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The effect of addition of calcium oxide (CaO) on the cultivation of *Litopenaeus vannamei* in freshwater

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ARTICLE INFO	ABSTRACT			
Keywords: Calcium_oxide Freshwater Vanammei	Indonesia, as the fourth-largest global producer of Vannamei shrimp, is facing increasing demand for shrimp, necessitating higher production. In response, the cultivation of Vannamei shrimp in freshwater has gained traction. However, freshwater environments are limited in essential calcium minerals required for shrimp growth. To address this issue, research on the addition of calcium oxide (CaO) in freshwater Vannamei shrimp cultivation was conducted. The main objective of this study was to assess the impact of calcium oxide (CaO) and determine the optimal dosage for the survival rate and growth of Vannamei shrimp in freshwater. The experimental method was employed, consisting of five different dosage treatments: seawater control, freshwater control, 40 ppm, 80 ppm, and 120 ppm of calcium oxide. The findings of this study revealed that the treatment with 120 ppm of calcium oxide exhibited the most favorable growth, with a weight of 16.40 g in the freshwater treatment. Additionally, the 80 ppm treatment demonstrated the highest survival rate, reaching 67% in the freshwater treatment.			
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1. Introduction

According to the data from the Ministry of Marine Affairs and Fisheries (2019), shrimp production in 2018 reached 717,094 tons. Based on the vannamei shrimp production graph from 2017 to 2019, there was an increase in production, but in 2019, it experienced a decline. In fact, in 2016, the production of vannamei shrimp was only around 265,000 tons per year, which decreased from 300,000 tons per year in 2014 due to disease outbreaks (Medistiara, 2017). Therefore, culturing vannamei shrimp in freshwater environments becomes an alternative choice to increase production yields. The implementation of cultivation technology in low salinity environments also opens up

opportunities to further expand vannamei shrimp aquaculture production (Febriani et al., 2018). Another advantage of culturing vannamei shrimp in low salinity media is the reduced risk of shrimp being infected by viruses and bacteria that commonly affect shrimp in brackish water (Fitriani et al., 2017).

Vannamei shrimp has several advantages compared to other shrimp species. Among these advantages, vannamei shrimp can be stocked at high densities because they live in the water column, have rapid growth, and are highly demanded in the global market (Velasco et al., 1999, cited in Yunus et al., 2020). Furthermore, the euryhaline nature of vannamei shrimp allows them to survive and thrive in a wide range of salinities, from 0.5 to 40 ppt (Febriani et al., 2018). This ability opens up opportunities for the development of this commodity in freshwater or inland water environments (Yunus et al., 2020).

In general, limiting factors for survival and growth of shrimp in low salinity environments are the inadequate mineral composition compared to optimal salinity conditions. Physiological processes within the body can function normally depending on the availability of certain anions (bicarbonate, chloride, carbonate, and sulfate) and cations (calcium, potassium, sodium, and magnesium). Various studies have revealed that calcium is a crucial mineral for the survival of vannamei shrimp in low salinity water. Calcium is one of the essential requirements, apart from feed, needed by shrimp for growth, including the molting process. Osmotic and ionic pressures of water are closely related to calcium, both in the internal and external media of the shrimp. The composition and concentration of ions in the body must be appropriate for the proper functioning of cells. One substance that contains calcium is calcium oxide (CaO). Additionally, salinity is also a factor that affects the calcium content in water. According to McNeely et al. (1979, cited in Kaligis, 2010), the calcium content in seawater is around 400 mg/L, while in freshwater, it is usually less than 15 mg/L. Therefore, minerals such as calcium are essential for shrimp growth and must be available in sufficient amounts in their rearing media. Based on these factors, the author is interested in studying the effect of calcium oxide (CaO) supplementation in freshwater cultivation media on the survival rate and growth of vannamei shrimp (Litopenaus vannamei).

