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Potential Reduction of Stress Levels and Improving Vaname (*Litopenaeus vannamei*) Growth Performance with Probiotic Application

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ABSTRACT

The aim of this research was to find the most effective type of probiotic for the growth, survival, and stress resistance of *L. vanamei*. *L. vanamei* shrimp measuring PL 13 with a density of 2 fish/L of water were used as subjects for this research, which was conducted at the Poniang Beach Fish Seed Center from July 27 to September 24, 2023. This study was conducted using a completely randomized design (CRD) and consisted of four treatments and three repetitions, namely the operational standard, *Bacillus* sp., *Lactobacillus* sp., and a combination of *Bacillus* sp. and *Lactobacillus* sp. with a dose of 20 ml/kg mixed into feed containing 30% protein. The results of the study showed that the use of probiotics had no significant impact ($p > 0.05$) on the growth and survival of *L. vanamei*.

Keyword: Growth, Probiotics, Survival, Stress resistance, White shrimp (*L. vanamei*).

INTRODUCTION

The *L. vanamei* or white shrimp, originates in Latin American waters under the family Penaidae. *L. vanamei* is one of the most developed species of Indonesian shrimps (Nuntung *et al.,* 2018). *L. vanamei* is one of the most profitable fishing commodities (Herawati and Endar, 2014). Since the decline in the production of tiger shrimp (*P. monodon*) in Indonesia, *L. vanamei* have become an alternative to aquatic breeding animals. This species is highly suitable for cultivation, thanks to its ability to thrive in high stocking densities and adapt to low-temperature environments, with a relatively short maintenance-to-harvest period of just 100 to 110 days (Fuady *et al.,* 2013). *L. vanamei* is more feeding-saving, environmentally resistant, grows faster than other shrimp, is more disease-resistant, and has a fairly high survival rate (Anita *et al.,* 2017), including euryhaline organisms that can tolerate a wide range of salinity (5–30 ppt) (Jeffer *et al.,* 2022).

However, the survival rate of the *L. vannamei* is still low at 40.13% (Pratama *et al.,* 2017). Efforts are needed to boost survival and growth through a feed management approach, one of which is the use of probiotics, to make this shrimp-growing effort more profitable. Probiotics are living microbes that help the host by keeping intestinal microbes inside the animal's digestive tract. *Bacillus* sp. and *Lactobacillus* sp. are bacteria that grow both in the small intestine and can be used to increase the rate of growth and maintain the balance of the intestinal flora (Fadri *et al.,* 2016). Previous studies showed that probiotics can boost growth of *L. vannamei* with a dose of 15 ml/kg can increase the 3% average of weight, reaching 7,184 ± 0,86 g/h, while without the addition of a probiotic, resulting in a mean

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weight of 4,634 ± 0,323 g/h (Nadhif, 2016). Furthermore, in a study conducted by Anwar *et al.* (2016), the administration of probiotic *Lactobacillus* sp. and *Bacillus* sp. on feed resulted in the highest growth rate of 1.38%, whereas without the probiotic administration, the rate of growth was 1.16%. the survival rate of *L. vannamei* can be enhanced by adding *Bacillus* sp. and EM4 to its package (Agustama *et al.,* 2021).

A critical factor in shrimp acquisition is optimal body condition, which equates to minimal stress levels. Stress can be defined as the internal hormonal response of an organism triggered by environmental or external factors, aimed at enhancing the physiological condition of the organism. In stressful situations, the chances of a shrimp to survive are usually very small because the appetite is disrupted and can lead to a decrease in susceptibility to disease. Probiotic administration is suspected to help develop shrimp (El-Saadony *et al.,* 2022). Probiotic administration can stimulate the growth of cellular defense cells in *L. vanamei*. These cellular defenses are responsible for phagocytosis, nodulation, and encapsulation. An increased number of hermosite cells can help the shell body fight bacterial infections. (Suwoyono dan Mangampa, 2010). *Vibrio* sp. bacteria can cause stress, appetite, and gastrointestinal damage in shrimp or cause hepatopankreas disease that causes shrimp to die (Wijayanto *et al.,* 2020). Research is needed to investigate how probiotics enhance growth, synthesis, and stress resistance in *L. vanamei*. The goal of this research is to identify the most effective type of probiotic for supporting the growth, stress resilience, and resistance of *L. vannamei.*

MATERIALS AND METHODS

Time and Place

This study lasted for 60 days conducted from July 27 to September 24, 2023, at Poniang Beach Fish Seed Hall (BBIP) in Tallu Banua Village, Sendana Prefecture, Majene District, West Sulawesi.

