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Rotifer *Brachionus rotundiformis* enriched with taurine for survival and growth of larval milkfish *Chanos chanos*

Rotifera *Brachionus rutondiformis* diperkaya taurin untuk kelangsungan hidup dan pertumbuhan larva ikan bandeng *Chanos chanos*

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ABSTRACT

This research evaluated the effect of taurine-enriched rotifers on the survival and growth of milkfish larvae. Twenty-five fertilized milkfish eggs were placed in a 250 L cylindrical fiber tank. This study used a completely randomized design with four treatments and five replications. Three days old larvae were given rotifers enriched with taurine at 0, 25, 50, or 75 mg/L. Larvae were cultured until they were 18 days old. The results showed that the taurine content in rotifers was higher in rotifer at 50 mg/L treatment, then decreased at the 75 mg/L treatment. Taurine content in the milkfish larvae aligned with taurine levels in rotifers. Furthermore, the taurine content in rotifers affected milkfish larvae' survival and body length. The highest larval survival and growth was obtained in the 50 mg/L taurine treatment. Thus, it can be concluded that taurine at 50 mg/L is the optimal rotifer enrichment dose for milkfish larvae.

Keywords: growth, milkfish larvae, notochord, survival rate, taurine

ABSTRAK

Penelitian ini dilakukan untuk mengevaluasi pengaruh pemberian rotifer yang diperkaya taurin terhadap kelangsungan hidup dan pertumbuhan larva ikan bandeng. Dua puluh lima butir telur ikan bandeng yang telah dibuahi ditempatkan pada tangki fiber berbentuk silinder berukuran 250 L. Penelitian ini menggunakan rancangan acak lengkap dengan empat perlakuan dan lima ulangan. Larva berumur tiga hari setelah menetas diberi rotifer yang diperkaya taurin dengan dosis 0, 25, 50, atau 75 mg/L. Larva dipelihara hingga berumur 18 hari. Hasil penelitian menunjukkan bahwa kandungan taurin pada rotifer tertinggi diperoleh pada perlakuan rotifer 50 mg/L, kemudian menurun pada perlakuan rotifer 75 mg/L. Kandungan taurin pada larva ikan bandeng sebanding dengan kandungan taurin pada rotifer. Kandungan taurin pada rotifer berpengaruh nyata terhadap kelangsungan hidup dan panjang tubuh larva ikan bandeng. Kelangsungan hidup dan pertumbuhan larva tertinggi diperoleh pada perlakuan dosis 50 mg/L. Dari hasil penelitian dapat disimpulkan bahwa pemberian taurin 50 mg/L pada pengayaan rotifer merupakan dosis yang optimal untuk diberikan pada larva ikan bandeng.

Kata kunci: kelangsungan hidup, larva bandeng, notochord, pertumbuhan, taurin

INTRODUCTION

The survival rate of milkfish (*Chanos chanos*) in hatcheries remains notably low. Reports from hatcheries in the Gerokgak area of Bali indicate that the average survival rate of milkfish larvae to the fry stage is only around 20%. This low survival rate is attributed to the insufficient availability of essential nutrients required by the larvae. Various strategies have been implemented to address this issue, such as enriching rotifers with highly unsaturated fatty acids (HUFA) and vitamin C (Gapasin *et al*., 1998), docosahexaenoic acid (DHA) (Ogata *et al*., 2006), and β-carotene (Ridwan, 2002). Despite these efforts have not been able to achieving a significant improvement in milkfish larvae survival.

Another effort to improve the survival of milkfish (*Chanos chanos*) larvae is to provide rotifers enriched with taurine. Taurine is an essential amino acid for many marine fish larvae (Banthani *et al*., 2019; Sampath *et al*., 2020). However, rotifers cultured with *Nannochloropsis* have a low taurine content, only 3.9% of their total free amino acids (Aragão *et al*., 2004), This free amino acid content is very low when compared to the amount of taurine in *Artemia salina* which can reach 63%. Increasing the taurine content in rotifers has been shown to increase the survival of humpback grouper larvae (*Cromileptes altivelis*) (Jusadi *et al*., 2015), increase the growth and survival of Atlantic bluefin tuna (*Thunnus thynnus*) (Koven *et al*., 2018), and sunu grouper larvae (*Plectropomus leopardus*) (Banthani *et al*., 2019).