2. Material and methods

This research was conducted in April-May 2021 at the Aquaculture Study Program Laboratory, Department of Fisheries and Marine Science, Faculty of Agriculture, Mataram University. The experimental containers used in this study had dimensions of L x W x H = $45 \times 30 \times 30 \text{ cm}^3$. The research employed an experimental method with a Completely Randomized Design consisting of 5 treatments and 3 replications. The treatment doses used in this study were as follows:

Treatment A: Without the addition of CaO / Seawater control

Treatment B: Without the addition of CaO / Freshwater control

Treatment C: Addition of 40 ppm CaO

Treatment D: Addition of 80 ppm CaO

Treatment E: Addition of 120 ppm CaO

2.1 Procedure

Preparation of research containers, consisting of 15 units of containers with a water capacity of 20 liters each. Preparation of test animals, which were vannamei shrimp larvae. The test animals were obtained from PT. Bibit Unggul Lombok Utara with a size of 20 post-larvae (PL). Acclimatization of test animals: Before the vannamei shrimp larvae were stocked, they were placed in temporary

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holding tanks containing seawater, followed by acclimatization (salinity reduction) until reaching 0 ppt.

- e. Preparation of solution and addition of calcium CaO: Calcium CaO was diluted in a separate container using strong agitation with a free-sized container.
- f. Feeding: Shrimp feeding was conducted with a frequency of 5 times a day, every 4 hours, at a rate of 6% of the shrimp's body weight.

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2.2 Parameters
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Survival Rate (SR)

The formula from Effendie (1997) as cited in Rakhfid (2019) can be used to calculate the survival rate of vannamei shrimp, as follows:

SR = Nt/(No) x 100%

Where:

SR = Survival rate of vannamei shrimp

Nt = Total number of vannamei shrimp alive at the end of the study (individuals)

No = Total number of vannamei shrimp alive at the beginning of the study (individuals)

Weight Growth

The absolute weight growth can be calculated using the formula proposed by Effendi (1979) as cited in Rakhfid (2019), as follows:

Wm = Wt - Wo

Where:

Wm = Absolute weight growth (g)

Wt = Average weight of shrimp at the end of the study (g)

Wo = Average weight of shrimp at the beginning of the study (g)

Length Growth

Absolute length increment can be calculated using the following formula (Nasir, M., & Khalil, 2016):

Where:

L = Length increment (cm)

Lt = Final length (cm)

Lo = Initial length (cm)

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Feed Conversion Ratio (FCR)
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According to NRC (1977) as cited in Supomo (2015), the feed conversion ratio (FCR) of shrimp can be calculated using the following formula:

FCR = F/Biomass

Where:

FCR = Feed conversion ratio F = Total feed consumed (kg)

Biomass = Shrimp biomass (kg)

Coefficient of Variation

The coefficient of variation represents the level of uniformity or variability in the size of organisms

during the study period. The coefficient of variation can be calculated using the formula (Fendjalang et al., 2016):

$$CV = S/Y \times 100$$

Where:

CV = Coefficient of variation (%)

S = Standard deviation

Y = Mean

Water Quality Parameters

The observed parameters include dissolved oxygen (DO), pH, temperature, salinity, ammonia, and calcium levels in the water. The determination of Ca++ concentration is done by the following calculation:

Calcium hardness (Ca++) = (ml titrant + M titrant \times 100.1 \times 1000) / ml sample

2.3 Data Analysis

Survival rate, weight growth, length growth, and feed conversion ratio are analyzed using analysis of variance (ANOVA). If significant differences are found at a 95% confidence level, Duncan's test is performed as a post hoc test to determine the best treatment. Meanwhile, the coefficient of variation for water quality data is analyzed descriptively.

3. Results and Discussion

3.1. Survival rate

The average survival rate values are presented in Figure 1. The results of the survival rate calculations over a period of 45 days show that the highest survival rate was observed in treatment A, while the lowest was observed in treatment E. The tabulated values from the analysis of variance (ANOVA) indicate that the survival rate data are significant (P<0.05). Treatment A significantly differed from all other treatments (P<0.05), and treatment B significantly differed from treatments A, C, and E (P<0.05), but did not significantly differ from treatment E (P>0.05).



Figure 1. Survival rate levels

Based on the research results, the survival rate of shrimp in this study ranged from 52% to 87%. The percentage of survival rate of vannamei shrimp is presented in Figure 1 (survival rate graph). The low survival rate in treatment E (+120 ppm CaO) is likely due to cannibalism among the shrimp. Arumsari et al. (2019) reported that molting in vannamei shrimp significantly affects their survival rate because shrimp become physically weak after the molting process. Asynchronous molting can lead to cannibalism among shrimp, as observations indicate that newly molted shrimp are physically weak, making them susceptible to attacks from their conspecifics and leading to death.

