Growth of Sunu Grouper (*Plectropomus leopardus* ) Larvae That Given Rotivera (*Bachionus rotundiformus*) Enriched with Taurine and Glutamine

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**Abstract.** This study aims to determine the effect of taurine and glutamine enriched Brachionus on the growth and survival rate of Sunu Grouper (*Plectropomus leopardus*). This research was conducted in Marine Research and Fisheries Extension Center Gondol, Bali. The study was conducted for 1 month on September 2017. The research method used was using Completely Randomized Design (CRD) consisting of 4 treatments and 4 replication, namely treatment A (without Taurine and Glutamine); (B) 0,5 gram Taurine / 10 L media; (C) 0,5 gram Glutamine / 10 L media; (D) 0,5 gram Taurine and 0,5 Glutamine / 10 L media. The age of Sunu Grouper was D-2 until D-10, parameters observed were Survival Rate, Average Daily Length, Relative Growth Rate, number of Brachionus in the digestive tract, and water quality. Which included DO, temperature and pH. Data were analyzed using analysis of variance with F test with 95% confidence level and the difference between treatments was tested by Duncan multiple-range test, then water quality was analyzed descriptively by comparing to related literature and water quality standard according to SNI 1999. The results showed that the use of 0,5 gram Taurine and 0,5 gram Glutamine enriched Brachionus, had an effect on the growth of Sunu Grouper.

**Keyword:** *Plectropomus leopardus*, brachionus, taurine, glutamine, growth, survival rate

1. **Introduction**

Sunu or leopard coral grouper (*Plectropomus leopardus*) is a grouper highly needed by Asian market and has important economic value for its relatively high price [1]. However, the development of sunu grouper farming in Indonesia is constrained by hardly continuous and adequate juvenile fish supply [2]. Aslianti [3] states that sunu grouper larvae survival rate is 0.15% - 0.27% and tends to die on day 14. This low survival rate requires appropriate natural feed supply at larva stadium, which is a very important initial phase for larva survival, with the appropriate feed is zooplankton Brachionus.

Brachionus is tiny with size of 50-250 µ[4][5], that it is suitable for the mouth opening of sunu grouper larva, with slow movement, easy to cultivate, easy to digest and easy to improve its nutrients with various fish oils [6]. The nutrients of Brachionus are inadequate to fulfill the nutritional need of sunu grouper larva, thus it needs to be enriched with various materials in support of rotifer’s nutrients, such as *Chorella sp.*, *Nannocloropsis sp.*, baker’s yeast and taurine [7].
The other attempts to improve rotifer’s quality are with enrichment using *Chlorella* sp, vitamin C and vitamin B complex [4]. Enrichment using fatty acid [8] and enrichment using β-carotene [9]. Addition of β-carotene is evidently not able to maximally improve the survival rate of larva at stadium D2-D20. According to to Jusadi *et al.* [1], humpback grouper larva D16 fed with rotifer enriched with amino acid taurine may improve its survival rate. Rotifer enrichment with glutamine for humpback grouper may significantly enhance larva growth.

Taurine is non-essential amino acid which contains sulfur, but is not of protein group since it does not have carboxyl group (-COOH) needed to form peptide bond. Taurine is found in high content in skeletal muscle, heart and white blood cell and central nerve system. Taurine may be found in various sources of food like meat and fish [10]. This amino acid exists about 79.5% in total amino acid in abalone’s muscle and in egg and larva of *Haliotis rubra* [6]. Taurine is known as non-essential amino acid which plays an important role in osmoregulation of marine invertebrates and a number of anaerobic energy metabolisms through formation of final product glycolysis, tauropine [11].

Glutamine is non-essential amino acid which is quantitatively free amino acid substantially found in blood plasma and muscle compared to other free amino acids. Glutamine is a source of energy which plays a role in biosynthesis of glucose, amino sugar and glutathione [12]. Additional glutamine in feed is expected to improve intestinal performance in feed digestion. This research aims at identifying the influence of enrichment and addition of taurine and glutamine to rotifer in support of sunu grouper larva’s survival and growth.

