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Effect of Dietary Taurine Enrichment Levels on Growth Performance, Survival and Metamorphosis of Humpback Grouper *Cromileptes altivelis*

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Abstract

The effect of feeding rotifers and *Artemia* enriched with taurine on the growth performance, survival and metamorphosis of humpback grouper *Cromileptes altivelis* larvae was investigated. Rotifers and *Artemia* nauplii were enriched with a commercial taurine supplement at four levels: 0 gL^{-1} (T-0), 1 gL^{-1} (T-1), 2 gL^{-1} (T-2), and 3 gL^{-1} (T-3). The larvae were fed the enriched rotifers from 2 days after hatching (DAH) to 20 DAH and enriched *Artemia* nauplii from 14 DAH to 60 DAH in triplicate. The growth of larvae was significantly higher in T-1 and T-2 than that of T-0. The survival rate of larvae was significantly higher in T-1, T-2 and T-3 than that of T-0. The percentage of flexion stage larvae of T-1 group was significantly higher than that of T-0 group at 15 and 20 DAH. Stage-1 larvae of metamorphosis firstly appeared in all of the groups at 30 DAH, however, at 45 DAH stage-2 larvae were significantly more abundant in T-1 than other groups. Further, at 55 DAH percentage of stage-3 larvae was significantly higher in T-1 group than that of T-3 and T-0 and thereafter (60 DAH) remained significantly higher than T-0. Therefore, the larvae fed taurine-enriched rotifers and *Artemia* nauplii of 1 gL^{-1} (T-1) showed the best growth performance, survival and metamorphosis which were significantly higher than those of other treatments ($P<0.05$). These results suggest that taurine is an essential nutrient for humpback grouper larvae and taurine enrichment of rotifers and *Artemia* is an effective method of enhancing the growth performance, survival and metamorphosis success of humpback grouper larvae.

***Keywords*:** Humpback grouper; taurine; rotifer; *Artemia*; growth; survival; metamorphosis.

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1. Introduction

Humpback grouper *Cromileptes altivelis* is one of the most valuable reef fish species of economically important. Juvenile of the spesies are prize targets for the aquarium trade throughout the species' distribution, and adults are among the most valued products in the Asian live reef fish trade [1]. *C. altivelis* is a grouper species as facing imminent threat of extinction based on IUCN criteria if threat trends, mainly uncontrolled fishing, continue [2]. To meet the demand of export markets and reduce excessive exploitation pressures on the population of humpback grouper as well as damage to the habitat necessary to increase production through aquaculture industry, both hatchery and grow-out.

Humpback grouper hatchery has been undertaken by some government and private agencies. The hatchery has contributed to the provision of seeds for humpback grouper culture, although not able to meet the needs of both domestic and export demand. It is caused by low productivity of humpback grouper hatchery due to the high mortality and slow growth in the larval until the juvenile stage.

One factor thought to cause high mortality and low growth rate in the larval to the juvenile stage of humpback grouper is nutrient needs unmet from feeding. Although morphological development is mainly genetically determined, critical physiological control processes can be strongly influenced by diet [3, 4]. Improper nutrition may delay and disrupt larval development, causes growth retardation, high frequency of malformations, and ultimately death [5]. During this time, live feed that used as first feed in the rearing of grouper larvae is rotifers, followed by *Artemia*. The use of rotifers as first feed on marine fish hatchery in general, because its maintenance system is simple and continue to grow until now [6, 7].

In the larval stage, the hull of humpback grouper has not been functioning [8], because its development has been perfect at 45 days after hatching [9], or after experiencing metamorphosis into the juvenile stage. Therefore, it necessary the first feed that can be digested easily, contains a lot of protein and short chain free amino acids [10, 11].

One type of free amino acid is deficient in rotifers [12] and contained relatively low in *Artemia* is taurine. Taurine contained only about 0.8-1.8 mg 100 g^{-1} in rotifers [13], or just around 0.08 ± 0.04% of the protein in the rotifers [14, 15]. Taurine or 2-aminoethanesulfonic acid is an amino acid metabolic end product that contains sulfur. Taurine is not incorporated into protein, nor degraded by mammalian tissues [16]. As in mammals, taurine is a free amino acid conditionally essential that plays a vital role in many physiological functions in fish [17] including conjugation of bile salts [18, 19] and enhancement of digestive enzyme activities [18, 20, 21, 22, 23].

