



## Combinations of $\beta$ -Carotene and Taurine Enhanced Growth and Eye Development of the Golden Rabbit Fish *S. guttatus*

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### ARTICLE INFO

#### Article History:

Received: May 15, 2024

Accepted: June 29, 2024

Online: July 19, 2024

#### Keywords:

$\beta$ -carotene and taurine,  
Combination,  
*S. guttatus*,  
Supplementation

### ABSTRACT

A high mortality rate in the gold rabbitfish larvae of *Siganus guttatus* has been found during the initial developmental phase. This condition is thought to be due to unprepared larvae obtaining exogenous feed. Various efforts have been conducted to prevent high mortality rates, such as nutrient enrichment. This study was performed to evaluate the combined effect of  $\beta$ -carotene and taurine on the growth performance and survival rate of the gold rabbitfish larvae of *S. guttatus*. This study had four treatments for rotifer enrichment, namely BT0 without  $\beta$ -carotene and taurine enrichment, B7.5 (7.5mg L<sup>-1</sup>  $\beta$ -carotene), T50 (50mg L<sup>-1</sup> taurine), and a combination of 7.5mg L<sup>-1</sup>  $\beta$ -carotene and 50mg L<sup>-1</sup> taurine (Com-BT). The *S. guttatus* larvae were reared for 10 days after hatching (DAH). The parameter observed was growth performance, which contained absolute growth (body length and weight), fin development (dorsal, pectoral, and caudal), visual development (eye diameter and retina), total prey intake, and survival rate. The results indicated that enrichment of the rotifers with  $\beta$ -carotene and taurine can improve the absolute growth rate, fin development, visual development, and total prey intake. These parameters obtained better values as both ingredients were combined together ( $P < 0.05$ ) Com-BT. Nutrient enrichment with  $\beta$ -carotene and taurine had no significant difference ( $P < 0.05$ ) on the survival rate of *S. guttatus* larvae, but significantly showed a better survival rate in the combination of  $\beta$ -carotene and taurine treatment (Com-BT). In conclusion,  $\beta$ -carotene and taurine can be applied to improve the growth and survival rate of *S. guttatus* larvae.

### INTRODUCTION

High mortality rate in the golden rabbitfish *Siganus guttatus* larvae has been found during the initial developmental phase (2-3 days after hatching) (Juario *et al.*, 1985). Mortality is thought to be due to larvae being unprepared to obtain

exogenous feed. Visual limitation in the larvae becomes one of the factors causing the unprepared larvae to perform predation activities. The visual capability of *S. guttatus* larvae, 1-2 days after hatching (DAH), is still highly limited in terms of visibility and range (Juario *et al.*, 1985) due to their underdeveloped eyes (Darsiani *et al.*, 2022).

Eyes are organs that directly associate with the environment and detect objects including feed. Highly developed eyes can support the total prey intake (Yufera *et al.*, 2014). The prey intake success can lead to the improvement of the larval growth and survival rate (Jacobsen *et al.*, 2022).

Various efforts have been conducted to overcome the larval death, namely nutrient enrichment in live feeds.  $\beta$ -carotene is a nutrient material that can support the visual development and increase the growth of organisms (Widomska & Subcyaski, 2018). Moreover,  $\beta$ -carotene can act as an active ingredient to increase the color pigmentation for organisms (Tachibana *et al.*, 1997; Gupta *et al.*, 2007; Madiara *et al.*, 2019). Thus,  $\beta$ -carotene is often used as a feed coloring agent for cultivars to obtain a higher amount of predation, and in turn can increase the growth and survival rate. In addition to  $\beta$ -carotene, taurine is also believed to have an important role in the growth (Jusadi *et al.*, 2012; Wei *et al.*, 2020) and visual development (Wei *et al.*, 2018). However, fish do not have the ability to synthesize  $\beta$ -carotene, therefore it is necessary to add this compound to their feed (Kaur *et al.*, 2016). The same thing also happens to taurine compounds. It is suspected that marine fish have limitations in synthesizing taurine, therefore it is necessary to add taurine to their feed to obtain better growth and survival (El-Sayed, 2013).

In the previous studies (Jusadi *et al.* 2012; Darsiani *et al.* 2023),  $\beta$ -carotene and taurine were used as feed enrichment ingredients of the rotifers for *S. guttatus* larvae. From the results, both ingredients could support the visual development and growth of *S. guttatus* larvae. Based on this condition, this study applied  $\beta$ -carotene (dose  $7.5\text{mg L}^{-1}$ ) and taurine ( $50\text{mg L}^{-1}$ ) as enrichment ingredients of the rotifers for *S. guttatus* larvae. Studies regarding the use of  $\beta$ -carotene and taurine in *S. guttatus* larvae are still very limited or has not ever been carried out before, especially the combination of both ingredients. Thus, a study regarding the use of  $\beta$ -carotene and taurine (combination) is highly necessary. At this stage, the experiment was carried out to evaluate the combination effect of  $\beta$ -carotene and taurine as rotifer enrichment ingredients on the growth performance (including eye development) and survival rate of the golden rabbitfish *S. guttatus* larvae.

## MATERIALS AND METHODS

### 1. Enrichment of rotifer

Type S rotifers (*Brachionus rotundiformis*) with a density of 500- 1000 individuals/ml (Jusadi *et al.*, 2015) were harvested from mass cultivation tanks, then enriched

according to treatment before being given to larvae. Procedures for enriching the rotifers refer to technical instructions (**Jusadi et al., 2012**). First, 10L of enrichment media was used using 0.5mL of A1 DHA Selco fish oil, 0.1g egg yolk, 0.25g baker's yeast, and the addition of PA Sigma beta-carotene and PA Sigma taurine according to the treatment dose. Next, all the ingredients were mixed with 200mL of water and emulsified using a blender for three to five minutes, then poured into a rotifer enrichment container. The rotifers were incubated for 2 hours and then harvested using a 50 $\mu$ m plankton net and then fed to the larvae.

## 2. Design of feeding trial

This study used a completely randomized design with four treatments and three replications, thus containing 12 experimental units. In this experiment, the rotifers were enriched with the combination of  $\beta$ -carotene and taurine, based on the best results from the previous study. The treatments applied in this experiment were:

BT0 = Control (without  $\beta$ -carotene and taurine)

B7.5mg L<sup>-1</sup> = The best dose of  $\beta$ -carotene obtained from the second study

T50mg L<sup>-1</sup> = The best dose of taurine obtained from the third study phase

Com-BT = The combination of 7.5mg L<sup>-1</sup>  $\beta$ -carotene and 50mg L<sup>-1</sup> taurine.

