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THE EFFECT OF THE ADDITION OF PAPAYA SEED FLOUR *Carica papaya* L. IN FEED ON THE IMMUNE RESPONSE AND GROWTH PERFORMANCE OF WHITELEG SHRIMP *Litopenaeus vannamei*

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ABSTRACT

The main issues in shrimp farming are disease and high feed prices. To overcome these issues, a safe and inexpensive substance that can improve health and growth is needed. Natural ingredients from plants such as papaya *Carica papaya* L. are widely available in Pekalongan. This study aimed to determine the effect of papaya seed flour as a substance addition to artificial feed on the immune response and growth performance of whiteleg shrimp. This experimental research employed a completely randomized design (CRD) consisting of five treatments and three replications. The test animals were whiteleg shrimp (PL21) post-larvae, while the test feed was supplemented with papaya seed flour at different doses for each treatment: 0 $g.kg^{-1}$ feed (A); 1 $g.kg^{-1}$ feed (B); 3 $g.kg^{-1}$ feed (C); 5 $g.kg^{-1}$ feed (D); and 7 $g.kg^{-1}$ feed (E). The parameters observed included PA (Phagocytic Activity), THC (Total Haemocyte Count), absolute biomass, SGR (Specific Growth Rate), and FCR (Feed Conversion Ratio). Treatment D was identified as the optimal dose, resulting in PA of 65.67%, THC of 48.93×10^6 $cells.mL^{-1}$, absolute biomass of 6.23 g, SGR of $6.09\%.d^{-1}$, and FCR of 1.18, with a 100% survival rate. Data analysis confirmed that the treatments had a significant difference ($P < 0.05$) on all parameters. The results showed that the use of papaya seeds can improve both the health and growth of whiteleg shrimp.

Keywords:
Growth
Papaya seed
Phagocytic
Total haemocyte cells

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INTRODUCTION

Shrimp is a highly valuable aquaculture commodity that accounts for a large portion of the monetary value of aquaculture (Robert et al., 2021). However, the cultivation of whiteleg shrimp has been inhibited due to inefficient feed consumption and disease outbreaks. Feed is one of the most important elements in aquaculture, determining the growth and survival of cultured fish. Ashour et al. (2024) noted that feed quality and its compliance with nutritional requirements are among the main issues as they have an impact on the shrimp development and general health. The composition of feed ingredients should be adjusted to the digestive ability. Similarly, Prakash et al. (2023) demonstrated that dietary components influence nutritional digestibility. According to Briggs et al. (2004), diseases have the potential to cause up to 100% mortality in shrimp within a short period. Initially, this disease severely damaged the shrimp aquaculture industry, inflicted considerable economic losses and resulted in average yearly losses of $4 billion in the Asian shrimp industry from 2009 to 2018 (Shinn et al., 2018). One of the diseases, vibriosis, is commonly found in shrimp ponds and can bring adverse impact, such as significant losses. In general, various additives can be used to improve the growth performance and health of aquatic biota. Vibrio species are responsible for the significant and devastating bacterial disease known as shrimp vibriosis, which affects a variety of shrimp species (Chandrakala and Priya, 2017). However, these additives can contain chemicals in the form of hormones and antibiotics that may carry considerable adverse effects, such as resistance (Baruah et al., 2008). Shrimp vibriosis is currently controlled by vaccination, water quality management, bacteriophage methods, biosecurity measures, herbal immunomodulators, and a number of other methods (Abdel-Latif et al., 2022).

The World Health Organization (WHO) recommends the use of natural or herbal ingredients as substitutes for chemicals, or to minimize their use. Papaya seed flour is one of the natural ingredients that can increase the growth of aquatic biota, such as reproductive performance in Nile tilapia (Charitra et al., 2021). The improvement of shrimp immune response can be assessed through the increase of total haemocyte cells, which could be described as the value of THC (Total Haemocyte Count) and phagocytic activity (PA). The results showed that the use of papaya seeds can improve both the health and growth of whiteleg shrimp. Increased THC strengthens the immune system, leading to the emergence of pathogen resistance in shrimp. Mahasri et al. (2018) stated that increased THC can be used as an indicator of the immune system reaction in shrimp. According to Hu (2022), immunological indicators, such as phagocytic activity, may also be useful to evaluate the effectiveness of shrimp feed additives that improve immunity. An increase in phagocytosis is an indication that the body is actively fighting infection or inflammation. By actively engulfing and destroying pathogens, phagocytes can prevent infection and reduce tissue damage (Rosales & Uribe-Querol, 2017). Furthermore, Xu et al. (2014) described that haemocytes have an important role both as a cellular response and as a humoral response in the shrimp's immune system mechanism.

