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Effect of Giving Api-Api (*Avicennia marina*) Mangrove Leaf Solution In Artificial Feed On The Immunity Response and Growth of Vannamei Shrimp (*Litopenaeus vannamei*)

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Abstract

This study aimed to determine the effect of giving *Avicennia marina* mangrove leaf solution on artificial feed to increase the immune response and growth of vannamei shrimp. The parameters observed in this study were the enhancement of PA value (Phagocytosis Activity), ADG (Average Daily Growth), FCR (Feed Conversion Ratio), and EPP (Efficiency of Feed Utilization). The vannamei shrimp used was PL-20 fry with a weight of 1,09 – 1,13 g·head^{-1} and density 1 shrimp L^{-1}. The method used in this study was experimental in the laboratory, with a completely randomized design (CRD) with 4 treatments and 3 replications. This research was conducted by adding *A. marina* mangrove leaf solution to the feed, with treatments of A (control), B (125 ‰), C (175 ‰), and D (225 ‰). The addition of *Avicennia marina* leaf solution to the feed significantly affected the increase of phagocytosis activity and growth of the shrimp with an F count of 30,773, which was higher than the F table of 4,06. Moreover, the best dose obtained in treatment D which increased PA was 61,9%, ADG was 0,22 g·day^{-1}, FCR was 1,29, and EPP was 79,04%.

INTRODUCTION

Fishery products are contributing to the world's supply of animal protein. FAO (2020) reported that fishery products accounted for 17% of the total animal protein consumed by humans. In the aquaculture sector alone, in 2020, the total production for human consumption reached 87,5 million tons (FAO, 2022), and among the prominent ones increasing yearly is shrimp.

Cultivation of shrimp experiences serious problems because of the existence of disease attacks, such as white spot syndrome virus, vibriosis, or AHPND (Acute Hepatopancreas Necrosis Disease) caused by *Vibrio* sp. bacteria and less maximum efficiency of feed utilization related to protein on fish diet. The optimal dietary crude protein level for small *L. vannamei* is 34,5 % and 32,2 % for adults based on broken line analysis for growth

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data (Lee and Lee, 2018). This disease can cause losses because of the high mortality. With the fast development of the intensive aquaculture industry, the emergence and outbreaks of disease become more often, which seriously threaten the development of shrimp cultivation (Xiong *et al.,* 2016). Weak feed management, such as a lack of nutritional content or not utilizing feed properly, can also have fatal impacts. This cannot be underestimated because 40-60% of the cost of farming activities is feed (Rao and Joseph, 2019).

Efforts that can be made to prevent the disease can be made by giving additional material/substance that can stimulate the body defense system of shrimp and at the same time, increase the shrimp growth. Phagocytosis activity is one of the most important mechanisms of shrimp body defense (Ellis *et al*., 2011). The phagocytosis mechanism is the immunity response of shrimp toward the incoming strange particle into the body. The activity value of phagocytosis will increase along with the efforts of the body to withhold dangerous particles. Saptiani *et al.* (2021) mentioned that adding n-butanol leaf extract mangrove *Acanthus ilicifolius* 300mg L-1 increased granulocytes, phagocytosis percentage, and propenoloxidase activity in *P. monodon* (Black Tiger shrimp) tested with *Vibrio harveyi.*

Furthermore, according to Vargas-Albores *et al.* (1998), feeding is more efficient if given additional capable material that can increase the growth rate. Extra material that contains active compounds like anti-oxidants such as flavonoids is believed to be capable of increasing immunity and also the growth of organisms. The mangrove plant of *A. marina* species is considered one of the best sources of bioactive chemicals, one of which is phenolics and flavonoids (Al-Mur, 2021). Flavonoids are used as immunopotentiators to reduce oxidative stress and growth (Li *et al*., 2018). Based on these effects, it can be known indirectly that the content of flavonoids can help in maximizing the utilization of the feed given to the shrimp.

The purpose of this study was to find out the effect of giving the *A. marina* mangrove leaf solution on artificial feed toward the activity of phagocytosis and growth of vannamei shrimp through daily growth parameters, FCR, and utilization feed efficiency.

