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Bioencapsulation of Artemia with Calcium from Crab Shells on Growth and Survival Responses of Crab Larvae (Portunus pelagicus)

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Crab is one of the main export commodities from Indonesia, but it is still very difficult to cultivate, especially in the larval stage. The problem faced is the low survival rate of larvae due to failed molting in the metamorphosis process. In the crab shell there is calcium which is the main element in the exoskeleton and tissue structure of the crab. The purpose of this study was to examine the response of growth and survival of crab larvae to bioencapsulated artemia using calcium from crab shells. Calcium from crab shells was added to the artemia media for 6 hours, then artemia was given to the crab larvae. Based on calcium analysis, there was an increase in the calcium content of artemia in each treatment (30,40,50 ppm), but did not show a significant effect ($p>0.05$) on the calcium content of crab larvae. Likewise with the growth and survival of crab larvae. Based on this study, it can be concluded that bioencapsulation of artemia with calcium from crab shells does not affect the growth and survival of crab larvae.

Keywords: Crab Larvae, Artemia Bioencapsulation, Calcium Enrichment.

1. Introduction

The main problem in crab seeding efforts is low survival in the zoea and megalopa phases. Zoea survival in several studies reached 12.5% (Thirunavukkarasu, 2014), 5-10% (Gunarto, 2016), Karim et al., 2003), 15-25% (Jantrarotai et all, 2002), 7.9% (Paran, 2021). Hamasaki et. al., (2002) reported that mass mortality was found in the final zoea phase to megalopa with a survival rate of 2.5%. The cause of low survival in the zoea - megalopa stage is the failure of molting during metamorphosis. In the ecdysis process, the old shell of the crab larva comes off and is replaced by a new shell. The main component of the exoskeleton and tissue structure is calcium, which functions to maintain the balance of osmotic pressure, metabolic processes, nerve transmission, muscle contractions and others (Akiyama et al., 1991; Davis, 1991). Crab larvae can absorb calcium available in seawater, but the content is very low at around 0.002

mg/l (Lall, 1989). Meanwhile, crustacean shells contain 22-27.8% calcium (Bobelmann et. al., 2007) and their meat contains 11.5 mg/100 g (Mohapatra, et. al., 2009). Fulfillment of calcium needs can be done through bioencapsulation of artemia. Artemia is a live feed that will encapsulate substances needed by fish.

2. Methods

The research was conducted in October - November 2023 at the Brackish Water Aquaculture Center (BPBAP) Takalar, South Sulawesi as a crab breeding location. Meanwhile, calcium analysis was conducted at the Feed Chemistry Laboratory, Faculty of Animal Husbandry, Hasanuddin University.

Materials and tools

The test animal is crab larvae (zoea-megalopa), the material used is calcium powder from crab shells which is given to Artemia. While the tools consist of a plastic water tank, aerator, refractometer, pH meter, DO meter, seser, microscope, petri dish, porcelain dish, measuring flask, filter paper, pipette, sample container, desiccator, furnace, Atomic Absorption Spectrophotometer (ASS).

Research Procedures

Container preparation

The container for maintaining crab larvae is a plastic tub with a capacity of 50 liters as many as 12 pieces, washed clean using soap then installed with an aeration installation. The filtered sea water is put into the tub as much as 40 L. Enrichment of artemia with calcium using 3 liter jars as many as 8 jars and equipped with an aeration installation.

Provision of calcium from crab shells

Calcium from crab shells is obtained from crab miniplant waste in Barru Regency. The shells are cleaned, washed and then dried under the sun for 5 days. The shells are then crushed using a blender. After that, it is calcined by burning at a furnace temperature of 800oC for 5 hours or until it becomes white ash.

Preparation of artemia

The artemia used is Mackay Marine produced in the USA with a hatching rate of 96%. Artemia is hatched in a cone-shaped hatching tank with a capacity of 250 liters and harvested after 24 hours. After that, the artemia is enriched with calcium from crab shells according to the treatment. After 6 hours of treatment, the artemia is ready to be given to the crab larvae.

Maintenance of crab larvae

The density of crab larvae is 50/liter. Larvae are maintained from zoea 1 to megalopa for 13 days. Crab larvae go through 4 sub-stages of zoea before entering megalopa. Artemia is given to larvae at Zoea 2 stage (5th day of maintenance) until megalopa stage.

Treatment

There were 4 treatments and 3 repetitions in this study, namely the addition of calcium from

crab shells to artemia as much as 0, 30, 40 and 50 ppm.