Taurine contributes to improved larval growth, supports oxidative reactions, strengthens the immune system, and enhances the overall survival rates of aquatic organisms (Kim *et al*., 2017; Koven *et al*., 2016; López *et al*., 2015; Nguyen *et al*., 2015; Richard *et al*., 2017; Poppi *et al*., 2018; Zhang *et al*., 2019; Rotman *et al*., 2017). However, excessive doses of taurine can have negative effects such as decreasing free amino acid levels and reducing the efficiency of amino acid utilization (Wang *et al*., 2015), pathological changes in the liver, disruption of the structure and function of the distal intestine (Liu *et al*., 2017), and suppressing the growth and survival of larvae (Jusadi *et al*., 2015; Koven *et al*., 2018). Taurine is a free amino acid widely found in brain tissue, retina, liver, muscles, and kidneys. According to (Andersen *et al*., 2016; Widiastuti *et al*., 2015; Bruździak *et al*., 2018; Hernandez *et al*., 2018) the ability of taurine can affect protein synthesis through amine groups in the fish body, involved in several amino acid metabolism pathways such as methionine, bile acid biosynthesis and sulfur metabolism which can accelerate nutrient absorption in the body of seawater fish.

Taurine is also an essential nutrient required by fish larvae stages (Sarih *et al*., 2019; Loekman *et al*., 2018). In general, taurine also plays a role in supporting the development of skeletal muscle function, the cardiovascular system, the central nervous system, and the retina (Hawkyard *et al*., 2015; Onsri & Srisawat, 2016; Adeshina & Abdel-Tawwab, 2020; Gaon *et al*., 2021). In protein synthesis, taurine has functional properties by protecting mitochondria against excess superoxide formation and increasing electron transport (Jong *et al*., 2012). In several species of freshwater and marine fish, taurine can increase the content of protease enzymes in fish, thereby increasing the protein levels in their bodies (Abdel-Tawwab *et al*., 2017). According to Shen *et al*. (2018) the addition of taurine in feed can increase amino acid absorption and accelerate mitochondrial protein synthesis and target of rapamycin (TOR) gene expression. Based on these research findings, providing feed in the form of rotifers enriched with taurine at specific doses is expected to improve the survival and growth of milkfish larvae.

This study aims to examine the role of taurine-enriched rotifers in improving the survival and growth of milkfish larvae *Chanos chanos*.

MATERIALS AND METHODS

Research design

This study used a completely randomized design, with milkfish larvae being fed rotifers enriched with taurine at concentrations of 0, 25, 50, and 75 mg/L. Each treatment was replicated five times. This study was conducted in two stages. The first stage of maintenance is feeding rotifers to milkfish larvae from hatching until they developed into fry (18 days). During this stage, evaluations were conducted on survival rates and larval body length of the larvae. The second stage of maintenance is feeding rotifers to milkfish larvae from hatching until the day 14 to observe bone development. In the second stage of maintenance, fish were periodically sampled for bone development observations.

Rotifer enrichment

Rotifers of the *Brachionus rutondiformis* species was cultured in a 100 liter fiber tub from the Center for Marine Cultivation Research and Fisheries Extension, Gondol. Rotifers cultivated using *Nannochloropsis* were put into a 10 L enrichment container at a density of 500 individuals/mL (Jusadi *et al*., 2012; Jusadi *et al*., 2015). Taurine at the respective treatment doses was blended together, then added 60 mg of β-carotene (Ridwan, 2002), 0.1 mg vitamin C, 0.1 g of chicken egg yolk, 0.5 mL of fish oil, 0.25 g of baker's yeast, and 200 mL of water. All ingredients were homogenized for three minutes, then added to the enrichment medium, followed by the addition of rotifers. Rotifers were enriched with taurine for four hours, referring to Jusadi *et al*. (2012). Some of the enriched rotifers were then collected and stored in a -20°C freezer until chemical analysis was performed.

