

Original Research Article

Performance of Whiteleg Shrimp *Litopenaeus vannamei* with Additional of Dolomite to the Feed

ABSTRACT

The objective of this study was to analyze the use dolomite in diet of whiteleg shrimp. It was conducted in June to August 2018 at the hatchery of Pangkep State Polytechnic of Agricultural. Experimental design used a completely randomized analysis with 4 treatments, A (control), B, C, and D with the additional of dolomite at dose 4, 8, and 12 mg/kg, respectively with 3 replications. Animal test were PL 25 from the shrimp hatchery in Barru District and acclimatized before stocking in aquarium (30l), with density of 20 ind., reared for 40 days, fed pellets (30% protein, 10% biomass, 5 times). The molting intensity (MI) determined by observing and counting the number of moulting every day. Observation of growth rate (GR) using a digital scale (0.0001 g) and survival rate (SR) by calculated the number of live shrimps at the end of the study. Water quality parameter included temperature, salinity, DO and pH. Analysis of Variance (ANOVA) was performed to evaluate the effect of treatment on MI, GR, and SR and followed with the Least Significant Difference (LSD) Test. ANOVA for MI, GR, and SR revealed that dosage have different significant ($P < 0.05$) and LSD test showed that the dose was not optimal (only differentiated between those given dolomite). The highest of MI (avg. 18 inds.), GR (1.82% /day), and SR (78.33%) were obtained in C and the lowest one obtained in A. Data of water quality obtained temperatures 29.87-31.03°C, salinity 25-30 ppt, DO 4.86-5.51 mg/l which were still in the proper range. The highest pH value was obtained in D was 7.22-7.75, while the lowest one obtained in A 6.95-7.16. The highest MI, GR, and SR were obtained in C, temperature, salinity, DO, and pH are still in the proper for the growth and survival of shrimps.

Keywords: calsium, moulting, dolomit, growth rate, whiteleg shrimp

1. INTRODUCTION

In Indonesia, whiteleg shrimp is a very important commodity because production from aquaculture has increased every year and currently dominates the total national shrimp production. The Ministry of Marine and Fisheries released data on national shrimp production of 1.21 million tons in 2021 with a value of IDR 79.21 trillion and an increase of 9.20% compared to 2020 of 1.11 million tons with a value of IDR 66.53 trillion. Furthermore, the production target in 2024 is increased to 2 million tons. In terms of exports, Indonesia is included in the top 5 in the world after Ecuador, India and Thailand [1]. Whiteleg shrimp has several advantages compared to other shrimp species. Among these advantages, it can be stocked at high densities because they live in the water column, have rapid growth, and are highly demanded in the global market [2].

In addition to quality feed, shrimp growth is also influenced by the fulfillment of several essential minerals. Calcium is one of mineral that very necessary to supporting shrimp performance. In shrimp, it mainly plays a role in the formation of the skin and carapace,

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especially in the transmission of nerve impulses, osmoregulation, muscle contraction and cofactor in several enzymatic processes [3]. Large amount of calcium must be accumulated from the environment to form the skeleton and consequently all these group have an active calcium metabolism. The requirement for calcium is particularly high in the crustacea as the skeleton is shed regularly to allow increase in body size. Calcium availability differs between these environments. The food again is an additional source of calcium [4].

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Studies on the importance of calcium for shrimp have been carried out including the source of calcium [4], the effect of calcium phosphorus on nutrient retention in tiger shrimp [3], the effect of additional Calcium Oxide (CaO) on the cultivation of whiteleg shrimp [2, 5]. Studies on the addition of calcium to diet are still rare. Therefore, research was conducted on the additional of dolomite (CaMg(CO₃)₂) to artificial feed is expected to increase the supply of calcium which is needed by shrimp for the moulting process, growth, and survival rate.

2. MATERIAL AND METHODS

2.1. Materials

The material used in this study were post larvae PL 22-25 as many as 240 individuals obtained from the shrimp hatchery in Barru District. The container used during this study is an aquarium with a capacity of 30 liters.

