Bioencapsulation Single Cell Protein from *Chlorella costatum* Extract on Survival rate and Mortality of Barramundi Larvae, *Lates carcarifer*, Bloch 1790

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**Abstract**- Barramundi is one of the cultivated commodities which is being developed at this time. One of the challenges in Barramundi Aquaculture is the low survival rate due to internal mortality during the larval phase. Adequate feed supplementation, both in quantity and dose can be a solution in preventing mortality in fish larvae. The research objective was to analyze the effect of SCP on survival and mortality in barramundi larvae. This study used a completely randomized design (CRD) method with 4 treatments and 3 replications. The treatment doses used were 0, 100, 200, and 300 mg / L. The results of the analysis of variance showed that the dose had a significant effect (P <0.05) on the survival and mortality of barramundi larvae. The results showed that using SCP at a dose of 100 mg / L increased the survival rate of 80.00% and significantly decreased the mortality rate of barramundi larvae.

**Key words**: Barramundi larvae, Bioencapsulation, *Chlorella costatum*, Singel cell protein, Supplementation.

**I. INTRODUCTION**

The main problem in the treatment of barramundi larvae (*Lates calcarifer*) during the production process is the low survival rate. Previous research from Nurmasiyith et.al., (2018) and Darosman et.al. (2019) obtained the results of research on stress factors arising from lack of nutritional needs due to low feed quality (Basford *et al.*, 2020), and environmental conditions that are less than optimal in larval rearing (Ribeiro *et al.*, 2015). Inadequate nutritional requirements in live food are an important contributor to mortality in larvae during the production period. To increase the survival rate in barramundi larvae, it is necessary to improve water quality and good maintenance management as well as supplementation of live food to improve quality. Supplementation of protein and lipid forms is needed by marine fish larvae to support normal growth and improved survival (Pangkey, 2011). Supplementation that has the potential to be done is through the application of single-cell protein.

**Single-cell Protein** (SCP) is a biotechnology product that refers to microbial biomass or protein extract that is extracted as a food additive (*Bharti et al.*, 2014). In addition to protein, SCP has the potential for lipid content in the form of fatty acids reaching 35.13% (*Kurnia et al.*, 2018). SCP has been successfully developed from several microalgae extracts such as *Spirulina* sp (*Sharma et al.*, 2019). However, the use of *Spirulina* sp as SCP is considered inappropriate due to high production and management costs. Therefore, an alternative is needed that could potentially to replace *Spirulina* sp. and *Chlorella costatum* can be recommended. Research on SCP has been carried out on several species of fish and shrimp larvae, among others is tilapia fish (*Hussein et al.*, 2013), zebra fish (*Şişman et al.*, 2013), salmon fish (*Eichner et al.*, 2018), and vannamei shrimp (*Hamidoghli et al.*, 2019). However, information on the use of SCP on barramundi larvae has not been found.

The utilization of SCP in barramundi larvae is hindered by the carnivorous nature of the fish, so methods such as bioencapsulation are necessary. Bioencapsulation is nutritional enrichment using additives to natural feed to improve the quality and quantity so that the nutritional quality of natural feed can be increased (*Sarmudianto et al.*, 2015). Based on this background, research on the benefits of *Chlorella costatum* as an SCP product needs to be done. This study aims to analyze the effect of SCP on survival and mortality in barramundi larvae.

**II. METHOD**

This research was conducted from June to September 2020 at the Center for brackish water fisheries development Air, Takalar regency, south Sulawesi province, Indonesia. Single- cell protein (SCP) production process at the Mini Fisheries and Water Quality Laboratory, Faculty of Marine and Fisheries Sciences, Hasanuddin University. Analysis of test samples at the Laboratory of Animal Feed Chemistry, Faculty of Animal Husbandry, Hasanuddin University.
This study used a plastic tub with a round shape, measuring 40 liters and filled with 25 liters of water for the treatment test of barramundi larvae. Treatment animals/objects used barramundi D-16 (16 days after hatched) and 50 larvae were stocked at a density of 2 Ind / L. The days of maintenance were obtained from Center for development Air, Takalar province, Indonesia. Live feed with Artemia salina. Cell Protein (SCP) from solvent using seawater with 30 ppm of salinity. SCP processing requires a starter material for microalgae (Chlorella costatum) and liquid tofu waste from the waste products of the tofu factory in the Karanganyar area, Mariso District, Makassar. The nutritional content of SCP is shown in Table 1.