For nutrient enrichment, the rotifers, *Brachionus rotundiformis*, were enriched with  $\beta$ -carotene and taurine following the treatments applied, and fed to the *S. guttatus* larvae for 10 days. The rotifers were fed to the larvae at 10- 20ind/ ml (**Juario et al., 1985; Duray, 1998**). Meanwhile, larvae were stocked at 20 individual/ L (**Duray & Kohno 1988; Lante & Muslimin, 2012**), and reared in a 3000-L tank filled with 2400L seawater at 20- 25ppt salinity. The tank and seawater used were initially sterilized. The seawater was exchanged, following **Duray and Juario (1988)**, and the water quality was measured twice a day at 07.00 and 17.00 WITA. Green water treatment was applied for larval rearing by supplying *Nannochloropsis* sp. at 1x10<sup>5</sup> individual/mL (**Duray & Juario, 1988**).

## 3. Samples collection and observed parameters

This study was performed in the hatchery installation for the tiger shrimp (IPUW) Barru, South Sulawesi on January 15 – March 30, 2022. The eyes histological samples were prepared in the Laboratory of Fish Health, Faculty of Fisheries and Marine Sciences, IPB University. Moreover, the  $\beta$ -carotene and taurine contents in the rotifers were determined in the Saraswanti Indo Genetech, Inc., Bogor, Indonesia.

Parameters observed in this study were obtained from the growth performance, namely absolute growth rate (body length and width), fin development (dorsal, pectoral, and caudal), visual development (eye diameter and retina), total prey intake, and survival rate. The 30 larvae were taken from the rearing tank for further development observation.

This observation was performed using the Olympus 40, Japan microscope at 4 times magnification. Histological observation was performed using the same microscope with 100x magnification. The microscope was equipped with scales and connected to the computer for documentation. For histological samples, the eyes of 10 larvae were taken from each rearing tank.

Absolute growth (length) was calculated using the formula (Mulqan *et al.*, 2017):

$$L = Lt - L0$$

To calculate the larval survival rate, all larvae were harvested (total harvesting) at the end of rearing (10 DAH). Survival rate was calculated using the following equation (Effendie, 2002):

$$SR (\%) = \frac{Nt}{N0} \times 100\%$$

SR = Survival rate (%)

Nt = Number of larvae at final day of the experiment

N0 = Number of larvae at initial day of the experiment

For  $\beta$ -carotene and taurine contents in the rotifers, the  $\pm 50$ - 100g samples of the rotifers were taken from each treatment and determined in the Saraswanti Indo Genetech, Inc., Bogor, Indonesia. For antioxidation activity in *S. guttatus* larvae, the larvae were harvested from each rearing tank and tested in the Laboratory of Fish Nutrition, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University, Bogor, Indonesia.

Biochemical analysis was carried out on the rotifers to determine the content of beta-carotene and taurine. The test used a high performance liquid chromatography working procedure (HPLC, Shimadzu 20 A, Tokyo, Japan). While testing the antioxidant content of glutathione peroxidase (GPx), it was analyzed spectrophotometrically using the Glutathione Peroxidase Assay Kit (Abcam UK, London).

#### 4. Data analysis

All data were analyzed using the analysis of variance (ANOVA). Data with significant difference were analyzed further using the W-Tukey test (Steel & Torrie, 1991). For statistical analysis software, the SPSS 22.0 was used. The eyes histological samples (in figures) and water quality parameters were analyzed descriptively based on the standard condition of the living golden rabbitfish *S. guttatus*.

## RESULTS

### 1. Absolute growth

The average of absolute growth rate (length and width) is presented in Table (1).

**Table 1.** Absolute growth rate of *S. guttatus* larvae (mean $\pm$ SD, n=3) after feeding with  $\beta$ -carotene and taurine-enriched rotifers

| Treatment | Absolute growth                 |                                    |
|-----------|---------------------------------|------------------------------------|
|           | Total length (mm)               | Body width ( $\mu$ m)              |
| BT0       | 0.850 $\pm$ 0.106 <sup>a</sup>  | 101.820 $\pm$ 4.972 <sup>a</sup>   |
| B7.5      | 1.159 $\pm$ 0.194 <sup>ab</sup> | 125.193 $\pm$ 11.772 <sup>ab</sup> |
| T50       | 0.882 $\pm$ 0.021 <sup>a</sup>  | 116.123 $\pm$ 6.114 <sup>ab</sup>  |
| Com-BT    | 1.257 $\pm$ 0.157 <sup>b</sup>  | 137.027 $\pm$ 13.682 <sup>b</sup>  |

Note: sd = standard deviation, different letters above the numbers indicate significant differences between treatment at the level of 5% ( $P < 0.05$ ). BT0 = Control (without  $\beta$ -carotene and taurine); B7.5mg L<sup>-1</sup> = The best dose of  $\beta$ -carotene obtained from the second study; T50mg L<sup>-1</sup> = The best dose of taurine obtained from the third study phase; Com-BT = The combination of 7.5mg L<sup>-1</sup>  $\beta$ -carotene and 50mg L<sup>-1</sup> taurine.

Table (1) reveals that the highest length and width absolute growth were obtained from the combination treatment (Com-BT) at 1.257mm and 137.027 $\mu$ m, respectively. Meanwhile, the lowest values were found in the treatment without  $\beta$ -carotene and taurine enrichment (BT0) with 0.850mm length and 101.820 $\mu$ m width.

Based on the ANOVA results, the rotifers enrichment with  $\beta$ -carotene and taurine obtained a significant difference value ( $P < 0.05$ ) on the absolute length and width growth rates. The W-Tukey test results indicate that the absolute length growth in Com-BT treatment was significantly different ( $P < 0.05$ ) from BT0 treatment. This condition was similar in absolute body width of the Com-BT treatment, which was significantly different ( $P < 0.05$ ) from the BT0 treatment, but showing no significant difference ( $P > 0.05$ ) with B7.5 and T50mg L<sup>-1</sup>, and both treatments were also insignificantly different ( $P > 0.05$ ) with BT0.

### 2. Fins

The average of fin sizes (dorsal, pectoral, and caudal) in *S. guttatus* larvae is presented in Table (2).

