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Supplementation of Crab (*Portunus pelagicus*) Shell Calcium Flour on Commercial Feed on Freshwater Lobster (*Cherax quadricarinatus*) Molting Frequency

Suplementasi Tepung Kalsium Cangkang Kepiting (Portunus pelagicus) pada Pakan Komersial Lobster Air Tawar (Cherax quadricarinatus) Frekuensi Molting

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Abstract

Freshwater lobster *(Cherax quadricarinatus)* is a type of crustacean (crustacean). Freshwater lobster is a type of shrimp that lives in shallow fresh inland waters, with a muddy substrate and lots of gaps and cavities to hide. The cultivation of freshwater lobsters is quite easy because the tolerance to the environment is quite high with relatively few disease problems. Molting is a natural process that occurs in freshwater lobsters as their bodies grow. Calcium is one of the mineral elements that lobsters really need to form new shells. In addition to calcium, lobsters will also undergo a mineralization process to form new membranes using calcium absorbed from the environment in which they live. The purpose of this study was to determine the effect of supplementation of calcium flour from crab shells on commercial feed on molting frequency, absolute weight and length, survival of freshwater lobster, and determine the appropriate dose of calcium flour supplementation of crab shells. This study used a completely randomized design (CRD) with 4 treatments and 4 replications with additional doses of calcium flour, 0%, 2%, 4%, and 6% per kg of feed. The stock density of freshwater lobster was 7 individuals per aquarium for 30 days of rearing. The results showed that the addition of shell calcium powder of as much as 4% (treatment C) was the best dose with 2 molts/ind and the lowest was in treatment A (control). The highest growth in absolute weight and absolute length was at a concentration of 4% (treatment C) 2.34g and 1.55 cm, the lowest value was obtained at treatment A (control) 1.63g and 0.95 cm. Supplementation of crab shell calcium flour in commercial feed for crayfish did not have a significant effect on the survival (SR) of freshwater lobster.

Keyword: Freshwater lobster, Molting, Calcium, Crab shell

Abstrak

Lobster air tawar (*Cherax quadricarinatus*) adalah sejenis krustasea (crustacean). Lobster air tawar adalah sejenis udang yang hidup di perairan pedalaman tawar yang dangkal, dengan substrat berlumpur dan banyak celah serta rongga untuk menyembunyikan diri. Budidaya lobster air tawar cukup mudah karena toleransi terhadap lingkungan cukup tinggi dengan masalah penyakit relatif sedikit. Moulting adalah proses alami yang terjadi pada lobster air tawar seiring pertumbuhan tubuhnya. Kalsium merupakan salah satu unsur mineral yang sangat dibutuhkan lobster untuk membentuk cangkang baru. Selain kalsium, lobster juga akan mengalami proses mineralisasi untuk membentuk membran baru dengan menggunakan kalsium yang diserap dari lingkungan tempat tinggalnya. Tujuan dari penelitian ini adalah untuk mengetahui pengaruh suplementasi tepung kalsium dari cangkang rajungan pada pakan komersial terhadap frekuensi molting, bobot dan panjang absolut, kelangsungan hidup lobster air tawar dan menentukan dosis suplementasi tepung kalsium cangkang rajungan yang tepat. Penelitian ini menggunakan Rancangan Acak Lengkap (RAL) dengan 4 perlakuan dan 4 ulangan dengan penambahan dosis tepung kalsium, 0%, 2%, 4% dan 6% per kg pakan. Kepadatan stok lobster air tawar adalah 7 ekor per akuarium selama 30-hari pemeliharaan. Hasil penelitian menunjukkan bahwa penambahan serbuk kalsium cangkang sebanyak 4% (Perlakuan C) merupakan dosis terbaik dengan 2 molting/ekor dan terendah pada perlakuan A (kontrol). Pertumbuhan berat mutlak dan panjang mutlak tertinggi pada konsentrasi 4% (perlakuan C) 2,34 g dan 1,55 cm, nilai terendah diperoleh pada perlakuan A (kontrol) 1,63 g dan 0,95 cm. Suplementasi tepung kalsium cangkang rajungan pada pakan komersial lobster air tawar (*Cherax quadricarinatus*) tidak memberikan pengaruh yang nyata terhadap kelangsungan hidup (SR) lobster air tawar.

