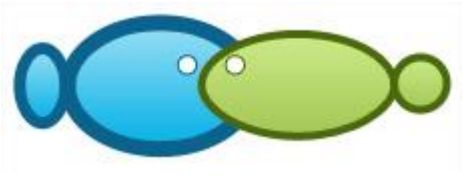

The effect of *Aloe vera* powder on phagocytosis activity and growth of *Litopenaeus vannamei*

Linayati Linayati, Muhammad Z. Yahya, Tri Y. Mardiana, Hayati Soeprapto

Department of Aquaculture, Faculty of Fisheries, Pekalongan University, Pekalongan, Central Java, Indonesia. Corresponding Author: L. Linayati, pattyana95ina@yahoo.co.id

Abstract. Some common issues in shrimp culture include slow growth and diseases outbreaks. *Aloe vera* contains polysaccharides, minerals, vitamins, amino acids, and proteases that can improve the immune and growth of shrimp. The shrimp immune system is reflected in phagocytic activity. This study analyzed the effect of the addition of *A. vera* powder to shrimp feed on the phagocytic activity and absolute biomass gain of white leg shrimp *Litopenaeus vannamei*. This experimental study was performed using a completely randomized design (CRD) consisting of 4 treatments with 3 replications. The test animals were *L. vannamei* (PL21), while the feed was supplemented with *A. vera* powder with different doses: 0 g kg^{-1} of feed (A), 20 g kg^{-1} feed (B), 40 g kg^{-1} feed (C), and 60 g kg^{-1} of feed (D). Parameters being observed included the phagocytic activity, absolute biomass gain, and survival rate (SR). This study was performed in 40 days. The results showed that treatment D produced the highest phagocytic activity and absolute biomass gain compared to other treatments. The highest phagocytic activity reached 68% and the absolute average biomass gain was 11.73 g. The survival rate of *L. vannamei* in all treatments was 100%.

Key Words: active ingredients, biomass, immune response, phagocytosis, white leg shrimp.

Introduction. White leg shrimp (*Litopenaeus vannamei*) is a very important commodity with high economic value in the global market. By March 2021, the average global *L. vannamei* export reached almost 2000 million USD (FAO 2021). However, constraints in the shrimp culture, including slow growth and disease outbreak often cause high shrimp mortality. The immune system of the shrimp relies on the innate or non-specific immune system, which generally sources from the hemocyte cells (Johansson et al 2000). Hemocytes play roles in phagocytic activity, which is an important process for eliminating harmful microorganisms or foreign particles, as well as in the melanization and encapsulation, which are important in *Penaeid* shrimp immune system activities (Aguirre-Guzman et al 2009). Increased phagocytic activity indicates an activation in the immune system of the shrimp. Setyawan et al (2021) stated that an increase in phagocytosis up to 98% after adding sodium alginate from *Sargassum* as a supplement indicates an increase in non-specific immune response.

Additional natural ingredients, such as *Aloe vera* containing active substances, can be used as preventive measures that can support the immune system, growth, and survival rate of white leg shrimp. Growth in shrimp must be supported by quality feed (Zaenuddin et al 2018).

A. vera has a variety of active ingredients and it is effective as an immunostimulant (Abdy et al 2017). Among the active ingredients, acemannan (polysaccharide) presents some antimicrobial properties (Alishahi & Abdy 2013). *A. vera* also contains some other active compounds, which support growth and increase appetite (Prasetio et al 2018).

A previous study on the utilization of *A. vera* in aquatic organisms was carried out by Novita et al (2020) on white snapper (*Lates calcarifer*). The study proves that the addition of *A. vera* resulted in a survival rate of up to 100%. Avenue of further research includes the effect of *A. vera* powder on the immune system and growth of *L. vannamei*. This research aims to determine the effect of adding *A. vera* powder on the phagocytic activity and growth of *L. vannamei.*

Material and Method. This research was carried out from December 1st, 2020, to January 20th, 2021, at the Marine and Brackish Water Laboratory, Faculty of Fisheries, Pekalongan University, Indonesia. The tools used include aquarium, digital scales, DO meter, pH meter, refractometer, thermometer, fishnet, siphon, aeration equipment, plastic, label paper, stationery, and a cellphone camera. This study used *L. vannamei* (PL21), water, *A. vera* powder, and a standard feed.