Papaya seeds can serve as natural insecticides as they contain active compounds, such as alkaloids, carpaines, flavonoids, saponins, tannins, polyphenols (Masfufatun et al., 2019), as well as papain enzymes (Owoyele et al., 2008). Papain is a proteolytic enzyme that acts as a polypeptide to simplify amino acid molecules by breaking down organic protein molecules (Amri and Mamboya, 2012). This supports the growth of fish and improves feed efficiency. Flavonoids are polyphenolic compounds found in plants that have bioactive properties beneficial for treating various diseases and other bioactivities (Zakwan et al., 2023). According to Li et al. (2019), flavonoids, as immunostimulants, are often administered as nutritional supplements to enhance growth performance, antioxidant function, immune response, and disease resistance. Furthermore, Ye et al. (2019) reported that alkaloids can increase crude protein and lipid deposits in Wuchang bream *Megalobrama amblycephala*. These active compounds act as antioxidants which have an important role in protecting cells from pathogens. Secondary metabolites play an important role in stress reduction, antioxidant activity, and immune system enhancement due to their ability to identify, bind to, catalyse, and change proteins and DNA (Tadese et al., 2022). Further research by Linayati et al. (2023) reported that mangrove leaf supplementation *Avecennia marina* can enhance the growth and disease resistance of whiteleg shrimp, especially against *Vibrio harveyi*, due to metabolic compounds such as flavonoids and alkaloids.

Previous research by Tomasoa and Azhari (2019) demonstrated that the use of papaya seed flour can enhance the growth and survival rate of tilapia. The optimal dose is 5 $g.kg^{-1}$ for a body weight gain of 547 g. The survival rate of blue tilapia reached 100% over 30 days of rearing. The same results were also reported by Syakirin et al. (2022) who found that the use of papaya seeds can increase the growth of cantang grouper. These results have encouraged other researchers to examine the effects of papaya seed flour on the growth and survival of whiteleg shrimp. In addition to seeds, papaya leaves can also improve growth performance in tilapia, leading to an average weight gain of 31.14 g (Hamid et al., 2022). Further research on the use of papaya plants to increase aquaculture productivity is needed. This study aimed to examine the effect of the addition of papaya seed flour, and to determine the optimal dosage to increase the growth and survival rate of whiteleg shrimp.

MATERIALS AND METHODS

The research was carried out from 2 September to 30 November 2022 at the Brackish and Marine Water Laboratory, Faculty of Fisheries, Pekalongan University. The study lasted 60 days, including preparation, a one-week adaptation period and 30 days of treatment.

A completely randomized design (CRD) with 5 treatments and 3 replications was used, with different papaya seed flour concentrations as follows:

A: No addition of papaya seed flour

B: Papaya seed flour addition of 1 $g.kg^{-1}$ feed

C: Papaya seed flour addition of 3 $g.kg^{-1}$ feed

D: Papaya seed flour addition of 5 $g.kg^{-1}$ feed

E: Papaya seed flour addition of 7 $g.kg^{-1}$ feed.

Those doses were recommended by Tomasoa and Azhari (2019) who found 5 $g.kg^{-1}$ feed as the optimal dose for the growth and survival rate of tilapia.

21 whiteleg shrimp post-larvae obtained from farmers in Pekalongan City were used as test animals, with ca. 10 shrimps in each chamber, each filled with 10 liters of water. The shrimp were first acclimatized to prevent stress and to help them adapt to the new environment. Whiteleg shrimps were weighed using a digital scale. The shrimp were then put into the rearing media, with 1 shrimp per 1 liter of water. Feeding was carried out four times a day (Zaenudin et al., 2019), based on 3-5% of biomass weight. The feed used in the study was commercial feed containing 35% protein. Papaya seed flour extract was diluted in 100 ml of water, then sprayed onto 1 kg of feed while stirring to ensure even distribution. The feed was then air-dried for several hours. Once dry, the feed was given according to the provisions.