Several studies have shown that feeding of rotifers or *Artemia* enriched with taurine can promote growth, development, and metamorphosis of marine fish larvae such as red sea bream *Pagrus major* [24], Japanese flounder *Paralichthys olivaceus* [25], Pacific cod *Gadus macrocephalus* [26], sole *Solea senegalensis* [27], cobia *Rachycentron canadum* [23], California yellowtail *Seriola lalandi* and white seabass *Atractocsion nobilis* [28], amberjack *Seriola dumerili* [29], and northern rock sole *Lepidopsetta polyxystra* [30]. However, the

effective enrichment level of rotifers and Artemia to promote the growth, development, and metamorphosis success of larvae is species specific [17], and the information is still limited. Therefore, this study aimed to evaluate the effective enrichment level of rotifer and Artemia with taurine in humpback grouper larvae.

2. Material and methods

2.1. Experimental systems and fish

Fertilized eggs of humpback grouper from the same batch, were obtained from the Institute of Brackish water Aquaculture Takalar, South Sulawesi. Eggs were randomly stocked at 25 eggsL^{-1} into one of four replicated systems comprising three 3000 L concrete tanks (n=3) and estimated hatching rate of 95%. Larvae were reared at 20 larvae L^{-1} in the concrete tank equipped with an installation of water, air, and lighting. Water quality parameters (ammonia, dissolved oxygen, temperature, salinity, pH, and alkalinity) were measured, and all were within a suitable range for humpback grouper larvae. The treatment in this study was enrichment dose of rotifers and *Artemia* with taurine consisting of four levels i.e., 0 gL^{-1} or control (T-0), 1 gL^{-1} (T-1), 2 gL^{-1} (T-2), and 3 gL^{-1} (T-3) of enrichment media.

2.2. Feeds and feeding

Chlorella sp and rotifers (*Brachionus rotundiformis*) were obtained from plankton mass culture division of the IBA Takalar. *Chlorella* sp + *Nanno chloropsis* sp (Nanno 3600, Reed Mariculture, Campbell, CA) used to form green water system (approx. 500,000 cell mL^{-1}) and *Brachionus rotundiformis* (SS- and S-type rotifers) were fed to larvae from 2 days after hatching (DAH) to 20 DAH at wich point only *Artemia* as live feed was offered. *Artemia* nauplii derived from hatching *Artemia* cysts (Supreme Plus Golden West *Artemia*, Great Salt Lake, USA) were fed to larvae from 14 DAH to 60 DAH. Co-feeding of larvae with Otohime marine weaning feeds (51% crude protein, 11% lipid; 250-650 µm; manufacturer's analysis; Marubeni Nisshin Feed, Japan) commenced at 14 DAH. The enrichment materials used in this research were DHA Protein Selco (Inve Aquaculture, Hoogveld, Dendermonde-Belgium) to increase the protein content of rotifer with an optimum of DHA/EPA ratio, A1 DHA Selco (Inve Aquaculture, Inve Thailand Ltd.) to increase unsaturated fatty acids (DHA and EPA) content of *Artemia*, and taurine (99%; Jiangyin Huachang Food Additive Co., Ltd.) to increase the taurine content of rotifers and *Artemia* nauplii.

The enrichment of live feed with taurine was conducted according to the treatment: rotifers were enriched at density of 500 individuals mL^{-1} with 0 gL^{-1} (T-0), 1 gL^{-1} (T-1), 2 gL^{-1} (T-2) and 3 gL^{-1} (T-3) of taurine for 20 hours with the addition of 0.35 gL^{-1} of DHA Protein Selco at the last six hours of the enrichment period. *Artemia* were enriched at density of 250-350 nauplii mL^{-1} with 0 gL^{-1} (T-0), 1 gL^{-1} (T-1), 2 gL^{-1} (T-2) and 3 gL^{-1} (T-3) of taurine with the addition of 0.6 gL^{-1} of A1 DHA-Selco for 24 hours.