The study used a completely randomized design (CRD) with 4 treatments and 3 replications. The treatments are as follows: A - feed without the addition of *A. vera* powder; B - 20 g *A. vera* powder kg^{-1} feed; C - 40 g *A. vera* powder kg^{-1} feed; D - 60 g *A. vera* powder kg^{-1} feed. The doses refer to Prasetio et al (2015), who determined that the best dose of *A. vera* powder was 40 g kg^{-1} feed in their experiment on fish. The acclimatization process for *L. vannamei* was done 1 week before treatments. This study used 12 aquariums with measurements of 40x25x25 cm. Each aquarium was filled with 10 L of water and 10 shrimps, with a density of 1 shrimp L^{-1}. Observations on shrimps were carried out for 40 days and 20% of water was exchanged every week. Feed was administered to needs, three times a day. During the study, the white leg shrimp condition has been monitored along with water quality measurements.

Obtaining A. vera powder. Obtaining *A. vera* powder was started by cutting *A. vera* into slices. They were washed with clean water and dried for 1-3 days in the open air (Prasetio et al 2018). After the *A. vera* was dried, it was then blended and sieved with a 100-200 mesh sieve for a fine powder (Sari et al 2012; Wahjuningrum et al 2012).

Mixing A. vera powder into feed. The mixing of *A. vera* powder with feed followed the required doses according to treatments. They were mixed with egg white, 2% of the weight of the feed (Prasetio et al 2018). Hence, they were stirred until homogeneous. Feed was administered three times a day, at 8 a.m., 1 p.m., and 5 p.m. (Akbary & Aminikhoei 2018). The artificial feed used has met feed standards for shrimp, especially for protein content (minimum 36%).

Phagocytic activity. 1 mL of hemolymph of *L. vannamei* was placed on a glass and smear preparations were made. The preparations were fixed with 100% methanol for 5 min and stained with Giemsa (10%) for 15 min. Running water was added slowly for about 5 min to remove the remaining Giemsa coloration. Observations were made under a light microscope with a magnification of 400x. Phagocytic activity (PA) was measured based on the percentage of cells showing phagocytosis (Cheng et al 2005).

PA = Active phagocytic cells/Phagocytic cells x 100

Absolute growth. The weight gain of *L. vannamei* was calculated using the formula of Effendi (1997):

W = Wt – Wo

Where: W - absolute weight growth (g); Wt – final average weight (g); Wo – initial average weight (g).

Survival rate (SR). The survival rate calculation of *L. vannamei* was calculated by the formula of Effendi (1997) as follows:

SR = Nt/No x 100

Where: SR - survival rate (%); Nt - number of live fish at the end of the study; No - number of fish at the beginning of the study.

Water quality observations. Parameters observed were dissolved oxygen (DO), salinity, temperature, and pH. All parameters were determined using appropriate tools three times a week.

Data analysis. Hypothesis testing used the analysis of variance (ANOVA). The data were first tested for normality using the Lilliefors test (Nasoetion & Barizi 1983) and for homogeneity using Bartlett's test (Sudjana 1996). Then, ANOVA was carried out. If the results were significant, then the Tukey test was carried out to determine the difference between treatments. Phagocytic activity and water quality were analyzed descriptively.

Results and Discussion

Phagocytosis activity. Data obtained shows that there is an increase in phagocytic activity from treatment A (27%) to D (68%) (Figure 1).

