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Evaluating the Effects of *Sonneratia alba* Leaf in Feed on Growth Performance and Clinical Manifestations in Milkfish (*Chanos chanos*) Exposed to *Vibrio harveyi*

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Abstract

Milkfish (*Chanos chanos*) cultivation faces challenges related to high feed costs and disease outbreaks caused by Vibrio bacteria. The use of natural ingredients containing bioactive compounds, such as *Sonneratia alba* mangrove leaves, as feed additives may serve as an effective solution. This study aimed to determine the effect of adding Pidada (*S. alba*) leaf extract at different doses to feed on growth performance and survival rates after infection with Vibrio harveyi. The experiment used a Completely Randomized Design (CRD) consisting of four treatments and three replications. The treatments included: A (control, artificial feed without extract), B (75 ppm per 500 g feed), C (125 ppm per 500 g feed), and D (170 ppm per 500 g feed). Treatment B produced the best biomass gain of 8.4 g, the best feed conversion ratio (FCR) of 1.16, and a feeding efficiency of 86.52 percent. The addition of S. alba leaf extract significantly improved growth and survival of milkfish infected with V. harveyi, with treatments B, C, and D maintaining a 100 percent survival rate after infection. Treatment A showed the lowest growth and survival, indicating that S. alba leaf extract positively supports fish health and enhances milkfish cultivation outcomes.

Keywords: Clinical symptoms, milkfish, *Sonneratia alba*, *Vibrio harveyi*.

INTRODUCTION

Milkfish (*Chanos chanos*) is a valuable brackishwater aquaculture species and a major commodity in Indonesian aquaculture, produced both for domestic consumption and as a source of foreign exchange (Ningsih *et al.*, 2024). Demand continues to rise for local markets, the fishing industry as bait, and export, which creates significant economic opportunities. According to the Ministry of Maritime Affairs and Fisheries (KKP),

Indonesia produced 785,719 tons of milkfish in 2022 with a value of IDR 16.53 trillion. This represents a 0.12 percent increase from 2021, when production reached 784,778 tons valued at IDR 15.56 trillion (KKP, 2023).

Feed is a primary determinant of fish growth (El-Hack *et al.,* 2022). Adequate feed provides essential nutrients, including carbohydrates, fats, proteins, and vitamins, which are required for energy and physiological functions (Manam, 2023). Feed accounts for 60 to 70 percent of total production costs in a cultivation cycle, particularly when commercial feed is used (Widyasari *et al.,* 2022). Improving feed efficiency by incorporating natural ingredients is therefore important. The use of natural ingredients that show positive results is also the provision of papaya seeds which can increase the immunity and growth of vaname shrimp (Linayati et al., 2025).

Mangroves represent one promising natural additive. The leaves of the *Pidada* mangrove species are elongated, ovate, and green, measuring 5 to 12.5 cm in length and 3 to 9 cm in width, with petioles 6 to 15 mm long (Giesen *et al.,* 2017). Mangroves contain bioactive compounds with antimicrobial and medicinal properties (Dai et al., 2017). Their leaves have also been identified as potential growth-promoting additives in aquaculture feed (Linayati *et al.,* 2024).

Mangrove plants contain various bioactive chemical compounds, including alkaloids, steroids, flavonoids, quinones, and saponins (Srinengri *et al.,* 2019). Alkaloids can support hemocyte function by reducing inflammation, treating infections, accelerating wound healing, and enhancing immune responses to pathogens (Sijuade, 2016). Saponins are effective against gram-positive bacteria because they increase cell membrane permeability, which leads to hemolysis (Herrialfian *et al.,* 2021).