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2.2. Experimental Design

Experimental design used a completely randomized design consisting of 4 treatment and 3 replications. The treatment dose used in this study were A without the additional of dolomite (control), B, C, and D with the additional of dolomite 4, 8, and 12 mg per kilograms diet, respectively. Observed variables included molting intensity, growth rate, survival rate, and water quality.

2.3. Research Procedure

The research materials were PL 25 (juvenile) shrimps obtained from the hatchery in Barru District and then acclimatized before stocking in aquarium (volume 30 liters). Shrimp reared for 40 days in an aquarium equipped with aeration with stocking density of 20 individuals in each container. The vannamei shrimp were first acclimatized in a fiber tub for two days. Before stocking one shrimp per liter container (30 liters container is stocked with 20 shrimp). The diet used in this study was commercial shrimp pellets with an amount of 10% of the shrimp body weight with a nutritional content of 30% protein, 5% fat, 4% fiber, 12% minerals and water. The shape of the feed is still round according to the shrimp's mouth opening. The pellets that have been added to the dolomite that will be given are weighed first according to the needs and immediately given to the vannamei shrimp. Rearing of test animals was carried out for 40 days. The frequency of feeding was carried out 5 times with a range of once every 4 hours, starting at 06:00, 10:00, 14:00, 18:00 and 22:00. Sampling was carried out to determine the intensity of molting shrimp, growth and survival of juvenile vannamei shrimp. Measurement or sampling is done by taking 4 test shrimp from each treatment. Sampling was carried out 4 times with an interval of 7 days. Sampling is done in the morning, with the aim that the shrimp are not easily stressed due to temperature changes. Sampling of shrimp in the container by using a scoop. After taking the shrimp sampling, the shrimp are weighed first on an analytical balance.

2.4. Research Parameters

Moulting intensity was determined by counting the molted shrimp every day. Growth Rate determined by using formula:

$$SGR = \frac{\ln W_t - \ln W_0}{t} \times 100\% \dots \dots \dots (1)$$

Where: SGR=specific growth rate (% per day), W_t=final body weight (g), W₀=initial body weight (g), t=rearing period (day)

$$SR = \frac{N_t}{N_0} \times 100\% \dots \dots \dots (2)$$

Where: SR(%), N_t=final population (individuals), N₀=initial population (individuals)

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Observation of growth using a digital scale of 0.0001 g and survival rate was calculated based on the number of live shrimps at the end of the study. Monitoring of water quality using a hand refractometer, thermometer, pH meter and DO meter.

2.5. Data Analysis

Analysis of variance (ANOVA) was performed to evaluate the effect of treatment on molting intensity, daily growth rate, and survival rate and followed with the Least Significant Difference Test (LSD) if the effect of treatments is significantly different.

3. RESULTS AND DISCUSSION

3.1. Moulting Intensity

The intensity of molting shrimp indicated the number of shrimp molting during the study which can be seen in the Table 1.

Table 1. Moulting Intensity of whiteleg shrimp (individuals)

Replication	A	B	C	D
1	13	14	19	18
2	10	16	16	16
3	12	16	20	17
Total	35	42	49	47
Average	13	15	18	17

Table 1 show that period of molting and total number of molting are different one each other. On the first day of rearing no molted shrimp were found, and on the second day only two molted shrimp were found in treatment C and thereafter molted shrimp were found every day until the end of rearing (DOC 40). During maintenance, molting shrimp were found every day and the highest number of molted shrimp was obtained 5 on they 13th. Unlike the case with treatment C, in treatments A, B, and D it was found that there were several days when no shrimp molted at all. For treatment B, the highest number of molting shrimp was 4 obtained on the 18th day, then in treatment D there were 4 on the 17th day, and in treatment A (control), as many as 4 individuals were obtained on the 21st day. Based on analysis of varians reveals that the treatment of adding dolomite with different doses to the feed has a significant effect on the intensity of molting in vannamei shrimp ($P < .05$). The results of the

LSD test reveals that treatment A was not different from treatment B and D ($P > .05$) but significantly different from treatment C ($P < .05$). Treatment C was significantly different from treatment A ($P < .05$) but not different from treatment B and D. Based on this, it shows that the dose of dolomite tested has not reached the optimal dose because the different doses of dolomite give the same response in treatments B, C, and D and are significantly different from A (control/without the addition of dolomite). The highest molting intensity was obtained at a dose of 8 mg kg of feed (C), which was an average of 18 ind.