Maintenance of fish larvae

Fish maintenance was conducted at the center for marine cultivation research and fisheries extension (BBRBLPP) Gondol. In the first stage of maintenance, milkfish larvae were maintained in 20 round fiber tanks with a volume of 300 L filled with 250 L of water equipped with an aeration installation. Fertilized eggs were spread at a density of 25 eggs/L in each fiber tank. Larvae were maintained for 18 days as previously explained. Water quality management was carried out through water changes and the provision of *Nannochloropsis* sp. phytoplankton. At the beginning of maintenance, no water changes were carried out. During the larval maintenance process, phytoplankton (*Nannochloropsis* sp.) was provided. Water changes were carried out starting from the larvae being 10 days old after siphoning as much as 10% of the total water volume. During maintenance, larvae were fed rotifers according to their treatment. Rotifers were provided starting from the three-day-old larval stage. The types and amounts of feed given during the study can be seen in Table 1.

The second stage of maintenance was carried out as in the first stage. During this maintenance period, starting from the third day, 50 fish samples were taken from each treatment, and fixed with 4% formalin (Greszkiewicz & Fey, 2018). Sampling was carried out until the 14^{th} day, assuming that all the fish's spines in all treatments are perfectly formed.

Growth and survival rate of larvae

The observations in this study include body length growth and larval survival rate. The observation of larval length was conducted by measuring the larvae using calipers for 30 individuals per replicate. Then the observation of larval survival rates was carried out by counting the number of larvae in each maintenance medium. The calculation of larval survival rates was performed using the following formula:

$$SR\ (\%) = \frac{N_t}{N_0} \times 100$$

Note:
SR = Survival Rate (%)
N_0 = Number of milkfish larvae at the start of maintenance (fish)
N_t = Number of milkfish larvae at the end of maintenance (fish)

Observation of bone development

The development of milkfish bones was observed using the double staining method (Walker & Kimmel, 2007). Fish larvae were immersed in alcohol with concentrations of 50%, 70%, 96% and acetone (every 24 hours). Then the specimen was soaked in an alcian blue solution for 24 hours and in alizarin red solution for another 24 hours. Rehydrated specimen was performed by immersing them in 0.5% potassium hydroxide (KOH) for three hours (observations were made every hour). The specimens were then transferred to 0.25% KOH for three hours, 0.50% KOH for four hours and 0.125% KOH for six hours (with hourly observations). Then destaining and bleaching were carried out by transferring the

Table 1. Feed management for milkfish larvae maintenance.

Feed	Day													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Nanochloropsis sp. (cell/mL)			5	5	5	10	10	10	10	10	20	20	20	20
Rotifers (individual/ mL)			2	2	2	3	3	3	3	3	3	3	3	3

specimens into a solution of 1% KOH and 100% glycerine (1:1). Furthermore, the specimens were put in a solution of 1% KOH and 100% glycerine (1:2) until the bones appeared sufficiently transparent. Observations were then conducted under a microscope with magnifications of 100× to 400×. The specimen was stored in a solution of 1% KOH and 100% glycerine with a ratio of 1:5.

Chemical analysis

Chemical analysis was conducted on samples of rotifers and milkfish larvae at the end of maintenance period. The analysis included the measurement of taurine levels using the high-performance liquid chromatography (HPLC) method at Saraswanti Indo Gentech, Bogor. Additional chemical analysis was performed at the Fish Nutrition Laboratory, Department of Aquaculture, IPB University. The chemical analysis of rotifers and larvae refers to AOAC (2023) guideline, covering water content, protein content and lipid content. Water content was measured by the heating method using an oven at a temperature of 105-110°C for four hours and weighing the sample until a constant weight was obtained. Protein analysis using the Kjeldahl method, while lipid content was determined using the Folch method.