Vibrio species are major pathogenic bacteria responsible for significant aquaculture losses, and vibriosis is one of the diseases most frequently associated with these pathogens (Ambat *et al.,* 2022). Vibriosis remains a persistent challenge in aquaculture and can affect nearly all marine fish species under cultivation (Krishnika & Ramasamy, 2014). Fish infected with Vibrio often show clinical symptoms such as frayed fins, scale loss, exophthalmia, pale internal organs, liver necrosis, and enlargement of the kidneys and spleen (Farisi *et al.,* 2021). This research aimed to evaluate the effect of adding *Pidada* mangrove leaf extract at different doses into feed on the growth performance, clinical symptoms, and survival of milkfish infected with *V. harveyi.*

MATERIALS AND METHOD

Research Setting

This research was conducted from 14 June to 30 July 2024 at the Brackish water and Marine Fisheries Research Laboratory, Faculty of Fisheries, University of Pekalongan. The research employed a Completely Randomized Design (CRD) with four treatments and three replications. The treatments consisted of the following doses of *Pidada* leaf extract:

A: Artificial feed without *Pidada* leaf extract
B: 75 ppm *Pidada* leaf extract per 500 g feed
C: 125 ppm *Pidada* leaf extract per 500 g feed
D: 170 ppm *Pidada* leaf extract per 500 g feed

These doses were adapted from Wijianto & Fahrurrozi (2023), who reported that 75 ppm of *Avicennia sp.* mangrove leaf extract in milkfish feed was the most effective concentration for promoting growth.

Research Procedure

Container Preparation

Twelve rearing containers were prepared, each equipped with aeration. The culture medium used water with a salinity of 15–25 ppt, following the conditions reported by Deran *et al.,* (2023).

Fish Preparation

The research used milkfish fry, stocked at a density of 1 fish per liter. Before the experiment began, the fry was acclimated to the rearing environment to minimize stress.

Preparation of *Pidada* Leaf Extract

The procedure for preparing *Pidada* leaf extract followed Junaidi *et al.,*(2018).

- Freshly collected leaves were cleaned, drained, and air-dried until wilted.
- Leaves were then oven-dried at 70°C until fully dehydrated.
- The dried leaves were ground and sieved to obtain a fine powder.
- The powder was macerated with 90 percent ethanol in a 5:1 ratio.
- The macerate was filtered using filter paper, and the filtrate was collected in a glass container.
- The filtrate was concentrated using a rotary vacuum evaporator at 60°C.

Addition of *Pidada* Leaf Extract

Pidada leaf extract was incorporated into the feed by weighing the extract and pellets according to the designated treatment doses. The extract solution was applied to the pellets by spraying or slowly pouring it while continuously mixing to prevent clumping and to ensure uniform distribution.

Water Quality Monitoring

Water quality parameters measured included temperature, pH, and salinity. Temperature was recorded with a thermometer, pH with a pH meter, and salinity with a refractometer.

Test Parameters

Biomass Growth

Absolute weight growth of milkfish was calculated using the formula proposed by Effendie (1997):

$$\mathbf{Wm = Wt - W0}$$

Where:
Wm = Absolute individual growth (g)
W0 = Initial biomass (g)
Wt = Final biomass (g)

Feed Conversion Ratio (FCR)

FCR was calculated according using the formula used by Effendie (1997):

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

Where:
F =Total feed consumed
Wt =Final biomass (g)
Wo =Initial biomass (g)

Feed Utilization Efficiency

Feed utilization efficiency was calculated following Effendie's formulation (1997) as follows:

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

Where:
Wt : Final biomass (g)
Wo : Initial biomass (g)
F : Total feed consumed

Survival Rate

Survival rate refers to the percentage of living biotas at the end of specific period, which was determined using Effendie's (1997) formula:

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

Where:
SR = Survival rate (%)
Nt = Number of fish at the end of the research)
No = Number of fish at the beginning of the research.

Challenge Test and Observation of Clinical Symptoms

Clinical symptoms were monitored for seven days following the challenge test with *Vibrio harveyi*. The bacterial culture, obtained from BBPBAP Jepara, was first inoculated into Alkaline Peptone Water medium, and its density was measured using a spectrophotometer. The challenge test was conducted by adding of *V. harveyi* at a density of 10^6 CFU/ml to each container. Observations focused on behavioral changes, such as reduced feeding response and decreased activity, as well as morphological signs including ulcers, reddish lesions, scale loss, fin erosion, and gill abnormalities.

Data Analysis

Data were analyzed using analysis of variance (ANOVA). Prior to analysis, normality and homogeneity were tested using Microsoft Excel. When significant differences were detected, Tukey's test was applied to compare treatment means.