However, the results of this study showed that the addition of dolomite to diet was effective in increasing the intensity of molting shrimp, especially in accelerating the molting process. The higher dose of dolomite given, the shorter time to reach molting and the greater the number of molted shrimps. Erlando et al. (2015) stated, 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 [6]. However, excessively high calcium levels in the water are also detrimental as high potassium levels can disrupt calcium ion homeostasis. The molting of crustaceans is determined by age, food and environmental factors. Crustaceans that are receiving optimal nutrition and environmental calcium levels will experience more frequent molting [7]. The addition of calcium will increase the calcium content in the water, where calcium is useful in the formation and hardening of new shrimp shells. Adequate calcium availability will make the shrimp molting process run smoothly and quickly. The occurrence of the molting process in crustaceans is controlled by organ X/sinus gland complex located on the eye stalk, which secretes molt inhibiting hormone (MIH), which is a neuropeptide that inhibits ecdysteroid production by Organ Y which is located in the cephalothorax and plays a role in controlling the process of molting and embryo development [8]. The use of calcium Ca(OH) as much as 30 mg/L was able to accelerate the rate of increase in skin calcium levels. A faster rate of increase in shell calcium levels has implications for increased feed consumption and has further consequences for increasing shrimp growth [5]. Moulting requires energy and involves a net loss of protein, lipid, carbohydrate and minerals. For example, *L. vannamei* required a minimal of 0.36% of its consumed energy for moulting during the trial period and this energy demand increased with higher moulting frequencies [9]. For hardening shrimp shells, high enough calcium is needed and in an environment with sufficient calcium levels, the mineralization process in shrimp shells is completed for 3 days [10].

3.2. Growth Rate

Shrimp growth is closely related to the molting process, where the more frequent and rapid the molting, the faster the shrimp growth. Shrimp can grow well if the molting process goes well and consumes enough feed [11]. The shrimp will undergo several molting processes to adjust to their body size. Growth is achieved in a stepwise fashion, whereby moulting or ecdysis occurs, followed by the rapid intake of water to enlarge the new cuticle before hardening and remineralization occur. Evaluation of the growth rate is carried out which is presented in Table 2.

Table 2. Growth Rate of Whiteleg Shrimp (% per day)

Replication	A	B	C	D
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1	1.350	1.370	1.830	1.470
2	1.010	1.240	1.720	1.580
3	1.190	1.330	1.910	1.220
Average	1.183	1.313	1.82	1.423

Based on Table. 2 it was found that the highest average weight growth rate was 1.820% obtained in treatment C and the lowest was in treatment A (control) of 1.183%. The results of the analysis of variance showed that differences in dolomite doses in feed had a very significant effect on the growth rate of vannamei shrimp weight ($P < .01$). The results of the LSD Test showed that treatment A (control) was significantly different from treatment C ($P < 0.05$) and did not differ from treatment B ($P > 0.05$) and D ($P > 0.05$). Treatment B was also significantly different from C ($P < 0.05$) and not different from A and D ($P > .05$). Treatment C was significantly different from treatment A, B, and D ($P < 0.05$). The daily growth rate of whiteleg shrimp reached 1,57% per hari at a dose of 3 mg/kg diet [12].

The results of this study explained that the response to a different growth rate was obtained in treatment C to treatments A, B, and D, which means that the best dose of calcium to support growth rate is 8 mg/kg of feed. A lower dose of calcium in the feed affects the growth rate where the resulting growth is lower than with a higher dose to a certain degree. According to Adegboye, low calcium levels will make it difficult for shell formation [13]. Meanwhile, high calcium levels also complicate the homeostatic process of calcium ions. Hypoionic or hyperionic conditions of the body's calcium will make it difficult for the body's calcium ion balance with the environment so that the energy for the continuation of this process will be greater. Therefore, the use of energy for growth will be hampered.

Research on the effect of calcium on growth was also conducted by Aisyah et al. [14], 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. [15] also presented research findings on the role of calcium in growth that a daily growth increase of 0.31% with the addition of 60 mg/L of calcium (hydroxide).