The data obtained in this research were first tested for normality and homogeneity. Subsequently, an analysis of variance (ANOVA) was performed to find out the differences among treatments. If the results were statistically significant, the Tukey test was performed. Water quality data were analyzed descriptively.

The following are some of the tests that were carried out.

Phytochemical Test (Qualitative Test)

Phytochemical tests were carried out to detect the presence of phytochemical compounds.

- Flavonoid
  A 1 mL ethanol extract of papaya seed was taken and supplemented with magnesium powder and 10 drops of concentrated hydrochloric acid. The presence of flavonoids was indicated by the formation of a black, reddish, yellow, or orange.
- Alkaloid
  A total of 4 mL of the sponge ethanol extract was placed in a test tube, followed by the addition of 2 mL of chloroform and 5 mL of 10% ammonia. Then, 10 drops of 2% sulfuric acid were added to clarify the separation. The formation of 2 different phases was observed. The upper part of the phase formed was taken, and then Mayer's reagent was added. The presence of alkaloids in the sample was indicated by the formation of a red precipitate.
- Saponin
  A foaming test was performed to determine the presence of saponins. The 2 mL ethanol extract was mixed with 2 mL of distilled water and vortexed for 1 minute. If foaming occurred, 2 drops of 1 N HCl were added and the solution was incubated for 10 minutes at a temperature of 37 °C. If the foam remained stable, the extract contained saponins.
- Tanin
  The papaya seed flour was heated in a water bath at a temperature of 50 °C for approximately 5 minutes. After heating, 1 drop $FeCl_3$ 1% solution was added. If the extract formed a blue-black precipitate, it indicated the presence of tannins.

Phagocytic activity

The 0.1 mL of individual *L. vannamei* hemolymph was smeared onto a glass slide, and smear preparations were made. The specimens were fixed in 100% methanol for 5 min and stained with Giemsa (10%) for 15 min. Running water was slowly added for about 5 minutes to remove the remaining Giemsa stain. Observations were made under a light microscope at 400× magnification. Phagocytic activity (PA) was measured based on the percentage of cells showing phagocytosis (Cheng et al., 2005). Blood sampling was performed at the end of the maintenance period.

$$\mathrm{PA} = \frac{\text{Active phagocytic cells}}{\text{Number of phagocytic cells}} \times 100$$

Total Haemocyte Count (THC)

The sampling was conducted at the end of the research. The total haemocyte count for each treatment was determined according to Immanuel et al. (2012). The amount of 0.5 mL hemolymph of five shrimps in each chamber was collected from the pleopod base on the abdominal segment near the genital opening, using a 1 mL syringe preloaded with anticoagulant solution (10% sodium citrate, pH 7.2). Then, the mixture of anticoagulant-hemolymph was gently mixed, and a single drop of the mixture was placed on and calculated using a haemocytometer (Neubauer chamber) and under a light microscope at 100 – 400x magnification. The calculation formula used is as follows:

$$\mathrm{THC} = \mathrm{ACC} \times \frac{(1)}{(\mathrm{HV})} \times \mathrm{DF}$$

THC = ACC×((1))/((HV))×DF

THC = Total haemocyte count

ACC = Average cell count

HV = Haemocytometer volume

DF = Diluent factor

Biomass growth

The parameter of the shrimp growth observed in this research was biomass growth. The measurement of biomass growth is based on the formula proposed by Lugert et al. (2014):

$$\Delta W = Wt - Wi$$

ΔW = Absolute biomass growth (g)

Wt = Final biomass growth (g)

Wi = Initial biomass growth (g)

Specific Growth Rate (SGR)

The specific growth rate is measured using the following formula (Jarmolowicz et al., 2012):

$$SGR = \frac{\text{Ln Wt} - \text{Ln W0}}{t} \times 100$$

SGR = Specific Growth Rate (% day^{-1})

Wt = Final biomass growth (g)

W0 = Initial biomass (g)

T = Rearing duration

Feed Conversion Ratio (FCR)