The SS-type rotifers were fed to larvae (5 rotifers mL^{-1}) from 2 DAH to 5 DAH; then the S-type rotifers were fed to larvae (5-10 rotifers mL^{-1}) from 6 DAH to 20 DAH. Rotifers were fed to larvae twice day^{-1} at 08:00 and 14:00. Rotifer density in the larval rearing tanks was checked by sampling 5 ml of the rearing water twice a day (7:00 and 13:00). Otohime marine weaning feeds started to be fed to the larvae at 14 DAH four times day^{-1}

(07:00, 09:00, 11:00 and 13:00) with feeding rate 5 ppm day^{-1}. *Artemia* was fed to larvae (1-3 nauplii mL^{-1}) once day^{-1} (16:00) from 14 DAH to 20 DAH, then twice day^{-1} (11:00 and 16:00) from 21 DAH to 60 DAH.

2.3. Sampling and biochemical analyses

Sampling of rotifers and *Artemia* for taurine content analysis was done after enrichment. The sampling of larvae for all test parameters observation was done at the age of 3, 5, 7, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 days after hatching. Samples were randomly collected as many as 20 larvae per tank. Larvae were rinsed with distilled water to remove salt, blotted dry, then preserved with 70% alcohol for further observation. At the age of 60 DAH, larvae samples for the taurine content analysis were taken, washed, dried and stored at a temperature of -20^{o}C until analysis, then survival rate calculated. To measure the larval total length (TL) and to determine the notochord flexion and metamorphosis state, digital images of larvae were taken under the Stereo Microscope (SteREO Discovery. V12, Carl Zeiss) by each predetermined sampling time, and an image analysis software (AxioVision imaging software, Carl Zeiss Microscopy GmbH 07745 Jena, Germany) was used for the measurement. The metamorphosis pattern of humpback grouper larvae was assessed and categorized in to three metamorphic stages [8, 31], according to the external morphology change: 1–The second dorsal fin spine and first pelvic fin spine start to shrink, and black spots appear at caudal peduncle, bases of dorsal and anal fins (beginning of metamorphosis); 2–The elongated fin spines almost regress to normal size, the fins as well as the body are covered with black dots as large as their eyes (beginning of juvenile stage); 3–Both the fin spines are fully reabsorbed and serration have disappeared and body turned to opaque (completion of metamorphosis). The taurine levels of larvae, rotifers, and *Artemia* were quantified according to [32] using HPLC after the samples were extracted and derivatized with dansyl chloride precolumn of amino acids. Samples were extracted with hot water (50-60^{o}C) for ±10 minutes then protein was precipitated with Carrez reagents I and II. Samples were diluted with aquabides, filtered through a No. 42 filter paper then a 0.45-µm microfilter and ready for derivatization. Next, 1.0 mL of hydrolysate, 1.0 mL buffer Na_2CO_3 and 1.0 mL dansyl chloride reagents were pipetted into vial tube. After vortexing, the mixture was added with 0.1 ml of methylamine HCl, mixed and vortexed until precipitation of flocculate. Furthermore, the mixture was centrifuged, and the supernatant was filtered through a membrane of 0.45 µm and ready to be injected.

2.4. Statistical analyses

All the data of measurement and analysis to compare among the four treatments (T-0, T-1, T-2 and T-3) were subjected to one-way ANOVA followed by Duncan's multiple range test. The homogeneity of variances was first checked using the Levene's test. Differences were considered to be statistically significant if $P < 0.05$. The data were presented as mean ± standard error of the mean (SEM) of triplicate tanks of each sampling stage on treatment group. All of Statistical analyses were carried out using the SPSS program version 16.0 (SPSS, Michigan Avenue, Chicago, IL, USA) for Windows.

3. Results

3.1. Taurine content of rotifer, Artemia and larvae

Taurine contents of rotifers, *Artemia* nauplii and larvae with different taurine enrichment dose are presented in Table 1. Taurine content of taurine enriched-rotifers and *Artemia* groups (T-1, T-2, T-3) was significantly higher than that of rotifers and *Artemia* without taurine enrichment (T-0). Taurine content of rotifers increased with enrichment dose up to 3 gL^{-1}, however, taurine content of T-2 rotifers was not significantly different from T-3 rotifers group. *Artemia* nauplii taurine content increased proportionally with enrichment dose. At the end of trial, the taurine content of fish fed taurine enriched-rotifers and *Artemia* groups (T-1, T-2, dan T-3) was significantly higher than that of fish fed control rotifers and *Artemia*, and there was no difference among taurine supplemented group.