Data analysis

The data collected were tabulated using Microsoft Excel 2013. Data analysis, including body length and survival rate was performed using analysis of variance (ANOVA) with SPSS version 20. Differences between treatments were further analyzed with Duncan's test with a 95% confidence interval. The analysis of bone development was conducted descriptively.

RESULTS AND DISCUSSION

Results

Total taurine in rotifers and larval bodies

Taurine content in rotifers and larval bodies is presented in Table 2. The taurine content in rotifers increased up to a dose of 50 mg/L and decreased at a dose of 75 mg/L. The lowest taurine content was found in the control treatment (0 mg/L taurine). This aligns with the taurine content observed in the bodies of milkfish larvae. The highest taurine content in milkfish larvae was obtained at a dose of 50 mg/L, while it decreased at a dose of 75 mg/L and the lowest content observed in the control treatment.

Bone development

The development of the spine of milkfish larvae on day 14 can be seen in Figure 1. In larvae up to 14 days old, the transparent notochord membrane is still visible and ossification has not yet occurred to form a vertebral column. Until the age of 14, only cartilage (marked with blue from alcian blue) has formed. The cartilage includes the spinal spine, haemal spine, and caudal fin.

Chemical composition of rotifers and larvae

The chemical composition of rotifers (protein and lipid) and larval meat (water, protein, and lipid content) are presented in Table 3. The highest rotifer protein content was in the 50 mg/L taurine treatment (46.35%), while the lowest value was in the 0 mg/L taurine treatment (36.75%). The highest lipid content in rotifers was observed in the 25 mg/L taurine treatment (3.67%), while the lowest value was found in the 75 mg/L taurine treatment (2.50%). The protein content of fish larvae followed a similar pattern as the rotifer protein content, with the highest protein content in the 50 mg/L taurine treatment (21.0%) and the lowest in the 0 mg/L taurine treatment (14.87%). The lipid content of larvae in the 25, 50 and 75 mg/L taurine treatments was the same, higher than the lipid of larvae in the 0 mg/L taurine treatment.

Larval growth performance

Milkfish larvae length data during the study are presented in Table 4. Larvae in the 50 mg/L treatment were longer ($P<0.05$) compared to the control and 25 mg/L treatments, but showed no significant difference compared to the 75 mg/L treatment. The survival rate of milkfish larvae increased with taurine-enriched rotifer feed.

Table 2. Total taurine in rotifers and larvae.

Total taurine (mg/kg)	Treatment			
	T0	**T25**	**T50**	**T75**
Rotifers	829	1497	2331	866
Larvae	118	137	216	188

Note: T0 (Taurine 0 mg/L), T25 (Taurine 25 mg/L), T50 (Taurine 50 mg/L), T75 (Taurine 75 mg/L).