Kata kunci : Lobster Air Tawar, Ganti Kulit, Kalsium, Cangkang Kepiting

1. Introduction

The crab (*Portunus pelagicus*) is a meat-eating sea bottom animal belonging to the family Portunidae (Hastuti *et al.*, 2012). Blue swimming crab is one of Indonesia's mainstay export commodities, accompanying tiger shrimp, tuna and seaweed. From an economic point of view, blue swimming crab is a marine product that has high selling value and is an export commodity (Agustina, 2014). The volume of blue swimming crab production will increase if it is followed by an increase in export demand. If crab production increase, the waste produced will continue to increase, for example in the form of crab shell waste. According to Setiawan *et al.* (2017), freshwater crab contains 19.97% calcium, and 1.81% phosphorus. Kusumawati (2014), crab waste is rich in protein (32.95%), crude fiber (10.89%), calcium (22.93%), and phosphorus (0.78%)

Calcium is a mineral needed by humans and animals. The function of calcium in humans is needed for the growth of bones and teeth, while in animals, for example, lobsters, calcium is used for the molting process. Molting is a process of changing the skin of crustaceans, especially lobsters, which occurs depending on the size of the lobster meat that continues to grow. One of the causes of molting failure is that the lobster fails in the gastrulation process, which is to absorb the calcium contained in the lobster's body. The role of calcium in the hardening of the shell is after the molting process occurs in lobsters where the calcium needed by lobsters can be sourced from feed and the environment. Mulis (2012), states that the frequency of molting in lobsters decreases with age. The frequency of molting in juveniles occurs once every 10 days, in pre-adults between 4-5 times/year, and in adult lobsters 1-2 times/year.

The important thing about lobster cultivation is paying attention to its growth, lobster growth is closely related to the lobster molting process. The slow molting process in lobsters certainly slows down the growth and reproduction of these lobsters. Handayani & Syahputra (2018), the addition of 2% nano calcium from oyster shells to commercial feed has a significant effect on growth and a very significant effect on the number of molting freshwater lobsters. Therefore, it is also necessary to conduct research on the use of calcium from crab shells that aims to determine the effect of calcium flour supplementation from crab shells in commercial feed on molting frequency, absolute weight and length, survival of freshwater lobster and to find out the right dosage of crab shell calcium flour supplementation.

2. Material and Method

2.1. Time and Place of Research

This research was conducted in June - July 2022 and the manufacture of crab shell calcium flour was carried out at the Aquaculture Laboratory, Faculty of Agriculture, University of Asahan.

2.2. Research Materials

2.2.1. Source of Calcium

The main material used as a source of calcium is a crab shell (*Portunus pelagicus*) obtained from the waters of Gambus Laut Village, Tanjung Tiram Regency, Batubara, North Sumatra Province, while freshwater lobster obtained from the freshwater lobster hatchery in Deli Serdang District, Province of North Sumatra. Other ingredients are commercial feed, caustic soda, distilled water and water.

2.2.2. Manufacturing of Calcium Flour

The stages of making calcium flour from crab shells are as follows.

a) The crab shells are washed with running water until clean.
b) The crab shells were boiled at 100^oC for 1 hour.
c) The crab shells are washed again with running water so that the fat and dirt attached to the crab shells are removed.
d) The crab shell is cut into pieces (5-10 cm) to get a smaller size and make the next process easier.
e) The crab shells were then hydrolyzed with 2.5 N NaOH for 4 hours.
f) The shells are washed using running water until neutral.
g) Dry the shells in the oven for 1 hour at 121^oC.
h) The shells were crushed using a mortar and then sieved with 100 meshes.

