Growth Performance and Osmoregulation of Vaname Shrimp (*Litopenaeus Vannamei*) with Cultivation Media Engineering

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Abstract*— Production of white shrimp (*Litopenaeus vannamei*) in 2024 is targeted by the Ministry of Fisheries and Marine Affairs to be increased by producing 2 million tons / year. However, in fact, in 2022 white shrimp production is 1.09 million tons, so to achieve the target, new technology and innovation are needed. The obstacle experienced with freshwater cultivation is the low mineral that plays an important role in the growth of white shrimp. To be able to cultivate white shrimp in fresh water, it is necessary to first conduct a study on the manipulation of aquaculture water with freshwater, salt and bittern components as mineral sources. The purpose of this study is to test the combination of components of artificial water media for white shrimp farming and analyze the mineral content. The study will be conducted from March to April 2024. The method used is an experimental method with Completely Randomized Design (CRD). Data collection is carried out by field observation and literature study. The research procedures carried out were bittern and salt collection and white shrimp sampling, media preparation, mineral test on water as culture media, white shrimp stocking, white shrimp rearing, morphology and survival rate tests and viscosity tests to review osmoregulation in white shrimp and data analysis. Data analysis was performed using Canonical Correlation Analysis (CCA) with PAST 4.03 software. The mineral content produced is directly proportional to the amount of dose given. The best dose of bittern in this study is in treatment C with a dose of 15 ml which has a calcium content of 45.2 mg/L, magnesium 155 mg/L, and potassium 73.89 mg/L.

Keywords— *Bittern, Cultivation, Vaname Shrimp, Mineral, Growth, Osmoregulation.*

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I. INTRODUCTION

Vaname shrimp (*Litopenaeus vannamei*) is one of the most farmed shrimp species and has promising prospects. Vaname shrimp has become Indonesia's leading commodity in the fisheries sector. In 2022, shrimp became the largest fishery export commodity, reaching USD 2.1 billion. This is in line with the KKP's 2024 programme to produce 2 million tonnes per year, but in reality, only 1.09 million tonnes were achieved in 2022. The obstacles encountered are related to the aquaculture media used. Vaname shrimp farming is generally carried out in brackish water ponds using seawater as the water source, while in areas far from the sea, this is rarely or never done [1]. There is still a lot of freshwater land available, but its use as a farming medium is still constrained by the low mineral content in freshwater, which plays an important role in the growth of vaname shrimp.

Low mineral content in shrimp culture media can cause mineral deficiency, which affects growth rate and osmoregulation rate, thereby increasing osmotic load, oxygen consumption, and stress levels. The minerals that play an important role in the growth rate of vaname shrimp are calcium (Ca), magnesium (Mg), and potassium (K) [3]. Calcium plays a role in carapace growth, which is absorbed through the gills. Mg minerals function to maintain metabolism and osmotic pressure balance in the shrimp's body. Potassium (K) plays a role in maintaining constant haemolymph conditions during salinity fluctuations and regulating the electrolyte balance of body fluids [1]. Although it is known that vaname shrimp can live in a wide range of salinity, the metabolic implications of these conditions are not yet well understood. It is known that the optimal growth medium for vaname shrimp in brackish water or seawater is at a salinity of 25-30 ppt [2].

The use of seawater and brackish water in household aquaculture is certainly inefficient, therefore it is necessary to conduct a study on the manipulation of water media for vaname shrimp aquaculture by combining several components, namely fresh water, bittern (water left over from salt production), and rock salt. The objectives of this study include testing combinations of components in artificial water media for vaname shrimp farming at different doses, analysing the level of osmosis in the farming media and vaname shrimp bodies, and analysing comparisons in the addition of minerals in pre-farming and post-farming media for vaname shrimp.

II. METHOD

A. Research Method

This research was conducted using an experimental method with direct testing and observation of the effects of growth and ormoregulation rates of vaname shrimp whose cultivation media were manipulated using a combination of fresh water, bittern and rock salt. The design used was a completely randomised design (CRD). This study was conducted with 4 treatments, 1 control, and 5 replicates. The combinations of bittern (BT), rock

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salt (GK), and fresh water (AT) doses in each treatment are presented in (Table 1).