3.3. Survival Rate (SR)

Survival rate (SR) is the percentage of the population of organisms that live for each particular maintenance period. The survival rate of vannamei shrimp juveniles during the rearing period it presented in Table 3.

Tabel 3. Survival Rate of Juvenil UdangVaname (%)

Replication	A	B	C	D
1	65.00	55.00	80.00	75.00
2	50.00	65.00	75.00	65.00
3	60.00	70.00	80.00	60.00
Average	58.333	63.333	78.333	66.667

Based on Table 3, it was found that the highest SR was 78.33% in treatment C and the lowest in treatment A (58.33%). The results of the analysis of variance showed that the dose of dolomite in the diet had a significant effect on the survival of juvenile whiteleg shrimp

($P < .05$). The results of the LSD test showed that treatment C was significantly different from treatment A and B ($P < .05$) but not different from treatment D ($P > 0.05$). The results of this study indicate that the addition of dolomite to feed not only affects the intensity of molting and growth but also the survival rate of juvenile whiteleg shrimp. The survival of shrimp is greatly affected by molting because the shrimp's body will be very weak after molting. Therefore, if the amount of calcium in the environment is low at the time of molting it will interfere with the process of forming a new carapace for the shrimp so that it has the potential to cause death in shrimp. Anggoro[16] explained that, the molting process which is not the same in shrimp will cause cannibalism of molting shrimp because their physical condition is very weak. The high SR is influenced by several factors, including the nutritional needs in the feed given have been fulfilled and cause the shrimp to grow optimally, it is given on time and the dose very sufficient so it can affect the survival rate of the shrimp. Vannamei shrimp require feed with a protein content of at least 35% of the total nutrients contained in the feed. Feed is a factor that dominantly influences the growth of aquatic biota (fish and crustaceans) because feed serves as a supplier of energy to stimulate growth and maintain survival.

3.4. Water Quality

Water quality is an important indicator in determining the success of aquatic organisms including shrimp. One of the keys to success in shrimp farming is maintaining good water quality and reducing contamination [17]. In the following, some water quality parameters obtained during the study are presented in Table 4.

Table 4. Range of Water Quality Parameters during the Research

Parameter	Treatment			
	A	B	C	D
Temperature (°C)	29,98-31,03	30,11-30,18	30,02-31,00	29,87-30,61
Salinity (ppt)	26.00-28.00	26.00-28.00	26.00-28.00	26.00-28.00
DO (mg/l)	4.86-5.33	5.05-5.21	5.01-5.11	5.12-5.51
pH	6.95-7.16	7.08-7.43	7.32-7.65	7.22-7.75

Temperature

Water temperature is one of the main variables affecting shrimp growth. Water temperature greatly affects the growth, activity, and appetite of white shrimp [18]. Water temperatures below 20°C will inhibit the growth of white shrimp. Temperature is also very much needed by white shrimp during spawning to maintain the survival of larvae, embryo development and hatching of eggs. Temperature greatly influences oxygen consumption, growth, survival of shrimp in aquaculture environments [19]. The temperature range obtained in this study (Table 4) for each treatment ranged from 29.87-31.03°C, meaning that it is still in the optimal category to support the growth and survival of vannamei shrimp. The best growth of vannamei juvenile obtained between temperature ranges from 25-35°C. Temperature has been shown to influence the sensitivity of sensory systems, including vision, hearing, and olfaction/taste, likely affecting feeding behavior [20].

Salinity

Shrimp will grow better in waters with a salinity range of 15-30 ppt and even up to 5 ppt [21] (Whetstone et al 1992). Salinity ranged from 25-28 ppt obtained the same in each treatment during this study (Table 4.). Salinity that is too high can cause the shrimp growth rate to decrease [22]. McGraw & Scarpa that vaname shrimp can live in the range of 0.5-45 ppt [23]. Furthermore, according to Ponce-Palafox et al. [20], vannamei shrimp reached best growth at salinities above 20 ppt.

