Effects of Glucose on Hatching of Fertilized Eggs and Activities of Acetyl-CoA Carboxylase and Fatty Acid Synthase in Yolk Sac Larvae of Hybrid Grouper (Epinephelus fuscoguttatus × Epinephelus polyphekadion) Postprint

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Abstract

To investigate the effects of glucose on the hatching of hybrid grouper fertilized eggs and the activities of acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) in yolk sac larvae, different concentrations [0 (control), 2, 4, 6, 8, and 10 mg/L] of glucose were added to the hatching water of hybrid grouper (brown-spotted grouper × orange-spotted grouper) fertilized eggs. Hatching rate and malformation rate were measured, along with changes in total length and ACC and FAS activities in 1-, 2-, and 3-day-old yolk sac larvae. The results showed that glucose concentration significantly affected both fertilized egg hatching rate and newly hatched larval malformation rate ($P<0.05$). The relationships between glucose concentration and fertilized egg hatching rate, as well as between glucose concentration and newly hatched larval malformation rate, could both be described by quadratic equations. Through regression analysis, the glucose concentration yielding maximum fertilized egg hatching rate was calculated to be 3.8 mg/L. When glucose concentration was below 6 mg/L, increasing glucose concentration led to increased fertilized egg hatching rate, decreased newly hatched larval malformation rate, and increased yolk sac larval total length. For 1–3 day-old larvae, ACC and FAS activities in glucose-supplemented groups were significantly higher than those in the control group ($P<0.05$). With increasing glucose concentration, both ACC and FAS activities exhibited an upward trend at the 1-day-old larval stage; at the 2–3 day-old larval stage, ACC activity displayed an "increase-decrease" pattern, while FAS activity showed an "increase-plateau" pattern. In summary, the addition of 6 mg/L glucose to the hatching water in hybrid grouper artificial seedling rearing can

significantly promote fertilized egg hatching and yolk sac larval development, and significantly enhance ACC and FAS activities in yolk sac larvae.

Full Text

Effects of Glucose on Fertilized Egg Hatch and Activities of Acetyl-CoA Carboxylase and Fatty Acid Synthase in Yolk-Sac Larvae of Hybrid Grouper (Epinephelus fuscoguttatus × Epinephelus polyphekadion)

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Abstract

This study investigated the effects of glucose supplementation on fertilized egg hatch, acetyl-CoA carboxylase (ACC) activity, and fatty acid synthase (FAS) activity in yolk-sac larvae of hybrid grouper (Epinephelus fuscoguttatus × Epinephelus polyphekadion). Different glucose concentrations [0 (control), 2, 4, 6, 8, and 10 mg/L] were added to the hatching water, and hatch rate, abnormality rate, total length, and ACC and FAS activities were measured at 1, 2, and 3 days post-hatch. The results demonstrated that glucose concentration significantly affected both hatch rate and abnormality rate ($P < 0.05$). The relationships between glucose concentration and hatch rate or abnormality rate were best described by quadratic equations, with the optimal glucose concentration for maximum hatch rate calculated as 3.8 mg/L. When glucose concentration was below 6 mg/L, hatch rate increased, abnormality rate decreased, and larval total length increased with rising glucose levels. For 1–3 day-old larvae, ACC and FAS activities in glucose-supplemented groups were significantly higher than in the control group ($P < 0.05$). At 1 day post-hatch, both ACC and FAS activities increased with glucose concentration. At 2–3 days post-hatch, ACC activity showed an "increase-decrease" pattern while FAS activity exhibited a "rising-plateau" trend. These findings indicate that adding 6 mg/L glucose to the hatching water can significantly promote fertilized egg hatch and yolk-sac larval development while enhancing ACC and FAS activities in hybrid grouper.