### Table 1. Proximate analysis of SCP

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>water</td>
<td>20.86</td>
</tr>
<tr>
<td>Protein</td>
<td>8.55</td>
</tr>
<tr>
<td>Fat/lipid</td>
<td>0.87</td>
</tr>
<tr>
<td>fiber</td>
<td>0.86</td>
</tr>
<tr>
<td>BETN</td>
<td>12.82</td>
</tr>
<tr>
<td>Ash</td>
<td>76.91</td>
</tr>
</tbody>
</table>

The study was designed using a completely randomized design with 4 treatments and 3 replications. As treatment using different doses of SCP 0, 100, 200, and 300 mg/L. The parameters observed were the retention of nutrients absorbed in the larvae of barramundi (dissolved protein, lipids, and energy), survival, and mortality during the treatment period for barramundi larvae.

**Dissolved protein retention** is calculated using the following formula (Dewi and Tahapari, 2018):

\[
R_{Pt} = \frac{P_{tb}}{K_{pt}} \times 100
\]

Note: \( R_{Pt} \) is dissolved protein retention (%), \( P_{tb} \) is the increase in dissolved protein in the body (%), and \( K_{pt} \) is the dissolved protein content in feed (%).

**Lipid retention** is calculated using the following formula (Dewi and Tahapari, 2018):

\[
R_L = \frac{L_{tb}}{L_{p}} \times 100
\]

Note: \( R_L \) is the lipid retention (%), \( L_{tb} \) is the increase in lipids in the body (%), and \( L_{p} \) is the lipid content in the feed consumed (%).

**Energy retention** is calculated using the following formula (Tung and Shiau, 1991):

\[
RE = \frac{(E_{end} - E_{start}) \times 100}{F_E}
\]

Note: \( RE \) is energy retention (%), \( E_{end} \) is final body energy (Kcal), \( E_{start} \) is initial body energy (Kcal), \( F_E \) is total feed energy given (Kcal).

**Survival rate** is calculated using the following formula (Gunadi et al, 2016):

\[
SR = \frac{N_T}{N_{0}} \times 100
\]

Note: \( SR \) is the survival rate (%), \( N_T \) is the total number of live fish at the end of the study (tails), and \( N_{0} \). The total number of fish at the beginning of the study (tails).

**Mortality** is calculated by comparing the total larvae at the start of the study with the total larvae that died at a certain period in the treatment period. Larval mortality data were obtained in 3 time periods, the period being the 4th day of maintenance (D-4),
the 7th day of maintenance (D -7), and the 10th day of rearing (D-10). Larval mortality was calculated using the following formula (Ribeiro, 2015):

$$PM = \frac{(N_{\text{dead}} / N_{\text{start}})}{\Delta t} \times 100$$

Note: PM is the mortality of larvae in a certain period (%), N_{\text{dead}} is the number of dead fish larvae in a certain period (tail), N_{\text{start}} is the total number of fish at the beginning of the rearing period (tails), and \(\Delta t\) is the time interval / maintenance period (days).

During maintenance, several parameters of the water quality of the maintenance media were measured, including thermal temperature, water temperature, salinity, dissolved oxygen, pH, and ammonia. Thermal temperature was measured using a thermal thermometer, water temperature using a water thermometer, salinity using a hand refractometer, dissolved oxygen using a Digital DO meter, pH using a portable Digital pH meter and ammonia measured using a spectrophotometer. Thermal temperature and water temperature parameters were measured three times a day during the morning, afternoon and evening. Salinity, dissolved oxygen, and pH were measured daily during treatment. Ammonia parameters were measured by taking samples 3 times during treatment at the beginning, half of treatment and end of treatment.

The data obtained included dissolved protein retention, lipid retention, energy retention and survival rate for barramundi larvae, analyzed using the Analysis of Variance (ANOVA) method. Because the results have a real effect so it is continued with the W-Tuckey Test. The SPSS version 23.0 package was used as a tool for analysis. The mortality data for barramundi larvae were analyzed descriptively based on the pattern on the graph showed.

### III. RESULTS

**Retention of dissolved protein, lipid and energy content in barramundi larvae**

Retention of dissolved protein, lipid and energy content in barramundi larvae showed in table 2.