TABLE 1.
COMBINATION TREATMENT

Treatment	Repetition				
	1	2	3	4	5
A	A1	A2	A3	A4	A5
B	B1	B2	B3	B4	B5
C	C1	C2	C3	C4	C5
D	D1	D2	D3	D4	D5
K (+)	K (+)1	K (+)2	K (+)3	K (+)4	K (+)5
K (-)	K (-)1	K (-)2	K (-)3	K (-)4	K (-)5

Note: fresh water (FW), bittern (BT), rock salt (RS)
Description:

A : 10 L FW + 35 ml BT + 50 gr RS
B : 10 L FW + 25 ml BT + 50 gr RS
C : 10 L FW + 15 ml BT + 50 gr RS
D : 10 L FW + 5 ml of BT + 50 gr RS
K (+) : 10 L seawater
K (-) : 10 L FW

B. Time and Place

The research was conducted at the Freshwater Fisheries Laboratory of Brawijaya University and the Water Quality Testing Laboratory of Perum Jasa Tirta in March-April 2024.

C. Research Data Analysis

The data collection techniques used in this study were observation for primary data collection and secondary data collection. Primary data collection was carried out by directly documenting the conditions in the field and supported by secondary data in the form of previous research journals, books and articles

D. Research Procedures

1. Collection of Bittern and Rosck Salt

The bittern and rock salt used in this study were sourced from salt ponds in Pademawu Village, Pamekasan Regency, East Java. Bittern is generally not used or is considered waste from the salt production process. Bittern collection was carried out before the salt harvesting process. The brine was collected directly from the surface of the salt ponds using a dipper and stored in mineral water gallons. The salt harvesting process involved removing the remaining brine from the ponds. The salt was then collected at one point using a wooden tool commonly used by salt farmers. Once collected at one point, the salt was scooped up using a shovel and placed in sacks.

2. Acclimatisation of vaname shrimp fry

The initial process involves preparing the container and medium for acclimatising the fry. The container used is a 100-litre aquarium. The container is cleaned with soap and then rinsed with water. The next step is to prepare the medium with a salinity of 5 ppt using fresh water, bittern and rock salt. Once the medium is ready, the fry are taken from the hatchery location, namely CV. Barakka Situbondo, East Java, at the PL 14 stage. After that, the fry are acclimatised in the prepared container for 7 days to reduce stress and mortality rates.

3. Preparation of research containers

The research containers used were 24-litre jars that had been cleaned beforehand, filled with water, and labelled with treatment codes. Next, an aeration system was installed using PVC pipes, hoses, and aeration stones to supply oxygen. The bottom of the container was lined with foam, and the top was covered with a tarpaulin to maintain a stable temperature.

4. Preparation of research media

The research container that is ready for use is filled with 10 litres of fresh water from a borehole, then bittern is added according to the treatment dosage and left for 24 hours. After being left for 24 hours, 50 grams of rock salt is added to each treatment and left for 24 hours to become homogeneous. The next step is to add probiotics at a dose of 5 ppm.

5. Mineral content test

This test is carried out to determine the macro mineral content in the research media. The minerals to be tested are calcium (Ca), magnesium (Mg), and potassium (K). The test is carried out by sending samples to PT. Jasa Tirta. This test was conducted before the fry were spread. One litre of water was taken and placed in a cleaned bottle and coded according to the treatment performed.

6. Spreading of vaname shrimp fry

The larvae released were acclimatised larvae. Before the larvae were released, their initial weight and length were measured, and then the larvae were placed in prepared containers. Larvae release was carried out in the afternoon at a density of 5 larvae per treatment.

7. Feed management

Feeding is carried out using the blind feeding method from start to finish, based on the results of previous shrimp sampling. The feed used is in powder form and has a protein content of 40%. Feeding is carried out four times a day. Feeding times are at 07:00, 10:00, 14:00 and 17:00 WIB.

8. Water quality management

Water quality is monitored regularly by measuring water quality, skimming, adding water, and administering probiotics. Water quality aspects that are measured regularly in the morning before feeding and in the afternoon include temperature, pH, dissolved oxygen (DO), and salinity. Water siphoning is performed daily in the morning using a small hose before feeding, amounting to 10% of the total water volume, to reduce leftover feed and shrimp waste. Water addition is performed every three days to maintain water quality.