Table 1: Taurine content of rotifer, *Artemia* nauplii and larvae

Treatments	Taurine content (g/kg^{-1} DW)		
	Rotifer	*Artemia* nauplii	Larvae 60 DAH
T-0	0.120 ± 0.006^{c}	7.088 ± 0.390^{d}	12.928 ± 0.714^{b}
T-1	2.747 ± 0.146^{b}	11.782 ± 0.509^{c}	16.533 ± 0.895^{a}
T-2	8.413 ± 0.413^{a}	22.783 ± 1.237^{b}	15.877 ± 0.815^{a}
T-3	8.534 ± 0.436^{a}	29.903 ± 1.704^{a}	16.388 ± 0.959^{a}

Values are expressed as means ± standard error of the mean. Different superscript letters within columns indicate significant differences among treatments ($P < 0.05$). DAH (days after hatching), DW (dry weight).

3.2. Growth performance and survival

Growth performance and survival of the larvae are presented in Table 1. The final total length and survival of larvae were significantly higher in T-2 group compared with T-0 group ($P<0.05$). There were no significant differences in total length and survival between T-2 group and T-3 group ($P>0.05$). The survival of larvae was significantly higher in T-3 group compared with T-0 group ($P<0.05$), but there was no significant difference in final total length between T-3 group and T-0 group ($P>0.05$). Fish larvae fed taurine-enriched rotifer and *Artemia* of T-1 had the best growth performance in final total length and survival, which were significantly higher than those in other treatments ($P<0.05$).

Table 2: Growth performance and survival rate of Humpback grouper *C. altivelis* larvae under rearing differently taurine-enriched rotifer and *Artemia* feeding

Treatment	Initial total length (mm)	Final total length (mm)	Survival rate (%)
T-0	2.319±0.015	22.324 ± 0.666^{c}	0.773 ± 0.040^{c}
T-1	2.340±0.058	26.755 ± 0.439^{a}	7.630 ± 1.020^{a}
T-2	2.340±0.006	23.581 ± 0.310^{b}	4.183 ± 0.150^{b}
T- 3	2.378±0.039	23.320 ± 0.541^{bc}	3.927 ± 1.220^{b}

Values are expressed as means ± standard error of mean. Initial total length and final total length were measured at 3 DAH and 60 DAH respectively. The 20 larvae per tank in a certain age were provided to the measurement

(total 60 larvae per each treatment in a certain age). Different superscript letters within columns indicate significant differences among treatments ($P < 0.05$). DAH (days after hatching).

3.3. Notochord flexion state

The comparison of humpback grouper larvae flexion state in certain age is presented in Figure 2. The flexion stage larvae began to appear at 10 DAH for experimental groups fed taurine-enriched rotifers (T-1, T-2, and T-3), whereas in the control group (T-0) larvae remained at preflexion stage. At 15 DAH, the percentage of flexion stage larvae increased to 73%, 62%, 67% and 68% in T-0, T-1, T-2, and T-3, respectively, while that of postflexion stage larvae began to appear for all of the experimental groups. At 15 DAH, the percentage of postflexion stage larvae was significantly higher in the T-1(38%) and T-2 (30%) groups than that of T-0 (7%) group, but that was not significantly different from that of T-3 (18%) group. At 20 DAH, the percentage of flexion stage larvae decreased to 43%, 0%, 17% and 11% in T-0, T-1, T-2, and T-3, respectively, while that of postflexion stage larvae increased to 57%, 100%, 83%, and 89% in T-0, T-1, T-2, and T-3, respectively, which was significantly higher in the T-1 group ($P<0.05$) than that of T-0 group.