**Table 2.** Fin size (dorsal, pectoral, and caudal) of *S. guttatus* larvae (mean±SD, n=3) after feeding with  $\beta$ -carotene and the taurine-enriched rotifers

| Treatment | Fin size ( $\mu\text{m}$ ) |                             |                            |
|-----------|----------------------------|-----------------------------|----------------------------|
|           | Dorsal                     | Pectoral                    | Caudal                     |
| BT0       | 55.788±0.242 <sup>a</sup>  | 98.637±0.463 <sup>a</sup>   | 63.694±1.660 <sup>a</sup>  |
| B7.5      | 58.712±2.211 <sup>ab</sup> | 103.457±2.168 <sup>ab</sup> | 64.320±1.372 <sup>ab</sup> |
| T50       | 58.194±1.449 <sup>ab</sup> | 103.367±1.741 <sup>ab</sup> | 64.260±2.628 <sup>ab</sup> |
| Com-BT    | 61.873±2.374 <sup>b</sup>  | 105.623±2.651 <sup>b</sup>  | 69.470±2.685 <sup>b</sup>  |

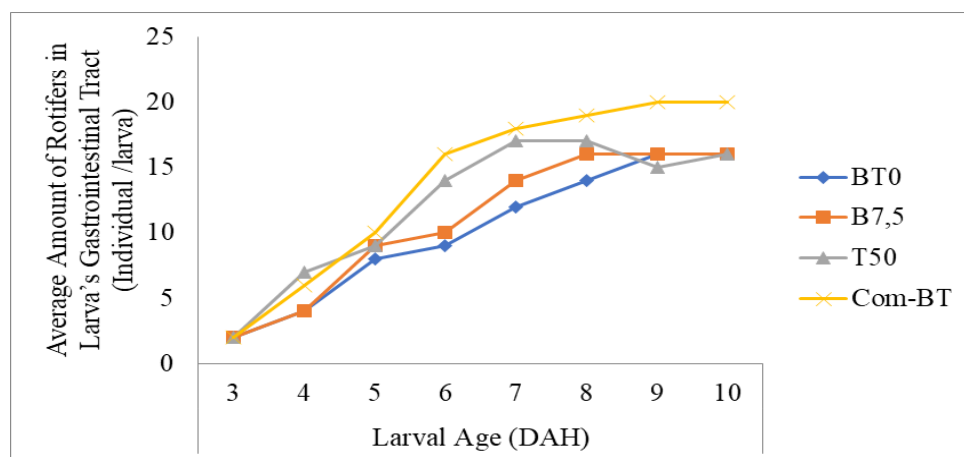
Note: sd = standard deviation, different letters above the numbers indicate significant differences between treatment at the level of 5% ( $P < 0.05$ ). BT0 = Control (without  $\beta$ -carotene and taurine); B7.5mg L<sup>-1</sup> = The best dose of  $\beta$ -carotene obtained from the second study; T50mg L<sup>-1</sup> = The best dose of taurine obtained from the third study phase; Com-BT = The combination of 7.5mg L<sup>-1</sup>  $\beta$ -carotene and 50mg L<sup>-1</sup> taurine.

Data presented in Table (2) show that the highest fin length (dorsal, pectoral, and caudal) of *S. guttatus* larvae was obtained from the Com-BT treatment at 61.873, 105.623, and 69.470 $\mu\text{m}$ , respectively. Meanwhile, the lowest values were obtained from the BT0 treatment.

Based on ANOVA results, enrichment with  $\beta$ -carotene and taurine had a significant effect ( $P < 0.05$ ) on fin development either in the dorsal, pectoral, or caudal fins. Following the continued statistical analysis test, enrichment of the rotifers in the Com-BT treatment was significantly different ( $P < 0.05$ ) from the BT0 treatment. Meanwhile, the B7.5 and T50mg L<sup>-1</sup> treatments were insignificantly different ( $P > 0.05$ ) from the BT0 treatment. The results of this study denote that the combination of  $\beta$ -carotene and taurine enrichment can have a better effect on fin development (dorsal, pectoral, and caudal) of *S. guttatus* larvae. As age increases, the body and fin size also increase.

### 3. Total prey intake

The total prey intake is an indicator for eyes capability in feed detection (visual detection). The average of total daily prey intake can be seen in Fig. (1).



**Fig. 1.** Daily prey intake in *S. guttatus* larvae at 3-10 DAH; BT0 = Control (without  $\beta$ -carotene and taurine); B7.5mg L<sup>-1</sup> = The best dose of  $\beta$ -carotene obtained from the second study; T50mg L<sup>-1</sup> = The best dose of taurine obtained from the third study phase; Com-BT = The combination of 7.5mg L<sup>-1</sup>  $\beta$ -carotene and 50mg L<sup>-1</sup> taurine

Fig. (1) shows that the highest prey intake on *S. guttatus* larvae was obtained in the D treatment at 17ind/ larvae, followed by B treatment = 14ind/ larvae, C treatment = 13ind/ larvae, and the lowest value was obtained in the A treatment (without  $\beta$ -carotene and taurine) = 12ind/ larvae. Based on the ANOVA results, the rotifers enrichment using  $\beta$ -carotene and taurine has a significant effect ( $P < 0.05$ ) on the total prey intake on *S. guttatus* larvae. The W-Tukey test showed that the combination of  $\beta$ -carotene and taurine was significantly different ( $P < 0.05$ ) from the treatment without  $\beta$ -carotene and taurine (A), but B ( $\beta$ -carotene) and C (taurine) treatments was insignificantly different from A and D treatments.

#### 4. Survival rate

The amount of individual until the final rearing period (10 DAH) is presented as the survival rate of *S. guttatus* larvae. The survival rate percentage of *S. guttatus* larvae is shown in Table (3).

**Table 3.** The survival rate of *S. guttatus* larvae (mean $\pm$ SD, n=3) after feeding with  $\beta$ -carotene and the taurine-enriched rotifers

| Treatment | Survival rate (%)              |
|-----------|--------------------------------|
| BT0       | 1.307 $\pm$ 0.338 <sup>a</sup> |
| B7.5      | 1.585 $\pm$ 0.994 <sup>a</sup> |
| T50       | 1.513 $\pm$ 0.571 <sup>a</sup> |
| Com-BT    | 1.622 $\pm$ 0.541 <sup>a</sup> |

Note: sd = standard deviation, different letters above the numbers indicate significant differences between treatment at the level of 5% ( $P < 0.05$ ). BT0 = Control (without  $\beta$ -carotene and taurine); B7.5mg L<sup>-1</sup> = The best dose of  $\beta$ -carotene obtained from the second study; T50mg L<sup>-1</sup> = The best dose of taurine obtained from the third study phase; Com-BT = The combination of 7.5mg L<sup>-1</sup>  $\beta$ -carotene and 50mg L<sup>-1</sup> taurine.