According to Anggoro (1992, cited in Orlando, 2015), molting at different times can cause cannibalism in shrimp, resulting in their death. Feed availability also affects shrimp cannibalism, as hungry shrimp often compete for food, and if the food supply is insufficient, shrimp are more likely to attack each other. Ali (2015) mentioned that starvation occurs when shrimp undergo the molting process, and as a result, their appetite increases after molting, leading to unavoidable competition for food.

The high survival rate of vanname shrimp in treatment D (+80 ppm CaO) in freshwater conditions indicates that the presence of calcium oxide (CaO) and the given dosage are sufficient to facilitate the rapid formation of a new exoskeleton during the molting process. Orlando (2015) stated that the addition of calcium oxide (CaO) under optimal conditions can meet the calcium requirements of shrimp during the formation of a new exoskeleton. The addition of calcium oxide (CaO) to the rearing media can supply calcium in the water, which is beneficial for the formation and hardening of the new exoskeleton in shrimp. A smooth and rapid molting process occurs when there is an adequate supply of calcium in the media because faster recovery during molting reduces the likelihood of cannibalism among shrimp. Furthermore, calcium in the rearing media helps in osmoregulation in vannamei shrimp and is an essential ion for their growth and survival. Tagwa (2011) stated that calcium and potassium ions are crucial for shrimp life. Deficiencies in potassium, magnesium, and calcium during salinity adaptation can jeopardize the survival rate of shrimp. In this study, treatment D (+80 ppm CaO) had a higher alkalinity (DKH) value in freshwater media compared to treatment E (+120 ppm CaO). This difference is likely due to the optimal calcium levels provided in treatment D (+80 ppm CaO), whereas treatment E (+120 ppm CaO) had an excessively high calcium content. The presence of calcium minerals from CaO is indeed necessary, but there is an optimal level. According to Orlando (2015), difficulty in forming a new exoskeleton in shrimp can occur due to low calcium levels, while difficulty in achieving homeostasis in shrimp can occur due to excessive calcium levels.

The influence of calcium (dolomite) on the survival rate of vannamei shrimp was also studied by Arumsari et al. (2019). The results showed that the survival rate of vannamei shrimp was 27% higher compared to the control group with the addition of calcium (dolomite) at a dosage of 3 mg/kg. Yulihartini et al. (2016) also presented their research results on the effect of adding calcium (hydroxide) on the survival rate of vannamei shrimp. The addition of calcium (hydroxide) resulted in a 9% higher survival rate compared to the control group with the addition of 60.

3.2. Weight growth

The average weight growth rate values in the study are presented in Figure 2 (weight growth graph), where the highest weight growth rate during the 45-day rearing period was observed in treatment A for seawater treatment. In the freshwater treatment, the highest weight growth rate was found in treatment E, while the lowest was observed in treatment B. Based on the ANOVA analysis with a 95% confidence interval, the absolute weight growth data showed significant differences (P<0.05). Treatment A showed significant differences with all treatments (P<0.05). Treatment B did not show significant differences with treatment C (P>0.05), but showed significant differences with treatments A, D, and E (P<0.05). Treatment C did not show significant differences with treatment D (P>0.05), but showed significant differences with treatments A, B, and E (P<0.05). Treatment E showed significant differences with all treatments A, B, and E (P<0.05).



Figure 2. Absolute weight growth

Shrimp growth is closely related to the molting process, where the more frequent and rapid the molting, the faster the shrimp growth. The high growth rate in treatment E (+120 ppm CaO) indicates the optimal dosage of calcium oxide (CaO) provided, which supports the calcium mineral requirement during the molting process of vannamei shrimp reared in low salinity media. The added calcium oxide does not directly influence shrimp growth, but it is beneficial during the formation of new carapace during molting, fulfilling the need for calcium minerals (Yulihartini et al., 2016). High growth rate was also observed in treatment A (seawater control), which is due to the optimal rearing conditions (salinity of 30 ppt), resulting in the shrimp's body being isoosmotic (osmotic pressure in balance with its body). The appropriate osmotic pressure in the shrimp's body affects the smooth osmoregulation process, which helps the shrimp in molting and promotes growth (Ali & Waluyo, 2015).