Treatment parameters

Analysis of calcium content in Artemia and crab larvae

A clean porcelain cup (50 ml) is sterilized at a temperature of 105oC for 2 hours then cooled in a desiccator for 30 minutes. Weigh 1 gram of artemia/crab larvae, store in a porcelain cup then put it in an electric furnace. The furnace temperature is 600oC burned for 3 hours until the artemia/crab larvae become ash. Cool and put in a desiccator for 30 minutes. Determination of ash content is done by adding 3 ml of concentrated HCl to the ash of artemia/crab larvae. Dilute with distilled water until the volume of the porcelain cup is full. Let stand overnight. Pour into a 100 ml measuring flask, rinse with distilled water until the line mark then shake. Filter with filter paper then inject into the AAS device.

Metamorphosis rate

Metamorphosis rate is the time (days) used to move to the next stage. Observations are made by observing changes in larval morphology either directly or using a microscope according to the characteristics of each stage. Measurement of the speed of larval metamorphosis uses the Larva Stage Index formula (Ipandri et. al., 2016):

$$LSI = \{\frac{(St \times nt) + (St\text{-}1 \times nt\text{-}1)}{N}\}$$

Growth in length of larvae

Megalopa (maintenance days 11 and 13) were observed under a microscope and their length was measured from the tip of the carapace to the tail, then the growth in length was calculated using the equation :

$$\Delta L = L_t - L_0$$

Survival

Larval survival is obtained by comparing the number of larvae alive at the end of the maintenance period with the number of larvae released, then the percentage is calculated using the equation:

$$SR = (N_t / N_0) \times 100\%$$

Data analysis

Data on calcium analysis, growth and survival of crab larvae were statistically analyzed using analysis of variance (ANOVA) and if the results obtained showed a significant effect, then continued with the W-Tukey further test.

3. Results and Discussion

Calcium Analysis

Based on the results of the analysis of artemia and megalopa calcium, the following calcium

content data was obtained:

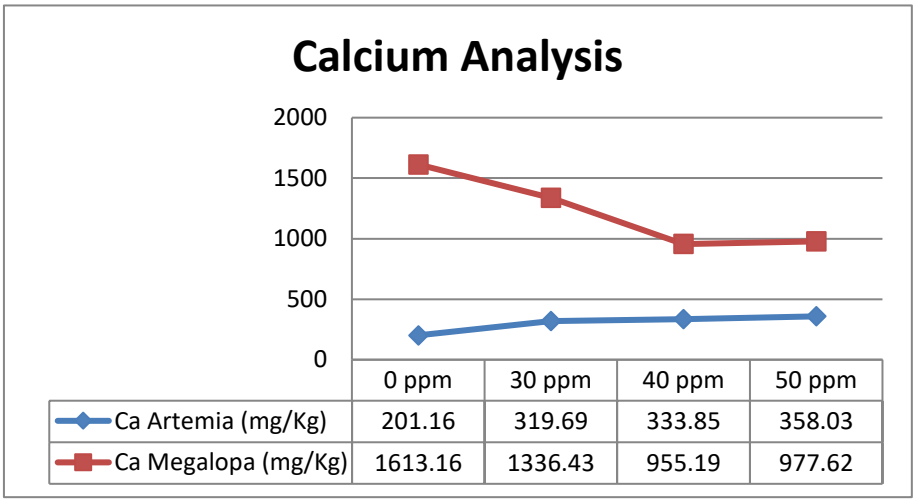
Table 1. Calcium Analysis of Artemia and Megalopa

Treatment	Ca Artemia (mg/Kg)	Ca Megalopa (mg/Kg)
0 ppm	201.16	1613.16
30 ppm	319.69	1336.43
40 ppm	333.85	955.19
50 ppm	358.03	977.62

Source: Feed Chemistry Lab, Faculty of Animal Husbandry, Hasanuddin University, 2023

The table above shows that there is an increase in the calcium content of artemia after being given calcium from crab shells.

Graph 1. Calcium Analysis



Artemia calcium increases with increasing calcium dose given. This indicates that there has been biotransformation of enrichment materials into the digestive system of artemia (Tocher et, al., 1997). Artemia absorbs calcium through simple diffusion. Calcium in the media penetrates through the mucous membrane in the osmoregulation process where the mucosa becomes the place for the osmosis and ion exchange process. Although there is an increase in calcium content in megalopa, the low calcium content of megalopa is in the 40 ppm and 50 ppm treatments. Calcium in megalopa is obtained from the results of bioencapsulation of artemia with calcium absorbed through the digestive tract (Wheatly, 1999) which is partly excreted as feces (Pratama et al., 2016) and maintenance media. Along with the increasingly complete and functional structure of the body organs of crab larvae, calcium is used by megalopa for the osmoregulation process, muscle contraction, nerve impulse transmission (Davis and Gatlin, 1996) and hardening of the exoskeleton (Zanotto, 2004).