METHODOLOGY

Ethical Approval

There was no animal harmed during this study. Animals were kept in good condition, and given appropriate feed, and sampling was done carefully. Water for rearing was maintained at optimal conditions for growth. Good maintenance of water quality parameters was performed, including temperature, salinity, pH, and dissolved oxygen. The research proposal also had the review and approval of the supervisory team at the Faculty of Fisheries, Pekalongan University.

Place and Time

This research was conducted from December 2022 to February 2023 at the Marine and Brackish Water Laboratory, Faculty of Fisheries, Pekalongan University. Phagocytosis testing was carried out at the Biology Laboratory, Pekalongan University.

Research Materials

The equipment in this study included a DO meter 5510 (Lutron, Taiwan) mercury thermometer (SP Bel-Art, China), refractometer (Atago, Japan), aquariums 40 x 25 x 25 cm (Nikita Star, Indonesia), digital scales with an accuracy of 0.01 g (i2000, China), closed net (Indonesia), air stone 40 (Rosston, China), spuit syringe (One Med, Indonesia)), glass tube (One Med, Indonesia), Mikroskop XSZ BN (Oregon, China), electric boiling pan (OEM, China), plastic bag (Golden, Indonesia), label paper No 107 (Panda, Indonesia) and a

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camera cyber shot DSC W810 (Sony, Japan). While the materials used in this study were vannamei shrimp from the shrimp pond center in Pekalongan City, *A. marina* leaf solution, and commercial shrimp feed (SI-01, CJ Feed Lampung).

Research Design

This study used a completely randomized design (CRD) with four treatments and three replications. These doses were used according to the recommendations of Rusadi *et al.* (2019), who mentioned that the best results for *L. vannamei* mortality were 250 ppm in addition to mangrove leaf extract. The arrangement of the treatment is as follows:

(A) Feed without the addition of *A. marina* mangrove leaf solution; (B) *A. marina* leaf solution 125 ‰·500 g^{-1} feed; (C) *A. marina* leaf solution 175 ‰·500 g^{-1} feed; (D) *A. marina* leaf solution 225 ‰·500 g^{-1} feed.

Sampling was performed 5 times at intervals of 7 days for the measurement of the observed parameters. The observed parameters were phagocytosis activity, Average Daily Growth (ADG), Feed Conversion Ratio (FCR), and feed utilization efficiency (EPP), while water quality was carried out as a supporting parameter.

Work Procedure

The test shrimps were initially acclimatized and fasted for 1 day to equalize the condition of the shrimp in terms of response to the feed that will be given during the maintenance and also to reduce stress levels. The container used in this study was an aquarium measuring 40 x 25 x 25 cm with a brackish water volume of 10 L. The culture medium used was brackish water that had been precipitated for 1 night. The medium was then filtered to prevent harmful particles from entering the water. The container was equipped with an aeration hose. Before filling the water, the container was washed and then rinsed clean, and then dried, after drying the water was filled and the aerator was given. The method was by providing feed for 6% of wet weight with a frequency of 4 times (7 AM, 11 AM, 4 PM, 8 PM) a day for 36 days which contains an optimal level of crude protein of 35,6% (Lee and Lee, 2018). Moreover, the first parameter measurement of PA, ADG, FCR, and EPP was obtained before treatment, while subsequent measurements were performed after treatment every week until the end of the study.

Test Parameters

Phagocytosis activity (PA)

One mL of hemolymph from *L. vannamei* was placed in a glass tube and prepared. The preparation was fixed with 100% methanol for 5 minutes and stained with Giemsa solution (10%) for 15 minutes. Flowing water was given slowly in preparation for 5 minutes to remove the remaining Giemsa stain. The observation was conducted by using microscope light with 400x magnification. Phagocytic Activity (PA) was measured based on cell percentage that showed phagocytosis (Cheng *et al.*, 2005).

$$PA = \frac{\text{Active phagocytic cell}}{\text{phagocytes cell}} x100$$

Average daily growth (ADG)

Average daily growth is based on the formula from Jensen (1992), which is as follows:

ADG=ABW Final-ABW initial

Feed Conversion Ratio (FCR)