Research Design

The study was designed using the Complete Random Design (CRD), which consists of four treatments with three repetitions for each treatment, so there are twelve experimental units in this study. One of the probiotic effects tested is as follows (Dian, 2021):

- Treatment A: Commercial feed (kontrol)
- Treatment B: B (*Bacillus* sp. 20 ml/kg)
- Treatment C: LB (*Labctobacillus* sp. 20 ml/kg)
- Treatment D: Com B+LB (combination between *Bacillus* sp. and *Labctobacillus* sp. 20 ml/kg, respectively).

Procedures

Animal Test

The research object was post-larva 13 (PL13) *L. vanamei* taken from the Mustika Benur Kupa hatchery in Barru district. Digital scales are used to measure the weight of shrimp. Before being used for research, the shrimp were acclimatized in a research container and fed for two days of cultivation.

Preparation of Containers and Water

Shrimp are reared using a 15 liter container. The containers used were 12 units. Then, sterilize the container with chlorine solutuion before putting water into the container. Sterilized containers are arranged according to the layout determined for the research. Each research container is equipped with an aerator. Seawater used as a maintenance medium is seawater that has been purified and taken from the Hatchery site. In order to optimize the water quality, before starting shrimp rearing, it's essential to ensure that the water container is equipped with an aeration system. This system helps maintain oxygen levels in the water, which is crucial for the health and growth of shrimp. Proper aeration prevents oxygen depletion, promotes circulation, and supports the biological processes within the container.

Nutrition and Probiotics Test

The feed used in this research is commercial feed with a protein value of 30%. The probiotics used in this study used *Bacillus* sp. and *Lactobacillus* sp. Feeding frequency of 4 times a day, at 07.00am; 12.00am; 05.00pm; and 09.00am WITA for 60 days of reared. During the rearing period of *L. vanamei*, waste food and feces are collected in each test container. The provision of probiotics in feed will be carried out using the following method. Firstly, to prepare the feed, start by weighing 100 grams of the basic feed and placing it in the feed box. Next, prepare a mixture of 2 milliliters of probiotics combined with 20 milliliters of mineral water. Spray the probiotics evenly over the weighed feed, stirring thoroughly with a spoon to ensure even distribution. Once mixed, allow the feed to air dry. After it has dried, place it back in the feed box, assign it a food code, and store it in the refrigerator until it is ready for use.

Parameters

In this study, the growth rate, survival rate, and stress resistance of *L. vanamei* was observed, besides several water quality parameters such as temperature, DO, pH, and salinity were also measured three times in everyday.

Absolute Weight Growth

The average weight growth of individuals in each treatment from the beginning to the end of maintenance is referred to as absolute weight growth, which can be calculated using the Effendie formula (Febri *et al.,* 2020):

$$W = Wt - Wo$$

Description:

W = Absolute weight growth (g)

W_t= Final average weight (g)

W_o= Initial average weight (g)

Survival Rate

Calculation of the survival rate of *L. vanamei* for each treatment was carried out by counting the number of white *L. vanamei* stocked at the start of rearing and then comparing it with the number of shrimp that were alive at the end of rearing. The formula created by Nami *et al.* (2023) can be used to calculate the survival of *L. vanamei*:

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

Description:

SR = Survival Rate (%)

N_t = Number of shrimp at the end of rearing (individuals)

N_0 = Number of shrimp at the start of rearing (individuals)

Stress Resistance

A stress resistance test was carried out at the end of the study to determine the physiological condition of *L. vanamei* after being given probiotic feed. Stress tests were conducted using increased salinity. Test salinity by taking 10 *L. vanamei* from each treatment, representing each sub-unit (rearing container), and then placing them in a container containing fresh water (0 ppt). Each treatment will be placed in a separate stress test container so that there are 4 stress test containers, each containing 10 shrimp, and starting by observing them simultaneously. Stress resistance, or CSI, is calculated by modifying the formula used by Ress *et al.* (1994) with the following formula:

$$CSI = D5 + D10 + D15 + \cdots + D60$$

Description:

CSI =Cumulative stress index (Cumulative Stress Index)

D = Number of stressed shrimp at a certain minute

The higher the CSI value, the lower the level of larval resistance, conversely, the lower the CSI value, the higher the level of larval resistance (Karim, 2000).