<table>
<thead>
<tr>
<th>Treatment dose (mg/L)</th>
<th>Retention (%)</th>
<th>Dissolved protein retention/ RPt</th>
<th>Lipid retention/ RL</th>
<th>Energy retention/ RE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0)</td>
<td>1.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.86 ± 1.85&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>1.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.54 ± 0.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.85 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>2.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.4 ± 0.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Values of the same row with different letters were significantly different (p<0.05)

The retention of dissolved protein content showed insignificant results (P<0.05) between treatments (Table 2). The ANOVA test results showed no significant effect or effect on the treatment given. The highest percentage value was obtained at the treatment dose of 300 mg / L with a value of 2.15% while the other treatments each obtained values of 1.26%, 1.07% and 0.30% for each treatment dose control (0), 100 mg / L and 200 mg / L.

Lipid retention in the body of barramundi larvae showed significant results between treatments (P<0.05) (Table 2). The results at the treatment dose of 100 mg / L showed a significant or significant difference to the treatment at the dose of 200 mg / L and 300 mg / L, but the control treatment did not give a significant impact. The highest lipid retention value was obtained at the SCP dose of 100 mg / L with a value of 3.54%, then respectively in the control treatment, 200 mg/L, and 300 mg / L resulted in retention values of 1.86%, 0.85% and 0.4%.

Energy retention in barramundi larvae showed insignificant results (P<0.05) between treatments (Table 2). The ANOVA test results showed no significant effect or effect on the treatment given. The highest percentage value was obtained at the SCP treatment dose 200 mg / L and 100 mg / L with a value of 7.39% and 7.20%. Then in the control dose treatment (0) and 300 mg / L, the percentage values were 3.47% and 3.44%.

**Survival of barramundi larvae**

Survival of barramundi larvae is showed in table 3
The value of the survival rate of barramundi larvae showed a significant difference between treatments (P < 0.05) (Table 3). The ANOVA test results show that there is a significant effect or effect on the treatment given. Treatment with a dose of SCP 100 mg / L can give a significant result or significantly different effect on control treatment (0). However, the results given did not show any significant results for other treatment doses of 200 mg / L and a dose of 300 mg / L. These results indicate that the treatment of SCP dosage as a feed supplement for barramundi larvae can give better results than treatment without SCP.

**Table 3. Survival in Barramundi larval**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0)</td>
<td>47.33 ± 9.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>100</td>
<td>80.00 ± 6.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>200</td>
<td>67.33 ± 3.05&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>300</td>
<td>68.00 ± 12.16&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values of the same row with different letters were significantly different (p < 0.05)

The development mortality of barramundi larvae is showed in Figure 1.

**Figure 1. Mortality in Barramundi larvae during treatment**

The development of mortality of barramundi larvae (Fig. 1) showed treatment with different doses of SCP can have an effect on mortality rates during larval rearing. The data show that the larval mortality rate has increased on the 7th day (D-7) of the maintenance period, where almost all treatments have resulted in a significant increase in mortality during that period. However, the treatment doses of 100 mg / L and 200 mg / L showed a decrease in the mortality rate during that period. The mortality of larvae at the treatment doses of 100 mg / L and 200 mg / L showed a trend or pattern that slowly decreased in each maintenance period including the D-7 period. In other treatments, the trend graph was showed to be different at the control dose (0) and the dose of 300 mg / L, where both treatments showed an increase in mortality on day 7 (D-7) of the maintenance period. This period as well as...
being the highest peak of mortality in that treatment during larval rearing. In the control treatment, the decrease in the mean mortality value obtained did not show a lower value than the initial maintenance period.

Environmental water quality treatment of barramundi larvae

Analysis of the quality of treatment water is shown in Table 4.