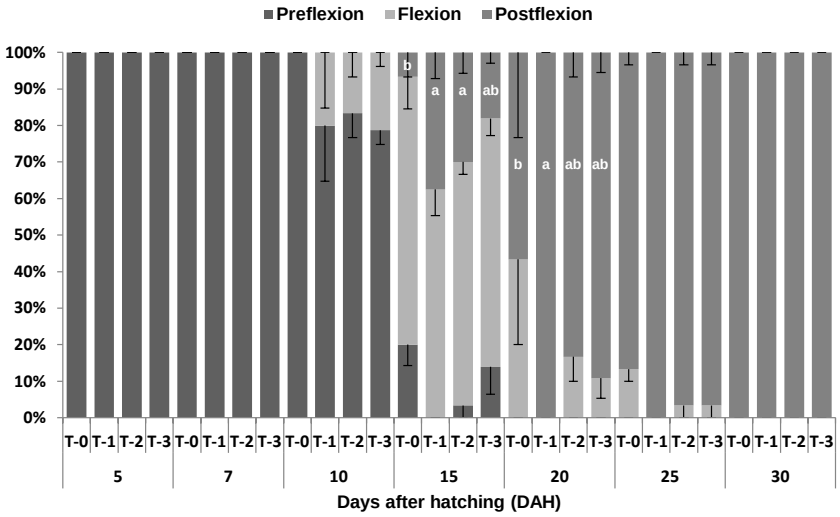

Figure 2: Comparison of humpback grouper larvae flexion state in certain age under rearing differently taurine-enriched rotifer and *Artemia* feeding. Results are expressed as percentage of each flexion state found at a certain age. The 20 larvae per tank in a certain age were provided to observation of flexion state (total 60 larvae per each treatment in a certain age). Vertical bars indicate standard error of mean values of triplicate tanks. Different letters within the same state represent significant differences among treatments at a certain age ($P < 0.05$).

3.4. Metamorphosis

The metamorphosis pattern of humpback grouper *C. altivelis* larvae under rearing differently taurine-enriched rotifer and *Artemia* feeding is presented in Figure 3. The stage-1 larvae began to appear at 30 DAH for all of the experimental groups. At 35 DAH, the larvae fed taurine-enriched rotifers and *Artemia* groups (T-1, T-2 and T-3) began to enter the stage-2 of metamorphosis, whereas the control treatment (T-0) larvae remained at stage-1 of metamorphosis. The percentage of stage-2 larvae increased to 45 DAH (100%) in the T-1 group which was

significantly higher than that of other groups. Further, the percentage of stage-2 larvae increased to 50 DAH (97% and 93%) in the T-2 and T-3 groups, respectively, and to 55 DAH (100%) in the control (T-0) group. At 55 DAH, the percentage of stage-2 larvae decreased to 37%, 60% and 73% in T-1, T-2 and T-3, respectively, while that of stage-3 larvae began to appear at a rate of 63%, 40% and 27% in T-1, T-2 and T-3, respectively, whereas in the control treatment (T-0) none of the larvae displayed these features, which showed significant difference among the treatment groups ($P < 0.05$). At 60 DAH, the rate of stage-3 larvae increased to 37%, 93%, 63% and 60% in the T-0, T-1, T-2 and T-3 groups, respectively, which was significantly higher ($P < 0.05$) in the T-1 group than that of T-0 group, but there was no significant difference among larvae fed taurine-enriched rotifers and *Artemia* groups (T-1, T-2 and T-3).