Dissolved Oxygen (DO)

Dissolved oxygen (DO) is also an important water quality parameter. Low concentrations have a negative impact on health which includes anorexia, respiratory stress, tissue hypoxia, weakness, and even mass death in a short time. If the DO level is 4 mg/l in the pond, the shrimp will continue to eat but the food is not utilized efficiently and the rest accumulates at the bottom of the pond. This triggers the growth of disease-causing agents. If the concentration drops to the level of 2-3 mg/l, the shrimp will stop eating and become weak, and then die if the level is <2 mgL⁻¹ [24]. The results of DO measurements during the study ranged from 4.86-5.51 mg/l, meaning that they were still in optimal conditions for growth and survival.

pH

The degree of acidity or pH is an indicator of the acidity and alkalinity of water. The pH value is an important factor because it can affect the process and speed of chemical reactions in water and in shrimp embryos/eggs. Shrimp eggs have a low tolerance to high pH. Water pH also plays a role in supporting shrimp growth. The pH value was found to be different in each treatment where the higher the dolomite content given to the feed, the higher the pH level of the water obtained. The highest pH value was obtained in treatment D where the largest dose of dolomite was 7.22-7.75, while the lowest pH value was obtained in treatment A (control/without adding dolomite to feed), namely 6.95-7.16 (Table 4). The degree of acidity (pH) of water is important to determine the water use-value for fisheries. The generally of tolerance limits of fish and their bodies for acidity range from 4.0–11.0. Besides, pH is also related to the ability to dissolve certain compounds. The pH level of the water column fluctuates according to the photosynthetic and respiratory activities that are taking place, which starts from low numbers at dawn to high in the middle of the afternoon [25].

4. CONCLUSION

Moult intensity (average 18 inds.), daily growth of (1.82% per day), and the highest SR (78.33%) were obtained in the treatment of adding dolomite to diet at a dose of 8 mg/kg feed (C) and the lowest on the same parameter was obtained in treatment A (control/no addition of dolomite). The results of measuring water quality during the study obtained temperatures ranging from (from 29.87-31.03°C, salinity 25-30 ppt, DO 4.86-5.51 mg/l which were still in the proper range (optimal for vaname shrimp). Unlike other parameters, there is a difference in the pH value obtained by adding dolomite. The highest pH value was obtained in treatment D (12 mg/kg diet) where the largest dose of dolomite was 7.22-7.75, while the lowest pH value 6.95-7.16 was obtained in treatment A (control/without adding dolomite to diet).