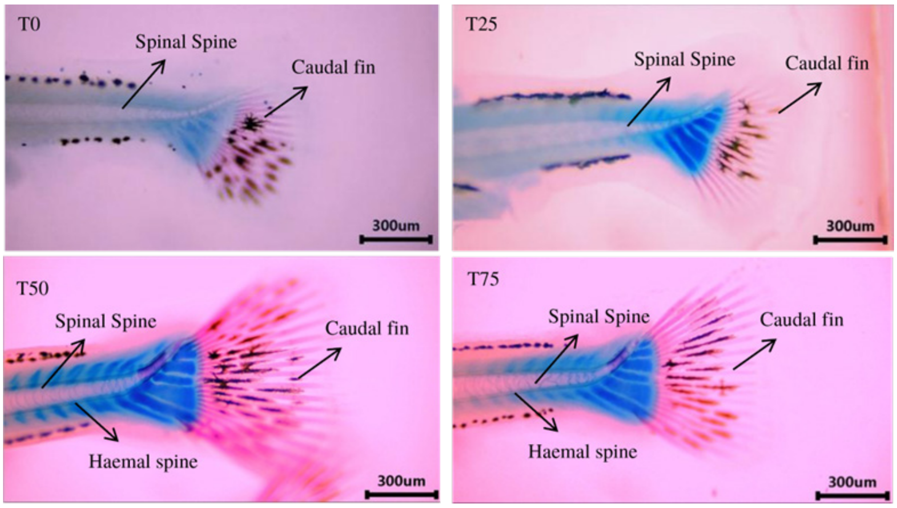

Figure 1. Notochord development in milkfish larvae (*Chanos chanos*). (T0) Taurine 0 mg/L, (T25) Taurine 25 mg/L, (T50) Taurine 50 mg/L, (T75), Taurine 75 mg/L.

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Table 3. Chemical composition of rotifers and milkfish larvae after maintenance.

Composition	Treatment			
	T0	T25	T50	T75
Rotifers				
Protein (%)	36.75 ± 2.47	42.87 ± 1.24	46.37 ±1.24	44.62 ± 1.24
Lipid (%)	2.83 ± 0.71	3.66 ± 0.47	2.83 ± 0.24	2,50 ± 0.24
Larvae				
Water (%)	62,00	68,0	68,0	68,6
Protein (%)	14.87 ± 1.23	17.5 ± 0.00	21.0 ± 0.00	18.37 ± 1.24
Lipid (%)	0.50 ± 0.24	0.83 ± 0.24	0.83 ± 0.24	0.83 ± 0.24

Note: Mean values ± standard deviations followed by different uppercase letters in the same row indicate significant differences (p<0.05). T0 (Taurine 0 mg/L), T25 (Taurine 25 mg/L), T50 (Taurine 50 mg/L), T75 (Taurine 75 mg/L).

Table 4. Length and survival of milkfish larvae fed taurine-enriched rotifers.

Parameter	Treatment			
	T0	T25	T50	T75
Length (mm)	15.4 ± 0.1[a]	15.4 ± 0.1[a]	15.6 ± 0.1[b]	15.5 ± 0.1[ab]
Survival rate (%)	32.9 ± 4.8[a]	42.1 ± 6.1[b]	61.6 ± 2.5[d]	54.4 ± 3.6[c]

Note: Mean values ± standard deviations followed by different uppercase letters in the same row indicate significant differences (p<0.05). T0 (Taurine 0 mg/L), T25 (Taurine 25 mg/L), T50 (Taurine 50 mg/L), T75 (Taurine 75 mg/L).

The highest survival rate was observed in the 50 mg/L taurine treatment group (61.6%), while the lowest was in the control group without taurine enrichment. The 75 mg/L taurine treatment showed that larval survival was greater than the 50 mg/L treatment.

Discussion

The best growth of milkfish larvae was obtained in the rotifer enrichment treatment with 50 mg/L taurine. This dosage is the same as that used for *Cromileptes altivelis* larvae (Jusadi *et al*., 2015). Previous studies have also shown that taurine effectively enhances the growth of yellow catfish *Pelteobagrus fulvidraco* (Li *et al*., 2016), red seabream *Pagrus major* (Gunathilaka *et al*., 2019), and various other marine fish (Li *et al*., 2022). The increase in taurine in rotifers after enrichment is attributed to their nature as filter for particles used in enrichment materials. Rotifers are omnivorous zooplankton that are unable to filter food selectively or non-selective filter feeders. However, a significant decline was observed at a dosage of 75 mg/L.