FCR (Feed Conversion Ratio) is calculated using the formula proposed by Tacon (1987), as follows:

$$FCR = \frac{\text{Total of Feed Consumption}}{\text{Final Weight} - \text{Initial Weight}}$$

Efficiency of Feed Utilization (EPP)

Calculation of the value of feed efficiency is calculated based on the formula Tacon (1987), as follows:

$$EPP = \frac{\text{Final Weight} - \text{Initial Weight}}{\text{Total of Feed Consumption}} x100\%$$

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Avecenia marina Leaf Solution

A. marina leaf 100 g is cleaned formerly and then dried by exposing it to air for several hours. After drying, it is weighed with digital scales to count the weight of the leaf in gram units. After that, the leaf is cooked in as much as 1 L of water until it boils and then is filtered. To obtain 175 ‰, it takes 0,175 g in 1 L of water (Rusadi *et al.,* 2019) and refers to the preparation of solution in ppm (Tumanggor *et al.,* 2023). The dose then follows existing provisions. The ready feed will be sprayed with a solution and then left until seeps before being given to the shrimp.

Data Analysis

The data obtained from the observations was calculated statistically, namely the normality and homogeneity test. Then, the data is tested through analysis of variance (ANOVA) to determine significant differences from treatment. Duncan's multiple region test is also performed to compare the average value that shows a significant difference with P<0.01. Data processing is using SPSS Statistics ver. 24 for Windows (IBM Corp., New York, USA).

RESULTS AND DISCUSSIONS

The results of statistical tests with ANOVA showed that the treatment of *A. marina* leaves solution in artificial feed has a significant effect on Phagocytosis activity, ADG, FCR, and EPP with F counts greater than F tables.

Phagocytosis activity

The phagocytosis activity level increased along with increasing the dose of *A. marina*. These values are shown in Table 1.

Table 1. The value of phagocytosis activity.

Treatments	Average of Phagocytosis Activity (%)
A. *A. marina* 0 ‰ 500 g-1 feed	45,59±0,92[c]
B. *A. marina* 125 ‰ 500 g-1 feed	47,16±1,66[c]
C. *A, marina* 175 ‰ 500 g-1 feed	53,02±2,72[b]
D. *A. Marina* 225 ‰ 500 g-1 feed	61,19±2,91[a]

*Different Superscript marks showing the difference between treatments (P<0.01)

Based on Table 1, The PA value can be known to be the highest achieved in treatment D with an average value of 61,19%. After that it is followed by treatment C with an average value of 53,02%, then treatment B with a value of 47,16%, and finally treatment A (control), which is 45,59%. There is no significant difference between treatments A and B which is indicated by the same superscript (c), for example, treatment A and B. Meanwhile, treatment D shows significant differences from all treatments due to different superscripts (a). This explanation also applies to the research results in other tables.

Average Daily Growth

There was a gradual increase in the average daily growth rate as the dose continued to be increased. The value shows the daily development of the shrimp body that continues to increase and this is clearly illustrated in Table 2 below.

Table 2. Average weight gain rate (g.day^{-1}).

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Treatments	Average ADG (g.day^{-1})
A. *A. marina* 0 ‰ 500 g-1 feed	0,16 ± 0,0037^{c}
B. *A. marina* 125 ‰·500 g^{-1} of feed	0,18 ± 0,0025^{bc}
C. *A. Marina* 175 ‰·500 g^{-1} of feed	0,19 ± 0,0008^{b}
D. *A. Marina* 225 ‰·500 g^{-1} of feed	0,22 ± 0,0202^{a}

*Different Superscript marks showing the difference between treatments (P<0.01).

Based on Table 2, it can be seen that the highest average daily growth is obtained in treatment D with an average value of 0,22 g. After that it is followed by treatment C with an average value of 0,19 g, then treatment B with a value of 0,18 gr, and finally treatment A (control), which is 0,16 g.

Feed Conversion Ratio

The feed conversion ratio is a value that indicates the amount of feed required to produce 1 kg of meat. The lower the FCR value, the better the description of feed digestibility. The value is shown in the numbers listed in Table 3.

Table 3. Vannamei shrimp feed conversion ratio.