2.3. Research methods

This research method is experimental using a completely randomized design (CRD), consisting of 4 treatments and 4 repetitions. The treatment given is

A = Feed without giving calcium flour (Control).
B = Feed with the addition of 2% crab shell calcium flour per kg.
C = Feed with the addition of 4% crab shell calcium flour per kg.
D = Feed with the addition of 6% crab shell calcium flour per kg.

Lobster maintenance was carried out in an aquarium with a density of 7 individuals per container. The loster seeds used are two months old with an average initial weight of 4-5 g/ind. Feed given as much as 3% of the weight of biomass per day with a frequency of feeding in the morning (25%) and afternoon (75%) for 30 days (Rihardi *et al.*, 2002).

2.4. Observation Parameters

2.4.1. Water quality

Water quality parameters measured in this study include dissolved oxygen (DO), pH, and temperature. Dissolved oxygen measurement can be done using a DO meter, pH can be measured using a pH meter and temperature can be measured using a thermometer. These variables were measured 4 times during the 30-day study period. Sipping of the water in the aquarium is done every 1-2 days or when the water looks cloudy and dirty.

2.4.2. Frequency of Molting

The percentage of molted skin that is calculated can be based on the ratio of the lobster population to the total number of original lobsters and molting. The percentage can use the formula

$$M = x\_molt/N\_tot$$

Description:
MFq : Molting frequency (times/ind)
x_molt : The number of molting lobsters (Ind)
N_tot : Initial number of lobsters (Ind)

2.4.3. Absolute Growth

Absolute growth has 2 categories, namely absolute weight growth and absolute length growth. Absolute weight growth can be interpreted as the difference in total lobster weight at the end and beginning of maintenance, and can be calculated based on the formula (Effendie, 1997).

$$GR = Wt - Wo$$

Description:
GR = Growth in absolute weight (g/day)
Wt = Average weight at the end of maintenance (g)
Wo = Average weight at the start of maintenance (g)

Lobster length measurements include the total length from the tip of the mouth to the tip of the tail. Can be calculated using the formula (Effendie, 1997):

$$Pm = Pt - Po$$

Description:
Pm : Growth in absolute length (cm/day)
Pt : LAT length at time t (cm)
Po : LAT length at time 0 (cm)

2.4.4. Survival Rate

Survival rate can be calculated between the comparison of the number of live lobsters at the end of the study and the beginning of the study. In observing dead lobsters, the total weight was recorded every day. According to Effendie (2004), the equation used to calculate the survival rate is:

$$SR = Nt/No \times 100\%$$

Description:

SR = Survival (%)

Nt = Number of fish at the end of rearing (ind)

No = Number of fish at the start of rearing (ind)

3. Result and Discussion

3.1 Observation of Water Quality

Water quality during the maintenance of freshwater lobster, the lowest temperature was 26.8^{o}C and the highest was 29^{o}C, overall within the range that can be tolerated by lobsters to live and grow (Table 1).

Table 1. Water quality during Maintance

Treatment	Water Quality Parameters		
	Temperature (^{o}C)	pH	Do (mg/L)
A(Kontrol)	26,9-28,9	7,2-8,5	4,3-5,7
B (2%)	26,8-28,	7,3-8,4	4,4-5,8
C (4%)	26,9-28,9	7,2-8,5	4,3-5,7
D (6%)	27-29	7,2-8,4	4,2-5,7
Optimum Value	24-29	6,5 – 9	5

This range is good for lobster growth as stated by Holdich & Lowery *in* Faiz *et al.* (2021). Freshwater lobster can live in wide water parameter intervals, freshwater lobsters tolerate very low dissolved oxygen content, but to grow and develop properly it certainly cannot be done in such conditions. The red claw type lobster will experience the best growth at 24-29℃ during 30 days of maintenance, the lowest pH value for lobster growth was 7.2 and the highest was 8.5. This is not much different from the statement by Holdich & Lowery *in* Faiz *et al.* (2021) that the growth of *C.quadricarinatus* is around 6.5-9. In accordance with the opinion of Mosigh *in* Faiz *et al*. (2021) freshwater lobster can live in a pH range of 6-9 and have an optimum range of pH 7-8. In accordance with the opinion (Hastuti *et al.*, 2016). The pH value can affect growth through the ability of physiological processes, biochemistry, and gill structure