9. Harvesting

Harvesting was carried out at the final stage of the study and included morphological testing, survival rate testing, mineral content testing, and viscosity testing. Morphological testing was carried out by measuring the final weight using analytical scales with an accuracy of 0.1 grams and measuring the final length using calipers. The survival rate was calculated by counting the number of vaname shrimp remaining at the end of the cultivation period. Mineral content testing is performed using the APHA3120B method with a spectrophotometer. The viscosity test was conducted to determine the free water content in the vaname shrimp's body to find out the osmolarity level of the vaname shrimp. The viscosity testing stage began by weighing 2.5 grams of harvested shrimp, then grinding them using a mortar and pestle, adding 5 ml of distilled water, and stirring evenly. A 1.5 mL sample of the liquid was taken using a micropipette and placed in a tube for centrifugation for 15 minutes at a speed of 1200 rpm and a room temperature of 29 °C. Next, the viscosity of the supernatant was checked using a refractometer. The data collection techniques used in this study were observation for primary data collection and secondary data collection. Primary data collection was carried out by directly documenting the conditions in the field and supported by secondary data in the form of previous research journals, books and articles

E. Data Analysis Method

The data obtained was analysed using analysis of variance (ANOVA) with the help of Microsoft Office Excel. The aspects tested included normality tests, followed by the Least Significant Difference (LSD) test if the data analysis results showed significant differences in each dose to determine the effect of adding different amounts of bittern and rock salt to freshwater on the growth rate of vaname shrimp. The next step was to analyse the data using the Canonical Correspondence Analysis (CCA) method. This was done by combining all the data to determine the factors that influence the growth, survival rate, osmoregulation, and viscosity of vaname shrimp. The CCA analysis was carried out using the PAST 4.03 application.

III. RESULTS AND DISCUSSION

A. Mineral Content

The mineral content was observed at the beginning and end of the study, as illustrated in (Figure 1).

The highest increase in calcium levels was observed in the K+ treatment at 347 mg/L, followed by treatment D at 281 mg/L, treatment C at 263 mg/L, treatment B at 216 mg/L, and the lowest in treatment A at 216 mg/L. The K+ treatment had the highest value because it was seawater that matched the shrimp's natural habitat. When looking at the treatments that were most effective for shrimp growth, treatments C and D had the highest weight. The bitter dosage in treatments C and D was able to produce results close to those of the seawater control. The calcium content required by shrimp is generally in the range of 40-60 mg/L to support growth, exoskeleton formation, and molting (4). When calcium requirements are not met, the hardening process of the shrimp carapace will be inhibited [5]. [6] stated that the calcium minerals required by vaname shrimp can also be obtained from the feed provided.

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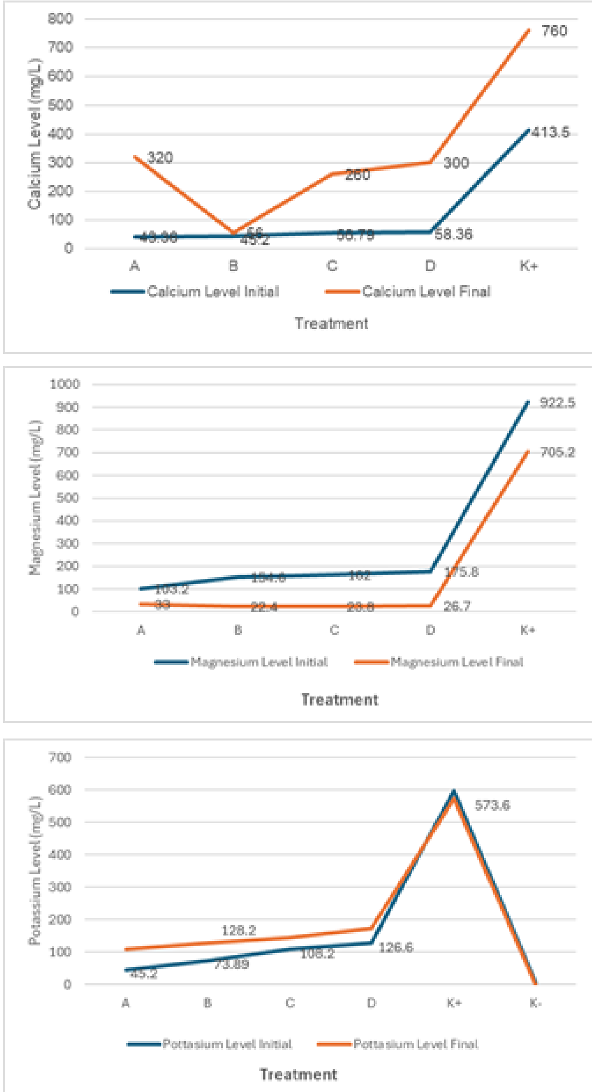