Treatments	FCR average
A. *A. marina* 0 ‰ 500 g-1 feed	1,43 ± 1,47^{b}
B. *A. marina* 125 ‰·500 g^{-1} of feed	1,40 ± 1,39^{ab}
C. *A. marina* 175 ‰·500 g^{-1} of feed	1,38 ± 1,39^{ab}
D. *A. marina* 225 ‰·500 g^{-1} of feed	1,29 ± 1,18^{a}

*Different Superscript marks showing the difference between treatments (P<0.01).

Based on the data obtained, the best FCR value for vannamei shrimp is obtained in treatment D with a value of 1,29. After that it is followed by treatment C with an average value of 1,38, then treatment B with a value of 1,40, and finally treatment A (control), which is 1,43.

Efficiency of Feed Utilization

Feed utilization efficiency shows an increasing number which means that the use of *A. marina* leaves solution on feed utilization has a significant effect. This indicates more efficient feed consumption with increasing treatment dose.

Table 4. Vannamei shrimp feed utilization efficiency (%).

Treatment	EPP Average (%)
A. *A. marina* 0 ‰ 500 g-1 feed	69,87 ± 0,02^{b}
B. *A. marina* 125 ‰·500 g^{-1} of feed	71,30 ± 0,01^{ab}
C. *A. marina*175 ‰·500 g^{-1} of feed	72,62 ± 0,03^{ab}
D. *A. marina* 225 ‰·500 g^{-1} of feed	79,04 ± 0,06^{a}

*Different Superscript marks showing the difference between treatments (P<0.01).

Based on the data obtained, the best EPP value for vannamei shrimp is obtained in treatment D with a value of 79,04%. After that it was followed by treatment C with an average value of 72,62%, then treatment B with a value of 71,30%, and finally treatment A (control), which is 69,87%.

The results of observations made during the study show that there were significant effects between the treatments with the addition of *A. marina* leaf solution to the feed which showed an increase in immunity response, in the form of PA value increase. The statistical test result shows the significant effect of giving *A. marina* leaf solution on the AF with an F Count of 30,773 which is higher than F table 4,06. Treatment A had the lowest results because the feed given did not contain feed additives such as alkaloids, flavonoids, steroids/triterpenoids, and

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tannins, so there was no active substance that could stimulate the immunity system as well as feed digestion optimally. Phagocytosis activity in shrimp increased by up to 61 % which was caused by the presence of antioxidants and active substances such as flavonoids (Munawaroh *et al.*, 2018) and other active substances such as terpenoid (Rohman *et al.*, 2007) that those active substances induced hyaline cells to do degranulation so that increasing phagocytosis activity. Following Giulianini *et al.* (2007), hyaline cell plays an important role in phagocytosis in shrimp immunity.

Phagocytosis activity is one of the non-specific immunity response forms that is included as the first defense mechanism system against the attack of harmful microorganisms (Widanarni *et al.,* 2020). Saptiani *et al*. (2021) mentioned that adding n-butanol leaf extract mangrove *A. ilicifolius* 300mg L^{-1} increased granulocytes, phagocytosis percentage, and propenoloxidase activity in *P. monodon* (Black Tiger shrimp) tested with *V. harveyi*. Since shrimps lack a distinct immune system, humoral and cellular immunity are essential innate immunological defenses against pathogens (Le *et al.,*2019). According to Rahmaningsih *et al.* (2021), the absorbed active substance from the Majapahit fruit is capable of increasing the phagocytosis activity of *L. vannamei* shrimp. Several active substances among them are alkaloids, pectins, tannins, flavonoids, and terpenoids which are also owned by the leaves of *A. marina*. *A. marina* leaf solution is a natural feed additive that can be used as an anti-inflammatory and antioxidant that can ward off free radicals strongly (Roy *et al.,* 2022).

Furthermore, Pês *et al.* (2018) mentioned that Quercetin, one type of flavonoid can arrange antioxidants enzyme gene expression and glutathione ingredients (GSH). That helps strengthen the defense system of the shrimp body. Shrimp survival rate can be increased up to 90% 7 days after *V. harveyi* infection by feeding *A. marina* mangrove leaf extract (Linayati *et al.,* 2023). Moreover, *A. ilicifolius* mangrove leaf extract containing flavonoids and alkaloids showed similar results, which increased the non-specific immune response in *P. monodon* and prevented mortality due to *V. harveyi* infection (Saptiani *et al.*,2021).