Water Quality Parameters

For additional data, during the research, several water quality parameters, including salinity, temperature, pH, and dissolved oxygen, were measured. Temperatures were measured using a thermometer, pH was measured with a pH meter, and salinities were measured with a refractometer. Measurements of temperature, PH, and dissolved oxygen were carried out three times a day: at 07.00 am, at 01.00 pm, and at 05.00 pm.

Data Analysis

The data obtained in the form of growth and survival of *L. vanamei* were analyzed using analysis of variance (ANOVA). Data showing a real effect were further carried out by the W-Tuckey test (Steel and Torrie, 1993). Statistical test, using SPSS version 23.0. Meanwhile, stress resistance, or cumulative stress index, and the range of measured water quality parameters were analyzed descriptively based on the viability of *L. vanamei*.

RESULTS AND DISCUSSIONS

Absolute Weight Growth

Based on the research, average value of absolute weight growth of *L. vannamei* using probiotic bacteria shows increased as the rearing age increases Figure 1. Figure 1 shows that Treatment B exhibited the highest absolute weight growth at 3.35 g on average, followed by treatment B+LB at 2.63 g, LB treatment at 2.59 g, and the control treatment with the lowest average growth at 2.43 g. The study found that adding probiotics to the feed did not significantly affect the absolute weight of *L. vanamei* larvae ($p > 0.05$). However, the results indicate that the probiotic dose used in the experiment enhanced body weight growth compared to the dose without probiotics.

Feed-given probiotics can increase digestibility through the ability of probiotics to secrete a number of digestive enzymes in the digestive tract of organisms that consume them (Renitasari *et al.,* 2021). In line with Widanarni *et al.* (2014), the provision of probiotic bacteria in feed can support the growth of organisms that consume them through adequate activation of digestive enzymes. Praditia (2009) stated the same thing: the presence of probiotics in the digestive tract can help increase the activity of digestive enzymes. Probiotics were able to trigger the growth of tiger shrimp (*L. monodon*) by increasing the absorption of feed nutrients in the body and increasing immunity (Basir, 2013). The best absolute weight was obtained in treatment B because the addition of probiotics in this experiment can increase the appetite of *L. vanamei*. Rachmawati *et al.* (2006) stated that high feed efficiency can result in better growth of *L. vanamei*. The presence of probiotics in the digestive tract of *L. vanamei* can help enzymes work better, this ensures a more efficient digestion process and promotes greater growth in shrimp (Praditia, 2009; Macey and Coyne, 2005; Nadhif, 2016).

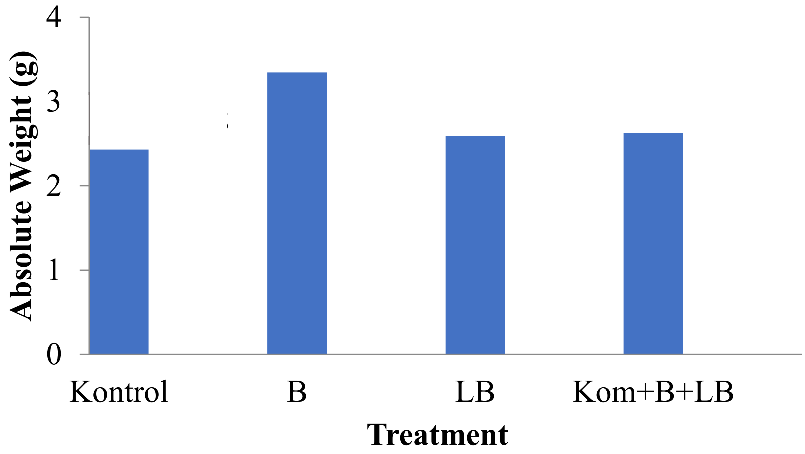

Figure 1. Absolute Weight Growth

The control treatment produced the lowest absolute weight with a value of 2.43 g. This happens because the feed does not contain probiotic bacteria, which can help metabolism. According to Macey and Coyne (2005), the addition of probiotics results in low digestibility. This condition occurs due to the absence of probiotics in the digestive tract, causing decreased enzyme performance. Low enzyme performance can cause increased energy requirements for metabolism to produce protein products.