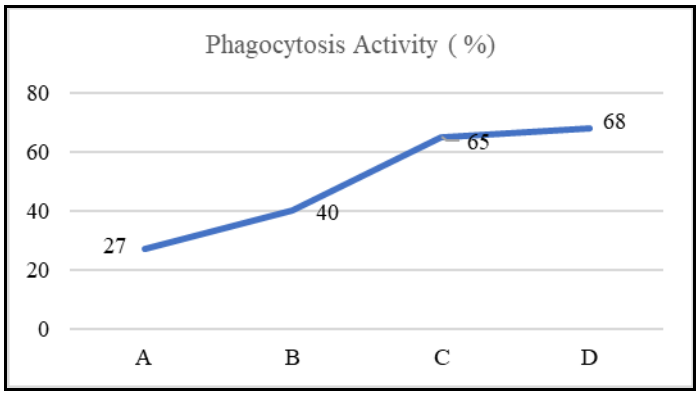

Figure 1. Phagocytosis activity.

Phagocytosis plays an important role in responding to infections in the body. According to Liu et al (2020), phagocytosis is an ancient, highly conserved process in all multicellular organisms, through which the host can protect itself by countering invading microorganisms and harmful particles from the environment. The phagocytic activity starts with the identification of foreign particles that enter the body by receptor cells (Xu et al 2014). The increase in phagocytic activity in this study occurred simultaneously with the increase in the dose of *A. vera* powder.

In treatment A (control without *A. vera*), the phagocytosis rate was 27%. The highest rate was obtained in treatment D (68%). The findings prove the merits of the active ingredients in *A. vera* that stimulate immune response increasing the phagocytic activity value. The active ingredients are polysaccharides that act as immunomodulators or substances that activate innate and adaptive immune systems. Several types of polysaccharides in *A. vera* are glucomannan, mannans (acetylated), and pectin (Pugh et al 2001). Glucomannan and mannan in *A. vera* can boost the immune system, activate macrophages, accelerate wound healing, and act as an antiviral and antibacterial agents (Dotta et al 2014). Aranda-Cuevas et al (2020) stated that the substances in *A. vera* can heal wounds in shrimp, while Trejo-Flores et al (2016) mentioned that *A. vera* can have potential as an anti-viral and anti-bacterial agent in shrimp culture and increase the survival rate. During the phagocytosis process carried out by hemocytes, SOD (superoxide dismutase), as well as Hsp70 (heat shock protein), are produced as a form defense mechanism for shrimp, against stress conditions or pathogen infection. Moreover, Trejo-Flores et al (2018) stated that *A. vera* has a potential effect to increase the immune response of shrimp, along with the expression of SOD, Hsp70, and panaeidin4. The administration of immunostimulators from seaweed (*Gracilaria verrucosa*) also increase phagocytosis as an indicator of an increased immune response of *L. vannamei* (Yudiana et al 2018).

Growth. The average absolute biomass gain of *L. vannamei* from the beginning to the end of the study (40 days of rearing) is presented in Table 1.

Table 1

Absolute growth (g) of *L. vannamei* fed with *A. vera* supplemented diets

Number	*Treatments*				*Total*
	A	*B*	*C*	*D*	
1	6.91	7.9	9.24	12.22	
2	6.55	7.96	10.14	11.04	
3	6.82	7.96	10.34	11.94	
Total	20.28	23.82	29.72	35.2	109.02
Average	6.76	7.94	9.91	11.73	

The highest average of absolute biomass gain of *L. vannamei* was obtained in treatment D (11.73 g), with the addition of 60 g kg^{-1} feed *A. vera* powder. The lowest average of absolute biomass gain of *L. vannamei* was obtained in treatment A (6.76 g), without the addition of *A. vera* powder. The normality test results using the Lilliefors test showed that the data were normally distributed. The homogeneity test also showed that the data variance was homogeneous. Hence, an ANOVA test was conducted. The results of the ANOVA test of the absolute biomass showed that the F-count (75.84) was greater than the F-table (4.07 and 7.59). Thus, there were very significant differences among the treatments regarding the absolute biomass gain of *L. vannamei*.