FCR (Feed Conversion Ratio) is calculated based on a formula proposed by Tacon (1987):

$$FCR = \frac{F}{Wt - W0}$$

FCR = Feed Conversion Ratio

F = Amount of feed consumed (g)

Wt = Final biomass growth (g)

W0 = Initial biomass growth (g)

Survival Rate (SR)

The survival rate is measured based on the formula proposed by Antunes et al. (2018):

$$SR = \frac{\text{Final population} \times 100}{\text{Initial population}}$$

SR = Survival rate (%)

Water quality parameters

The parameters of water quality observed in this research are temperature, pH, DO, and salinity. Water quality was monitored three times a week to obtain accurate results. Water quality management was carried out by changing 20-30% of the water every three days and ensuring good aeration to supply oxygen at all times.

An extract from papaya seed flour was obtained using the procedure recommended by Manila et al. (2009). In short, the papaya seeds were crushed into small bits and left to air-dry for 5 – 7 days. The raw preparation commenced with measuring 200 g of dried papaya seed flour, macerated thrice for 24 hours, each in 200 ml of 70% ethanol solution. The mixture was subsequently agitated for 1 hour at 45 °C employing a hot plate equipped with a stirrer. The extracts were then filtered with filter paper and evaporated under reduced pressure using a rotary evaporator for 2 hours at 40 °C.

After ensuring that the data were normally distributed and homogenous, an analysis of variance (ANOVA) was carried out using SPSS. If the addition of papaya seed flour showed a significant difference compared to the control ($P < 0.05$), a Tukey test was conducted. All data were processed using SPSS software. Meanwhile, the water quality data were analyzed descriptively.

RESULTS

The results of the phytochemical tests showed the presence of bioactive compounds of flavonoids, alkaloids, tannins, saponins, and steroids with a positive sign (Table 1).

Table 1. Phytochemical Test Result of Papaya Seed Flour

Phytochemical Constituent	Result
Flavonoid	Positive (+)
Alkaloid	Positive (+)
Tanin	Positive (+)
Saponin	Positive (+)

Normality and homogeneity tests for all parameters showed that the data were normally distributed and homogenous with p value > 0.05. The results of the analysis of variance of PA, THC, biomass, SGR and FCR obtained the same result (F count > F table). These results indicate a significant difference between groups in all research parameters ($P < 0.05$). The results of the Tukey test are indicated by the superscript shown in the figures. Moreover, the highest shrimp biomass and specific growth rate (SGR) were observed in treatment D, while the lowest FCR was also recorded in treatment D (Table 2).

There is an increase in phagocytic activity with a higher dose of papaya seeds. The highest value was recorded in treatment D (5g.kg^{-1}) at 65.67%, followed by a decrease in treatment E (Fig. 1).

There is a decrease in THC value in treatment E as the dose increases (Fig. 2).

The observation values for water quality are presented in Table 3.

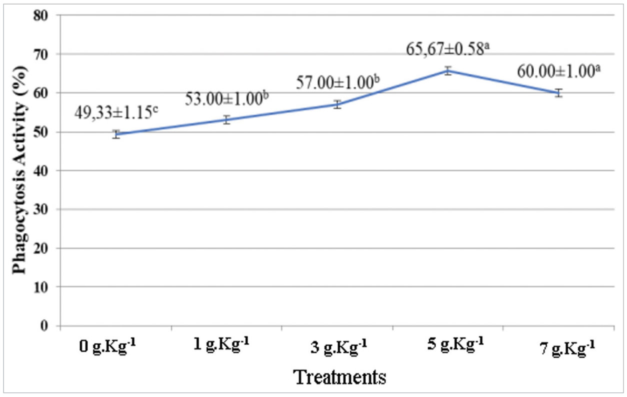
Table 2. The result of growth performance and survival rate of whiteleg shrimp

