Growth performance of *Litopenaeus vannamei* in low salinity cultivation with different natural feeds

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Abstract. The development of vanamei shrimp farming in freshwater aims to expand economic and social opportunities. However, raising shrimp outside their natural habitat presents challenges. This study aimed to identify the best acclimatization method, the lowest salinity level that supports shrimp growth, and suitable natural feeds for low-salinity conditions. The acclimatization process involved five treatments with three repetitions, gradually reducing salinity by 2 g/L and 1 g/L daily to reach 0, 3, 5, 8, and 10 g/L. A total of 1,000 postlarvae-10 were reared in aquariums for a month, followed by 300 shrimp in 1.5-meter tarpaulin ponds for two months. Shrimp were fed pellets, earthworms, and trash fish. The results showed that the best growth and survival rates at the lowest salinity were achieved at 5 g/L, with a survival rate of 90% and a length of 5.9 cm by DOC-30. Feeding shrimp with African Nightcrawler earthworms resulted in higher average body weight (ABW) and average daily growth (ADG), with survival rates and feed conversion ratios (FCR) comparable to those fed pellets and trash fish.

1 Introduction

Vannamei shrimp (*Litopenaeus vannamei*) is a seawater crustacean living in waters with high salinity/salt content (more than 30 g/L). Vaname was introduced to Indonesia from Central America to replace tiger prawn cultivation (*Penaeus monodon*), which often experiences failure, especially due to disease [1]. Vaname has been widely cultivated since 2000. Until now, it has become the most widely cultivated crustacean in Indonesia, and Has become the largest aquaculture commodity, placing Indonesia as the fifth producer in the world [2]. The government is targeting an increase in production of up to 250% by 2024 [3]. Two approaches can be taken to achieve this production target: intensification and extensification. Intensification characterized by the application of high stocking densities carrying capacity.

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The environment is also a limitation of intensification efforts, which causes different productivity levels in each cultivation area/region [6].

The second approach to increasing production is extensification, namely by increasing the size of cultivation areas (new ponds) in coastal/brackish areas or by utilizing new land that was previously not possible to use as vanamei shrimp cultivation land (freshwater) [7]. Cultivating vanamei shrimp outside their ideal habitat is not easy engineering. Several efforts have been reported, but with low productivity levels, they cannot be said to be feasible [8]. The low productivity of freshwater name cultivation is caused by shrimp failing to adapt to low salinity, osmotic pressure stress, failure to molt, low levels of feed efficiency [9], and low pigmentation so that levels of survival rate low, namely < 30%. Apart from being intended to increase production through extensification, freshwater name cultivation is expected to be an alternative to prevent farmers from the risk of shrimp diseases, for example (AHPND) [10][11][12], which is currently a serious scourge for farmers. Even though these engineering efforts face many obstacles, cultivating vaname in freshwater has the potential advantage that shrimp will be free from diseases caused by pathogens such as bacteria (*Vibrio* sp.) [13], fungi (*Aspergillus*) [14], viruses (WSSV, IHHNV) [15][16] which mostly attack sea/brackish water vaname.

Based on the results of several limited-scale preliminary tests, freshwater vaname cultivation (salinity 0 g/L) in ponds with a diameter of 2-3 meters has been successfully carried out with a survival rate of 60%, ADG 1.2% (average daily gain), ABW 15 g (average body weight) on DOC 60 (day of culture). However, production performance still needs to be improved through further tests that are more specific, measurable, and accurate on a larger scale.

Development of vaname shrimp cultivation technology (*Litopenaeus vannamei*) in fresh water on a household scale" is an engineering technology that will be realized through a series of research divided into two stages, namely based on shrimp stages and cultivation stages. The first stage (first year) focuses on engineering the post-larval stage with sub-research, namely 1) The effect of low salinity on the morphology, physiology, and growth of vaname post larvae, 2). The growth performance of vaname shrimp post-larvae fed with natural food differs at low salinity; 3) The growth performance of vaname shrimp juveniles at different densities.