This causes organisms to allocate energy for metabolism to the exclusion of energy for growth. As a result, shrimp growth is slow or even stunted, and this could result in mortality. Jamila (2021) posits that enzymes, which are protein-based chemical compounds, serve as biocatalysts in metabolic processes, facilitating the production of protein products without necessitating substantial energy expenditure. A similar thing was reported by Pradita (2009), without the addition of probiotics, the absolute weight obtained was lower compared to the treatment with the addition of probiotics. This happens because probiotics are very good at supporting the effectiveness of an organism's digestive process.

Survival Rate

The survival rate is the percentage ratio between the number of organisms initially stocked and the number of organisms alive at the conclusion of the rearing period in a container, as defined by Setiawati (2013). When rearing *L. vanamei* for 60 days, the addition of probiotics resulted in the highest survival of white *L. vanamei* compared to the addition without probiotics. Figure 2 shows the average survival value of *L. vanamei* for each treatment in the experiments carried out. Based on the results of the ANOVA analysis of variance, it was found that the addition of probiotics had no significant impact on white shrimp survival ($p > 0.05$). Treatment C have the highest survival rate of 71.67%, followed by treatment D at 66.67%, and treatment B achieved the lowest survival rate of 63.33%. Treatment without probiotics achieved the lowest survival rate of 58.33%. The highest survival was found in the C treatment. Lactic acid supplementation by *Lactobacillus* sp. and some of its enzymes reduces death rates from disease.

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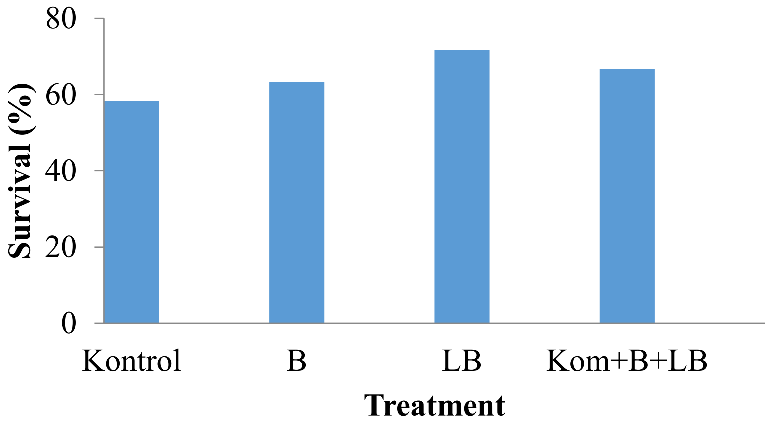

Figure 2. Survival rate of *L. vannamei* during research for 60 days

Miranda (2019) showed a similar thing that the bacteria *Lactobacillus* sp. produces lactic acid and functions in the digestive system by converting proteins into amino acids (simpler compounds). Stated that adding probiotics to shrimp feed can increase the shrimp's immune response and eliminate *Vibrio* bacteria that enter the digestive tract, and indirectly increase the survival and growth of *L. vannamei* (Fadilah, 2023). Treatment without probiotics showed the lowest white shrimp survival at 58.33%.

This is because there are no probiotic bacteria added, which can reduce the survival of *L. vannamei* and cause the death of *L. vannamei* because the shrimp's immune system decreases. Hartini *et al.* (2013) stated that giving probiotics can increase the survival rate of *L. vannamei* compared to treatment without probiotics. This happens because probiotics can improve the immune system, which allows *L. vannamei* to achieve good health status, which in turn results in higher survival. Cahyono *et al.* (2018) emphasized that survival can be influenced by abiotic and biotic components, in addition to feed nutrient components and immune status abiotic factors include the physical and chemical factors of waters, while biotic factors are all elements that live and influence the aquatic environment. These factors are more commonly known as water quality factors. Good water quality can help biota's physiological processes run well, which results in a high level of survival. Furthermore, Qi *et al.* (2009) stated that *L. vannamei* cultivation can maintain the water quality of the rearing media by using probiotics. Good water quality is very important for the survival of *L. vanamei*.