	Parameter			
	Biomass (g)	**SGR (%.day^{-1})**	**FCR**	**SR (%)**
0 g.kg^{-1} of feeds	4.36 ± 0.04^{b}	4.90 ± 0.02^{c}	1.37 ± 0.02^{c}	100 ± 0.00^{a}
1 g.kg^{-1} of feeds	4.46 ± 0.25^{b}	4.98 ± 0.13^{bc}	1.34 ± 0.02^{c}	100 ± 0.00^{a}
3 g.kg^{-1} of feeds	6.01 ± 0.39^{a}	5.97 ± 0.03^{a}	1.21 ± 0.02^{ab}	100 ± 0.00^{a}
5 g.kg^{-1} of feeds	6.23 ± 0.35^{a}	6.09 ± 0.14^{a}	1.18 ± 0.01^{a}	100 ± 0.00^{a}
7 g.kg^{-1} of feeds	4.91 ± 0.52^{b}	5.30 ± 0.20^{b}	1.24 ± 0.01^{b}	100 ± 0.00^{a}

* Values with different superscript letters between treatments indicate a significant difference (*P* < 0.05)

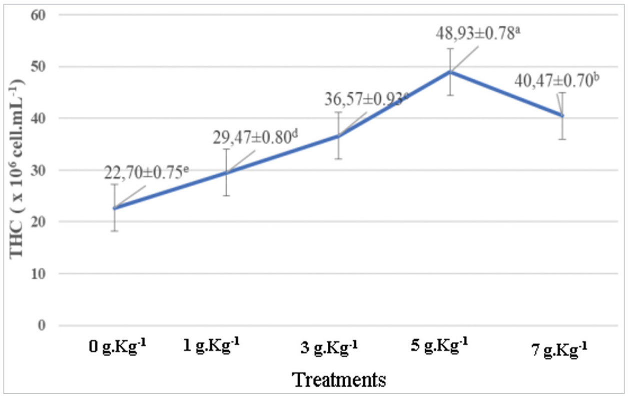
Table 3. Water Quality Parameters During Study

Parameter	Result of Observation	Ideal Value	Reference
Temperature (°C)	29 – 30	28 – 32	Durai et al. (2021)
Acidity (pH)	7.8 – 8.0	7.0 – 8.3	Durai et al. (2021)
DO (ppm)	5 - 6	5.5 - 7.5	Harlina et al. (2022)
Salinity (ppt)	20 - 21	15 - 25	Huang et al. (2019)



* Values with different superscript letters between treatments indicate a significant difference (*P* < 0.05)

Fig 1. Phagocytic activity during the study



* Values with different superscript letters between treatments indicate a significant difference (*P* < 0.05)

Fig 2. THC (Total Haemocyte Count) Value of Shrimp

DISCUSSION

Phagocytic cells, which actively engulf and destroy pathogens, can prevent the transmission of infection and reduce tissue damage (Rosales and Uribe-Querol, 2017). According to Liu et al. (2020), phagocytosis is an ancestral and highly conserved process in all multicellular organisms that allows the host to protect itself against invading microorganisms and harmful agents from the environment. Likewise, alkaloids have the potential to act as immunomodulators (Azwari et al., 2021), and to increase phagocytosis in microglial cells (Xia et al., 2024). The presence of active compounds contributed to the increased phagocytic activity. This finding is in line with Ginwala et al. (2019) who reported that the addition of flavonoids could increase PA by up to 40%, especially in immune cells such as macrophages. It was reported that the addition of aloe vera powders containing flavonoid compounds showed positive results by increasing PA of whiteleg shrimp by up to 68% (Linayati et al., 2022).

As shown in Figure 2, THC values differed significantly between treatments over 30 days. Treatment A had the lowest value at 22.7 x 10^6 cell.mL^{-1}, which increased to 48.93 x 10^6 cell.mL^{-1} in treatment D, before decreasing in treatment E. The increase in THC value suggests that the haemocyte cell is stronger in its effort to defend the cell from a strange or harmful substance. Based on the research by Prayitno et al. (2020), the increasing THC value has a close correlation with the ingestion of foreign particles into the body. According to Johansson et al. (2000), the haemocyte cell has an important role in the defence mechanism of shrimp, with the function to destroy the foreign substance particle, including the recognition stage, phagocytosis, melanisation, cytotoxicity, and cell communication. Active substances in the papaya seed, namely flavonoids and alkaloids, are able to stimulate the formation of haemocyte cells, which in the end could increase the shrimp's immune system. In line with Braak (2002), the increase in total haemocytes indicates an improvement in the cellular immunity of the shrimp due to the haemocyte cells being part of the defence mechanism of the shrimp. Flavonoids also stimulate macrophage activation, which results in increased phagocytosis as an important immune response (Middleton et al., 2000). The latest development was reported by Chen et al. (2023) who found that an increased immune response, as indicated by the total haemocyte count, correlated positively with the regulation of digestive enzymes in the intestinal tract. This provides new information about the relationship between immune response and shrimp growth. In line with Novriadi et al. (2021) who explained that dietary nucleotide supplementation in feed for Pacific whiteleg shrimp *Litopenaeus vannamei* showed positive results in immune responses, especially total haemocyte count and survival rate, after being tested against *Vibrio harveyi* as pathogen.