2 Materials and Methods

This research consisted of 2 stages, namely the first stage, testing the adaptation of vaname shrimp to low salinity. The next stage is testing the provision of different natural feeds to vaname culture at the best salinity based on the first test's results. The test animals used in this research were vaname shrimp at the post-larvae stage (PL-10 days), free from certain pathogens (Specific Pathogen-free/SPF). P.L., as many as 15,000 individuals were cultured in hatchery ponds with a salinity of 25 g/L at P.T. Summa Benur, Situbondo, Indonesia. The following is the experimental stage for low salinity white vaname shrimp. During the experiment, seawater was taken from the waters of the Indian Ocean, which has an average salinity of 34 g/L. Sea water is stored in a 10 ton capacity tank, and then the water is sterilized using 30 ppm chlorine. Water with low salinity can be used by mixing sea water (34 g/L) with fresh water (0 g/L) in a certain ratio to obtain the desired salinity.

2.1 Acclimatization rate

The next stage of the experiment is the maintenance of the juvenile phase. A total of 5000 individuals were reared in water with a salinity of 5 g/L, which is the lowest salinity and the best results from the first experiment. Likewise with the acclimatization method. However, this culture was carried out en masse in a tarpaulin pool with a diameter of 2 meters. As many as 300 individual Vaname shrimp were cultured, namely DOC-30, which were then transferred to each of the 9 round tarpaulin ponds with a diameter of 1.5 meters. The experiment consisted of 3 treatments and three replications. Treatment P1 is shrimp that are given natural food in the form of commercial pellets. The commercial feed used consists of 3 variants according to shrimp size with a protein content of 30%, fat 5%, and fiber 4%. Treatment P2 is shrimp that are given natural food in the form of African Nightcrawler (ANC) (*Eudrilus eugenia*) type earthworms. The P3 treatment is shrimp that are given natural food in the form of trash fish, namely a mixture of pelagic caught fish. Feed is given randomly satiation through anco, and the amount of feed consumed is recorded. Every week, weight and length growth, survival, and water quality parameters are sampled. Every day, siphoning is carried out, and the water is changed every three days by 20%.

2.2 Data analysis

The results were analyzed using analysis of variance (ANOVA) to see the significance of each treatment in the experiment. Then, if there is a significant difference, a further test will be carried out in the form of the Least Significant Difference (BNT) test

3 Results and Discussion

3.1 Acclimatization rate

During maintenance, vaname postlarvae died at all levels of salinity. The percentage of P.L. that are still alive increases as the salt content in the water increases, where the lowest percentage of P.L. at the end of rearing is those reared at a salinity of 0 g/L and the highest is at a salinity of 8 g/L. The ANOVA test results showed that the P.L. survival rate at salinity 3, with an average of 67%, was significantly higher than at salinity 0, with an average of 58%. Meanwhile, survival rates at salinities of 5, 8, and 10 g/L were also significantly higher than at salinities of 3 g/L. The test results show that increasing the salinity or salt content in the water will further support P.L.'s life. Water with higher salt content provides near isotonic conditions for P.L. which were originally maintained at high salinity of 25-30 g/L.

Differences in salt levels force P.L. to adapt to osmoregulation. P.L.s that are unable to adapt will experience a severe hypotonic condition that can damage the outer cells, for example, the gills, and cause death [17]. This is confirmed by a number of deaths in the first week of maintenance. Meanwhile, other P.L.s experienced death gradually. The slow death of P.L. is caused by P.L. not being able to continuously carry out osmoregulatory adaptations, which directly drain energy. Based on the large survival rate value, it can be concluded that salinities of 0 and 3 g/L are not good for use as culture media. Meanwhile, culture media with salinities of 5, 8, and 10 g/L are good for use for P.L. maintenance. The following Figure 2 shows a graph of the P.L. survival rate at different salinities.