2. Method

The instruments used in this research are graduated cylinder, Pasteur pipette, bucket, ruler, plankton net, thermometer, fiber vessel 1000 L, blender, analytical balance, microscope, light meter and hose. The materials are Rotifer (*B. rotundiformis*) in size of (80-120 μm) serving as natural feed with addition of chicken egg yolk, baker’s yeast, fish oil, taurine and glutamine as enriching materials.

This research is conducted by employing a Completely Randomized Design (RAL) consisting of four treatments and four repetitions:

1. **Treatment A**: Larva is given with rotifer only (without taurine and glutamine).
2. **Treatment B**: Larva is given with rotifer enriched with 0.5 g taurine per 10 L of rotifer media.
3. **Treatment C**: Larva is given with rotifer enriched with 0.5 g glutamine per 10 L of rotifer media.
4. **Treatment D**: Larva is given with rotifer enriched with 0.5 g taurine and 0.5 g glutamine per 10 L of rotifer media.

The survival rate is the comparison between the number of live larvae at the end of research and the number of larvae at the beginning research.

\[ SR(\%) = \frac{\sum\text{larvae at } t_1}{\sum\text{larvae at } t_0} \times 100\% \]

Larvae relative length-growth is the comparison between the addition of larvae length during the research to larvae initial length. According to Takeuchi [13], relative growth rate may be stated in the following formula.

\[ RGR = \frac{W_t - W_o}{W_o} \times \frac{t}{100\%} \]

2.1. The Number of Rotifers in Fish Digestive Tract

The number of rotifers in fish larva’s digestive tract is counted to predict the amount of consumed feed. The counting is performed one hour after feeding. Five of larvae are taken from each nurturing media and observed under microscope by putting them one by one onto microscope slide, in which the number of rotifers in digestion tract is counted.
2.2. Water Quality
Water quality test needs to be performed to observe the influence of enrichment materials on nurturing media, covering pH, temperature, Nitrite, and Ammonia. This water quality test is conducted at the beginning and at the end of research.

2.3. Data Analysis
A completely randomized design (RAL) is employed in this research with 4 treatments and 4 repetitions for each treatment. F test is conducted at level of 5% on the survival and larva’s total final length parameters to examine whether the treatments influence the parameters, and in case of significant difference, the Duncan test will be conducted.

3. Result and Discussion
3.1. Survival Rate of Larvae Sunu Grouper
The research result shows that treatment A has survival rate of 2.12 ± 0.16 %, treatment B has survival rate of 2.29 ± 0.09 %, treatment C has survival rate of 2.40 ± 0.17 %, and treatment D has survival rate of 2.38 ± 0.16 %. Rotifer is enriched with addition of 0.5g taurine, 0.5g glutamine and mixture of 0.5g taurine and 0.5g glutamine. The survival rate data are presented in Table 1.

| Treatment | Taurine and Glutamine (gr) | Survival Rate (%) |
|-----------|---------------------------|-------------------|
| A         | Control                   | 2.12 ± 0.16       |
| B         | 0.5 Glutamine             | 2.29 ± 0.09       |
| C         | 0.5 Taurine               | 2.40 ± 0.17       |
| D         | 0.5 Glutamine + 0.5 Taurine | 2.38 ± 0.16    |

The analysis of variance results show that amino acid taurine and glutamine additions do not significantly influence (Fhit < F table) in Duncan test at level 5% on sunu grouper larvae’s survival. The reason is the function of taurine and glutamine as the neurotransmitter in the central nerve system. Neurotransmitter accelerates message delivery to the nerve system in the brain and facilitates communication between brain cells [14]. The chemical compound neurotransmitter and adequate energy supply from taurine or glutamine and other important roles of taurine and glutamine in larva’s body may expectedly speed message delivery through nerve cells to the nerve system in the brain, thus the organogenesis process controlled by the central nerve system occurs more smoothly. In addition, the other important roles of taurine in larva’s body function like vision, brain growth and heart function supposedly make larva has better body growth which may eventually improve the larva’s survival. This conforms to the statement of Russheim [15] that taurine also plays an important role in vision, brain, nerve system, heart function and also serving as bile acid conjugator.