The decrease in the THC value in treatment E was caused by high bioactive compounds in the feed. The high dose of papaya seed flour was in line with the increase in bioactive compound content, including flavonoid. According to Christine and Martyn (2000), if there is an adverse impact on the high flavonoid dose, then the flavonoid acts as a mutagen, a pro-oxidant producing the free radical, and as an enzyme inhibitor involved in the hormonal metabolism process in the body. The unbalanced metabolism decreased the condition of shrimp, resulting in a smaller number of haemocyte cells. Another bioactive compound, namely alkaloid, also has the potential to become a toxicant for brine shrimp *Artemia salina* if it exists in high concentrations (Coe et al., 2010). Generally, the common THC value in the study is still within the normal range. According to Chang et al. (1999), the THC value in shrimp is around 20 x 10^6 to 40 x 10^6 cells.mL^{-1} or with a minimum limit of 16.4 x 10^6 cell.mL^{-1} (Song et al., 2003). Previous research with the method of immersion of whiteleg shrimp in papaya leaf extract at a concentration of 30 mg.L^{-1} gave positive results of an increase in phagocytic activity and total haemocytes, in line with the improvement of the non-specific immune response of shrimp (Monica et al., 2017).

Treatment D with the addition of 5g.kg^{-1} feed of papaya seed flour resulted in the highest growth average of 6.23 g. The addition of papaya seed flour in this treatment is believed to increase the appetite of whiteleg shrimp and add more nutrients to the feed. Papaya seed flour contains several active compounds: alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, polyphenols, sugar, proteins, and fat (Madinah et al., 2015), which can act as antioxidants, protecting cells from pathogens and supporting the formation of new cells. The formation of new cells supported the growth. Arisa et al. (2021) also explained the results on the use of papain enzyme for shrimp growth, reporting that adding 1% papain and 1% bromelain enzymes to feed could increase the growth of whiteleg shrimp.

Previous studies have shown that adding 1% papain powder, 1.25% raw papaya fruit, and 2.5% papaya leaves can result in significantly greater growth performance of carp than the control (Tewari et al., 2018). Papaya seeds maintain the optimal function of the intestines in the process of absorption and digestion, which then improves the growth value. According to Mahmoud et al. (2023), papaya seed extract could enhance growth and health through the modulation of innate immunity and have a positive effect against disease in fish. Furthermore, the presence of the enzyme papain plays an important role in decomposing complex proteins into simpler forms so that they are easily absorbed. Rachmawati et al. (2020) mentioned there was an increase in the growth of Sangkuriang catfish *Clarias gariepinus* which were given papaya in their feed because it improved the digestive enzyme activity. Adding papain supplements to feed has the effect of increasing enzymatic antioxidant protection and increasing pepsin and lipase activity significantly, so that they greatly affect growth, especially in feed utilization and feed digestibility (Wiszniewski et al., 2022). Kang et al. (2010) argued that shrimp fed with the addition of papaya seeds were able to grow up to two-fold when cultured in an open space area. Research on papaya plants has also shown positive results in the use of papaya sap for shrimp culture. Malahayati et al. (2022) explained that papaya sap extract at concentrations of up to 2% in feed was able to increase whiteleg shrimp growth by 23.34 g.

Linayati et al. (2021) stated a similar view regarding the use of plants for fish, as they found that herbal plants, such as *Curcuma zanthorrhiza* at a dose of 15 g.kg^{-1} of feed, resulted in an average growth yield of 8.04 g in Nile tilapia. Curcumin, flavonoids, essential oils, proteins, carbohydrates, fats, and even essential minerals in the plant contributed to improved growth yield. Herbal ingredients can also prevent fish from contracting diseases, reduce stress, and prevent infections. Herbal ingredients also act as immunostimulants that can increase both specific immune responses and non-specific immune responses in fish (Pandey et al., 2012). These effects appear stronger when the dosage is optimal (Kamble et al., 2014).