Stress Resistant

In a shrimp's effort to maintain body balance (homeostasis), it can encounter stressful conditions. Stress is the body's physiological response to environmental or external factors that can alter the body's normal functions. Stress can cause a decrease in appetite and an increase in the likelihood of disease (Afrianto *et al.,* 2015). According to Syawal and Syafriadiman (2008), stress is shown by shrimp as a physiological response to defend themselves and adapt to environmental changes. Figure 3 shows the cumulative stress index data for *L. vanamei* that were reared for 60 days.

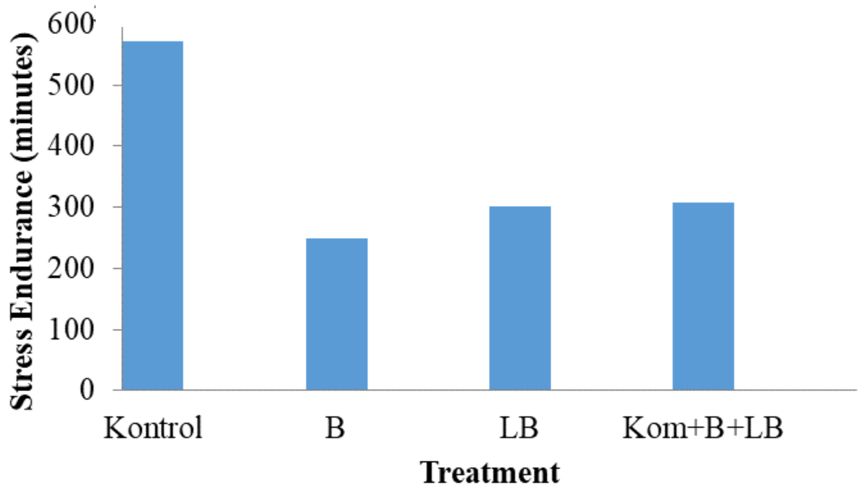

Figure 3. Resilience Stress *L. vanamei*

Stress test data with fresh water with a salinity of 0 ppt showed that probiotic treatment B, treatment C, and treatment D) increased the resistance of *L. vanamei* larvae to stress (changes in media salinity), with a lower CSI value than the control treatment. This shows that adding probiotics to white *L. vanamei* feed can help reduce stress by improving the *L. vanamei* immune system. This is in line with what Ahmadi and Kurniawati (2012) said, who stated that probiotics can work in the intestines of fish to encourage a non-specific immune system in fish. Probiotics can improve host health. Table 1 shows changes in the behavior of *L. vanamei* during stress level testing.

Table 1. Observation change act in demand shrimp based on shock osmotic

Treatment	Act in demand
Control	- First minute the larva moves normally - 40th and 45th minutes there were 2 larvae that had settled at the bottom or dead, some larvae still some are active moving, up and down and circling - 50th minute of larval movement start slowed down and there was 1 dead shrimp - The 50-100th minute, some of the larvae are gone is at the bottom container and there are 2 dead shrimp - Minutes to 100-190 larvae already is at the bottom container and not Again move or dead.
B	- First minute the larva is still move normally - 50 minutes still move active however there is some have start rotating on the surface of the water - 120 minutes, some of the larvae are still there spinning around on the surface of the water and there are some at the bottom settles or dead. - Minutes 190 larvae circling on the surface its movement already start slow. - 220 minutes still moving larvae were found however already abnormal or slowed down and still there are also those who survive - 305 minutes already no there is again larval interactions and all has settles to the bottom or dead.

LB	- First minute shrimp Still move normally - At 100 minutes some of the larvae begin to rise and fall and some Still move normally and that's it there are dead larvae - 130 minutes of movement of remaining larvae there are more and more active up and down - At 200 minutes some of the larvae fell to basic, go around and start move slow and partial start No moving (dead) - Minutes 300 all larvae no There is Again movement (off)
Comm+B + LB	- First minute the larva is still move normally - 90 minutes still move active however already someone is there at the bottom no moving (dead) - At 135 minutes some of the larvae were moving normally and some of the larvae were moving up and down and were found shrimp are not again moving (dead) - Minutes 210-210 all the larvae are circling on the surface then larval movement begins slow down dang, dang, dang to base waters - At 315 minutes all the larvae had settled and no there is again movement (off)