The lower growth rates in treatments B and C compared to treatment D were due to the small number of alkaloids, flavonoids, steroids/ triterpenoids, and tannins. Therefore the digestion process could not be absorbed optimally, because the amount of nutrients in the feed is still lacking. The high growth of vannamei shrimp in treatment D with a daily growth yield of 0,16 g given the feed is suspected because the content of *A. marina leaves* had a positive effect on shrimp growth. Thus, according to Sanchez *et al.* (2005) can contribute to minimizing the leaching of nutrients and feed waste caused by selective shrimp-eating behavior. *A. marina* mangrove contains essential amino acids such as GABA or γaminobutyric acid (Hinokidani *et al.,* 2020) and glutamine and, alanine (Tsuchiya *et al.,* 2013), so the more protein is hydrolyzed into amino acids, the more amino acids are absorbed and available for use by the body. The amino acid has a function in growth, cell repair, and as a source of energy (Linayati *et al.,* 2022).

Flavonoid content is used as an immunopotentiator to reduce oxidative stress and support growth (Li *et al.,* 2018). Flavonoid and polyphenol compounds can be prebiotic (Alves-Santos *et al.,* 2020) which support growth bacteria digestion like *Lactobacillus casei.* The presence of *L. casei* can increase nutrient absorption, feed consumption, and growth by stimulating endogenous enzymes for production. Combining immunostimulants in feed can provide

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good growth and increase non-specific defense in shrimp.

The results of FCR observations during the study showed that the best treatment was in treatment D with a value of 1,27, followed by treatment C with a value of 1,38 treatment B with a value of 1,40, and treatment A with a value of 1,43. The low FCR value was found in treatment A with a conversion ratio of 1,43. The low value of feed conversion is suspected of the absence of feed additives such as flavonoids, alkaloids, and tannins.

Treatment with an efficient feed conversion value was in treatment D with a value of 1,27, it is suspected that there was a feed additive content so that the feed could be used for the growth of the shrimp. In addition, the nutritional content in the feed with the addition of *A. marina* leaf solution provided energy for metabolic activity and was also used to meet the needs of vannamei shrimp to grow (Linayati *et al.,* 2022).

The role of flavonoids contained in *A. marina* leaf have functions in giving a positive effect on shrimp in utilizing the feed given. Furthermore, Sakuma *et al.* (2017) mentioned that Flavonoids can regulate various metabolisms in cells and can inhibit the growth of adipose tissue through the anti-angiogenesis pathway. It causes the metabolism can be maximally used for growth.

Factors that affect the efficiency of a feed are the type of nutrient source and the amount of each component of the nutrient source in the feed. *A. marina* leaf contains 11,04% protein, 69,2% water content, 14,91% ash content, and 2,21% fat content (Sari *et al.,* 2021). The lowest feeding efficiency value in this study was in treatment A 69,86%. This is presumably due to the absence of the addition of *A. marina* mangrove leaf solution which resulted in fish absorbing protein better than the treatment containing *A. marina* mangrove leaf. A high EPP value indicated that a small amount of feed nutrients was overhauled to meet energy needs and the rest was used for growth.

CONCLUSION

The addition of *Avicennia marina* mangrove leaf solution to the feed had a very significant effect on the increase of immunity response in the form of increasing in phagocytosis activity, daily growth, FCR, and EPP of vannamei shrimp. The best dose of adding *A. marina* mangrove leaf solution to feed toward PA, and vannamei shrimp growth was in treatment D (225 ‰.500 g^{-1} of feed) with a PA value of 61,9%, with a daily growth of 0,22 g·day^{-1}, FCR 1,27 and EPP of 79,04%.

CONFLICT OF INTEREST

The authors would like to declare that there was no conflict of interest in the research or the writing and publishing of this manuscript.

AUTHOR CONTRIBUTION

Linayati is the principal researcher, and thanks to Tri Yusufi Mardiana and Wutti Rattanavichai for research assistance and writing the discussion, Leonardus Bayu for data collection, and Muhammad Zulkham Yahya for analyzing data.

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