In treatment E, the addition of a 7 $g.kg^{-1}$ dose of papaya seed flour resulted in less optimal growth due to excessive flavonoid and alkaloid contents. The addition of high concentrations of phytochemicals in plants may serve as a vibriocidal agent but can also have lethal properties that are not beneficial for shrimp growth (Kenconojati et al., 2023). Flavonoids and alkaloids are antibacterial agents that help prevent bacterial infection and protect cells from diseases; however, in excessive amounts, these compounds can damage the cells, thereby negatively affecting the growth. Mukti et al. (2012) explained that at certain levels, alkaloid and flavonoid compounds become toxic and can lead to the mortality of the organism. Furthermore, Ishaku et al. (2019) stated that the active substances of papaya, including carpine and papain, are toxic and can lower the pulse and nervous system. In line with Hajra et al. (2013), the negative effects due to high doses of phytochemical substances may cause decreased health and adverse physiological effects.

The highest SGR of 6.09%.d^{-1} was found in the use of a dose of 5 $g.kg^{-1}$, followed by treatment C with 5.97%.d^{-1}, treatment E with 5.30%.d^{-1}, treatment B with 4.98%.d^{-1}, and the lowest in treatment A (control) with 4.90%.d^{-1}. The content of papain enzymes in papaya seed powder given to post-larval shrimp can increase protease activity and growth performance (Manush et al., 2013). Furthermore, the presence of *G. elegans* alkaloids increases the lipid content, crude protein, and amino acids in the whole muscle (Ye et al., 2019), thereby increasing growth performance. The best FCR was found in treatment D (5 $g.kg^{-1}$ feed) of 1.18. This low feed conversion is due to the role of essential oils that acted as an anti-stress agent for shrimp. Essential oils have sedative functions (Chakraborti et al., 2013). The sedative effect can calm the fish from stress so that the metabolic process can run well. In addition, this effect also influences the physiological process of remaining good, increasing growth in general and reducing mortality. Moreover, Souza et al. (2016) stated that stress had been suspected to cause mortality, affecting fish production with the consequence of economic loss. A study by Tewari et al. (2018) on carp treated with papain powder, unripe papaya fruit, and papaya leaves showed that the fish with the best growth performance were very responsive to the feed, developed normal skin color, and were able to swim very fast. Zhang et al. (2020) also explained that essential oils can increase secretion and enzymatic activity, such as amylase, protease, and lipase.

The best treatment was treatment D, with a feed utilization efficiency of 84.49%, followed by treatment C at 82.05%, treatment E at 80.17%, treatment B at 74.93%, and finally treatment A at 72.93%. In this study, the results of feed utilization efficiency were higher than those reported by Larasati et al. (2021), yielding a value of 70.56% - 74.54%. Flavonoids may influence the composition of the gut microbiota by acting as carbon substrates that promote the growth of beneficial bacteria (Wang et al., 2023). Essential oils also play a crucial role by suppressing harmful bacteria and allowing microflora to dominate the intestine, which improves the digestive process (Thapa et al., 2012). Additionally, Patil and Singh (2014) reported that freshwater giant prawns fed with feed supplemented with 1% papain showed the best growth and feed consumption efficiency.

The average survival rate (SR) is 100%, in which the addition of papaya seed flour did not affect the survival value of whiteleg shrimp. Similar findings were proposed by Farrag et al. (2013) who reported that tilapia treated with papaya seed meal for 60 days had a slightly different SR. The high SR in shrimp could be due to the well-maintained water quality and the selection of quality feed. According to Carbajal-Hernandez et al. (2012), water management in shrimp farming is important for the proper growth of organisms, especially to reach standards for good quality and quantity of the final product.