Keywords: glucose; hybrid grouper; hatching rate; yolk-sac larvae; acetyl-CoA carboxylase; fatty acid synthase

Introduction

During embryonic development and the yolk-sac larval stage, marine fish rely entirely on endogenous yolk nutrients for growth and development. As a crucial yolk component, lipids serve as energy substrates, structural components

for membrane biogenesis, and participate in cellular differentiation and organ formation, playing a decisive role in larval growth and survival. Carbohydrates, widely distributed organic compounds, are essential metabolic nutrients and energy sources in fish. Recent studies have demonstrated that nutrient supplementation via solution immersion can be applied during the embryonic and pre-feeding larval stages of aquatic animals. For instance, Li (2007) reported that 1.5% glucose in the hatching solution significantly promoted embryonic development and improved larval survival in zebrafish (*Danio rerio*). Xue (2008) found that 2 mmol/L glucose in the incubation medium significantly reduced abnormality and developmental arrest rates in tetraploid scallop (*Chlamys farreri*) embryos. Xiong et al. (2014) and Jiang et al. (2014) showed that 15 g/L glucose during fertilized egg incubation significantly shortened hatching time, increased hatch rate, and promoted ACC and FAS synthesis and secretion during embryonic development in gibel carp (*Carassius auratus gibelio*). These studies collectively demonstrate that appropriate glucose concentrations in the water can provide nutritional supplementation and promote early development, though such effects appear species-specific.

Most research on glucose supplementation in marine aquaculture species has focused on juvenile fish using dietary addition methods, as seen in studies on large yellow croaker (*Pseudosciaena crocea*), orange-spotted grouper (*Epinephelus coioides*), and cobia (*Rachycentron canadum*). However, no reports have examined glucose effects on early developmental stages of marine fish. Grouper represents an important marine aquaculture species widely cultured in coastal China and Southeast Asia due to its excellent taste and nutritional value. The industry' s sustainable development is constrained by germplasm degradation and large-scale seed production challenges. Hybridization technology has been introduced to address these issues, producing hybrid groupers with superior traits, and transcriptome analyses have revealed the molecular mechanisms underlying their heterosis. Our research team has successfully developed a hybrid between brown-marbled grouper (*Epinephelus fuscoguttatus*) and camouflage grouper (*Epinephelus polyphekadion*), investigating salinity effects on egg hatch and larval morphology, as well as path analysis of morphological traits on body mass. International reports indicate this hybrid exhibits faster growth than its parents, demonstrating heterotic advantages. However, artificial seed production technology remains unstable, characterized by low embryonic hatch rates and larval survival. Therefore, this study examined the effects of glucose supplementation on hatch rate, abnormality rate, and ACC and FAS activity changes during yolk-sac larval development, aiming to provide references for improving artificial seed production success.

Materials and Methods

1.1 Experimental Materials The experiment was conducted in May 2016 at the Fish Culture Laboratory of Guangdong Ocean University. Hybrid grouper maternal stock (brown-marbled grouper) consisted of third-generation fish (11-

14 kg body weight) bred by our research team, while paternal stock (camouflage grouper, 5–7 kg) was provided by Hainan Junhong Industrial Co., Ltd. Hybrid grouper fertilized eggs were obtained through artificial induction and dry fertilization. Eggs were initially incubated in natural seawater (salinity 31) at gradually decreasing temperatures to 22 °C, then surface eggs were collected with 150-mesh silk netting and packaged. Double-layered nylon bags with pure oxygen were used for low-temperature transport to the laboratory at a density of 10 g/L. Upon arrival, water temperatures were equilibrated before transferring eggs to incubation tanks (70 cm × 50 cm × 60 cm) with gentle aeration. Microscopic examination confirmed embryos had reached mid-gastrula stage, at which point the experiment commenced.