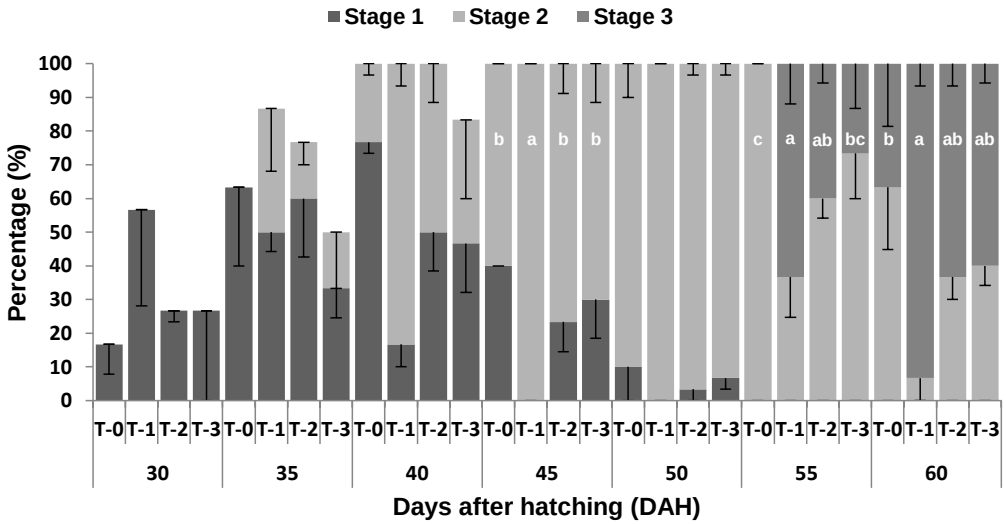

Figure 3: Metamorphosis pattern of Humpback grouper *C. altivelis* larvae under rearing differently taurine-enriched rotifer and *Artemia* feeding. Results are expressed as percentage of each metamorphic stage found at a certain age. The 20 larvae per tank in a certain age were provided to observation of metamorphic stage (total 60 larvae per each treatment in a certain age). Vertical bars indicate standard error of mean values of triplicate tanks. Different letters within the same stage represent significant differences among treatments at a certain age ($P < 0.05$).

4. Discussion

In this research, we investigated the effect of dietary taurine enrichment levels on growth performance, survival, and metamorphosis of humpback grouper *Cromileptes altivelis* larvae. The results indicated that rotifers and *Artemia* enrichment with taurine 1 gL^{-1} led to total length and survival rate wich were significantly higher than those of other treatments and it significantly shortened the larval postflexion stage and metamorphosis stage periods compared to the control treatment (T-0). The positive effects of dietary taurine on the growth have also been reported in marine fish larvae such as red sea bream *Pagrus major* [24, 33], Japanese flounder *Paralichthys olivaceus* [25], Pacific cod *Gadus macrocephalus* [26], and Senegalese sole *Solea senegalensis* [27], amberjack *Seriola dumerili* [29], *Nibea albiflora* [34], California yellow tail *Seriola lalandi* [35], northern rock sole *Lepidopsetta polyxystra* [36]. Although growth is essentially muscle protein deposition [37], taurine is

not incorporated into muscle protein. Thus, its effects on amino acid metabolism are expected to be related to indirect regulatory and/or metabolic functions. The taurine content of the larvae is suggested to affect their retention of amino acids. In Senegalese sole, amino acid retention increased when the larval body had a higher taurine content [27]. In the present study, the taurine content of larvae fed the taurine enriched-rotifers and *Artemia* groups (T-1, T-2, and T-3) was significantly higher than that of control larvae (T-0). Thus, the improved growth performance of larvae fed this rotifer may be attributed to the increased amino acid retention. In the current study, taurine content of control (T-0) larvae and T-1 larvae was higher than that of T-0 *Artemia* and T-1 *Artemia*, which indicates that taurine may also be obtained from weaning feeds. Conversely, body taurine level of T-2 larvae and T-3 larvae was lower than that of T-2 and T-3 *Artemia*, which suggests that taurine excretion becomes active when the net accumulation of taurine in the fish body exceeds a certain level [38]. The dietary taurine has been suggested to affect survival in certain fish species such as cobia *Rachycentron canadum* [23], *Nibea albiflora* [34], northern rock sole *Lepidopsetta polyxystra* larvae [36] larvae. In cobia *Rachycentron canadum* larvae, the survival rate of fish fed rotifers and *Artemia* nauplii enriched with taurine was higher than that of fish fed rotifers that had not been enhanced with taurine [23]. In the present study, the survival rate of humpback grouper larvae fed rotifers and *Artemia* nauplii enriched with taurine up to the 3 gL^{-1} of enrichment level was significantly higher than that of larvae fed rotifers and *Artemia* without taurine enrichment (T-0). However, the optimum enrichment level of taurine in this trial was 1 gL^{-1}, which resulted in the highest survival rate that significantly greater than that of 2-3 gL^{-1} (T-2 and T-3). The considerable low survival of larvae fed rotifers and *Artemia* without such taurine enrichment (T-0) suggests that taurine is an essential nutrient for humpback grouper larvae. The positive effect of dietary taurine supplementation on the period of flexion and metamorphosis stages have been reported in several fish species such as red sea bream *Pagrus major* [33], northern rock sole *Lepidopsetta polyxystra* [36], and *Solea senegalensis* [27] larvae. Feeding of taurine-enriched rotifers to the red sea bream larvae improved growth and shortened the period of flexion stage in the fish larvae [33]. Furthermore, the effect of feeding taurine-enriched rotifers on growth and development of *Lepidopseta polyxystra* larvae have been evaluated [36]. The results showed that northern rock sole *Lepidopsetta polyxystra* larvae fed taurine-liposome enriched rotifers grew significantly larger than larvae in the control and taurine-dissolved low treatment. In addition, the most developed larvae, regarding flexion, were observed in the taurine-liposome and taurine-dissolved high treatments. Conversely, dietary taurine supplementation did not affect larval growth performance and metamorphosis during the pelagic phase of Senegalese s\ole larvae, however, by the end of the trial, Senegalese sole previously fed taurine supplemented microcapsules had a significantly higher growth performance and metamorphosis completion success than larvae fed control microcapsules [27]. In the present study, enrichment dose of rotifers and *Artemia* with taurine 1 g L^{-1} produced the best growth and survival which were significantly higher than those of the other treatments. Increasing of the dietary taurine enrichment level to 2-3 gL^{-1} (T-2 and T-3) did not provide any further enhancement in the growth and survival, but they were still significantly higher than those of the control treatment except the growth in the T-3. The depressed growth and survival by excessive taurine supplementation have been reported in tongue sole, *Cynoglossus semilaevis* post larvae [39]. In addition, the reduction of growth and feed intake by excessive dietary taurine supplementation have also been reported in turbot [40], Japanese flounder [41] and rainbow trout [42]. It was presumed that the low level of feed intake was associated with lower feed palatability caused by the high acidity of excessive taurine accumulated in the feed [43]. However,

