Black tiger shrimp larvae (*Penaeus monodon*) that received eggshell powder in diet exhibit decreasing of growth and survival rate

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Abstract. The objective of the present study was to investigate the effect of eggshell powder supplementation in diet on the growth and survival rate of black tiger shrimp, *Penaeus monodon* larvae. A total of 600 shrimp larvae (PL 12) was used with five treatment levels and four replications. The shrimp fed with 42% of crude protein, feeding frequency three times a day at 08.00; 14.00; 18.00 pm and 5% feeding rate. The dietary treatments were A (0%), B (3%), C (6%), D (9%) and E (12%) fed for thirty days. Results showed that the supplementation of eggshell powder in diet had a significant effect on weight gain, length gain, specific growth rate, and survival of black tiger shrimp larvae. Duncan's advanced test showed that the optimum dose of eggshell powder was found in treatment A (0% eggshell powder) with weight gain, length gain, specific growth rate, and survival rate at 0.0301±0.0013 g, 12.41±0.56 mm, 12.82±0.16 %/day, and 94.17±1.66%, respectively. Hence, it can be concluded that the supplementation of eggshell powder may decrease the growth and survival rate of black tiger shrimp larvae.

1. Introduction
Indonesia has abundant of fish and shrimp biodiversity on its region [1, 2, 3]. Black tiger shrimp (*Penaeus monodon*) is an export commodity that is important for the Indonesian economy [4]. The success of intensive development of tiger shrimp cultivation depends on the level of minerals in feed. Minerals are organic materials needed by shrimp to form body tissues, metabolic processes, and osmotic balance. Shrimp obtain minerals from direct absorption through gills, absorption through the digestive tract, and skin. Minerals are needed for growth because during their development shrimp will lose some parts of minerals in the body during moulting.

One important component in black tiger shrimp cultivation is growth. During the growth process, tiger shrimp will make exoskeleton replacements (moulting) because the exoskeleton does not grow when the tiger shrimp grows larger. After moulting, tiger shrimp will do calcification or a new exoskeleton hardening process. Imperfect calcification can lead to a longer period of soft exoskeleton hardening. Tiger shrimp is still weak in these conditions so that it is indirectly more vulnerable to similar attacks or cannibalism. The calcification process is influenced by the availability of calcium (Ca) in the waters and Ca can also affect the maximum size of crustaceans [5].

Along with the increasing consumption of chicken eggs by the people of Banda Aceh, the waste from chicken eggs in the form of eggshells will certainly also increase. The eggs are used for purposes in restaurants, bakeries, and street vendors who sell eggs and omelets as raw material for making food. In addition, Acehnese omelets is also used as an additional ingredient in other foods such as Acehnese noodles, burgers and fried rice. Until now the utilization of waste in the form of chicken egg shells has not shown maximum results. However, this waste still has high mineral content which has not been utilized optimally.

In general, the eggshell structure consists of three layers, namely the cuticle layer, the sponge layer, and the lamellar layer. The cuticle layer is the outermost surface that contains a number of proteins. The sponge and lamellar layer forms a matrix formed by protein fibers bound by calcium carbonate in the
Egg shells represent 11% of total egg weight and are composed of calcium carbonate (94%), calcium phosphate (1%), organic material (4%), and magnesium carbonate (1%) [6]. So far, efforts have been made to increase calcium in black tiger shrimp feed by utilizing pure calcium, which is relatively expensive and difficult for farmers to obtain. Therefore another alternative is needed to increase calcium levels for tiger shrimp at a low cost and easily available. One way to increase calcium levels in black tiger shrimp feed can be done by utilizing and processing eggshells which have been considered as community waste to be a valuable waste for the growth of black tiger shrimp. Therefore, it is necessary to investigate the administration of eggshell flour to feed to increase growth and survival of black shrimp seeds.

2. Materials and Methods

2.1 Time and place
This research was conducted in June-July 2017 at the Ujung Batee Brackishwater Aquaculture Center (BPBAP), Aceh Besar District. Eggshell waste was obtained from several locations in Banda Aceh City.

2.2 Research design
This study used an experimental method that was carried out with Completely Randomized Design (CRD) 5 levels of treatment and 4 replications.

- Treatment A = without addition of eggshell flour
- Treatment B = addition of eggshell flour 3%
- Treatment C = addition of eggshell flour 6%
- Treatment D = addition of eggshell flour 9%
- Treatment E = addition of eggshell flour 12%

The container used was an aquarium with 20 units. Before use, all containers were washed with detergent and rinsed until the smell and foam were gone, then dried in the sun to dry. After drying, the aeration installation was attached to the entire container. Then filled with 10 liters of water. The black tiger shrimp larvae used came from the hatchery of the Ujung Batee Brackishwater Development Center (BBAP) in Aceh Besar District, Aceh Province. Tiger shrimp larvae used PL 12 stage with 3 ind / L stocking densities in each maintenance container. Before the implementation of the research the shrimp was acclimatized for 2 hours. After the acclimatization period was complete, the test shrimp was fasted for 24 hours. Black tiger larvae were kept for 30 days.