Table 4. water analysis treatment

<table>
<thead>
<tr>
<th>Parameter test</th>
<th>Control (0)</th>
<th>Treatment dosage (mg/L)</th>
<th>SNI 6145.3:2014 (BSN, 2014)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermal temperature (°C)</td>
<td></td>
<td>28.5 - 41.2</td>
<td></td>
</tr>
<tr>
<td>Water temperature (°C)</td>
<td>27.5 - 33.5</td>
<td>2.71 - 33.1</td>
<td>27.5 - 33.1</td>
</tr>
<tr>
<td>Salinity (mg/L)</td>
<td>31.32</td>
<td>31.32</td>
<td>31.32</td>
</tr>
<tr>
<td>Dissolved oxygen (mg/L)</td>
<td>5.2 - 6.3</td>
<td>5.3 - 6.5</td>
<td>5.3 - 6.7</td>
</tr>
<tr>
<td>Water pH</td>
<td>6.8 - 7.8</td>
<td>6.6 - 7.4</td>
<td>6.5 - 7.7</td>
</tr>
<tr>
<td>Water ammonia (mg/L)</td>
<td>0.001</td>
<td>0.002</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Based on Table 4, the water quality measurement results obtained did not show any significant differences in each treatment. The value recorded in each parameter shows the results by the standards for managing the water quality of barramundi fish larvae that have been set by BSN following SNI number 6145.3:2014 which was published in 2014. For thermal temperature parameters in raising barramundi larvae have not been standardized by BSN.

IV. RESULT

Based on the results of the study in Table 2 regarding the retention of nutrients such as dissolved protein, lipid and energy, it is indicated that the administration of SCP dosage as a bioencapsulation material for *Artemia salina* on certain parameters did not show a significant effect on treatment (P <0.05). As in the result of the dissolved protein retention value parameter, this can be influenced by the substitution value of the protein contained in SCP 8.55% or 0.085 g (Table 1). The result of this protein content is much less than the production of SCP without the compaction/drying process with a percentage value of 52.24% (Putri et.al, 2018). This result is also lower than the protein hydrolyzate value in *chlorella* sp in powder form, which is 46.3 g / 100g or 0.46 g (Grossmann et.al, 2018).
The results of the energy retention in Table 2 also did not show a significant effect on treatment (P <0.05). This can occur as a result of the high value of ash content in the resulting SCP, which is 76.91% (Table 1). Ash content is a collection of minerals that have properties as inorganic materials. Where this substance is not completely absorbed by the body. In the process, the main obstacle in mineral absorption is the ability of food to present enough minerals in ionized form to the apical membrane of the enterocytes for transcellular absorption, so that not all mineral nutrients can be absorbed (Goff, 2018). As a reference, one of the studies related to bioencapsulation using a microalgae source with the type of *Spirulina* sp has a fairly low ash content value of 2.99% (Suyanto et al., 2019). The ash content produced in this study is very high.

The results of the lipid retention test in Table 2 showed a significant effect on treatment (P <0.05). The value of lipid retention is thought to have a significant effect between treatments due to the effect of lipid substitution on SCP, although the resulting lipid value in this study was still low at only 0.87% (Table 1). When compared with SCP products with microalgae sources from *spirulina* sp, it can produce a lipid percentage of 7.4% (Moreira et al., 2011). However, the resulting lipid value is proven to be used properly as a nutrient in the bioencapsulated feed for barramundi larvae. part of the lipids that are important for the body and can directly play a role in the formation of body energy are fatty acids, one of which plays an important role in the body is EPA (Suyanto et al., 2019).

Based on the survival rate data in Table 3, the survival value in the SCP dose treatment ranges from 67.33-80.00%. In terms of the results of research related to the test of the benefits of microalgae as an enrichment material in barramundi larvae, it shows the percentage of survival rate of larvae ranging from 42.75 - 55.50 % (Darosman et al., 2019). Then another study showed that the average survival rate obtained in barramundi larvae obtained values ranging from 20.75 - 72.50 % (Nurmasiyatoh et al., 2018). Based on the results showed from previous studies, the results achieved in this study showed a much higher increase in survival rate in barramundi larvae.

Survival rate in barramundi larvae is caused by many factors. The factors that cause it can come from the adequacy of nutrients from the enrichment process of food and environmental factors that contribute to each other (Kusumawati et al., 2019). Enrichment in food/feed, especially lipid levels and energy retention levels in the body of barramundi larvae can be used as a reference in determining the effectiveness of increasing survival rate in larvae. The results of lipid retention or lipid absorption in the body of barramundi larvae that were given different doses of SCP resulted in a percentage range of 0.40% - 3.54% (Table 2). Then the lipid retention values commonly found in juvenile barramundi fish range from 0.45 to 1.59%. (Phan et al., 2019). Specifically, there is no reference regarding lipid retention found in the larval phase of barramundi, but based on the reference value of retention found in the juvenile phase, in this study the lipid retention value obtained was quite high.