The dissolved oxygen content during this study was good enough for cherax growth, namely the lowest was 4.2 mg/L and the highest was 5.8 mg/L or an average of 5 mg/L. In accordance with the opinion of Boyd (1982) in Faiz *et al.* (2021) the optimum range of dissolved oxygen values for crustacean growth is above 5 mg/L, thereby providing good growth and survival.

3.2. Molting Frequency

The frequency of molting freshwater lobster during the study was best obtained in treatment C at a dose of 4% per kg of feed with a value of 1.29 times/day, followed by treatment D with a dose of 6% per kg of feed with a value of 1.14 times/day, treatment B of 0.93 times/day, and treatment A 0.86 times/ind (Figure 1).

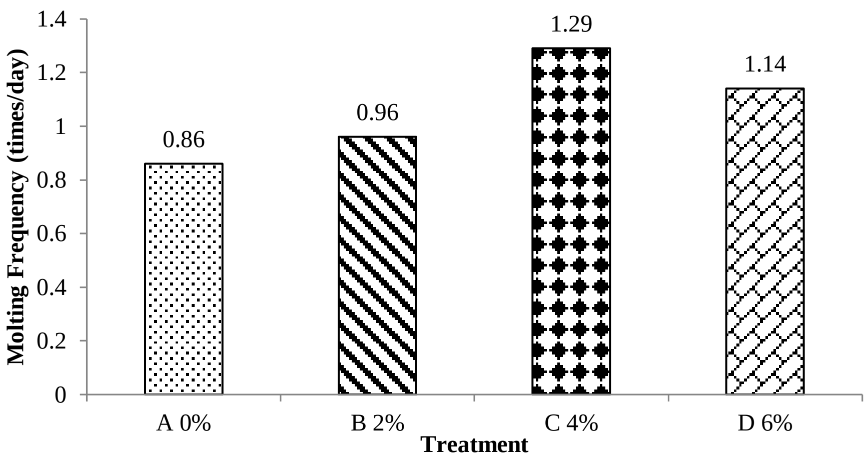

Figure 1. Molting Frequency

Molting is the molting of the skin and is an important process in the growth of crustaceans. Growth in freshwater lobster will occur if they have enough energy and then will carry out the process of changing the skin or replacing the exoskeleton, in this process the exoskeleton does not grow as the lobster grows older/bigger, so the old exoskeleton will be released so that the freshwater lobster continues to grow. During the 30 days of the study period, the results obtained showed that the addition of calcium crab shell powder to feed up to 4% was the best result for molting frequency. The higher the molting frequency, the more often the freshwater lobster changes its skin or new skin, making the freshwater lobster grow faster. Calcium which has been added to the feed and the environment can function to accelerate the process of hardening the new carapace so that cannibalism can be minimized and can prevent gastrulation failure. Gastrolissation occurs where Calcium is stored and accumulated in the Hepatopankreas organs and gastroliths (white round plates) found in the stomach

The success of the molting process is driven by the nutritional factors of the feed and the levels of calcium contained in the water. freshwater lobster h that get the appropriate food and have high calcium levels from the environment optimally experience more frequent molting. Low calcium levels will make it difficult for shell formation. The process of recovering or repairing body tissue in lobsters after the molting process will increase the growth of lobsters. Because the appetite after molting lobsters can be double satisfied, to meet the lobster's appetite decreases during molting, that is, the growth of the growth value of lobsters increases well.