Rate of metamorphosis

Observation of the rate of metamorphosis is used to determine how fast the larvae grow or move to the next stage. The results of the study showed that the development index of crab larvae is as follows:

Table 2. Metamorphosis Rate of Crab Larvae

Treatment	Maintenance day												
	1	2	3	4	5	6	7	8	9	10	11	12	13
0 ppm	Z1	Z1	Z1	Z1	Z2	Z2	Z2	Z3	Z3	Z4	Z4	Z4	M
		1	1,3	1,5	2,4	2,3	2,5	3,3	3,3	3,9	4,3	4,4	4,7
30 ppm	Z1	Z1	Z1	Z2	Z2	Z2	Z3	Z3	Z3	Z4	Z4	Z4	M
		1	1,3	1,6	2,5	2,4	2,6	3,3	3,3	3,6	4,2	4,3	4,6
40 ppm	Z1	Z1	Z1	Z2	Z2	Z2	Z3	Z3	Z3	Z4	Z4	M	M
		1	1,4	1,7	2,3	2,4	2,5	3,4	3,5	3,7	4,3	4,6	4,7
50 ppm	Z1	Z1	Z1	Z2	Z2	Z2	Z3	Z3	Z3	Z4	Z4	M	M
		1	1,5	1,7	2,3	2,5	2,6	3,4	3,5	3,7	4,5	4,6	4,9

Source: BPBAP Takalar

From the table above, it can be seen that crab larvae with 40 ppm and 50 ppm calcium addition treatments entered the megalopa stage faster than the 0 ppm and 30 ppm treatments. The 40 ppm and 50 ppm treatments entered the megalopa stage on the 12th day, while the 0 ppm and 30 ppm treatments on the 13th day. Meanwhile, according to Hartanto, et.al. (2017), it takes about 10 days for crab larvae to transform from zoea 1 to megalopa. This condition shows that the rate of metamorphosis of crab larvae during the study was slower when compared to ideal conditions. This is due to the long dry season which causes high seawater salinity during the maintenance period, namely 35-37 ppt.

Growth of crab larvae

Megalopa growth is measured based on the length of the megalopa from the chepalothorax to the tail. Based on the observation results, the growth of crab larvae on the 13th day of maintenance is as follows:

Table 3. Growth of Crab Larvae

Treatment	Growth (mean $\pm$ SD)
0 ppm	0.53 ± 0.22
30 ppm	0.53 ± 0.09
40 ppm	0.62 ± 0.21
50 ppm	0.75 ± 0.18

Source: BPBAP Takalar

The results of the analysis of variance (ANOVA) showed that bioencapsulation of artemia with calcium from crab shells did not show any significant effect ($P>0.05$) on the growth of crab larvae. However, the 50 ppm treatment gave the best growth effect, namely 0.75 mm compared to other treatments, namely 40 ppm (0.62 mm), 30 ppm and 0 ppm (0.53 mm). This is because the metamorphosis rate of the 50 ppm treatment is faster than other treatments, so that the growth of crab larvae is greater. Calcium is utilized during the calcification process of the postmolt period, making the larvae quickly stable and survive compared to other treatments.

Survival rate of crab larvae

Survival of crab larvae at the end of maintenance is presented in the following table:

Table 4. Survival Rate of Crab Larvae

Treatment	SR (%) (Mean ± SD)
0 ppm	13.30 ± 3.15
30 ppm	13.18 ± 1.46
40 ppm	14.93 ± 3.78
50 ppm	15.98 ± 3.02

Source: BPBAP Takalar

Based on the results of the ANOVA test shown in Table 4, there was no effect between treatments on the survival rate of crab larvae (P>0.05). Although it did not have a significant effect, bioencapsulation of artemia with a calcium dose of 50 ppm gave the highest survival rate of 15.98% compared to other treatments, namely 40 ppm treatment of 14.93%, treatment 0 of 13.30% and 30 ppm of 13.18%). In the study of Permadi, et.al., (2020), the addition of 50 ppm calcium showed the best survival rate. This is due to an increase in the number of hemocytes and phagocytosis activity. Calcium at a dose of 50 ppm is needed to harden the shell after molting. During pre-molt, calcium absorption occurs through the gills and stomach but then increases during postmolt. However, during ecdysis, crab larvae will lose about 87% of their calcium because they are attached to their old shells

4. Conclusion

Bioencapsulation of Artemia with calcium, which is given to crab larvae can increase the calcium content in artemia and crab larvae. This is because calcium in artemia is utilized by crab larvae for the process of metamorphosis, growth and survival. Although bioencapsulation of artemia with calcium from crab shells did not have a significant effect on the growth and survival of crab larvae, the 50 ppm treatment was the best dose because it provided the highest growth and survival among other treatments.

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