High weight gain is also influenced by feed management. The quality and quantity of feed provided can contribute to optimal growth for shrimp. Shrimp growth and survival can be disrupted if feed is given excessively. Besides causing excessive feed remnants and resulting in decreased water quality, excessive feeding also increases production costs due to wastage of feed. The addition of calcium oxide in the media can also increase feed consumption, as reported by Arumsari et al. (2019), indicating that the optimal addition of calcium can improve feed utilization and achieve optimal growth for vannamei shrimp.

Low growth in treatment B (freshwater control) is caused by mineral deficiency, which slows down the molting process and affects shrimp growth. Additionally, mineral deficiency in the rearing media also affects shrimp physiological processes. Kaligis (2010) stated that the low mineral composition in low salinity water is one of the limiting factors for vannamei shrimp growth. The smooth functioning of physiological processes in the body depends on the availability of minerals in the rearing media, including specific anions and cations. Slow growth is also attributed to the shrimp's non-isoosmotic (hypotonic) condition, which requires energy expenditure to balance its body with the environment, resulting in an energy deficit for growth.

According to Orlando (2015), the formation of new carapace proceeds smoothly when the calcium level in the water is optimal, as low calcium levels hinder the process of new shell formation. However, excessively high calcium levels in the water are also detrimental as high potassium levels can disrupt calcium ion homeostasis. The energy expenditure obtained from feed depends on the hypoionic or hyperionic calcium condition in the shrimp's body since shrimp with non-isoosmotic body conditions expend most of their energy to maintain balance between their body and the environment. The addition of CaO calcium to the rearing media is closely related to the activity of Na+K+ATPase enzymes and osmoregulation of shrimp in freshwater media. In freshwater media,

vannamei shrimp expends most of its energy on osmoregulation to balance the ionic fluids between intracellular and extracellular compartments.

Research on the effect of calcium on growth was also conducted by Aisyah et al. (2017), where the addition of calcium (dolomite) at a dosage of 3 mg/kg of feed resulted in a growth increase of 1.15 g, larger than the control group. Yulihartini et al. (2016) also presented research findings on the role of calcium in growth. The results showed a daily growth increase of 0.31% with the addition of 60 mg/L of calcium (hydroxide).

3.3. Length Growth

The average length growth values in the study are presented in Figure 3 (absolute length growth graph), where the highest length growth value during the 45-day rearing period was observed in treatment A for seawater. In the freshwater treatment, the highest length growth was found in treatment E, while the lowest was in treatment B. Based on ANOVA analysis with a 95% confidence interval, the length growth data showed significant differences. Treatment A significantly differed from all other treatments (P<0.05). Treatment B significantly differed from all other treatments C did not significantly differ from treatment D (P>0.05) but significantly differed from treatments A, B, and E (P<0.05). Treatment E significantly differed from all other treatments (P<0.05).



Figure 3. Length Growth Levels

The high length growth in treatment E (+120 ppm CaO) is attributed to the additional minerals from calcium oxide (CaO), which facilitate the body's metabolic processes and fulfill one of the crucial minerals for the molting process. Moulting intensity plays an important role in shrimp body length increment, as more frequent moulting leads to higher length growth, as growth occurs successfully after molting. This is consistent with Kaligis's statement (2010) that after the molting process (skin shedding), shrimp experience periodic growth (length and weight increment).

The low growth in treatment B (freshwater control) is due to salinity stress, resulting in a lack of essential minerals needed for survival and growth, and a greater utilization of feed for osmoregulation processes. Ali (2015) stated that shrimp maximize the energy obtained from feed for osmoregulation or to sustain their lives when they are exposed to suboptimal salinity levels, thereby unable to fully utilize energy for growth.

The addition of calcium mineral (CaO) had a better effect compared to the control without calcium oxide mineral supplementation. This is because calcium minerals facilitate and enhance the body's metabolic systems in vannamei shrimp. During intermediate metabolism or the essential

metabolism of amino acids, adenosine triphosphate (ATP), phosphoprotein, and phospholipid, calcium plays a crucial role in ensuring the smoothness of these processes (DA & DM, 1991).