Figure 1. Mineral level

The highest increase in magnesium levels was observed in the K+ treatment at 217 mg/L, followed by the D treatment at 149 mg/L, the C treatment at 138 mg/L, the C treatment at 132 mg/L, and the A treatment at 70 mg/L. [7] stated in their study that vaname shrimp can survive optimally at a magnesium mineral concentration of 140-180 mg/L in freshwater culture media. This concentration meets the requirements for vaname shrimp growth in terms of metabolism and osmoregulation processes. [8] also stated that if crustaceans, including vaname shrimp, live in an environment with mineral levels that meet their requirements, they can survive well.

The highest potassium level was found in treatment A at 64 mg/L, followed by treatment B at 54 mg/L, treatment D at 46 mg/L, treatment C at 37 mg/L, and treatment K+ at 24 mg/L. Potassium minerals in the rearing medium are essential for shrimp to maintain their physiological condition at low salinity [9]. Potassium minerals regulate osmoregulation of Na+/K+-ATPase enzyme activity during fluctuations in low salinity conditions [10]. [11] stated in their study that by adding 50–100 mg/L of potassium mineral under low salinity conditions with a value of 5 ppt, shrimp were able to grow optimally.

B. Mean Body Weight (MBW)

The MBW data for vaname shrimp after a 30-day cultivation period is presented in the (Table 1) below.

TABLE 1.
MEAN BODY WEIGHT

Treatment	Repetition (gr)					Average (gr)
	1	2	3	4	5	
A	2.27	2.56	2.28	2.56	2.56	2.44
B	2.98	3.45	2.97	2.76	2.91	3.01
C	3.91	3.68	3.02	3.68	3.36	3.53
D	3.27	3.67	3.57	2.94	2.68	3.23
K+	3.86	3.27	3.30	4.09	3.42	3.59
K-	0.00	0.00	0.00	0.00	0.00	0.00

Based on the table, MBW values ranged from 2.44 to 3.59 grams. The highest MBW was found in K+ with a value of 3.59 grams, followed by treatment C with a value of 3.53 grams, while the lowest was in treatment A with a value of 2.44 grams. The ANOVA results show that all treatments applied are significantly different, where Fcount is 106.84, which is greater than Ftable (5%) of 2.62. Next, a BNT test was conducted with the result that treatment A is significantly different from the other treatments. Treatments B and D are not significantly different. Treatments D and C were not significantly different but were significantly different from treatment K+. Treatments C and K+ were not significantly different. The growth of vaname shrimp with the addition of bittern in treatment C had conditions close to the natural habitat of vaname shrimp, namely treatment K+. This was thought to be due to the addition of minerals contained in 15 ml of bittern, which was able to support the growth of vaname shrimp. With this dose of bittern, the minerals produced in treatment C showed balanced levels, namely calcium 155 mg/L, magnesium 92.9 mg/L, and potassium 110.8 mg/L. Research conducted by [12] proved that the addition of calcium ($CaCO_3$) minerals to freshwater can support the growth of vaname shrimp. The addition of calcium (Ca) minerals to freshwater media for vaname shrimp cultivation can increase growth and maintain molting of vaname shrimp during the maintenance period. [13] stated that the addition of potassium minerals is very influential in the growth of vaname shrimp in order to maintain electrolyte balance, nerve impulse conduction, and release energy from proteins, fats, and carbohydrates. state that magnesium (Mg) minerals can play a role in maintaining osmotic pressure balance and regulating the pH of blood, haemolymph, urine, and body fluids during growth and development. [14] stated that low mineral content in vaname shrimp culture media can cause shrimp to be unable to survive and lead to high levels of cannibalism.

C. Average Daily Growth (ADG)

The Average Daily Growth (ADG) value is calculated to determine the daily growth rate of shrimp during the rearing period by calculating the average weight of shrimp divided by the rearing period. The ADG data for vaname shrimp is described in the (Table 2) below.

