6. Ariadi et al, (2019) supports these findings by indicating that the optimal pH range for promoting Pacific White Shrimp growth is between 7.0 and 8.5.

CONCLUSIONS

The addition of *A.marina* mangrove leaf extract positively affected the

growth and survival of Pacific White Shrimp following *V.harveyi* bacteria challenge test with the best dose obtained at 275 ppm/500 g feed (D).

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