The results of temperature observations in the whiteleg shrimp rearing medium for 30 days ranged from 29 to 30 °C. Durai et al. (2021) recommended an ideal temperature range between 22 and 32 °C. Moreover, monitoring of DO shows that it was still in the good range for shrimp, around 4-5 ppm. According to Re and Díaz (2011), who studied juvenile whiteleg shrimp, the optimum growth is obtained at a DO level of 5.5 ppm. Similarly, Harlina et al. (2022) reported that the dissolved oxygen value for shrimp *Litopenaus vannamei* was in the range of 5.5-7.5 ppm. Therefore, water salinity during maintenance is regarded as ideal for the growth of whiteleg shrimp. The pH value in the rearing media ranged from 7.8 to 8.0 (ideal). Durai et al. (2021) reported that the optimal pH for whiteleg shrimp growth ranged from 7.0 to 8.3, and that low pH could reduce the growth rate of the shrimp. The salinity of the water in the rearing media in this research ranged from 20 to 21 ppt. This salinity level falls within the optimal range and still supports the growth of whiteleg shrimp. Huang et al. (2019) also suggested that the ideal salinity for the growth of whiteleg shrimp was 3–30 ppt. The papain enzyme in papaya seed flour does not negatively affect the physical and chemical properties of the rearing media, thereby preventing the fish from getting stressed out (Tewari et al., 2018). Moreover, the environment has a major impact on the condition of organisms. In line with Linayati et al. (2024), stable conditions could influence the clinical appearance of the disease.

CONCLUSION

The addition of papaya seed flour to the feed had a statistically significant improvement in phagocytic activity, total haemocyte cells, and the growth of whiteleg shrimp. The optimal dose was found in treatment D (5 $g.kg^{-1}$ feed), which resulted in an average of PA of 65.67%, THC of 48.93 x 10^6 $cells.mL^{-1}$, FCR of 1.18, ADG of 6.09 %.d^{-1}, an average weight of 6.23 g and a 100% survival rate. In summary, utilizing immunostimulants derived from papaya seed to address shrimp immunity demonstrates encouraging possibilities. Although the results of this study show positive effects, further research is still needed on a larger scale, especially at a shrimp farm level, so that clear information on production costs can be obtained.

UTJECAJ BRAŠNA SJEMENKI PAPAJE *Carica papaya* L. KAO DODATAK HRANI NA IMUNOLOŠKI ODGOVOR I RAST BIJELIH KOZICA *Litopenaeus vannamei*

SAŽETAK

Glavni problemi u uzgoju bijelih kozica su bolesti i visoke cijene hrane. Kako bi se prevladali ovi problemi, potrebna je sigurna i jeftina tvar koja može poboljšati zdravlje i rast. Prirodni sastojci iz biljaka poput papaje *Carica papaya* L. široko su dostupni u Pekalonganu. Cilj ove studije bio je utvrditi učinak brašna sjemenki papaje kao dodatka umjetnoj hrani na imunološki odgovor i rast bijelih kozica. Ovo eksperimentalno istraživanje koristilo je potpuno randomizirani dizajn (CRD) koji se sastojao od pet tretmana i tri ponavljanja. Pokusne životinje bile su bijele kozice (PL21) nakon ličinki, dok je testna hrana nadopunjena brašnom sjemenki papaje u različitim dozama za svaki tretman: 0 g.kg^{-1} hrane (A); 1 g.kg^{-1} hrane (B); 3 g.kg^{-1} hrane (C); 5 g.kg^{-1} hrane (D); i 7 g.kg^{-1} hrane (E). Promatrani parametri uključivali su PA (fagocitnu aktivnost), THC (ukupni broj hemocita), apsolutnu biomasu, SGR (specifičnu stopu rasta) i FCR (omjer konverzije hrane). Tretman D identificiran je kao optimalna doza, što je rezultiralo PA od 65,67%, THC-om od 48,93 x 10^6 stanica·mL^{-1}, apsolutnom biomasom od 6,23 g, SGR-om od 6,09% d^{-1} i FCR-om od 1,18, sa stopom preživljavanja od 100%. Analiza podataka potvrdila je da tretmani imaju značajnu razliku (P < 0,05) na svim parametrima. Rezultati su pokazali da korištenje sjemenki papaje može poboljšati i zdravlje i rast bijelih kozica.

Ključne riječi: rast, sjeme papaje, fagocit, ukupne hemocitne stanice

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