Growth in crustaceans is a change in length and gain that occurs periodically after molting (Pratiwi et al 2016). Administration of *A. vera* powder with the highest dose (60 g kg^{-1} feed) in treatment D gave the highest increase in shrimp biomass gain with an average gain of 11.73 g. According to Prasetio et al (2018), amino acids function for cell growth and repair as well as an energy source. Moreover, calcium supports the growth of *L. vannamei.* Restari et al (2019) further assert that calcium is needed in gastrulation. It helps the molting process of shrimp as part of the growth phase.

Other substances such as vitamins also function as growth supporters and appetite stimulants. According to Amalia et al (2013), vitamins act as catalysts in metabolism. Vitamin C also plays a role as a catalyst that functions to accelerate reactions in the body (Kursistiyanto et al 2013). Thiamin (B1) functions to stimulate appetite and the use of carbohydrates as well as plays a vital role in the nervous system (Nuringtyas 2016). Vitamin B6 plays an important role in supporting energy formation (Ginting & Sembiring 2019). *A. vera* also contains protease, an enzyme that breaks down protein into amino acids. Referring to Susanto et al (2017), protease breaks down feed proteins with advantageous direct effects for growth.

The increase in growth from the treatments was due to the addition of *A. vera* powder that affected the increase in the nutrients absorbed by the shrimp. According to Widanarni et al (2012), a greater amount of nutrients digested by the fish means a greater amount of nutrients utilized for growth. In the absorption of nutrients, the polysaccharides in *A. vera* act as prebiotics that help maintain homeostasis of the intestinal microbiome and host health (Tremaroli & Backhed 2012) by influencing the pathogenic intestinal microflora (Citarasu 2010; Yu et al 2018). Polysaccharides also stimulate amylase, trypsin, and lipase activities (Gabriel et al 2017), thereby increasing feed digestibility and availability of nutrients and accelerating feed absorption (Platel & Srinivasan 2004). The improvement of growth performance in aquatic organisms fed with *A. vera* as a dietary supplement has also been demonstrated in previous studies. Gabriel et al (2015) stated that *A. vera* powder incorporated in fish fed at 1%, 2% kg^{-1} feed significantly enhanced weight gain and blood cell profile of tilapia (GIFT) such as red blood cells, hematocrit (Hb), hemoglobin (Hb), and white blood cells count (WBC). Thus, the active ingredients contained in *A. vera* have a positive effect on fish and shrimp culture.

Survival rate. The observation results of the survival rate of the *L. vannamei* are presented in Table 2.

Table 2

Survival rate of *L. vannamei* fed with *A. vera* supplemented diets

Number	*Treatments*				*Total*
	A	*B*	*C*	*D*	
1	10	10	10	10	
2	10	10	10	10	
3	10	10	10	10	
Total	30	30	30	30	120
Percentage	100%	100%	100%	100%	

According to Widigdo (2013), the survival rate is categorized as good if the SR value is higher than 70%, the SR is in the moderate category when it is between 50-60%, and in the low category, if it is lower than 50%. In another study, it was stated that the SR of shrimp reached 94,64 %, based on the polyculture system (Susilowati et al 2014). SR was 100% in all treatments 100% (Table 2). The addition of *A. vera* powder did not affect the SR of shrimp.

The high SR of shrimp could be due to the water quality being well maintained and the quality of feed (Samocha et al 2020). Quality feed is required for good shrimp culture, not only for growth and survival, but also to counter the negative impacts of the shrimp culture (Xu et al 2018). There is a possibility of stress due to crowding and poor water quality at high stocking densities. Quality feed will be absorbed well by shrimps, and the feed waste would be lower. According to Afrianto et al (2015), a good habitat will help fish be more resilient, whereas a lacking habitat will stress the fish. An ecological condition such as water quality is essential for the growth of shrimp. Carbajal-Hernadez et al (2013) stated that water quality parameters are the main factor affecting shrimps. Stress can have additive effects on the growth rate, survival rate, metabolism, and fecundity of aquatic organisms (Crain et al 2008).