TABLE 2.
AVERAGE DAILY GROWTH

Treatment	Repetition (gr/day)					Average (gr/day)
	1	2	3	4	5	
A	0.07	0.08	0.07	0.08	0.08	0.08
B	0.10	0.11	0.10	0.09	0.09	0.10
C	0.13	0.12	0.10	0.12	0.11	0.11
D	0.11	0.12	0.12	0.09	0.09	0.10
K+	0.13	0.11	0.11	0.13	0.11	0.12
K-	0.00	0.00	0.00	0.00	0.00	0.00

The ADG values obtained from all treatments ranged from 0.08 to 0.12 grams/day. The highest ADG value was found in the K+ treatment with a value of 0.12 grams/day, followed by the C treatment with a value of 0.11 grams/day. The lowest ADG value was found in the A treatment with a value of 0.08 grams/day. The ANOVA test results showed that the Fcount of 100.80 was greater than the Ftable (5%) of 2.62, indicating that the administration of bittern at different doses had a significant effect on the daily growth rate of vaname shrimp. The BNT test results showed that treatments K- and A were significantly different from the other treatments, while treatments C, D, and K+ were not significantly different. Treatment C produced the best results for average daily growth, in line with the MBW value. This was due to the combination of the main minerals calcium (155 mg/L), magnesium (92.9 mg/L), and potassium (110.8 mg/L), which were within the optimal range for the daily growth of vaname shrimp. [15] stated that the addition of Ca, K, and Mg minerals is essential for crustaceans, including shrimp, to support basal metabolism and growth. [16] state that sufficient calcium mineral requirements will reduce osmotic performance load in shrimp, enabling them to redirect energy that would otherwise be used to maintain osmotic pressure towards growth. According to [6], calcium minerals added to the cultivation medium. [16] stated that adequate calcium mineral requirements will reduce the osmotic performance load on shrimp, enabling them to allocate energy that would otherwise be used to maintain osmotic pressure to growth. According to [6], calcium minerals added to the cultivation medium can accelerate mineralisation in shrimp, thereby accelerating their growth rate.

D. Survival Rate (SR)

The survival rate is a parameter that indicates the percentage of individuals remaining in the cultivation process. Survival rate data is presented in (Table 3) below.

TABLE 3.
SURVIVAL RATE

Treatment	Repetition (%)					Average (%)
	1	2	3	4	5	
A	100	80	80	80	80	84
B	80	80	100	100	100	92
C	80	80	100	80	80	84
D	80	80	100	80	80	84
K+	100	80	80	100	100	92
K-	0	0	0	0	0	0

Based on the table above, the SR value range obtained is 82-92%. The highest SR is found in treatment K+, followed by treatment B with a value of 92%, followed by treatments A, C, and D with a value of 84%. The ANOVA test results show that the Fcount of 80.17 is greater than the Ftable (5%) of 2.62, indicating that all treatments applied are significantly different. This indicates that the administration of bittern at different doses has a significant effect on the survival rate of vaname shrimp. The BNT test results showed that treatments A, B, C, D, and K+ were not significantly different. This indicates that administering different doses of bittern does not affect the SR level in vaname shrimp. In treatment A, the higher dose of bittern may have resulted in an excess of minerals. Excess minerals will disrupt the molting process in vaname shrimp, which can lead to cannibalism. [17] stated that excessive calcium mineral content will cause shrimp to experience difficulties in the calcium ion homeostasis process and will inhibit calcium transfer to the body from the environment. [4] stated that low calcium levels can cause death because the shrimp are unable to harden their carapaces, causing them to weaken and eventually die. The growth of vaname shrimp with the addition of bittern in treatment B, with calcium content of 175 mg/L, potassium of 114.2 mg/L, and magnesium of 88.5 mg/L, had the same value as treatment K+, which is the natural habitat of vaname shrimp. The medium in treatment K+ is seawater with a salinity of 35 ppt. This is in accordance with the statement by [18], where vaname shrimp can grow optimally at a salinity of 20-35 ppt with an SR value of more than 85%. The addition of bittern to freshwater used as a medium for vaname shrimp proves to be feasible because bittern supplementation can meet the mineral requirements needed by vaname shrimp to facilitate the hardening process of their shells during molting. [19] stated that the molting and carapace hardening processes can be accelerated if the calcium and magnesium mineral content in the water is sufficient. [11] stated that the addition of potassium to the vaname shrimp cultivation medium can maintain high salinity fluctuations and reduce the osmotic load on vaname shrimp

E. Free Water Content in Vaname Shrimp

Free water content relates to the portion of water that can move relatively easily within cells and between tissues, in contrast to bound water, which is bound to biomolecular molecules such as proteins and carbohydrates [20]. Free water content is illustrated in the graph below.