1.2.1 Hatching Rate and Abnormality Rate Glucose (analytical grade, Guangdong Guanghua Sci-Tech Co., Ltd.) was added to hatching water at concentrations of 0 (control), 2, 4, 6, 8, and 10 mg/L. Fertilized eggs (200–250) were placed in 1,000 mL beakers containing 700 mL of the respective glucose solutions, with three replicates per group (incubation density 0.3 eggs/mL). Gentle continuous aeration maintained slight egg movement. To minimize temperature fluctuations, incubation was performed in a digital water bath (HH-6, Bangxi Instruments Technology Co., Ltd.). Water quality parameters were maintained at: temperature 27.5 ± 0.5 °C, salinity 29–31, dissolved oxygen 5.5–5.8 mg/L, light intensity 600–800 lx, and 12 h light:12 h dark photoperiod. Ammonia and nitrite were undetectable. Hatching progress was monitored continuously, and hatching time was recorded. At 6 h post-hatch, all newly hatched larvae and unhatched eggs were fixed in 3% formaldehyde prepared with seawater. Hatching rate was calculated as the percentage of hatched larvae relative to total fertilized eggs. Abnormality rate was determined based on spinal curvature and oil globule displacement or abnormal numbers. Statistical analysis revealed significant glucose concentration effects on both parameters ($P < 0.05$), with quadratic equations best describing these relationships. Regression analysis indicated the optimal glucose concentration for maximum hatch rate was 3.8 mg/L.

1.2.2 Larval Total Length and Lipid Metabolic Enzyme Activity Glucose concentrations of 0 (control), 2, 4, 6, 8, and 10 mg/L were established in incubation tanks (70 cm × 50 cm × 60 cm) for fertilized egg incubation and yolk-sac larval rearing. Initial egg density was 500 eggs/L with gentle continuous aeration. Water parameters were maintained at: temperature 28–29 °C, salinity 29–30, pH 8.1, dissolved oxygen >5 mg/L, light intensity 600–800 lx, and 12 h light:12 h dark photoperiod. Ammonia and nitrite remained undetectable. Water was replaced every 12 h with fresh glucose solution of the same concentration (one-third volume exchange). Three replicates were established per treatment. Developmental progress was monitored microscopically and timing was recorded. Samples were collected at 1, 2, and 3 days post-hatch (3-day-old larvae began feeding on *Brachionus* sp. at ~20 individuals/mL filtered through 200-mesh silk netting). For total length measurement, 10 larvae per group were

sampled with a rubber-tipped dropper and measured under a microscope with a micrometer. For enzyme analysis, 500 larvae per sampling point were collected, blotted dry with filter paper, placed in 1.5 mL centrifuge tubes, and stored at -80 °C.

Protein content was determined using the Coomassie brilliant blue method with a protein quantification kit (A045-2, Nanjing Jiancheng Bioengineering Institute). ACC and FAS activities were measured spectrophotometrically using enzyme-linked immunosorbent assay kits (H232 and H231, Nanjing Jiancheng Bioengineering Institute) following manufacturer protocols. ACC activity was defined as 1 unit = 1 mol inorganic phosphorus produced per hour per mg tissue protein. FAS activity was defined as 1 unit = 1 mol NADPH oxidized per minute per mg protein at 37 °C.

1.3 Statistical Analysis Data are presented as mean ± standard deviation ($\bar{x} \pm SD$). One-way ANOVA and Duncan' s multiple comparison tests were performed using SPSS 11.0 software. Significance was set at $P < 0.05$.

Results

2.1 Effects of Glucose on Fertilized Egg Hatch Rate and Newly Hatched Larval Abnormality Rate Hatch rate and abnormality rate of hybrid grouper under different glucose concentrations are shown in [Figure 1: see original paper] and [Figure 2: see original paper]. Hatch rate exhibited an "increase-decrease" pattern while abnormality rate showed a "decrease-increase" trend with rising glucose levels. The 4 mg/L glucose group showed a 6.17% higher hatch rate ($P > 0.05$) and 21.03% lower abnormality rate ($P < 0.05$) compared to the control. The 6 mg/L glucose group demonstrated a 12.18% higher hatch rate ($P < 0.05$) and 33.85% lower abnormality rate ($P < 0.05$) relative to the control. One-way ANOVA confirmed significant glucose concentration effects on both parameters ($P < 0.05$). Quadratic equations effectively described the relationships between glucose concentration and hatch rate or abnormality rate. Regression analysis determined the optimal glucose concentration for maximum hatch rate and minimum abnormality rate to be 3.8 mg/L.