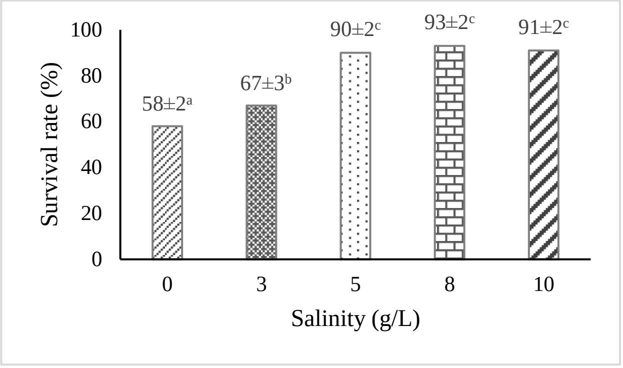

Fig. 1. Survival rate of postlarvae (PL-10) maintained until DOC-30 in water media, where the salinity was gradually reduced until it reached a salinity of 0, 3, 5, 8, and 10 g/L.

Total body length was measured from the rostrum to the base of the telson. The experimental results showed that the lowest total length value of 4 cm was found in the treatment with a salinity of 0 g/L. Meanwhile, the highest length growth, namely 6.3 cm, was in the treatment with a salinity of 10 g/L. The following Figure 3 shows the total length values of P.L. maintained at different salinities. The ANOVA test showed that the total length of P.L. reared at a salinity of 0 g/L was not different from that maintained at a salinity of 3 g/L but was significantly different from that of shrimp reared at a salinity of 5, 8, and 10 g/L. This shows that there is a correlation between salinity and growth.

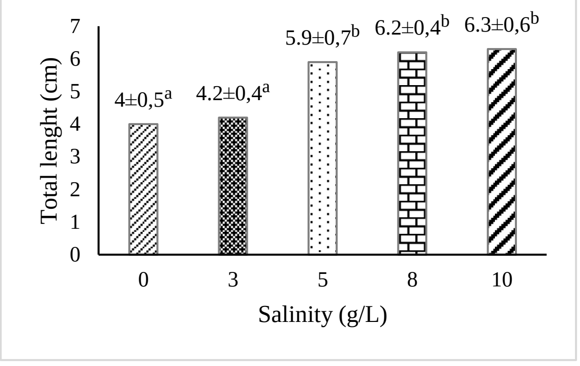

Fig. 2. Total length of postlarvae (PL-10) reared up to DOC-30 in water media, where the salinity was gradually reduced until it reached a salinity of 0, 3, 5, 8, and 10 g/L.

These results are consistent with previous studies highlighting a positive relationship between water salinity and shrimp growth. A study by [12] found that higher salinity can improve the growth and development performance of vaname shrimp larvae. This research notes that optimal salinity can increase feed utilization efficiency, nutrient availability, and metabolic processes, ultimately affecting shrimp growth.

Water with higher salinity has a higher salt ion content, which affects the osmotic pressure between the water and shrimp cells. Different osmoregulatory adaptations at each salinity level can explain differences in white shrimp growth at different salinities. Osmoregulation is an important process for aquatic organisms to maintain the water and salt balance in their bodies. In vaname shrimp, changes in water salinity trigger physiological responses involving regulating osmotic pressure and ion balance in the body [18]. The hypotonic condition experienced forces P.L. to osmoregulate passively and actively. Osmoregulation will require energy, so energy cannot be used optimally to support growth. The higher the difference in osmotic pressure, the more energy will be used, so growth will be more disturbed.

At low salinity (0 ppt), vaname shrimp face greater osmoregulatory challenges due to the high difference in water concentration inside and outside the body. The osmoregulation process at low salinity requires significant energy use to overcome osmotic pressure and maintain ion homeostasis in the body [19]. As a result, more energy that should be used for growth is directed to support this osmoregulation process.

In contrast, at higher salinity (10 ppt), vaname shrimp may experience fewer osmoregulatory challenges due to lower water concentration differences. This can result in energy savings that can be allocated to more efficient growth. Research by [13] revealed that certain shrimp species can experience increased osmoregulatory efficiency at higher salinity, resulting in better growth. It can be concluded that salinities of 5, 8, and 10 g/L provide the same growth performance and survival rate, so the lowest salinity that will be used in the second experiment is 5 g/L.