Table (3) presents the highest survival rate in the Com-BT treatment at 1.622%, followed by the B7.5mg L<sup>-1</sup> treatment at 1.585%, the T50 mg L<sup>-1</sup> treatment at 1.513%, and the smallest value was shown in the BT0 treatment at 1.307%. Based on the ANOVA results, enrichment of the rotifers using  $\beta$ -carotene and taurine had no significant effect ( $P > 0.05$ ) on the survival rate of *S. guttatus* larvae. However, the results also showed a better survival rate at the Com-BT treatment than other treatments.

## 5. Eye diameter

The average of eye diameter in *S. guttatus* larvae in each enrichment dose is presented in Table (4).

**Table 4.** Eye diameter of *S. guttatus* larvae (mean $\pm$ SD, n=3) after feeding with  $\beta$ -carotene and the taurine- enriched rotifers

| Treatment | Eye diameter<br>( $\mu\text{m}$ ) $\pm$ SD |
|-----------|--|
| BT0       | 114.637 $\pm$ 2.098 <sup>a</sup>           |
| B7.5      | 117.027 $\pm$ 1.311 <sup>a</sup>           |
| T50       | 117.464 $\pm$ 4.392 <sup>a</sup>           |
| Com-BT    | 125.794 $\pm$ 0.912 <sup>b</sup>           |

Note: sd = standard deviation, different letters above the numbers indicate significant differences between treatment at the level of 5% ( $P < 0.05$ ). BT0 = Control (without  $\beta$ -carotene and taurine); B7.5mg L<sup>-1</sup> = The best dose of  $\beta$ -carotene obtained from the second study; T50mg L<sup>-1</sup> = The best dose of taurine obtained from the third study phase; Com-BT = The combination of 7.5mg L<sup>-1</sup>  $\beta$ -carotene and 50mg L<sup>-1</sup> taurine.

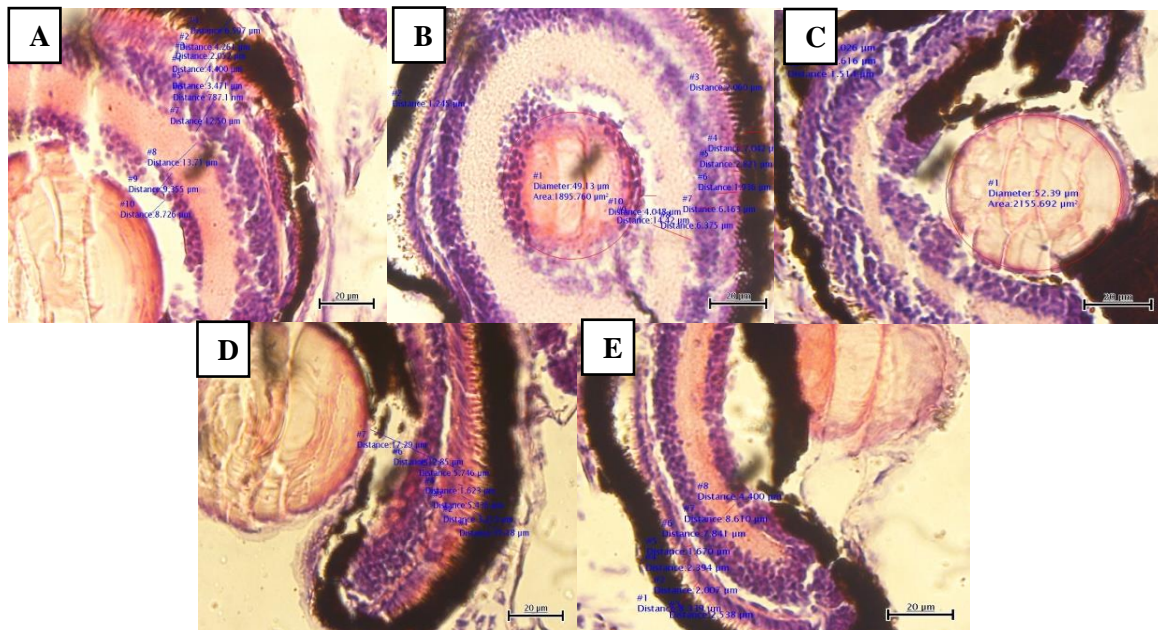
In Table (4), the greatest eye diameter was obtained from the Com-BT treatment at 125.794 $\mu\text{m}$ , followed by the taurine enrichment (T50mg L<sup>-1</sup>) at 117,464 $\mu\text{m}$ ,  $\beta$ -carotene enrichment (B7.5mg L<sup>-1</sup>) at 117.027 $\mu\text{m}$ , and the tiniest value was obtained from the enrichment without  $\beta$ -carotene and taurine (BT0) at 114.637 $\mu\text{m}$ .

Based on the ANOVA results, the  $\beta$ -carotene and taurine enrichment had a highly significant effect ( $P < 0.01$ ) on the increased eye diameter (eye differentiation) of *S. guttatus* larvae. A further statistical analysis test results showed that the combined enrichment of  $\beta$ -carotene and taurine (Com-BT) was highly significantly different ( $P < 0.01$ ) from treatment without  $\beta$ -carotene and taurine enrichment (BT0). Meanwhile, between BT0 and B-C treatments, no significant difference ( $P > 0.05$ ) occurred. The results of this study indicate that the combined enrichment of  $\beta$ -carotene and taurine enrichment produce a better eye development/differentiation than without both enrichment ingredients (A).