The state of the lobster will be very vulnerable if the lobster changes its skin, because a few hours before molting, the lobster is in a weak state and usually stays still. When the freshwater lobster shell is released, the inner body is no longer protected. This is a big opportunity for the lobster to be eaten by its partners and lobsters are cannibals (Arumsari, 2019). The death of freshwater lobsters is caused by cannibalism and competition. Cannibalism experienced by lobsters generally occurs due to molting. Based on observations, one of the causes of death is caused by the failure of the molting process. Meanwhile, the competition factor is influenced by the level of ability of an organism in obtaining space to live and eat. However, the addition of sufficient calcium for feed and the environment accelerates the hardening of the shell because the process of hardening of the shrimp shell after molting can occur properly due to the presence of sufficient calcium (Handayani & Syahputra, 2018)

Yulihartini (2016), stated that the small number of molting lobsters was affected by the low dose of calcium given, but at the highest dose of 6% per kg of feed, the number of molting lobsters also increased. amount of calcium. High concentrations also complicate the homeostatic processes of calcium According to (Arumsari, 2019), hypotonic conditions make it difficult for the balance between calcium ions in the body and the environment to create energy because the continuity of this process is greater.

3.3. Absolute Growth

The absolute growth in weight is the difference between the body weight of the freshwater lobster at the end of rearing and the body weight of the lobster at the beginning of rearing, while what is meant by the growth in absolute length is the difference between the body length of the freshwater lobster at the end of rearing and the body weight of the lobster at the beginning of rearing. Based on observations during the study, the absolute growth (weight and length) of crawfish for 30 days was an increase in the absolute growth (weight and length) of freshwater crab (Figure 2).

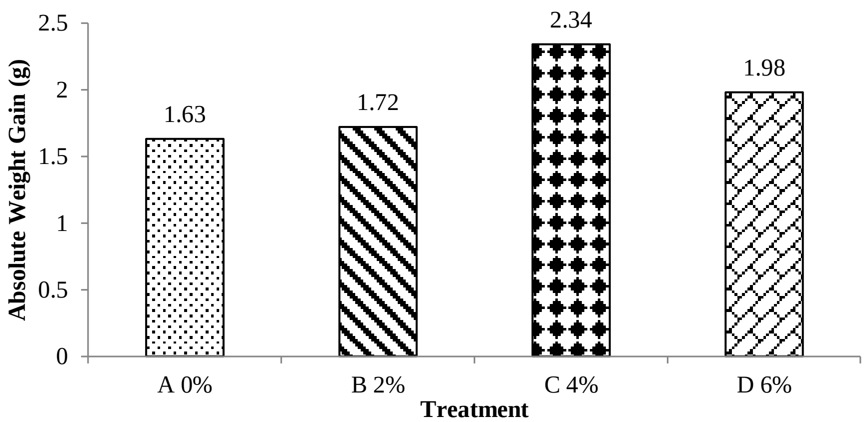

Figure 2. Absolute Weight Gain of Freshwater lobsters

Based on the picture above, it can be seen that the growth in absolute weight of freshwater lobster during the study was best obtained in treatment C with a dose of 4% per kg of feed with a value of 2.34 g, followed by treatment D with a value of 1.98 g, followed by treatment B with a value of 1.73 g and ends with treatment A, namely control or 0% per kg of feed with a value of 1.63 g. The results of the statistical analysis of the One WayAnova test show a value ($p = 0.028 < 0.05$), which means that H1 is accepted and H0 is rejected. H1 was accepted, namely the provision of crab shell calcium flour had a significant effect on the absolute weight growth of freshwater lobster. Fcount value (4.305) < FTable (3.49) and a significant value of 0.028 means $p \leq 0.05$ This means that there is an effect of various treatments on the growth of freshwater lobster weight.

The Least Significant Difference (LSD) test can also be said to be a test that can see the relationship between treatments. Based on the BNT test, it was known that treatment C with a dose of 4% per kg of feed was significantly different from other treatments, while the 2% and 6% treatment of crab shell calcium flour was different. The growth of absolute length of freshwater lobster during the research can be seen in the following figure.