3.2 Natural feeding trials

The second stage of the experiment tested the effect of different natural feeds on name shrimp cultured in the best salinity media from the first stage of the experiment, namely 5 g/L. In this experiment, two types of natural feed were tested with commercial pelleted feed as a comparison/control. Figure 4 shows the survival rate percentage where the three types of feed provide the same or insignificant survival rate value, with the lowest survival rate being 83% and the highest being 86%. This shows that providing natural feed in the form of ANC-resistant worms and trash fish can support the nutritional needs of juvenile shrimp, with performance that is as good as commercial feed, which is usually used in high-salinity cultivation ponds.

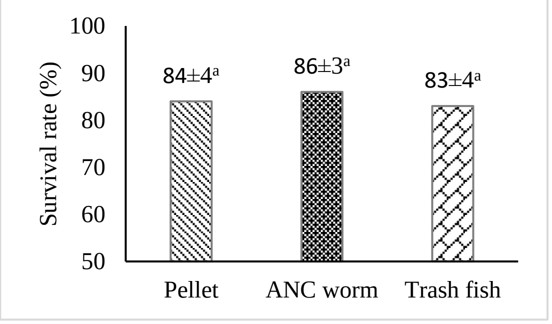

Fig. 3. Survival rate of juvenile vannamei reared for 60 days in water with a salinity of 5 g/L with different feed.

The average body weight of vaname shrimp during rearing can be seen in Figure 5. The graph shows the increase in shrimp body weight as the rearing time (DOC) increases. Shrimp body weight, initially 3 grams, increased to 19 grams for pellets, 22.2 grams for worms, and 20.5 grams for trash fish feed. The results of the ANOVA test showed a significant difference in the final weight of shrimp-fed ANC worms, which was higher than shrimp-fed pellets. This shows that the nutrients contained in ANC worms can support shrimp growth.

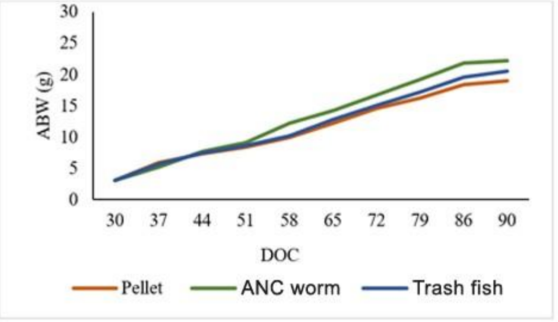

Fig. 4. Average body weight of shrimp during rearing in water with a salinity of 5 g/L.

Daily growth of vaname shrimp (*Litopenaeus vannamei*) reared at five ppt salinity with three different feeding treatments, namely pellets, worms, and trash fish, showed interesting variations in body weight gain. Data analysis shows that the highest average daily weight gain for vaname shrimp occurred in the worm treatment (0.3%), followed by trash fish (0.28%) and pellets (0.25%) see Figure 6. Treatment with Administration of worms resulted in the highest daily growth, indicating that worms can provide more complete and easily digestible nutrition for shrimp. A study by [14] states that providing natural food, including worms, can increase the growth and survival of shrimp, which is in line with the results of this study.

The results of the ANOVA analysis showed a significant difference in the daily growth of shrimp-fed pellets compared to ANC worms. The nutritional content of ANC worms has been proven to support the nutritional needs of a name shrimp raised in low-salinity water. Likewise, food in the form of trash fish also showed ADG values that were not significantly different from ANC worms. Research by [20] notes that types of feed derived from animal protein sources, such as trash fish, can positively contribute to shrimp growth. However, it is necessary to pay attention to the nutritional balance and level of digestibility. Meanwhile, feed in the form of pellets shows a lower ADG, which is thought to be influenced by the lower amount of feed consumed and poorer water quality conditions due to the remaining feed dissolved in the water.