## 6. Eye histology

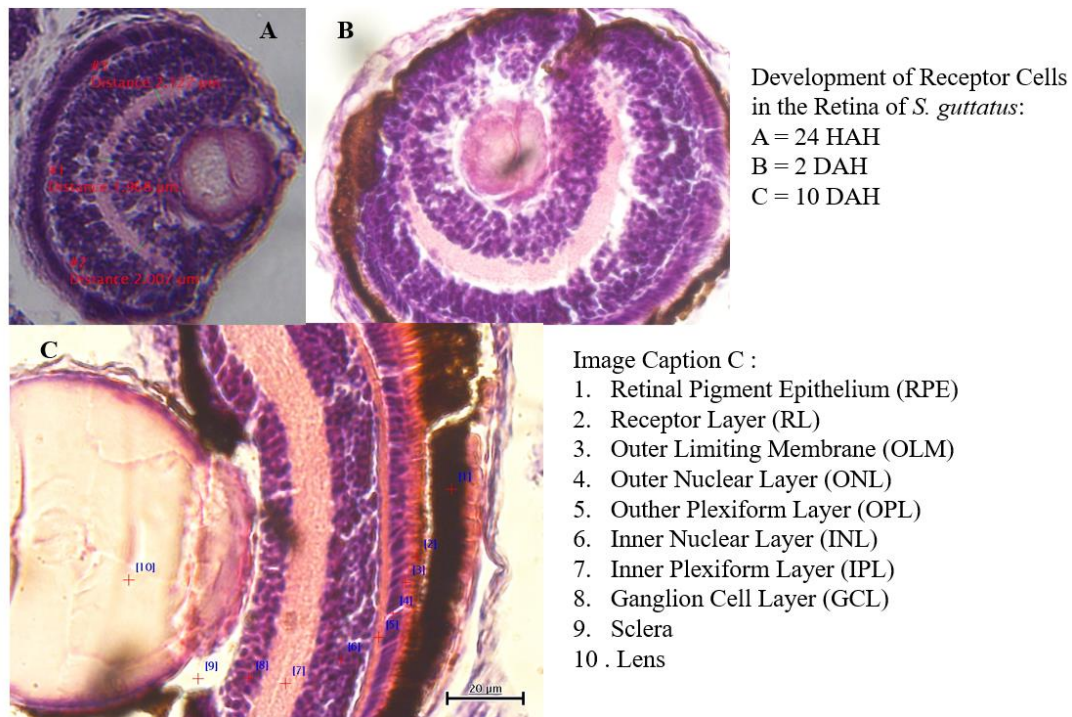
The eye histological samples were observed to determine the effect of  $\beta$ -carotene and taurine enrichment on eye development of *S. guttatus* larvae. The histological samples of *S. guttatus* eyes can be seen Figs. (2, 3).



**Fig. 2.** Eye histology of *S. Guttatus* larvae. A. 2-DAH larvae, B-E. 10-DAH larvae (B = BT0, C =  $7.5\text{mg L}^{-1}$ , D =  $T50\text{mg L}^{-1}$ , and E = Com-BT)

From Fig. (2), the photoreceptor cell thickness on 2-DAH larvae is  $1.755\mu\text{m}$  and lens diameter is  $46.709\mu\text{m}$ . In 10-DAH larvae (A, B, C, and D treatment larvae), the average photoreceptor thickness in the treatments is  $3.235$ ,  $4.017$ ,  $4.271$ ,  $5.161\mu\text{m}$ , respectively. Meanwhile, the average lens diameter is observed at  $57.222$ ,  $68.444$ ,  $73.006$ ,  $78.018\mu\text{m}$ , respectively.

Fig. (3) presents the eye difference of *S. guttatus* larvae 12 hours after hatching (12 HAH, A), 2 days after hatching (2 DAH, B), and 10 days after hatching (10 DAH, C). In 10 DAH, the eye parts can be differed.



**Fig. 3.** Retinal development of *S. Guttatus* larvae

## 7. $\beta$ -carotene and taurine contents in the rotifers

In this study,  $\beta$ -carotene and taurine contents in the rotifers were determined as the larval samples for further test was insufficient. The  $\beta$ -carotene and taurine contents in rotifers can be seen in Table (5).

**Table 5.**  $\beta$ -carotene and taurine contents in rotifers after the enrichment

| Treatment | Content ( $\text{mg g}^{-1}$ ) |         |
|-----------|--------------------------------|---------|
|           | $\beta$ -carotene              | Taurine |
| BT0       | 6                              | 1,953   |
| B7.5      | 322                            | 1,017   |
| T50       | 4                              | 2,210   |
| Com-BT    | 876                            | 920     |

Table (5) presents the highest  $\beta$ -carotene content in the rotifers at the combined treatment (D) with the value of  $876\text{mg g}^{-1}$ . Meanwhile, the taurine content in the rotifers is presented in the C treatment at  $2,210\text{mg g}^{-1}$ .

## 8. Water quality

The water quality parameters measured during the *S. guttatus* larval rearing are presented in Table (6).

**Table 6.** Water quality during *S. guttatus* larval rearing

| Parameter                   | Measured range |
|-----------------------------|----------------|
| DO (ppm)                    | 3.15-5.21      |
| Salinity (ppt)              | 23.0-25.0      |
| pH (ppm)                    | 7.00-8.30      |
| Temperature ( $^{\circ}$ C) | 27.0-29.0      |
| Ammonia (ppm)               | 0.0006-0.0631  |

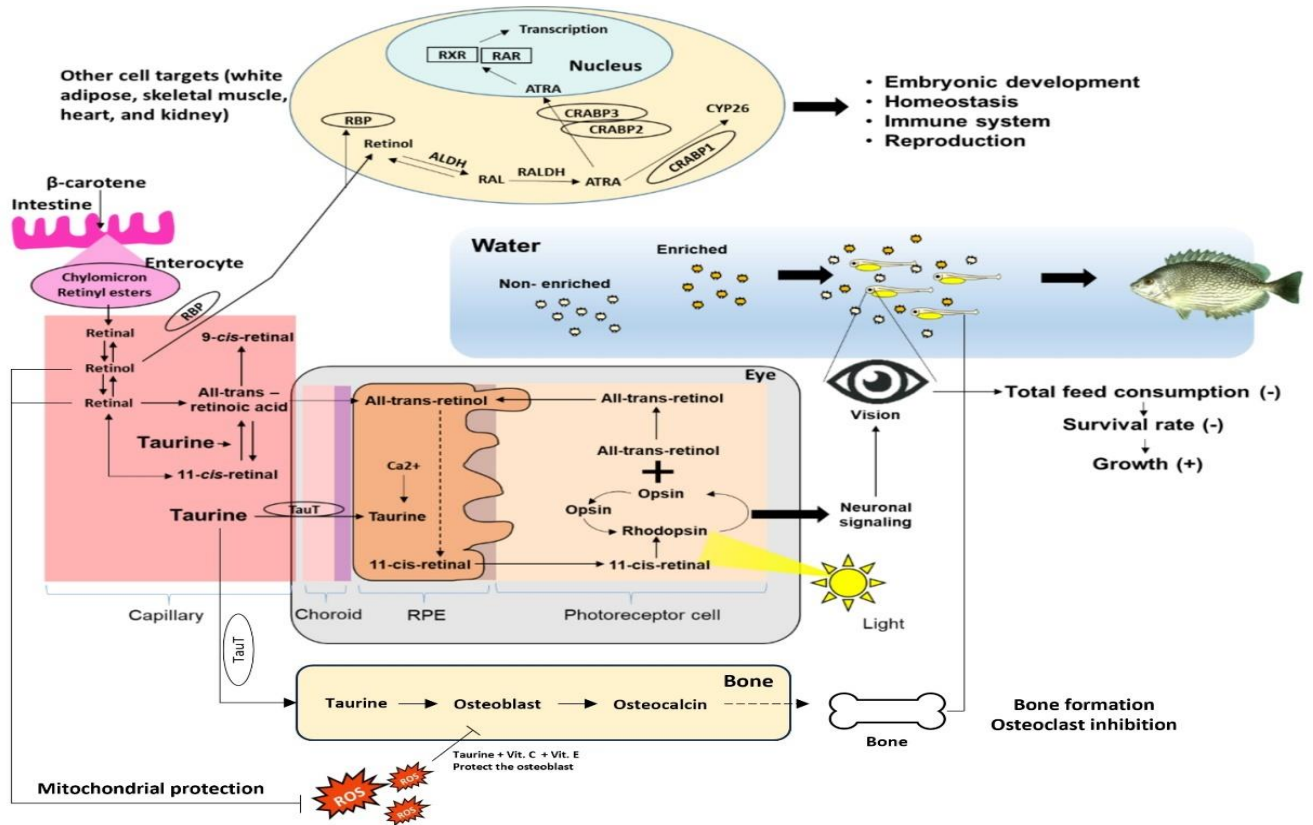
Based on Table (6), the dissolved oxygen (DO) level ranged from 3.15-5.21ppm. Salinity fluctuated between 23.0 & 25.0ppt, pH was measured at 7.00-8.30ppm, temperature was measured at 27.0- 29.0 $^{\circ}$ C, and ammonia ranged from 0.0006- 0.0631ppm.