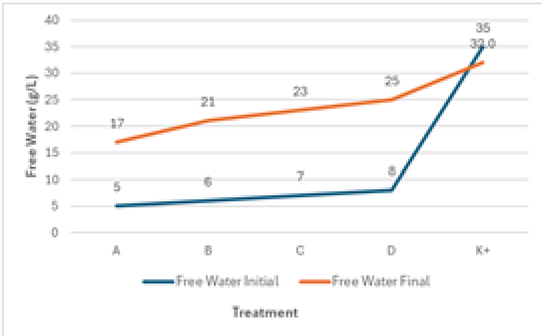

Figure 2. Free Water

The highest increase in free water content was found in treatment D at 17 g/L, followed by treatment C at 16 g/L, treatment B at 12 g/L, and treatment K+ at 3 g/L. Free water functions to maintain tissue moisture, support enzymatic function, and maintain homeostasis in shrimp [21]. The ANOVA test results showed that Fcount was 78.06, which was higher than Ftable (5%) of 2.87, indicating that the administration of bittern at different doses had a significant effect on the free water content of vaname shrimp. The BNT test results showed that treatment K+ was significantly different from treatments A, B, C, and D. Treatment K+ yielded the best results in this study. This indicates that osmoregulation in vaname shrimp is isoosmotic, thereby optimising the metabolism and growth of vaname shrimp. Isoosmotic is a condition where the concentration of body fluids is the same or close to the concentration of the medium [22], [23] stated in their research that the optimal balance between the medium and free water content in vaname shrimp ranges from 25-30 ppt. [24]; [25], in their research stated that minerals such as calcium (Ca), magnesium (Mg) and potassium (K) dissolved by free water will be transported into the body, thereby playing a role in increasing shrimp growth and osmotic performance, assisting in the synthesis of carbohydrates, proteins and fats, and also increasing shrimp metabolism.

F. The Relationship Between Mineral and Growth Parameters

The relationship between mineral content in the cultivation medium and the growth parameters of vaname shrimp is illustrated in the (Figure 3) below.

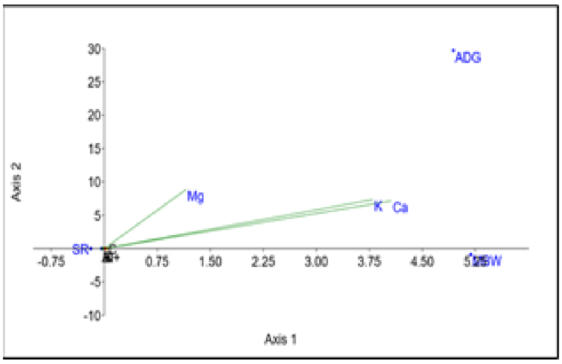

Figure 3. CCA Between Mineral and Growth

The CCA graph shows the relationship between mineral content and growth parameters (MBW, ADG, and SR), namely that mean body weight (MBW) tends to be influenced by high calcium (Ca) mineral content, followed by potassium (K) and magnesium (Mg) mineral content. This is related to the function of calcium in enhancing and accelerating the molting process as well as its role in the physiological processes of vaname shrimp [25]. Average daily growth (ADG) tends to be influenced by high magnesium (Mg) content, followed by potassium (K) and calcium (Ca) mineral content. [3] stated that the presence of magnesium minerals in the vaname shrimp cultivation media will balance the osmotic pressure in the environment and in the shrimp's body, or what is called isoosmotic. The survival rate (SR) tends to be influenced by moderate mineral content. [7] stated that the survival rate of vaname shrimp is influenced by the balance of mineral ions in the environment and those contained in the shrimp's body.

IV. CONCLUSION

The combination of bittern dosage significantly affected the growth performance and osmoregulation of vaname shrimp. Treatment C, with a bittern dosage of 15 mL, provided an optimal mineral balance of calcium (45.2 mg/L), magnesium (155 mg/L), and potassium (73.89 mg/L), thereby supporting the best growth (highest MBW and ADG) compared to other dosages. The osmolality levels in treatments A, B, C, and D were hypoosmotic. Under these conditions, the shrimp utilised the mineral content obtained from the bittern to balance their internal metabolism. The proportional addition of minerals via bittern before and after cultivation increased the main mineral content in the medium and the shrimp's bodies, which improved the growth efficiency and survival rate of vaname shrimp.

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