RESULTS AND DISCUSSION

Biomass Growth

Milkfish biomass was measured every seven days, as shown in Figure 1.

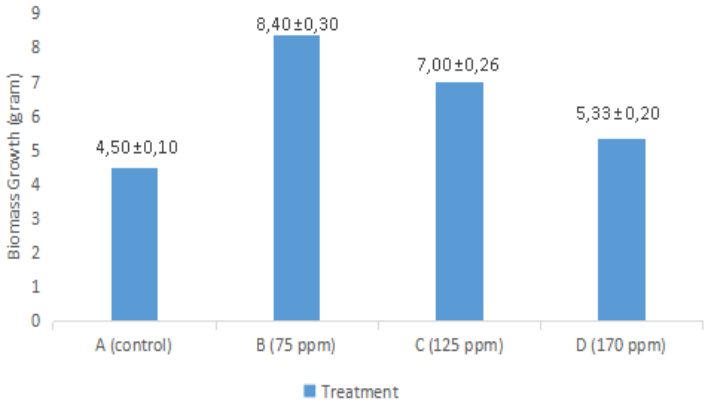

Figure 1 Milkfish Biomass Growth

The analysis of variance (ANOVA) showed that the calculated F value (170.141) exceeded the F-table values at the 0.05 and 0.01 levels (4.07 and 7.59). This result indicates that the addition of mangrove leaf extract had a significant effect on biomass growth. Prior normality and homogeneity tests confirmed that the data were normally distributed and homogeneous.

Figure 1 illustrates that biomass increased across all treatments. The highest growth occurred in Treatment B, while the lowest was observed in Treatment A (control). The incorporation of mangrove leaf extract likely enhanced feed quality by supplying additional nutrients needed to support optimal growth. However, increasing the extract dose beyond 75 ppm resulted in reduced biomass, which aligns with findings by Arghifari *et al.,* (2019).

Mangrove leaf extract contains tannins, steroids, and saponins. Saponins can form complexes with cell membranes, disrupt permeability, and lead to cell death (Supu et al., 2020). Flavonoids enhance feed digestibility and nutrient absorption, although some may produce adverse effects at higher concentrations (Peterson *et al.,* 2015). Linayati *et al.*, (2022) reported that flavonoids in aloe vera can improve growth and disease resistance in fish.

According to Yudhistira *et al.*, (2015), flavonoids improve growth by increasing the nutritional value and digestibility of feed components such as crude fiber and protein. In addition, saponins have antibacterial properties that contribute to overall fish health and growth (Linayati *et al.,* 2022).

Feed Conversion Ratio (FCR)

Feed conversion ratio data for milkfish during the culture period are presented in Figure 2.

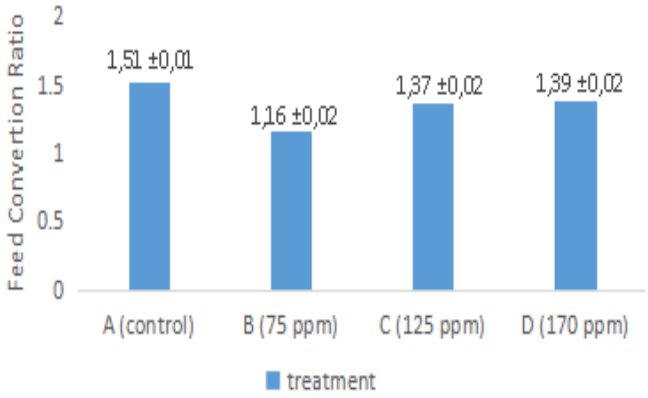

Figure 2. Feed Conversion Ratio

Treatment B produced the most efficient FCR value (1.16). Feed efficiency reflects how effectively fish convert feed into biomass (Wijianto et al., 2022). FCR can be affected by several factors, including feed quality and quantity, fish species and size, and water quality conditions (Muslim and Yonarta, 2017). Lower FCR values indicate more efficient feed use, reduced feed costs, and increased economic returns (Sopha *et al.,* 2015).