This is in accordance with Jusadi *et al*. (2012) that taurine absorption by rotifers will be maximum at a certain level if it is considered to have reached the saturation point according to the absorption capacity of the rotifer. Further increases in taurine concentration in the medium (beyond the saturation point) will reduce the metabolism associated with taurine absorption and incorporation to prevent toxicity or imbalance within the rotifers. Taurine plays a very important role in larval development. It modulates intracellular calcium in the larval body, thereby enhancing the initial bone growth in fish larvae. This is in accordance with Sampath *et al*. (2020) that taurine has multiple functions, such as bile acid synthesis, cell volume regulation, central nervous system cytoprotection and intracellular calcium modulation.

Calcium is essential in the early development of the notochord in fish larvae. The notochord formation process begins with the formation of straight threads, haemal arches and body edges lines in the initial phase. Furthermore, the neural increases until it extends towards the back. The neural arch appears on the dorsal side of the spinal cord and extends towards the stomach. Dorsal flexion occurs at this stage, during which the paraphyses appear for the first time, and the neural and haemal arches are completely formed. Then, the urostyle (tail fin) is formed.

In the final stage, ossification of the entire structure is completed (Faustino & Power, 1998). With taurine's ability to modulate calcium, notochord formation improves significantly. This is shown in the study (Table 4) that the 50 mg/L treatment produced the best larval length. However, taurine treatment at lower or higher dosages than 50 mg/L resulted in lower taurine concentrations in rotifers, causing calcium modulation for notochord formation to decrease. This aligns with Wang *et al*. (2015), who reported that excessive taurine dosages can have negative effects, such as significant loss of free amino acids, decreased amino acid utilization efficiency, and suppressed larval growth and survival (Jusadi *et al*., 2012; Koven *et al*., 2018).

Another function of taurine is its role in enhancing lipid emulsification, making it easier for lipid to be distributed into cells where they are needed as an energy source. According to Nguyen *et al*. (2015) and Li *et al*. (2016), taurine plays a crucial role in lipid metabolism, digestion, and absorption in fish. Studies by Moura *et al*. (2019) and Sampath *et al*. (2020) taurine has an important role in lipid metabolism in fish, including bile acid synthesis, lipid emulsification, lipid digestion and absorption, and body lipid deposition. Taurine increases fish lipid metabolism by optimizing lipid digestion and metabolic regulation (Shen *et al*., 2018). Research by Richard *et al*. (2017) shows that taurine supplementation can increase the lipid emulsion process and lipid absorption through bile salts in several teleost fish species. With the availability of non-lipid protein energy, the use of protein as a biofunction component increases.

This is indicated by the high protein content observed in larvae treated with 50 mg/L taurine. In addition, this study shows that the structural lipid deposition in the bodies of larvae given taurine was higher than the control treatment. The role of lipid as a cell membrane will be higher with the increasing of lipid deposition, which can help anticipate oxidative stress. The role of taurine as an antioxidant where taurine will increase the antioxidant capacity of larvae. By increasing oxidative capacity, taurine helps mitigate oxidative stress, leading to higher survival rates in larvae. In this study, enrichment with a 50 mg/L dosage resulted in the highest survival rate.

According to research by Coutinho *et al*. (2017), Hu *et al*. (2018), Zhang *et al*. (2019), and Xu *et al*. (2020), taurine has antioxidant properties due to its influence on oxidative enzymes, including SOD, CAT, T-SOD, T-AOC, GSH-px, and antioxidant genes in the liver and intestines. Taurine deficiency in an organism can cause oxidative stress due to disruptions in the electron transport chain to mitochondria, leading to the formation of superoxide anions (Jong *et al*., 2012). In other conditions, taurine deficiency can reduce growth performance, certain physiological abnormalities and increase the need for vitamins C and E in the body (Shen *et al*., 2018; Izquierdo *et al*., 2019).

CONCLUSION

Rotifers enriched with 50 mg/L taurine produced the best survival rate in milkfish larvae. Feeding larvae with taurine-enriched rotifers also significantly increased their length. Until the age of 14 days, the vertebral segments had not yet formed in milkfish larvae.

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