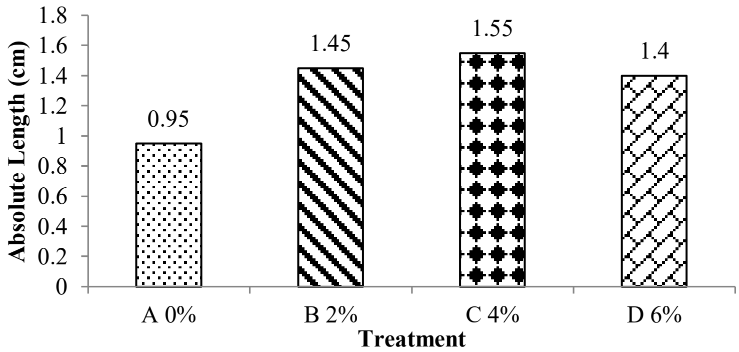

Figure 3. Absolute Length of Freshwater Lobsters

Based on the picture above, it can be seen that the absolute length growth of freshwater lobster during the study was best obtained in treatment C with a dose of 4% per kg of feed with a value of 1.55 cm, followed by treatment B with a value of 1.45 cm, followed by treatment B with a value of 1.4 cm and ends with treatment A, namely control or 0% per kg of feed with a value of 0.95 g.

Growth is the process of increasing length and weight in a certain period of time. Growth can be influenced by several factors including internal and external factors. Internal factors include heredity, sex, age, parasites, and disease. While the external factors are food and water temperature can affect growth (Rihardi *et al*., 2013). The absolute growth of freshwater lobster is related to the molting process. Increased growth in lobsters is associated with accelerating the recovery process in lobsters because after molting, the appetite of lobsters will increase in height to satisfy their decreased appetite before molting (Yulihartini, 2016). Providing optimal calcium in feed can ensure adequate feed intake and maximum absolute growth of lobsters.

The increase in the absolute weight of freshwater lobster is also related to the rate of gastric emptying where the lobster's appetite will increase when the stomach is empty so that it can use it. Rihardi *et al.* (2013) stated that feeding freshwater lobster twice a day is a better study because at very far feed frequencies it can make the lobsters a little hungry and when feeding the lobsters will be more aggressive in receiving food and that in accordance with the length of the gastric emptying rate that occurred for 12 hours. The best growth from the results of the study was aimed at treatment C, namely 4% per kilogram of feed where the result was an increase in length of 1.55 cm and a weight of 2.34 g. This can be influenced by the total calcium content of crab flour in treatment C at a dose of 4% per kg of feed of 10.45 g of calcium. This was obtained by calculation according to Faiz *et al.* (2009) through the results of the calcium test when the hydrolysis of the crab shells for 4 hours produced 26.13% calcium.

Environmental factors, especially regarding water quality, can also greatly affect the growth of freshwater lobster. Biological factors include food, predators, density, age, and maturity while abiotic environmental factors that affect the growth of lobsters include temperature, latitude, photoperiod, water quality (especially dissolved oxygen, calcium, and pH), nutrient level, and habitat composition, while from (Aiken & Waddy, 1992). Lobster appetite is closely related to water quality, so during the research, the water quality was maintained as well as possible so that the lobster appetite was quite good. This can be seen from the pellet feed that is always spent. The existence of good feed and supported by good water quality also causes the growth of lobsters during treatment that get more calcium because the feed can provide stimulation or stimulate molting in lobsters.

3.4. Survival Rate

The survival of freshwater lobster during the study was 92.85%, of which only 2 of the 28 freshwater lobster that were reared died. This shows that the administration of crab shell calcium flour to the feed has no effect on the survival of freshwater lobster (Figure 4).

The results of the statistical analysis of the One WayAnova test show a value ($p = 1{,}000 < 0.05$), which means that H_0 is accepted and H_1 is rejected. H0 was accepted, namely, the administration of crab shell calcium flour had no significant effect on the survival of freshwater lobster. F_{count} value (0.000) < F_{table} (3.49) and a significant value of 1,000 means $p \geq 0.05$ this means that there is no effect of various treatments on the growth of freshwater lobster length.