The lowest result is of treatment A (control) without taurine or glutamine addition. According to Guffron and Kordi [16], that a farming biota’s survival is low is caused by some factors, one of which is inappropriate feed nutrition. This may be caused by no supplementation of amino acid taurine or glutamine to rotifer, thus larva’s low amino acid absorption will make nutrient availability for protein synthesis and energy production in treatment A lower than that of treatment B, C, and D.

3.2. Average of Larvae Sunu Grouper Length
Taurine and glutamine supplementation to rotifer may result in different growth rate in sunu grouper larvae between the treatments during the 10 days of nursery, ranging between 12.07% - 8.40%. The highest growth of leopard coral grouper larva is presented by treatment A (0.5g taurine and 0.5g glutamin) of 12.07%, treatment C (0.5 taurine) of 11.57%, and treatment B (0.5 glutamin) of 10.17%.
Meanwhile, treatment D (control), which is not supplemented with taurine and glutamine, has the lowest growth rate of 8.40%. The relative growth rate data are presented in Figure 1.

![Figure 1](image-url)

**Figure 1.** The Relative Growth Rate of Sunu Grouper Larvae.

Based on the research results, rotifer enriched with taurine and glutamine may result in sunu grouper larvae’s different growth from treatment without taurine and glutamin supplementation. Table 2 show average of larvae sunu grouper length, treatments A, B, and C have significant growth compared to the control treatment (D).

| Treatment | Taurine and Glutamine (gr) | Length (cm)   |
|-----------|--------------------------|--------------|
| A         | Control                  | 2.84 ± 0.02a |
| B         | 0.5 Glutamine            | 3.01 ± 0.10c |
| C         | 0.5 Taurine              | 3.15 ± 0.06b |
| D         | 0.5 Glutamine + 0.5 Taurine | 3.20 ± 0.08d |

Factor which influences growth is good farming management, such as high stocking density, feed quality, water quality, parasite or disease. In addition, fish growth is influenced by internal and external factors. Internal factor includes heredity, resistance to disease, age, and capability to exploit feed, while external factor includes temperature, feed quality and quantity, and movement space [16]. Fish will have slow growth and small size in case of inadequate feed. When body organ’s functions and body physiological functions operate well, larvae body’s metabolism rate will increase, thus larvae digestion speed also increases. Free amino acid does need hydrolysis since it can be directly absorbed into larva body’s digestion tract. Larvae’s increased digestion speed makes larva absorbs nutrients faster for its body. This makes larvae of treatments B, C, and D stronger so that they can find feed faster and more than larvae of treatment A. The higher content of amino acid in rotifer because of enrichment process with taurine and glutamine makes the source of energy in larva body available faster since the free amino acid absorbed in the digestion tract is transferred 3.5 times faster to larva body’s tissue than in protein form.

3.3. Rotifer in Digestive Tract

Rotifers eaten in larvae’s digestive tract are observed using microscope one hour after rotifers are given, which at 13.00. The number of rotifers in digestive tract of larvae data are presented in Figure 2.
The results of observation of the number of Brachionus consumed by larvae one hour after feeding show an increase of the number of rotifers in digestion tract each day. However, from day 6 and 7, the number of rotifers eaten in treatments B, C and D tends to be more than that in treatment A. The mean number of rotifers consumed by larvae of treatments D, C and B is higher than that of treatment A. This is expectedly related to taurine and glutamine supplementation to the enrichment media which may improve larva’s appetite. This conforms to the research conducted by Aprilia [17], that rotifer added with taurine and glutamine is able to improve larva’s appetite compared to control treatment. The high amino acid absorption by larva will result in nutrient availability for protein synthesis and energy production with treatments B, C and D is higher than that of treatment A. The correlation with source of energy is that marine fish absorbs amino acid more than protein [18]. The reason is that amino acid may be directly absorbed by larva digestion system and does not require enzyme to break peptide bonds.