Water quality

Water quality parameters such as temperature, salinity, pH, and DO were measured. The values for all these parameters are presented in (Table 2). During the study, temperatures ranged from 28.1 - 33.4 °C. This temperature is still within the threshold that can be tolerated by *L. vannamei*. The ideal temperature for growing *L. vanamei* is 26 - 32°C (Briggs and Smith, 2007; Nadhif, 2016). During the research, the DO (dissolved oxygen) range was 7.2–7.3 ppm, suitable for *L. vanamei* cultivation. According to Fegan (2003), adequate dissolved oxygen (3-8 ppm) supports the maintenance of *L. vannamei*. The process of respiration (breathing) requires a lot of oxygen in the water. If the dissolved oxygen concentration is low or high outside the ideal range, it can cause slowed growth and death (Suwoyono and Mangampa, 2010). The salinity measurement results ranged from 30 to 40 ppt during the research (60 days). This range value is suitable for the growth of *L. vanamei*. According to Subaidah (2005), *L. vannamei* can grow well at a salinity of 29–34 ppt, and according to Se *et al.* (2023), *L. vannamei* can grow well at a salinity of 0-45 ppt but are optimal at a salinity of 15–25 ppt and optimal at a salinity of 10–30 ppt at the age of 1-2 months. Molting is a complex process and difficult to avoid (Lusiana *et al.,* 2021). Syukri and Ilham (2016) stated that high salinity (>35) can inhibit shrimp growth because the process is difficult because it requires a lot of energy, so it can drain energy and cause larval death. In conditions of energy deficiency, organisms require energy from the body to meet their energy needs, so that body weight decreases. If it occurs over a long period of time, it is possible that it can cause cell death or damage (apoptosis), which can have fatal consequences, causing a decrease in organ function and even causing the death of the organism.

Table 2. Measurement results Water Quality During the Maintenance Period

Water Quality Parameters	Range Value	Optimum Range
Temperature (°C)	28.1–33.4	11-30°C (Briggs and Smith, 2007)
DO (mg/L)	7.2-7.4	3-8 ppm (Fegan, 2003)
Salinity (ppt)	30-33	29-34 ppt (Subaidah, 2005)
pH (mg/L)	7.2-7.5	7.7-8.7 (Purba, 2012)

During the research, there were fluctuations in water quality parameters, namely salinity and temperature, so to handle these conditions, new water was added to the maintenance media. At the beginning of maintenance, the measured salinity was 30 ppt and increased to 40 ppt. Although handling changes in water quality have been carried out, it is suspected that fluctuations in salinity and temperature can affect the metabolic performance of the shrimp's body and can directly affect the survival of *L. vannamei*. According to Padusung *et al.* (2012), the ability of each organism to respond to changes in salinity is different. In conditions of salinity that do not match the concentration of environmental salt in the body, it requires organisms to make efforts to adapt to their environment (osmoregulation). Changes in salinity are related to the environment and cause salt content to tend to come out of an organism's body, while body fluids in an organism influence the environment, which can inhibit the process of concentrating body fluids in an organism (Fujaya, 1999).

According to Buwono (1993), salinity affects osmoregulation and molting in shrimp. If salinity exceeds normal limits, it can inhibit molting and make shrimp sensitive to disease. However, changing salinity turns out to have its own benefits, but it must be done under controlled conditions. According to Tobing (2019), to stimulate molting and increase shrimp growth, this can be done by changing the salinity regularly. The pH range measured during the experiment shows the range of values that can be tolerated by *L. vanamei*. According to Ferreira *et al.* (2011), optimal growth can be achieved by shrimp at a pH ranging from 6 to 9.

CONCLUSION

Bacillus probiotics at a dose of 20 ml/kg feed could significantly support better *L. vanamei* growth and minimize stress levels, resulting in higher survival. It is recommended to use *Bacillus* at a dose of 20 ml/kg feed as a probiotic candidate to increase the survival of *L. vannamei*. It is hoped that in the future similar research can be carried out with the application of probiotics at different age levels. It is thought that *L. vannamei* will respond differently to the administration of probiotics.

AUTHORS' CONTRIBUTIONS

Each author's contribution is as follows: DD, CUA, ZZ, DY, and CRM collected data, prepared the manuscript, and designed tables and graphs. DD, TFH, SPF, AH, and SSQT; processing data, writing manuscripts, and proofreading manuscripts. All authors contributed to the final manuscript.

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

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