3.4. Feed Convertion Ratio

The average feed conversion ratio (FCR) values in the study are presented in Figure 4, where the highest FCR in the freshwater treatment was observed in treatment B, with a value of 2.19, followed by treatment C with 2.00, then treatment D with 1.32, and the lowest was in treatment E with 0.89. Meanwhile, in the seawater treatment, the FCR value was 0.65. Based on ANOVA analysis with a 95% confidence interval, the oxygen consumption rate data showed significant differences. Treatment A did not significantly differ from treatment E (P>0.05), but significantly differed from treatments B, C, and D (P<0.05). Treatment B did not significantly differ from treatment C (P>0.05), but significantly differed from treatments A, D, and E (P<0.05). Treatment D did not significantly differ from treatment E (P>0.05).



Figure 4. Feed Convertion Ratio Levels

Feed conversion ratio is the ratio between the total feed given and the shrimp's weight increment (Ridlo & Subagiyo, 2013). The FCR values in this study are presented in Figure 10 (FCR graph). The low FCR in treatment E (+120 ppm CaO) is closely related to the added calcium oxide in the rearing media, where calcium aids in the molting process, resulting in rapid growth and a positive correlation with FCR. Additionally, the management of feed quality and quantity also influences optimal shrimp growth (Hidayat et al., 2014).

The low FCR value in treatment A (seawater control) is due to the rearing being conducted in seawater media, which provides optimal salinity for shrimp growth and feed utilization, as all consumed feed is used for growth purposes only. This is in contrast to treatment B (freshwater control) where shrimp experience salinity stress, causing most of the ingested feed to be used for osmoregulation processes and only a small portion for growth. According to Qurata'ayun (2009), shrimp feeding behavior can be influenced by the salinity level of the media, with higher salinity resulting in lower feed conversion. The salinity level of the media is closely related to shrimp osmotic pressure. The osmotic pressure of the media (salinity) becomes a burden for vannamei shrimp if the osmotic pressure of the rearing environment differs significantly from the osmotic pressure of their bodies, as shrimp require a considerable amount of energy to maintain the balance between environmental and body osmotic pressures. Therefore, it can be said that energy expenditure for osmoregulation and feed consumption rates in shrimp are influenced by salinity.

According to Effendi (2004) cited in Fendjalang et al. (2016), species, water quality, and feed are factors that can affect feed conversion. The higher the feed conversion ratio, the more feed is converted into shrimp biomass. The feed conversion ratio values in the study are directly proportional to the calcium level provided. The higher the calcium oxide (CaO) level, the lower the FCR value

obtained. This indicates that the addition of calcium oxide (CaO) influences the FCR value obtained. The calcium oxide (CaO) level indirectly affects the FCR value, but it does affect shrimp weight growth. The higher the shrimp weight influenced by calcium oxide (CaO), the lower the FCR value obtained.

3.5. Coefficient of Variation

The average coefficient of variation values in the study are presented in Figure 5, where the highest coefficient of variation was found in treatment B at 17.56, followed by treatment D at 15.44, then treatment E at 13.60, next treatment C at 9.88, and the lowest was in treatment A at 8.31. Based on ANOVA analysis with a 95% confidence interval, the coefficient of variation data showed non-significant differences (P>0.05).



Figure 5. Length Coefficient of Variation Levels



Figure 6. Weight Coefficient of Variation Levels

The average coefficient of variation values in the study are presented in Figure 6, where the highest coefficient of variation was found in treatment D at 65.3, followed by treatment E at 51.32, then treatment A at 30.15, next treatment B at 27.77, and the lowest was in treatment C at 24.20. Based on ANOVA analysis with a 95% confidence interval, the coefficient of variation data showed non-significant differences (P>0.05).

The coefficient of variation is expressed as a percentage and is interpreted as a measure of variance used to compare data distributions with different units. The coefficient of variation is also defined as a normalized measure of dispersion of a probability distribution. The absolute value of the coefficient of variation is sometimes referred to as the relative standard deviation (RSD). The ratio of the standard deviation to the mean is a concise definition of the coefficient of variation (Setiawan, 2012).