the exact mechanism through which excess taurine intake resulted in a reduction in growth performance remains unclear. Excessive taurine has an adverse impact on larval morphological development, so the optimal taurine enrichment level in live feed for fish larvae should be estimated. In the present study, the enrichment of *Artemia* with taurine 2–3 gL^{-1} (T-2 and T-3) might lead to excessive taurine. These treatment resulted in the taurine content much higher in T-2 and T-3 *Artemia* compared with the taurine content of T-2 and T-3 larvae at the end of the trial. Three levels of taurine in rotifers fed to Japanese flounder (*Paralichthys olivaceus)* showed an improvement in growth when taurine have increased from 0.5 to 1.7 g kg^{-1} dry weight of rotifers, but a further increase to 3.0 g kg^{-1} did not give any further enhancement in growth [25]. Taurine has long been associated with bile salts [44, 38]. Lipases that have been isolated from pyloric caeca of cod Atlantic [45, 46] and from hepatopancreas of red sea bream, *Pagrus major* [22] had similar characteristics with bile salt-activated lipase (BAL) in mammals. Besides that, gene expression level and specific activity of BAL found in haddock fish (*Melanogrammus aeglefinus*) larvae during ontogeny have also been determined and they showed that the haddock fish larvae were capable of digesting lipids at the time of mouth opening [20]. These results indicate that the possibility of the lipase enzyme found in Atlantic cod, red sea bream and haddock fish is bile salt-activated lipase (BAL) and may be the only lipolytic enzymes in these fish and other species of marine teleosts [46, 22, 20]. If so, lipase activity increased by taurine-enriched feeding in marine fish larvae such as cobia *Rachycentron canadum* [23], could be explained by a higher BAL activity as a result of more taurine-derived bile salts. In carnivorous marine teleosts, it is generally thought that BAL plays a more important role in lipid digestion [46] due to the composition of natural feeds, which primarily contain wax esters and triacyl glycerols high in unsaturated fatty acids that are relatively resistant to hydrolysis by pancreatic lipase [47, 48]. The increased digestive enzyme activities would enable better nutrient availability, thus increasing the growth and survival of larval fish fed rotifers and *Artemia* with taurine supplementation [23]. However, the effect of taurine on the activity of digestive enzymes of humpback grouper larvae still needs to be studied.

5. Conclusion

In conclusion, the results of the study suggest that taurine is an essential nutrient for humpback grouper larvae, and taurine enrichment of rotifers and Artemia is an effective method of enhancing the growth performance, survival and metamorphosis success of humpback grouper larvae.

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