## DISCUSSION

The highest absolute growth (length and width) of *S. guttatus* larvae was found in the Com-BT treatment (Table 1). The smallest absolute growth was obtained in the BT0 treatment. The greatest eye diameter and the thickest photoreceptor cells in retina were all obtained from the Com-BT treatment. Thus, the growth achieved is directly proportional to the increased eye and the thickness of the retinal receptor cells. The size of eye diameter has a direct effect on the eye ability to view objects. Increased eye visibility can be proven from the amount of feed consumed by *S. guttatus* larvae (Fig. 1). The enrichment of  $\beta$ -carotene and taurine can increase growth and support the eye development of *S. guttatus* larvae. Furthermore,  $\beta$ -carotene and taurine combination has no opposing effects, resulting in better growth and eye development of *S. guttatus* larvae, as shown in the Com-BT treatment. The effect of using  $\beta$ -carotene and taurine compounds together (combination) in this study is explained in Fig. (4).

The flow of utilizing  $\beta$ -carotene and taurine at different doses for *S. guttatus* larvae arranged based on the physiological response of the larvae is shown in Fig. (4) (modified results).  $\beta$ -carotene added to the rotifers can increase the color intensity of the rotifers so that they are easier for larvae to see. The digested  $\beta$ -carotene enters the enterocytes with the help of chylomicrons and is converted into retinyl ester. Retinyl ester can be stored in hepatocytes, converted to retinal, and distributed back to the blood. In blood vessels, retinal goes through a series of reactions to produce all trans retinoic acid and 11-cis retinal (Conserva *et al.*, 2019). All trans-retinoic acid is then transported into the eye, namely to the retinal pigment epithelium (RPE), and converted into 11-cis retinal. 11-cis retinal will be transferred to photoreceptor cells and converted into rhodopsin. Rhodopsin is a protein that binds to receptors and acts as a biomarker for thinning and degeneration of the outer nuclear layer of the eye (Cvekl & Wang, 2009; Medina *et al.*, 2019; Lenahan *et al.*, 2020). Increasing the amount of rhodopsin can increase the visibility of

*S. guttatus* larvae when looking for food, so that it is easier for the larvae to see food, and the amount of food consumed can increase.



**Fig. 4.** Prediction of the mechanism of utilization of  $\beta$ -carotene and taurine in the golden rabbitfish larvae *S. guttatus* (modification based on several reference literature)

. Increasing the amount of feed consumed can meet the energy needs of larvae, and thus they can survive and grow. Apart from that, retinal, which is converted into retinol, will also be delivered to target cells, such as adipose cells, skeletal muscles, the heart, and the kidneys and play a role in embryonic development, homeostasis, the immune system, and reproduction.  $\beta$ -carotene, and its derivative compounds actively inhibit ROS production in mitochondria, leading to a decrease in ROS production (Siems *et al.*, 2005; Widomska & Subczynski, 2018; Yunarsa & Adiatmika, 2018) including the golden rabbit larvae.

Taurine plays a role in improving vision in two ways, namely through all trans retinoic acid or directly transported into the retinal pigment epithelium (RPE with the help of TauT (the taurine transporter). Taurine that enters the RPE binds to calcium and inhibits the production of free calcium, so that the activity of guanylate cyclase and rhodopsin kinase increases and can increase an individual's ability to see in low-light conditions. Taurine has a role in the formation and development of osteoblasts (bone

cells) by activating the performance of osteocalcin in bones in binding calcium and indirectly suppressing ROS in bones (**Izquierdo *et al.*, 2018**).

$\beta$ -carotene is a pro-vitamin A compound that can support the retinal development, specifically RPE (retinal pigment epithelium). The vitamin A metabolite compound, namely 11-cis retinal, can help RPE differentiation and prevent abnormal cell growth (neoplasm) in the RPE (**Cvekl & Wang, 2009; Medina *et al.*, 2019**).  $\beta$ -carotene is also functioned to maintain the macula condition in retina (**Yunarsa & Adiatmika, 2018**). The macula is located behind the retina and acts as a neurosensory organ in retina. The middle part of the macula contains carotenoids, lutein, and zeaxanthin. Carotenoids have a role as antioxidants and filter light waves, hence they can prevent eye damage. Dysfunction of the macula can interfere with central vision or straight-ahead vision (**Gupta, 2021**).

A previous research we conducted showed that enriching the rotifers using  $\beta$ -carotene can increase the color contrast of the rotifers so that they can be more easily seen by the golden rabbitfish larvae and can further increase the amount of food predation. Apart from supporting eating activities,  $\beta$ -carotene also adds nutrients that can support growth performance, especially eye development. The addition of  $\beta$ -carotene can increase the diameter of the eye, the thickness of photoreceptor cells in the retina, and the diameter of the eye lens of the golden rabbitfish *Siganus guttatus* larvae (**Darsiani *et al.*, 2023**).