3.4. Water Quality
The temperature observation result shows that during the research taken in three different times, there is a range of temperature between 26.3°C – 28.6°C, as, for clarity, presented in Table 3. There is an increase of temperature during research for 2°C – 3°C. Meanwhile, according to Aslianti [3], the change in minimum-maximum temperature is 1°C - 1.5°C. This value does not conform to the standard quality of temperature for leopard coral grouper larvae of 28-30°C. The reason is that the research location is semi-outdoor, thus the optimal temperature cannot be achieved. The unstable ambient temperature makes the larvae grow abnormally and their survival cannot be enhanced. It is this change in temperature which makes it difficult for larvae to adapt. The temperature observation data are presented in Table 3.

| Treatment          | Time     | Time     | Time     |
|--------------------|----------|----------|----------|
| A (Control)        | 07.00    | 12.00    | 17.00    |
| B (Glutamine 0.5gr)| 26.2 – 27.7| 26.5 – 28.2| 28.6 – 27.7|
| C (Taurine 0.5 gr) | 26.3 – 27.2| 26.5 – 28.1| 27.7 – 28.8|
| D (Glutamine 0.5gr + Taurine 0.5 gr)| 26.4 – 27.4| 26.3 - 28.1| 28.6 – 27.6|

Based on the observation results of light parameter during research taken in three different times, the range is 101 – 4803 Lux, as, for clarity, presented in Table 4. Light is an important factor in the survival of larvae, especially of grouper. The light parameter value obtained in this research ranges about 101 -
4803 Lux. The light parameter in this research has different value from the standard quality of light of 500-700 lux. The reason is that the research location has high light intensity. Excessive light intensity will get the fishes stressed, since as nocturnal animal, sunu grouper are used to doing activities, including eating in dim condition. Consequently, an environment with high light intensity will disrupt their eating activity and result in stress. The light observation data on the nurturing media are presented in Table 4.

Table 4. Light Parameter During Research (Lux).

| Treatment          | Time            |
|--------------------|-----------------|
|                    | 07.00          |
| A (Control)        | 50 - 1651      |
| B (Glutamine 0.5gr)| 53 - 630       |
| C (Taurine 0.5 gr) | 55 - 596       |
| D (Glutamine 0.5gr + Taurine 0.5 gr) | 50 - 550 |
|                    | 12.00          |
| A (Control)        | 391 - 4803     |
| B (Glutamine 0.5gr)| 391 - 2270     |
| C (Taurine 0.5 gr) | 116 - 2830     |
| D (Glutamine 0.5gr + Taurine 0.5 gr) | 144 - 4040 |
|                    | 17.00          |
| A (Control)        | 52 - 160       |
| B (Glutamine 0.5gr)| 41 - 182       |
| C (Taurine 0.5 gr) | 38 - 192       |
| D (Glutamine 0.5gr + Taurine 0.5 gr) | 39 - 186 |

Based on the observation, pH parameter is 7.82 - 8.19mg/L, nitrite parameter is 0.75 - 1mg/L and ammonia parameter is 0 - 0.75mg/L. The measurement of pH, nitrite, and ammonia parameters in sampling is conducted before changing the water, thus the value is not too far different from the standard quality. The data of pH, Nitrite and Ammonia observation are presented in Table 5.

Table 5. Observation Result of pH, Nitrite, Ammonia During Research (mg/L).

| Treatment          | pH       | Nitrite | Ammonia |
|--------------------|----------|---------|---------|
| A (Control)        | 7.84 – 7.86 | 0.88 - 1 | 0 – 0.563 |
| B (Glutamine 0.5gr)| 7.98 – 8.20 | 1       | 0 – 0.625 |
| C (Taurine 0.5 gr) | 7.88 – 7.92 | 0.75 - 1 | 0 – 0.625 |
| D (Glutamine 0.5gr + Taurine 0.5 gr) | 7.83 – 7.88 | 0.75 - 1 | 0 – 0.563 |

4. Conclusion

Based on the research results, we may conclude that giving rotifers enriched with glutamine and taurine does not enhance the survival of sunu grouper larvae, but giving rotifers enriched with 0.5g taurine and 0.5g glutamine may enhance larvae’s growth during the 10 days of nursery.

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