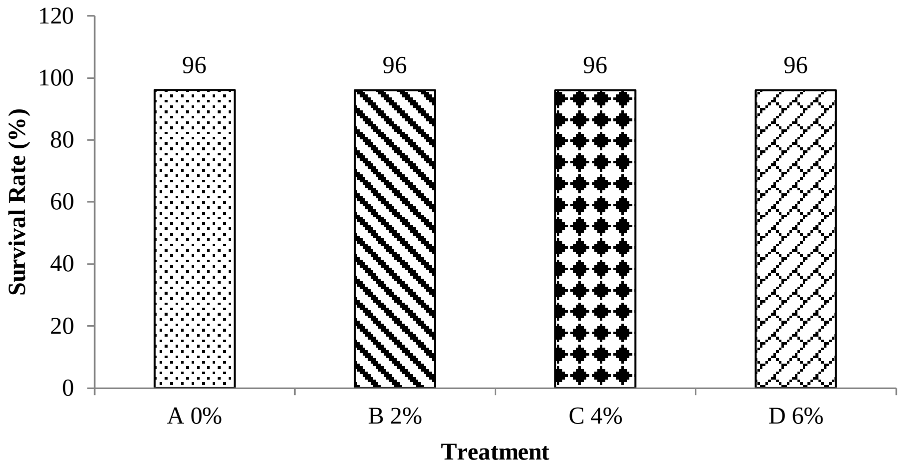

Figure 4. Survival Rate of Freshwater Lobsters

Survival is related to mortality, namely death that occurs in a decreasing population of cultural organisms. While the results of death in lobsters cannot occur because they are unable to make changes to the skin, this is suspected to be due to natural factors. The percentage of lobster survival is greatly influenced by molting because the lobster's body will be very weak after molting. If the amount of calcium is less in the environment at the time of molting, it will interfere with the process of forming a new lobster carapace, causing the death of the lobster. Based on the results of this 30-day study, showed that each treatment had no effect on the mortality rate of the test biota, the survival rate during the study reached 96% for each treatment, in which each treatment contained 28 freshwater lobsters and each treatment only had a mortality rate only 2 of them. Each of the treatments during the study period supported the handling of environmental impacts and excellent water quality as well.

According to Wickins & Lee (2002), that survival can be influenced by biotic and abiotic factors. Biotic factors consist of the age and ability of the lobsters to adapt to the environment, while abiotic factors include the availability of food, stocking density, and the quality of the living media. The availability of food in this study was sufficient to meet the needs of freshwater lobster for self-defense, and the water quality of the culture media was still within the feasibility range so that it could support the increase in the survival of freshwater lobster.

The death of freshwater lobster seeds occurred during the study, presumably because of the cannibalism that occurs when the freshwater lobster molts. Freshwater freshwater lobster is a cultivation that has cannibalistic characteristics and in general, freshwater lobsters that are in their molting stage are very weak and vulnerable to attacks from each other. freshwater lobster that have just changed their skin (molting) need a place to hide or take shelter, considering that when they just molt, their physical condition is very weak and the freshwater lobster have the nature of preying on each other (Setiawan, 2006)

4. Conclusions

From the research, results show that: 1) Supplementation of crab shell calcium flour in commercial feed for freshwater lobster has a significant effect on molting frequency, where the highest value is produced in treatment 3 (P3) which is 1.29 times/ind and the lowest is in treatment 1 (control) with value of 0.86 times/ind, and gave a real influence on the growth of absolute weight and absolute length, where the highest value was produced in treatment 3 (p3) namely 2.34 g and 1.55 cm, the lowest value was in treatment 1 (control), namely 1.63 g and 0.95 cm, 2) Supplementation of crab shell calcium flour in commercial feed for freshwater lobster did not have a significant effect on the survival of freshwater lobster.

5. Suggestion

Based on this research the authors suggest, there is a follow-up study with a rearing period of 45 or 60 days for freshwater lobster to see growth with the addition of calcium crab shell flour to more effective feed.

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