The coefficient of variation for weight and length of vannamei shrimp in this study can be seen in Figure 5 (length CV graph) and Figure 6 (weight CV graph). The range of coefficient of variation values for shrimp length is 8.31% to 17.56%. The range of coefficient of variation values for shrimp length in this study falls into the low score category because the coefficient of variation values are below 20%. According to Suwoyo (2014), the following criteria can be used to determine the level of biota coefficient: coefficient of variation values from 0% to 20% are classified as low, values from 20% to 50% are classified as high, and values greater than 50% are classified as very high. The height or lack of variation in the measured population can be observed from the coefficient of

variation, where a population with narrow variation (homogeneous) will have a low coefficient of variation, while a population with a high coefficient of variation indicates that the population has wide variation (heterogeneous). The range of coefficient of variation values for shrimp weight ranges from 22.27% to 35.46%. The coefficient of variation for shrimp weight in the study falls into the high size coefficient category. Hermawan (2013) reported that the quality of postlarvae can be assessed by their size variation. The coefficient of variation for size is considered low (score 10) if the coefficient of variation is equal to or less than 15%, moderate variation (score 5) if the coefficient of variation is petween 15% and 25%, and high variation (score 0) if the coefficient of variation is greater than 25%.

Table 1. Wa	ater Qualit	у				
Parameters	А	В	С	D	Е	Optimum (ref.)
Temperature	28.08°C	28.06 °C	28.08 °C	27.97 ℃	28.04 °C	28-30 °C (Yulihartini et al., 2016)
Dissolved oxygen	6.9	7.9	8.0	7.8	7.9	4-8 mg/l (Bahri et al., 2020)
pH	7.9	8.0	8.3	8.2	8.3	7.5-8.5 (Erlando et al., 2015)
Salinity	30 ppt	0 ppt	0 ppt	0 ppt	0 ppt	2 ppt < 40 ppt (Yulihartini et al., 2016).
Ammonia	0.02	0.03	0,05	0.05	0.06	≤ 0.1 mg/L (Anna, 2010)
Calcium	400 ppm	14,02 ppm	19,42 ppm	21,57 ppm	23,08 ppm	

3.6. Water Quality

The results of water quality parameter measurements in the study include dissolved oxygen (DO), salinity, ammonia, temperature, and pH, as presented in Table 1. The salinity value in this study was 0 ppt, which is far from the optimal salinity for shrimp. The optimum salinity level for vannamei shrimp cultivation ranges from 15 to 25 ppt. Vannamei shrimp is known as an extreme euryhaline organism capable of surviving in a wide range of salinity. This is consistent with the statement by Bray et al. (1994) as cited in Kaligis (2010) that vannamei shrimp is euryhaline, meaning it can live in a wide range of salinity, approximately 0.5-40 ppt. This euryhaline characteristic is one of its advantages. In low salinity, mineral availability is limited, but the addition of calcium in the form of CaO can enhance water mineral content and increase shrimp molting frequency. Salinity is one of the water quality parameters that significantly affect shrimp molting due to its relationship with osmoregulation processes in shrimp. Suboptimal salinity levels have a considerable impact on the osmotic pressure of water, which is influenced by the dissolved electrolytes. Osmotic pressure is closely related to osmoregulation, as it is the osmotic pressure of water that drives the osmoregulatory processes. High salinity levels result in a higher concentration of electrolytes, leading to increased osmotic pressure ('Aisyah et al., 2017).

pH, as a physicochemical property of water, is an important parameter to be examined during the study because it controls the rate and type of reactions of various substances in water. During the 45-day rearing period, the pH remained relatively stable and within the optimal range for vannamei shrimp cultivation, which is 7.9 for seawater treatment and 8.0-8.3 for freshwater treatment. According to Erlando (2015), a pH range of 7.5-8.5 is considered optimal for vannamei shrimp cultivation. The addition of calcium oxide to the culture medium reacts with H+ ions, resulting in an increase in pH. This occurs because calcium oxide has a neutralizing effect on the acidity of

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the culture medium (Roy & Boyd, 2006). This is evidenced by the difference in pH between the treatment without calcium oxide (CaO) and the treatment with CaO. The stable pH values observed in each treatment during the 45-day period can be attributed to regular siphoning activities (removal of shrimp waste and leftover feed) conducted every morning at 6 o'clock, preventing the accumulation of shrimp feces or decaying feed that could cause an increase in pH. Yunus (2020) reported that the decomposition of accumulated media waste (feces and leftover feed) is a major cause of pH increase in the rearing system, which can be detrimental to shrimp if the pH rises to extreme levels.