$\beta$ -carotene is a pro-vitamin A compound. Vitamin A metabolite compounds, namely All-trans and 9-cis retinoic acid, have the ability to increase growth through the activation of the nuclear hormone receptors, called RXR (retinoid X receptor) and RAR (retinoid acid receptor). The function of RXR and RAR receptors is to transcribe DNA into RNA. The presence of vitamin A metabolite compounds can act as electron donors (ligands) at the transcription stage of DNA into RNA. In conditions where ligands are unavailable, the receptor will bind to co-repressors (nuclear hormone co-repressor-NcoR, and silencing mediator for retinoid and thyroid hormone receptor-SMRT), and ultimately causes gene transcription process inhibition (**Militante & Lombardini, 2002**).

Meanwhile, the regulation of taurine as a nutrient enrichment ingredient helps increase eye growth and development through the central tissue system. Taurine facilitates the transmission of signals through nerves in the brain, accelerating the organogenesis process, thus the growth is achieved (**Wei *et al.*, 2018**). Taurine is an essential amino acid that can be used as an energy source directly by the body to achieve growth (**Jusadi *et al.*, 2012**). According to **Lombardini (1983)**, **Lombardini (1991)** **Militante and Lombardini (2002)**, taurine can support eye development by calcium regulation in the retinal pigment epithelium (RPE). Taurine can inhibit the production of free calcium in the RPE. High calcium in retina can disrupt the performance of CGMP to channel  $\text{Na}^+$  from outside the cells, whereas  $\text{Na}^+$  plays a role in binding free calcium in the RPE. Free calcium in RPE can also inhibit the performance of guanylate cyclase

(GC). GC is a phototransduction, that can convert light into electrical signals to the brain. In addition, the presence of free calcium can also inhibit the performance of rhodopsin kinase (RK), limiting the individual capability in viewing objects at low light conditions.

The growth of *S. guttatus* larvae is characterized by changes in physical size (length and width) over a certain period of time. According to **Mulqan *et al.* (2017)**, several factors that can influence growth include feed quality, feed quantity, water quality, and fish age. **Chavez *et al.* (2021)** added that growth is influenced by environmental factors. An environmental factor that can directly influence growth is the light intensity. Appropriate light intensity can support the success of predation activities. Feed predation activity is positively correlated with growth.

Furthermore, **Ruchin (2020)** stated that the development and growth of fish larvae is influenced by the photic environment, both vertically and horizontally. This condition is associated with the eye's ability to receive light (stimuli) that penetrates into the water column. Limited vision in certain lighting conditions will affect the ability of fish larvae to obtain food. The amount of light captured by the eyes can directly stimulate the performance of growth hormones in fish, but the use of light for organisms has a tolerance limit, namely certain lighting condition (dim or bright) (**Widomska & Subczynski, 2018**).

Although the environmental carrying capacity is adequate, including light, predation fails to proceed if the eyes that detect food are unwell developed. Indicators of visual organ development (differentiation) can be seen from the amount of prey intake. Furthermore, the visual development can be determined through the visual organ histology, which can provide an overview of development parts that constructs the eyes.

Based on the eyes histology observation (Figs. 2, 3), enrichment of the rotifers using  $\beta$ -carotene and taurine has a good effect on eyes development. At 2 DAH, the average thickness of photoreceptor cells is 1.755 $\mu\text{m}$ , and the lens diameter is 46.709 $\mu\text{m}$ . At 10 DAH (A = BT0, B =  $\beta$ -carotene, C = taurine, and D = Combination of  $\beta$ -carotene and taurine), the average thickness of photoreceptor cells is 3.235, 4.017, 4.271, and 5.161 $\mu\text{m}$ , respectively. Meanwhile, the average lens diameter is 57.222, 68.444, 73.006, and 78.018 $\mu\text{m}$ , respectively. The highest lens diameter and thickest receptor cell at 10 DAH were obtained in the Com-BT treatment.

The increased eye development of *S. guttatus* larvae continues along with the increasing age. If the eye size increases, the retinal parts in the eyes of *S. guttatus* larvae become clearer (Fig. 3). Based on the histological image at 24 HAH, eye pigmentation is still absent and the retinal parts are hard to differentiate. At 2 DAH, the eyes look darker and begin to experience pigmentation, and the retina appears to have an imperfect layer. The retinal layers become more visible and can be differentiated when the *S. guttatus* larvae are at 10 DAH. **Hernan *et al.* (2022)** reported a similar condition in the tuna *Thunnus thynnus* larvae. A newly hatched tuna had no retinas. Eye pigmentation and retinal formation occurred at 2 DAH with an imperfect layer. At 16 DAH, the retina

experienced a rapid development, namely the formation of photoreceptor cells (cones and rods). This eye differentiation can be an indicator that the vision ability of *Thunnus thynnus* larvae has increased.

Eye pigmentation is required to support vision. Eye pigmentation occurs in the retina, specifically in the retinal pigment epithelium (RPE) layer. Pigmentation in the RPE prevents the reflection and spread of light in the eyeball, thus light absorption can be maximized. If light cannot be absorbed by the eye completely, then visibility decreases (**Afitah et al., 2020**). A similar condition was reported by **Gupta (2021)**, that RPE dysfunction causes an incomplete light absorption by retina, causing a reflected light in all directions, so the image is unclear. Apart from absorbing light, RPE also has the ability to maintain the structural integrity of retina through the action of phagocytes on photoreceptor cells damaged by free radicals, photo-oxidative and light energy, thus photoreceptor cells (con and rod) can be renewed.

Retina consists of 10 layers, namely pigment epithelium (RPE), rod and cone cell layer (photoreceptor cells), external limiting membrane, outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer, ganglion cell layer, nerve fiber layer, and internal limiting membrane. Moreover, the retina functions to concentrate images and process information before being transmitted to the brain for translation. Then, photoreceptor cells, a part of retina that plays an important role in vision, have two types of cells that work under certain conditions, namely cone cells for maintaining bright conditions (cone cells) and rod cells for maintaining low light conditions (**Ruchin, 2020; Gupta, 2021**).

In this study, cone cells and rod cells in photoreceptor cells were almost indistinguishable in the retina, both in *S. guttatus* larvae at 2-10 DAH. This condition is thought to be because the eyes do not develop properly at the age of 2-10 DAH (Fig. 3).