Extracts from *Rhizophora mucronata* leaves, which contain flavonoids and tannins, have been shown to improve growth performance in whiteleg shrimp. These bioactive compounds support digestive and metabolic processes by functioning as antibacterial and antioxidant agents (Linayati *et al.,* 2024)

Feed Efficiency

Feed utilization efficiency during the 30-day culture period is presented in Figure 3.

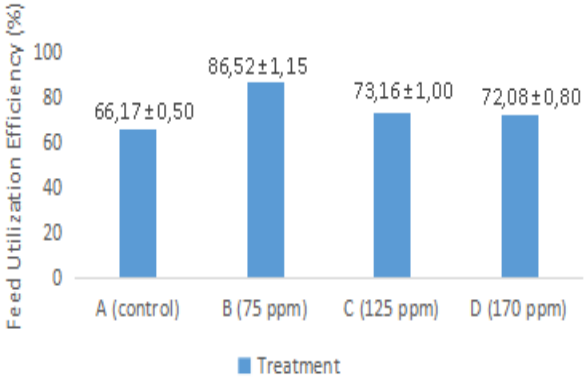

Figure 3. Feed Utilization Efficiency

The highest efficiency was recorded in Treatment B, reaching 86.52 percent. Higher feed efficiency indicates more optimal nutrient use and reflects good feed quality (Arisa *et al.,* 2020). According to Puspasari *et al.,* (2015), feed is considered effective when utilization efficiency exceeds 50 percent or approaches 100 percent.

Flavonoids can enhance feed efficiency and nutrient absorption by increasing metabolic activity. Higher metabolism accelerates the breakdown of glucose and fatty acids, thereby improving fish health and allowing dietary protein to be allocated for growth (Linayati *et al.,* 2023). Alhaddad *et al.,* (2019) also noted that flavonoids facilitate protein breakdown into amino acids, making them easier to digest.

Survival Rate

The survival rate of milkfish before and after the challenge test is shown in Figure 4.

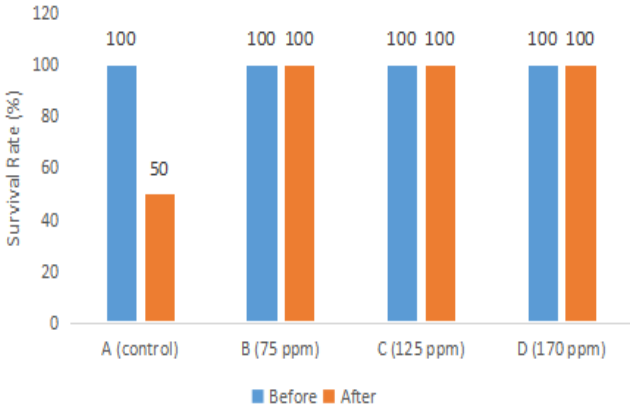

Figure 4. Survival Rate

Before the challenge test, all treatments exhibited a 100 percent survival rate, indicating that the addition of mangrove leaf extract did not affect baseline survival. Fish survival is influenced by internal factors such as genetics and immunity, as well as external factors such as feed quality, stocking density, and water conditions. High survival is achieved when both nutritional and environmental factors are optimal (Prasetio *et al.,* 2018). The presence of bioactive compounds in Pidada leaf extract supports growth and aligns with Wulansari *et al.,* (2020), who reported positive effects of mangrove leaf extract as a feed additive.

After exposure to *V. harveyi*, survival differed among treatments. Treatments B, C, and D maintained a 100 percent survival rate, while Treatment A (control) showed only 50 percent. No mortality occurred in treatments receiving leaf extract during the seven-day post-challenge period, which is likely related to the flavonoid, tannin, and alkaloid content of the extract. Flavonoids in mangrove leaves act as immunostimulants that inhibit pathogenic bacterial growth.

Manuhuttu & Saimima (2021) reported similar findings for *Sonneratia alba* leaf extract, which exhibits antimicrobial and immunostimulant properties. Flavonoids also promote the growth of beneficial gut bacteria (Linayati *et al.,* 2022), and other secondary metabolites enhance immune function and support resistance against pathogens (Yahya *et al.,* 2025).