Water quality. Water quality in the form of DO, salinity, temperature, and pH were assessed. The results of the measurements of water quality parameters for each treatment during the study are presented in Table 3.

Table 3

Water quality parameters

Parameter	*Treatments*				*Optimal range reference*
	A	*B*	*C*	*D*	
DO (mg L^{-1})	5.9-6.3	6.1-6.3	6-6.5	5.7-6.2	>4 (Venkateswarlu et al 2019)
Salinity (ppt)	30	30	30	30	3-30 (Huang et al 2019)
Temperature (^{0}C)	30-31	30-31	30-31	30-31	22-35 (Babu et al 2014)
pH	7.5-7.8	7.5-7.7	7.6-7.9	7.5-7.9	7.2-8.5 (Karuppasamy et al 2013)

Note: DO - dissolved oxygen.

Water quality is essential in the success of cultivation. In this study, the water quality was maintained to minimize the fish mortality rate. According to Carbajal-Hernandez et al (2013), during cultivation, the overall water quality is important for the comfort of the farmed animal life. Shrimps are animals that have their entire life cycle in the water. Hence, it requires water of good quality. DO during the study ranged from 5.6 to 6 mg L^{-1}. The ranges are following Venkateswarlu et al (2019), who stated that the optimal DO in shrimp rearing should be higher than 4 mg L^{-1}. Thus, it can be concluded that the DO

level during rearing supports shrimp survival. This is confirmed by Junior et al (2020), who stated that the DO content in water for shrimp should be in the range of 5.6 to 7.2 mg L^{-1}. This is in line with Fernandes et al (2019), who noted that the best DO levels for *L. vannamei* ranged from 4.4 to 6.8 mg L^{-1}. Salinity is related to shrimp osmoregulation. Whenever the salinity of the water is near the isosmotic level of the organism, less energy is needed to regulate osmotic pressure for more optimal growth (Huang et al 2018). During the study, the salinity of the water was 30 ppt. Huang et al (2019) stated that salinity in the maintenance of *L. vannamei* can be in the range of 3-30 ppt. Temperature is a factor that affects the appetite, the feed effect, the metabolism, and the growth of shrimp (Abdelrahman et al 2019). During the study, the temperature measurements ranged from 30 to 31°C. According to Babu et al (2014), a good temperature for *L. vannamei* cultivation in the tropics ranges from 22 to 35° C. The pH is a vital environmental parameter in physical processes (Venkateswarlu et al 2019). During the study, the pH ranged from 7.5 to 7.9, and was suitable for *L. vannamei* cultivation. Karuppasamy et al (2013) state that the optimal pH for shrimp growth ranges from 7.2 to 8.5.

Conclusions. The addition of *A. vera* powder to the feed affects the growth of *L. vannamei*. The best dose of *A. vera* powder for the growth of *L. vannamei* is 60 g kg^{-1} feed. *A. vera* supports shrimp growth and development and increases phagocytic activity.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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Authors:
Linayati Linayati, Department of Aquaculture, Faculty of Fisheries, Pekalongan University, 3 Sriwijaya St., 51117 West Pekalongan, Pekalongan, Indonesia, e-mail: pattyana95ina@yahoo.co.id
Muhammad Zulkham Yahya, Department of Aquaculture, Faculty of Fisheries, Pekalongan University, 3 Sriwijaya St., 51117 West Pekalongan, Pekalongan, Indonesia, e-mail: zulkhamy33@gmail.com
Tri Yusufi Mardiana, Department of Aquaculture, Faculty of Fisheries, Pekalongan University, 3 Sriwijaya St., 51117 West Pekalongan, Pekalongan, Indonesia, e-mail: yusufihanum@yahoo.co.id
Hayati Soeprapto, Department of Aquaculture, Faculty of Fisheries, Pekalongan University, 3 Sriwijaya St., 51117 West Pekalongan, Pekalongan, Indonesia, e-mail: hayatisoeprapto@gmail.com

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