The important component in lipids/lipids is lipid acid, where barramundi fish larvae require intake of lipid acids such as DHA with the recommended amount of 10 g / Kg in feed (Glencross and Rutherford, 2011). However, excess nutrients in the form of lipid acids such as DHA and ARA which are given in feed substitutions where there is also one or more nutrients that do not meet the minimum nutritional needs of barramundi larvae, it can cause a decrease in survival rate in barramundi larvae (Thépot *et al.*, 2016). Therefore the dose in the substitution of feed supplements has a very important role for the larvae of barramundi. There is no more specific reference regarding the correct SCP dose in barramundi larvae, so based on the findings of this study, a treatment dose of 100 mg/L can be recommended.

Based on the data in graph 1, death during the treatment of barramundi larvae can be caused by many factors. According to Ribeiro, (2015) the factors that cause mortality in fish are due to the heterogeneity of growth so that it can cause quite high cannibalism and death from wounds and suffocation. These factors are also the causes of death found in this study. The cause of death in this study indicated a link with the nutritional substitution in larvae feed. Nutrients such as dissolved protein can be indicated to play a role in the development and life of larvae. Dissolved protein is a source of nutrition consisting of oligopeptide bonds that are easily absorbed by the organism's body (Mardhika, et al, 2020). The Oligopeptide content has the properties or functions as antihypertensive, antimicrobial, immunomodulatory and antioxidant which is quite prominent (Jia et al., 2010) As immunomodulators and antioxidants, when substituted in sufficient quantities, it can improve health performance in fish (Dwiniati and Sasanti, 2019). Therefore, it is strongly suspected that the protein substituted by administering SCP at a certain dose can supply sufficient oligopeptides into the body of the larvae.

Based on the evidence in Figure 2, it shows that in this study the mortality that contributed the most was indicated as a result of cannibalism that occurred. This is based on findings on days at the 7th rearing period (D-7) or at the age of larvae 20-22 (D-21 to D-22) days. Besides, other findings in this study also indicated that the cannibalism that occurs mostly has the same pattern as the study from Fehér *et al.*, (2013) no findings of dead fish (carcasses) where cannibalism is responsible for the death of the barramundi fish. Several studies have shown cases of cannibalism in barramundi fish are generally found to occur in the juvenile phase where the larval age ranges from 67-112 days (after hatching) (Jesu Arockiaraj and Appelbaum, 2011). Recent studies have showed cases of cannibalism begin to appear in the juvenile phase when the fish are 35 (D-35) days after hatching (Pham *et al.*, 2020). The results regarding cannibalism in susceptible larvae aged 20-22 days after hatching have not been found in several articles regarding studies that have been conducted on barramundi fish. This is new information, especially in raising barramundi larvae.

Based on the data in table 4 regarding the test results of the water quality value parameters in the rearing of barramundi fish larvae, they comply with the standards set by BSN. Recent research shows the suitability of the results of water quality parameters

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in raising barramundi larvae where the water quality parameter values are deformed, such as water temperature in the range of 26 - 28.5 ° C, pH values in the range of 7.5 - 8.3 and salinity levels with range 32 - 34.5 and dissolved oxygen levels> 5 (Darosman dkk., 2019). The value obtained in this study indicates the conformity of the water quality value obtained, although parameters such as water temperature in the research conducted are slightly higher. The thermal temperature has not been standardized by BSN. However, the thermal temperature tolerance value has been tested on barramundi fish in open waters. The tolerance value is obtained at the highest value of 40 ° C (Newton et al., 2010). Barramundi fish can tolerate a wide temperature range of 14 - 40 ° C, where this species tends to be cultivated in water with a temperature range of 22 to 35 ° C (Tucker, et al., 2002; Thépot and Jerry, 2015). The intensive cultivation of barramundi sometimes exposes individuals to temperatures that reach above thermal tolerance temperatures. So with this reference, the value of room temperature or thermal temperature obtained in the range of 28-42 ° C can be tolerated by the larvae of barramundi, although in some conditions such as during the day the temperature value showed is 2 ° C higher than the tolerance value stated (Newton et al., 2013).

V. CONCLUSIONS

The conclusions in this study indicate that administering SCP doses to barramundi larvae by bioencapsulation method gives the best results at a dose of 100 mg / L. Treatment with SCP 100 mg / L can provide a survival rate of up to 80% and a decrease in mortality during treatment.

REFERENCES


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