The source of calcium was the eggshell obtained from several locations in Banda Aceh City, Aceh Province. Egg shells were first soaked with 80°C water for 15-30 minutes. Then cleaned and dried. Then it was soaked with phosphoric acid, then the process was carried out. Egg shells that had been processed into flour, then mixed with other feed ingredients and given to tiger shrimp larvae for 30 days. The feed given to tiger shrimp larvae was artificial feed with a protein content of 42%. Frequency of feeding as much as 3 times a day, namely at 08.00, 14.00 and 18.00 WIB. Feeding was given 5% of body weight. Adjusting the amount of feed needed was done every week. Dirt and leftovers were cleaned by shiponed once a day.

Sampling data on shrimp weight growth was carried out by sampling as many as 10 ind per container. Every seven days a shrimp was scaled using an LCS-B digital scale. Before weighing the water found on the surface of the body of the shrimp was absorbed first with tissue. The measurement of shrimp began before the shrimp stocked into a maintenance container.

2.3 Research Parameters

Absolute weight gain
Calculation of absolute weight gain used the formula [7] as follows:

\[ \Delta G = W_t - W_0 \]

Information:
\( \Delta G = \text{Weight gain (g)} \)
Wt = Shrimp weight at the end of the experiment (g)
W0 = Shrimp weight at the beginning of the experiment (g)

Specific growth rate (LPS)
According to [8, 9, 10, 11], the calculation of specific growth rates is as follows:
\[ \text{LPS} = \frac{(\ln \ Wt - \ln \ W0)}{t} \times 100 \]

Information:
LPS = Specific growth rate (% / day)
Wt = Biomass of test shrimp at the end of the study (g)
W0 = Biomass of test shrimp at the beginning of the study (g)
t = Maintenance time (days)

Absolute length gain
The growth of the absolute length of tiger shrimp was calculated by the formula [12] as follows:
\[ L = L_t - L_0 \]

Information:
L = Growth of absolute length (mm)
L_t = Final average length of study (mm)
L_0 = Average initial length of study (mm)

Survival rate
Survival rate was calculated using the formula [9, 10, 11, 12] namely:
\[ \text{SR} = \frac{(N_0 - N_t)}{N_0} \times 100 \]

Information:
SR = Survival rate (%)
N_0 = Number of shrimp that live at the beginning of maintenance (g)
N_t = Number of shrimp that die during maintenance (g)

Water quality management
Water quality parameters measured during the study were: temperature, pH and dissolved oxygen. Measurement of water quality parameters was carried out in each unit of experiment with a frequency of 3 days during the 30 days of the study period. Water change is done every 3 days as much as 50%. Squeezing the rest of the feed and feces is done once every day in the morning.

2.4 Data analysis
Data obtained at each treatment was carried out by analysis of variance (ANOVA). If the CC (Coefficient correlation) score was above 10%, a follow-up test was conducted, the CC score below 10% was carried out by the SSD further test (the Smallest Significant Difference) and the CC score below 5% was carried out by the PD (Pure Difference) test to see the difference between treatments and get the best treatment the Social Scoring Statistics software package (SPSS version 22).

3. Results and Discussions
The ANOVA test results showed that the addition of eggshell flour in black tiger shrimp feed with different doses significantly affected absolute growth, absolute length growth, specific growth rate and survival rate of black shrimp seeds (P <0.05) (table 1).

| Test parameters | Absolute weight gain | Absolute length gain | Growth rate specific (%) / day | Survival rate |
|-----------------|----------------------|----------------------|-------------------------------|--------------|
| Treatment       |                      |                      |                               |              |

Table 1. Absolute weight gain, specific growth rate, absolute length gain and survival rate of black tiger shrimp added to eggshell flour on feed at different doses.
Description: The average value in the same column with the addition of eggshell flour in black shrimp feed with different doses showed significantly different (P < 0.05). A = 0%; B = 3%; C = 6%; D = 9%; E = 12%.

Based on research carried out for 30 days of maintenance, black tiger shrimp larvae fed with eggshell flour on feed experienced growth inhibition. Data from the research showed that the best growth occurred in treatment A (control) without the addition of eggshell flour.

**Table 2.** Data on measurement of water quality parameters for 30 days maintenance of black shrimp larvae (Penaeus monodon)

| Treatment Code | Treatment                  | Temperature (°C) | pH    | DO (mg/L)  | Salinity (ppt) |
|----------------|----------------------------|------------------|-------|------------|----------------|
| A              | Control                    | 27.00-30.00      | 8.0-8.6 | 6.00-6.07  | 30-35          |
| B              | 3% eggshell flour          | 29.00-30.03      | 8.0-8.9 | 6.00-6.04  | 31-35          |
| C              | 6% 3% eggshell flour       | 28.02-30.05      | 7.9-8.5 | 6.00-6.04  | 29-35          |
| D              | 9% 3% eggshell flour       | 28.00-30.03      | 7.8-8.3 | 5.00-5.04  | 30-35          |
| E              | 12% 3% eggshell flour      | 29.00-30.07      | 8.0-8.8 | 5.00-5.06  | 31-35          |