Temperature in the culture medium significantly affects the condition of vannamei shrimp, particularly their survival rate and growth. The optimal range for the life and growth of vannamei shrimp is reported to be between 28°C and 30°C (Yulihartini et al., 2016). Bahri (2020) also reported that temperatures below 23°C and above 30°C can result in slower growth and a decrease in growth rate for vannamei shrimp. Temperature influences several aspects of vannamei shrimp's life, including metabolism, molting, growth, survival rate, reproduction, and behavior. The temperature range in the culture medium during the experiment varied from 27.97°C to 28.08°C, indicating that the temperature in the study exhibited fluctuations that were not detrimental to the shrimp. Under optimal temperature conditions, the metabolic processes in shrimp occur at a faster rate, leading to an increased demand for oxygen. However, when the temperature deviates from the ideal range, it affects the shrimp's appetite, causing a decrease in their feeding activity (Sawito, 2019). The increase in temperature in the culture medium is usually caused by the shrimp's own metabolic activity and the rising room temperature. Conversely, the decrease in temperature in the research medium is due to a decrease in room temperature and excessive dissolved oxygen caused by overly strong aeration. Zonneveld et al. (1991) as cited in Budiardi (2005) reported that during the process of food metabolism, the chemical energy obtained from the ingested feed is transformed into ATPase, while the remaining catabolic process is lost as heat.

Dissolved oxygen (DO) is another important physicochemical parameter of water that needs to be examined in this study. The metabolic processes in the aquatic environment are closely related to the level of dissolved oxygen in the medium. Vannamei shrimp have varying requirements for dissolved oxygen, depending on their size and activity level. A low concentration of dissolved oxygen in the culture medium can lead to a decrease in shrimp's feeding activity, indirectly affecting their growth. The range of dissolved oxygen during the study was 6.9-8.0 mg/L, which falls within the optimal range for vannamei shrimp in terms of survival and growth. According to Bahri (2020), a dissolved oxygen range of 4-8 mg/L is considered suitable for shrimp's life. The high dissolved oxygen values observed in the study were due to the strong aeration system added to the research setup. This was done with a dual purpose: to increase the oxygen supply in the medium and, specifically for this study, to help dissolve the calcium CaO, which might not have fully dissolved in the solvent medium.

The ammonia content in the culture containers ranged from 0.02 to 0.06 mg/L, which falls within the range of ammonia levels considered suitable for the cultured biota. This is consistent with Anna's (2010) statement that ammonia values below 0.1 mg/L are within the tolerable range for vannamei shrimp. Ammonia in the culture medium is primarily a byproduct of shrimp excretion and serves as the main nitrogenous waste material. This waste material exists in two forms: ionized form (ammonium) and non-ionized form (ammonia).

Based on the research findings regarding the calcium (Ca) concentration in the culture medium of vannamei shrimp, it can be observed that the highest calcium content in the freshwater treatment is seen in Treatment E (+120 ppm calcium CaO) at 23.08 ppm, followed by Treatment D (+80 ppm

calcium CaO) at 2157 ppm, Treatment C (+40 ppm calcium CaO) at 19.42 ppm, and the lowest calcium content is observed in Treatment B (0 ppm calcium CaO) with a calcium level of 12.47 ppm, while the seawater calcium treatment A is at 400 ppm. The high calcium content in Treatments D, E, and C is positively correlated with the growth rate of vannamei shrimp in those treatments. The addition of calcium oxide to the culture medium has a significant impact on the growth rate of vannamei shrimp during the 45-day culture period, as the added calcium mineral functions in the formation of exoskeletons and cuticles. Kaligis (2009) states that calcium plays several roles in the shrimp's body, including being an essential factor in structural tissues, blood clotting, nerve transmission, osmoregulation processes, muscle contraction, and as a cofactor in enzymatic processes are also reported by David et al. (1991). Among the approximately 20 types of minerals, calcium and phosphorus play vital roles in the shrimp's body (Akiyama et al., 1991).

4. Conclusion

Based on the research findings, it can be concluded that the addition of calcium CaO to the culture medium enhances the growth and survival of vannamei shrimp cultured in 0 ppt salinity. The optimal dosage in this study was found to be 120 ppm CaO for growth and 80 ppm for the survival rate of vannamei shrimp reared in 0 ppt salinity media.

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