During the *S. guttatus* larvae rearing, the larval behavior was observed every day. At 2- 4 DAH, *S. guttatus* larvae were observed to carry out more swimming activities on the the rearing media surface. At 5- 6 DAH, the larvae began to swim more frequently in the rearing media columns. Based on the behavior of *S. guttatus* larvae, cone cells in the eyes of *S. guttatus* larvae were formed earlier than the rod cells. Several studies reported similar conditions, as cone cells were formed earlier, followed by the formation of rod cells. **Ruchin (2020)** stated that the cone cells in the eyes were formed first, as observed in the fish larvae that are more active on the water surface and receive more light or sunlight. Similarly, **Stuart (2013)** reported that cone cells were formed earlier than rod cells. As the organisms become older, the rod cells will be formed. Based on **Yufera et al. (2014)**, the *Thunnus thynnus* larvae experience eyes pigmentation at 3 DAH and showed to have formed cone cells. At 17 DAH, rod cells began to form, which can be used as an indicator of the larva's ability to catch prey in low light conditions. Disruption of cone cell differentiation in fish larvae can cause visual disorientation, which results in the larvae misdirection (often hitting the walls) in the rearing container.

The use of  $\beta$ -carotene as an enrichment for the rotifers had a positive effect on increasing the amount of prey intake in the golden rabbitfish *S. guttatus* larvae, since  $\beta$ -carotene has an active function of providing color to the organisms (Tachibana *et al.*, 1997; Ridwan, 2002; Effendi *et al.*, 2004; Malide *et al.*, 2018; Madiara *et al.*, 2019), including the rotifers, so that the rotifers can be more easily visible as a prey for *S. guttatus* larvae. Efforts to create contrast between the rotifers and the rearing tank wall color are an appropriate act of environmental manipulation for the larvae due to limited visibility in fish larvae (Hara *et al.*, 1986; Yufera *et al.*, 2014). Providing colorant to live feed can help improve the larval eyes to detect the presence of feed. The detected feed will be more easily captured by larvae, and will directly influence the amount of prey intake.

The success of predation activities can directly influence the growth and survival rate of *S. guttatus* larvae. The results of this study showed that the survival rate of *S. guttatus* larvae was better in the Com-BT treatment. The Com-BT treatment (D),  $\beta$ -carotene (B) and taurine (C) were insignificantly different from the BT0 treatment, but could significantly increase the survival rate of *S. guttatus* larvae. A similar condition was reported by Warastuti *et al.* (2022); they elucidated that the dietary supplementation of  $\beta$ -carotene in the maru fish *Channa maruloides* has an effect on growth, but showing an insignificant effect on the survival rate (SR). This condition was thought to be caused by other factors, namely the carrying capacity of a stable environment for the niche of the maintained maru fish.

From the study results, an enrichment with  $\beta$ -carotene and taurine (Com-BT) resulted in a better fin size increase (dorsal, pectoral, and caudal fins) than other treatments (Table 2). The increased body size is in line with the increased fin size. Good fin development will support the fish movement in the water column including assisting the predation activities easier. Therefore, the amount of prey intake is an indicator that the fish activity has no problems found. Based on Rahardjo (2020), the general function of fins is to support fish movement activities in the water.

The amount of prey intake is the amount of prey consumed and found in the digestive tract of the test animal. The amount of prey consumed is positively correlated with fish growth and survival rate (Jacobsen *et al.*, 2022). Based on the results, the use of  $\beta$ -carotene and taurine as the rotifer enrichments (Fig.1) can increase the amount of prey intake, and has a better effect if both ingredients are combined.

The results of  $\beta$ -carotene and taurine in the rotifers (Table 5) indicate that the rotifers naturally contain  $\beta$ -carotene and taurine. After the enrichment of  $\beta$ -carotene and taurine, the  $\beta$ -carotene and taurine contents increased. In the Com-BT treatment, the value of  $\beta$ -carotene content increased and the value of taurine decreased. The decrease in taurine content in the rotifers at the Kom-BT treatment was thought to be ascribed to the easiness in the use of taurine as an energy source for the rotifers to proliferate, than  $\beta$ -carotene, causing a greater decreased taurine concentration, than  $\beta$ -carotene



concentration. Taurine is a neutral or simple amino acid that comes from the metabolism of sulphur-containing amino acids, which can be directly used by the body (Jusadi *et al.*, 2012; McBean, 2017; Wei *et al.*, 2018).

The rotifers are living creatures that need energy to survive and reproduce. If the energy is unable to fulfill from the food consumed, the rotifers will use the energy stored in their bodies. Hence, the rotifers often experience nutritional deficiencies although fish larvae really need their nutrition. Therefore, it is highly recommended that the rotifers should be enriched with certain ingredients before being applied as feed for fish larvae (Li *et al.*, 2017; Swari *et al.*, 2019). The rotifers experience deficiencies in vitamin A and a number of amino acids (Parma *et al.*, 2013) including free amino acids (taurine). The rotifers contain taurine at 0.8- 1.8mg/ 100g (Takeuchi, 2001).

The water quality is a highly important environmental component and an indicator for good water for fish rearing. Changes in water quality can affect the sustainability of the resources. Water quality parameters include physical, chemical, and biological parameters (Roy *et al.*, 2021). In this study, only DO, salinity, pH, temperature, and ammonia were measured. Ammonia content was only measured three times, at the beginning of rearing, the 5th day of rearing, and the 10th day of rearing.

The dissolved oxygen (DO) content was between 3.15- 5.21ppm. Salinity ranges were from 23.0- 25.0ppt, pH value was 7.00- 8.30ppm, temperature was 27.0- 29.0°C, and ammonia was 0.0006- 0.0631ppm. The water quality parameters measured in this study could still be tolerated by *S. guttatus* larvae (Darsiani *et al.*, 2022), and were categorized as suitable for *S. guttatus* larvae.

According to Roy *et al.* (2021), good water conditions can be characterized by the survival of organisms. The survival level of the organisms can be an indicator of a suitable niche for the organism (Warastuti *et al.*, 2022). To obtain better survival rate, maintaining the stability of the maintenance media is necessary. In this study, green water system was used to stabilize the water quality. Based on Aly *et al.* (2023), *Chlorella* sp. in green water system has the advantage of maintaining water quality parameters. An advantage of green water is that it can absorb heavy metals and convert them into essential metals for metabolic purposes, besides stabilizing the water quality parameters, i.e., pH.

## CONCLUSION

From the research results, it can be concluded that, the combination of  $\beta$ -carotene and taurine can support the growth performance and survival of the golden rabbit fish larvae *S. guttatus*.

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