Based on research carried out during 30 days of maintenance, showed that the highest absolute weight gain was obtained in treatment A dose of 0% (without the addition of eggshell flour) which was 0.0301 ± 0.0013 grams and the lowest absolute weight gain in treatment E (12% dose) at 0.0073 ± 0.0008 grams.
grams. Addition of calcium sourced from eggshell flour with different doses on black tiger shrimp larvae provided the opposite results, where with the higher dose of eggshell added, the growth of tiger shrimp will be lower. This is different from what was stated by [13] that the method of adding calcium to feed is one of the effective methods to provide additional calcium for lobsters, because with this method calcium can be digested directly along with the feed given. So that all of the calcium given to the feed can be used properly for the calcium reserves stored in the lobster's body. This calcium reserve will then be used for the process of hardening the shell again after the lobster has experienced moulting. According to [14] the role of moulting is very important in the growth of lobsters, because lobster can only grow through moulting.

Specific growth rates for 30 days of maintenance showed the highest growth in treatment A dose of 0% (without the addition of eggshell flour) which was 12.82 ± 0.16 % / day and the lowest specific growth at treatment E dose 12% was 8.32 ± 0.35%/day. It is suspected that black tiger had not been able to absorb the calcium content in egg shells. It was assumed that egg shells do not only contain calcium, but there are other chemicals that might affect the growth of tiger shrimp. As stated by [6] that egg shells represent 11% of total egg weight and are composed of calcium carbonate (94%), calcium phosphate (1%), organic material (4%), and magnesium carbonate (1%). Another thing that might be the cause of the low specific growth of the treatment carried out was the addition of eggshell flour because the eggshell flour is not so smooth that it affects the ability of shrimp larvae to digest. While the use of pure calcium which has been applied so far is in very subtle conditions compared to calcium sourced from egg shells.

The growth of shrimp body length is influenced by the amount of moulting that occurs because moulting is a process of growth in the weight and length of shrimp. The results of measurements of the length of tiger shrimp seeds during the study obtained the highest long growth in treatment A dose of 0% (without the addition of eggshell flour) which was 12.41 ± 0.56 mm, while the lowest length growth in treatment E 12% dose added eggshell flour is 1.52 ± 0.38 mm. This might be influenced by the inability of tiger shrimp to absorb calcium from egg shells. As stated by [13] that good calcium absorption will stimulate the crustaceans to do moulting. It is explained that growth in crustaceans is the increase in length and weight of the body that occurs periodically shortly after moulting [15].

Survival is a comparative value between the number of living organisms at the end of the study and the number of organisms at the beginning of the study expressed in percent. Observation of the survival of black tiger shrimp larvae added to eggshell flour on feed was observed for 30 days of maintenance, the survival rate of black tiger shrimp larvae showed different results in each treatment. Treatment A dose of 0% (without the addition of eggshell flour) was a treatment that showed the highest survival rate of black shrimp larvae, which was 94.17 ± 1.66%. Then the lowest survival at treatment E was 50.00 ± 2.72% with a 12% dose of addition of eggshell flour. The mortality of tiger shrimp during maintenance was due to the cannibalism that occurs when moulting (changing the skin). Tiger shrimp that are changing the skin are in the weakest condition due to the amount of energy released. According to [16], when a shell change occurs (changing skin) is when prone to tiger shrimp because when the skin is released the body inside is not protected anymore so it will be easier for other shrimp to prey it. This cannibalism process is proven by the discovery of pieces of tiger shrimp in a maintenance container.

Water quality is one of the important factors that determines the success in maintaining tiger shrimp larvae, because water quality is closely related to the growth and survival of reared tiger shrimp larvae. Water quality for 30 days of maintenance did not change significantly and was still within the normal range. Water temperature measured during the study ranged from 27.00-30.07 °C, pH ranged from 7.8-8.9, DO ranged between 5.00-6.07 ppm and measured salinity ranged from 29-35 ppt. According to [11] optimal temperature of shrimp larvae growth between 26-32°C, optimal pH range maintenance of tiger shrimp larvae ranged between 7.8-8.8 and optimal salinity range maintenance of 24-35 ppt for tiger shrimp larvae. It is stated that the suitable temperature for the growth of tiger shrimp between 25-32 °C [18], the best acidity (pH) for shrimp farming is 7.4-8.9 and the range of salinity that is suitable for shrimp is 15-34 ppt. According to [19] the suitable temperature for the growth of shrimp larvae ranges from 25-32 °C, a good degree of acidity (pH) for shrimp farming is 7.4-8.9 and a good range of salinity
for shrimp hatching is 28-35 ppt. Therefore, the water quality parameter during experiment was within optimal range for shrimp larvae.

**Conclusion**

It can be concluded that eggshell flour in feed can inhibit the growth and survival of black tiger larvae (*Penaeus monodon*). The addition of fermented eggshell flour may be an alternative for future research.

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