The antibacterial properties of the extract also contribute to protection against *V. harveyi*. Saponins can lyse bacterial cells by damaging the cell wall (Khan *et al.,* 2018), while tannins disrupt the bacterial polypeptide wall, leading to cell death (Sapara *et al.,* 2016).

Clinical Symptoms Observation

Observations from day 0 to day 30 showed that fish behavior remained normal until the introduction of *Vibrio harveyi*. Early in the infection period, fish continued to swim normally. Clinical symptoms then appeared, characterized by peeling scales and reduced activity, with most fish becoming immobile or passive. These symptoms were observed in all fish receiving *Pidada* leaf extract (treatments B, C, and D).

In fish that did not receive the extract (treatment A), peeling scales and weak movement were also present, along with additional damage to the caudal fin. A red dish brown discoloration appeared on the body surface of fish in treatments B, C, and D, although the color change was limited to the head or operculum region (Figure B1). In contrast, fish in treatment A showed discoloration across nearly the entire body (Figure B3).

Clinical symptoms appeared on the second day after infection in treatment A and on the third day in treatments B, C, and D. Survival was 50 percent in treatment A, while all fish survived in the other treatments. This difference is likely due to the presence of flavonoids in *Sonneratia alba* leaf extract, which function as antimicrobial, antibacterial, and immunostimulant compounds (Manuhuttu & Saimima, 2021). Alkaloids in the extract also act as antibacterial agents that help fish overcome bacterial infections (Masithoh *et al.,* 2019).

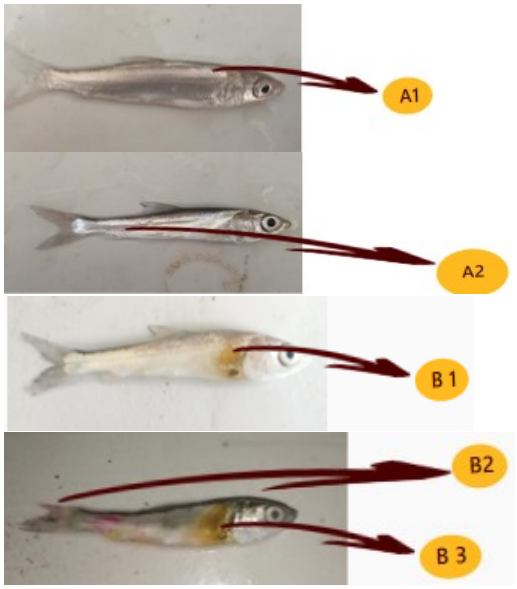

Figure 6. Clinical Symptoms Before (A) and After Infection (B)

Where:
A1 : Scales still intact
A2 : Fish color is shiny
B1 : Scales peeling
B2 : Damages to the tail
B3 : Doscoloration and damage to the surface of the fish's body

Water Quality

Water quality plays an important role in fish growth. Clean water with sufficient oxygen is essential for maintaining the health and development of fish (Shamsuddin *et al.,* 2022). Water serves as the primary environment for milkfish, and its quality directly influences both growth and survival. According to Effendie (2002), fish survival is affected by internal and external factors. Fluctuations in parameters such as temperature, pH, and salinity can lead to fish mortality. Linayati *et al.,* (2021) also noted that a decline in water quality can trigger disease outbreaks and reduce fish survival. Poor water conditions can further lead to slower growth rates.

Table 1. Water Quality Parameters

Parameter	Water Quality	Optimal Score	Reference
Salinity	15-20 ppt	15-30 ppt	Yunita (2023)
Temperature	27-29°C	29-30°C	Yahya *et al*., (2022)
pH	5.61 – 8.1	6.8 – 8.7	Yahya. *et al*. (2022)

CONCLUSION

The addition of *Sonneratia alba* (*Pidada)* leaf extract influenced the growth and survival of milkfish infected with *Vibrio harveyi*. A dose of 75 ppm per 500 g of feed produced an average biomass increase of 8.40 g and a survival rate of 100%. Clinical symptoms were seen better in fish fed with pidada leaf extract.

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