REFERENCES

1. The Ministry of Marine and Fisheries. Ministry of Marine and Fisheries in Figures. Center for Data, Statistics and Information. 2021; 356 p.
2. Juniarti, H.A., N.Diniarti, and A.R. Scabra. The effect of addition of calcium oxide (CaO) on the cultivation of *Litopenaeusvannamei* in freshwater. *International Journal of Tropical Aquaculture*. 2022; 5(1): 50-63.
3. Zainuddin. Effect of calcium-phosphorus with different ratios on nutrient retention and changes in body chemical composition of juvenile tiger shrimp (*Penaeus monodon*fabr.) *Journal of Tropical Marine Science and Technology*. 2012; 4(2) 208-216.
4. Greenaway, P. Calcium balance and moulting in the crustacea. *Biological reviews of the Cambridge Philosophical Society*. 1985; 60: 425-454.
5. Zaidy, A. B. and W. Hadie. The effect of adding calcium to the media on the molting cycle and growth of giant prawn biomass, *Macrobrachiumrosenbergii* (de man). *Aquaculture Research Journal*. 2009; 4(2):179-189.
6. Erlando, G., Rusliadi, &Mulyadi. Increasing Calcium Oxide (Cao) To Accelerate Moulting And Survival Rate Vannamei Shrimp (*Litopenaeusvannamei*). 2015. University of Riau.
7. Ling, S.W. General Account on the biology of the Giant Freshwater Prawn *Macrobrachiumrosenbergii*and its rearing and culturing. FAQ. 1976.
8. Hasamani,N., R. B Srinivasa and R.P. Ramachandra. Crustacean molting: regulation and effects of environmental toxicants. *Journal of Marine Science Resources*. Dev 2017; 7: 5
9. Truong, H.H., B. M. Hines., M. G. Emerenciano, D. Blyth, S. Berry, N. A. Bourne, N. Wade, E. J. Simon. Mineral Nutrition in penaeid shrimp. *Reviews in Aquaculture*. 2022;1–19.
10. Wang, W.N., Wang, A.L., Wang, D.M., Wang, L.P., Liu, Y., & Sun, R.Y. Calcium, phosphorus and adenylate levels and -ATPase activities of prawn, *Macrobrachiumnipponense*, during the moult cycle. *Comparative Biochemistry and Physiology- Part A: Molecular & Integrative Physiology*. 2003;134(2): 297-305.
11. Allen, P.G., C.W.,Botsford, A.M., Schuur, and W.E. Johnston. *Bioeconomics Aquaculture. Ecological Applications*. 1984; 8(3): 833-845.
- 12.Arumsari,C. , Rusliadi,and Mulyadi. Effect of dolomitCaMg(CO₃)₂ as pellet for intensity of moulting, growth and survival rate of shrimp vanname (*Litopenaeusvannamei*). University of Riau Indonesia.2019.
13. Adegboye, JD. Calcium Homeostatic in The Crayfish. In: Goldmann RC (Editor). PaPer From The 5th International Symposium on Freswater Crayfish. Davis, California, U.S.A. 1981;115-123.
14. Aisyah, N., Agus, M., &Mardiana, T. Y. Analysis of the Utilization of dolomite in feed on vaname shrimp (*Litopenaeusvannamei*) molting period in unique ponds. *Pena Akuatika*. 2017;16(1), 95–102.
15. Yulihartini, W., Rusliadi, and H. Alawi.Effect of additional of Calcium Hidroxyid(Ca(OH)₂) on moulting, growth, and survival rate of vannamei shrimp (2016). (Vol. 1).
16. Anggoro, S. Osmotic Effect of Various Level Salinity on Hatching Rate and Vitality Hatching Rate and Vitality of Black Tiger Shrimp Larval*Penaeusmonodon* Fabricius. Dissertation. Faculty of Pascasarjana, IPB. Bogor. 1992. 127 hlm.
17. Smith, D.M., M.A.,Burford, S.J. Tabrett,, S.J. Irvin, and L. Ward. The effect of feeding frequency on water quality and growth of the black tiger shrimp (*Penaeus monodon*). *Aquaculture*. 2002;207(125-136).
18. Fast A.W &Lester L.J. Pond Monitoring and Management Marine Shrimp. Culture Principle and Practise. Elsevier Science Publisher Amsterdam. Netherlands. **1992**.
19. Pan-Lu-Qing, B. Fang, J. Ling-Xu, and Liu-Jing. The effect of temperature on selected immuneparameters of white shrimp*Litopenaeusvannamei*. *Journal of the World Aquaculture Society*. 2007;38 (2),326-332.

20. Ponce-Palafox, C.A.Martinez-Palacios, L.G. Ross. The effect of salinity and temperature on the growth and survival rates of juvenile white shrimp *Penaeus vannamei*, Boone, 1931. *Aquaculture*. 1997; 157 (107-115).
21. Whetstone, J.M., G.D. Treece., C.L. Browdy and A.D. Stokes. Opportunities and Constraints in Marine Shrimp Farming. SRAC Publication No 2600. 1992.
22. Boyd C.E, A.W Fast. Pond Monitoring and Management. *Marine Shrimp. Culture-Principles and Practices*. Elsevier. Amsterdam. 1992; 862 p.
23. Mc Graw, W.J., and J. Scarpa. Determining ion concentration for *Litopenaeusvannamei* culture in freshwater. *Global Aquaculture. Advocate*.2002; 5 (3): 36-37.
24. Sunusi, H. Effect of ablation and repeated spawning of female broodstock on the quality of eggs and larvae in white shrimp *Fenneropenaeusmerguinsis*de Man 1888. Dissertation. Hasanuddin University. 2012. 203page.
25. Chamberlain, W. G. Review of shrimp pond management, in the *Principles of Shrimp Cultivation Management*. Technical Bulletin. Thing. 1988; 48-64

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