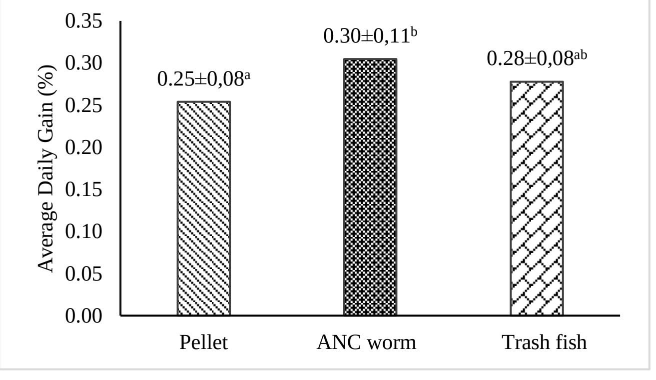

Fig. 5. Average daily weight gain for shrimp fed different foods.

The graph in Figure 7 shows the average feed conversion ratio value of the three feed types, with the lowest FCR value being ANC worms, namely 1.72, and the highest FCR being trash fish feed. Even though the results of the ANOVA analysis showed that the three were not significantly different, there was a tendency for worm food to be more efficient. This is related to the remaining feed that has not been consumed. Based on shrimp eating behavior, natural food in the form of worms and trash fish appears to dissolve little in the water, in contrast to pelleted food, which has a higher dissolution potential. An FCR value of 1.72 means that to increase the weight of vaname shrimp by 1 gram, 1.72 grams of ANC worms are needed. This experiment proves that feed in the form of ANC worms and trash fish can be an alternative to artificial feed with their respective advantages and disadvantages.

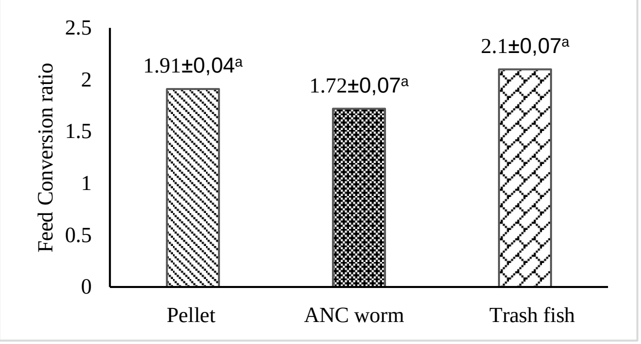

Fig. 6. Efficiency of feed use based on feed conversion ratio.

Water quality parameters were measured for 60 days. The observation results showed no spikes or extreme fluctuations in temperature, pH, D.O., ammonia, and total organic matter (TOM) parameter values. Parameter values between treatments are the same or not significant. The five parameters are also still within the optimum range for vaname shrimp cultivation based on SNI 8037.1:2014. TOM and ammonia levels in ponds fed with pellets were higher compared to ponds fed with ANC worms and trash fish. The ammonia and TOM content in the water can affect shrimp growth [21]. This is because some of the pelleted feed is destroyed and dissolved in water. Meanwhile, feed in the form of ANC worms and trash fish leaves less dissolved food residue. It can be concluded that the water quality from all treatments is still within the optimum range and suitable for vaname shrimp cultivation.

Table 1. Average data on experimental pond water quality.

Treatments	Salinity g/L	Temp. °C	pH	DO mg/L	Ammonia mg/L	TOM mg/L
Pellet	5	28.5	7.5	8.5	0.12	40
Worm ANC	5	29	7.8	8.6	0.07	24
Trashfish	5	29	7.7	8.3	0.08	33

4 Conclusion

PL-10 name shrimp can be cultivated in low salinity, namely 5 g/L, by gradual acclimation by reducing salinity levels by 1 to 2 g/L every day. Meanwhile, white DOC-30 shrimp, which have adapted to a salinity of 5 g/L, can be cultivated by feeding them ANC worms and regularly controlling water quality.

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