2.2 Effects of Glucose on Total Length of Yolk-Sac Larvae Total length measurements of yolk-sac larvae under different glucose concentrations are presented in . At 1 day post-hatch, no significant difference was observed between the 2 mg/L glucose group and the control ($P > 0.05$), while all other glucose groups were significantly larger ($P < 0.05$). At 2 days post-hatch, all glucose-supplemented groups showed significantly greater total length than the control ($P < 0.05$). At 3 days post-hatch, the 8 and 10 mg/L groups did not differ significantly from the control ($P > 0.05$), while other glucose groups remained significantly larger ($P < 0.05$). When glucose concentration was below 6 mg/L,

total length increased with concentration across all ages; however, at concentrations exceeding 6 mg/L, total length decreased.

2.3 Effects of Glucose on ACC Activity in Yolk-Sac Larvae ACC activity changes during yolk-sac larval development under different glucose concentrations are shown in . At 1–3 days post-hatch, all glucose-supplemented groups exhibited significantly higher ACC activity than the control ($P < 0.05$). At 1 day post-hatch, ACC activity increased with glucose concentration, with no significant differences among the 6, 8, and 10 mg/L groups ($P > 0.05$) but significant differences among other groups ($P < 0.05$). At 2–3 days post-hatch, ACC activity showed an "increase-decrease" pattern, rising significantly when glucose concentration was below 6 mg/L ($P < 0.05$) but decreasing significantly at 8–10 mg/L compared to 6 mg/L ($P < 0.05$). The 6 mg/L glucose group showed ACC activity increases of 2.8-fold, 3.2-fold, and 2.9-fold at 1, 2, and 3 days post-hatch, respectively, compared to the control.

2.4 Effects of Glucose on FAS Activity in Yolk-Sac Larvae FAS activity changes during yolk-sac larval development under different glucose concentrations are presented in . All glucose-supplemented groups showed significantly higher FAS activity than the control ($P < 0.05$), with activity increasing with glucose concentration at 1–3 days post-hatch. At 1 day post-hatch, FAS activity increased with glucose concentration, with no significant difference between the 8 and 10 mg/L groups ($P > 0.05$) but significant differences among other groups ($P < 0.05$). At 2–3 days post-hatch, FAS activity exhibited a "rising-plateau" pattern, increasing significantly when glucose concentration was below 6 mg/L ($P < 0.05$) but showing no significant change at higher concentrations ($P > 0.05$). The 6 mg/L glucose group demonstrated FAS activity increases of 2.1-fold, 1.7-fold, and 1.5-fold at 1, 2, and 3 days post-hatch, respectively, relative to the control.

Discussion

3.1 Effects of Glucose on Early Development of Hybrid Grouper Glycogen reserves accumulated during oocyte development serve as important energy substrates and membrane components, playing crucial roles in fertilization, embryonic development, and energy metabolism in fish. Marine fish depend entirely on endogenous nutrients during embryonic and yolk-sac stages. Previous research indicates that fish embryos sequentially utilize carbohydrates, proteins, and lipids as energy sources during development. Although glucose is not the primary energy source, its direct absorption without decomposition makes it more efficiently utilized than other carbohydrates, contributing significantly to embryonic development. Studies by Li (2007), Xue (2008), and Xiong et al. (2014) demonstrated that glucose added to incubation media can enter embryos and exert effects. In this study, hatch rate increased gradually with glucose concentration up to 6 mg/L, with the 6 mg/L group showing significantly higher rates than the control. This may be attributed to two factors: first, the

vigorous shaking of embryos within the chorion during hatching requires substantial energy, and appropriate glucose concentrations provide supplementary energy to facilitate emergence; second, this may relate to the metabolic characteristics of early development and the presence of oil globules in eggs. Lipids remain the primary metabolic energy source during marine fish embryonic development, and neutral lipid synthesis occurs during early stages, possibly resulting from carbohydrate conversion. Appropriate glucose supplementation may promote lipid anabolism, increase lipid accumulation, and provide more energy for hatching. Additionally, absorbed glucose serves as a cellular component and precursor for non-essential amino acid synthesis, likely explaining the larger size of larvae in glucose-supplemented groups. While optimal glucose concentrations vary among species—15 g/L for gibel carp, 1.5% for zebrafish, and 2 mmol/L for tetraploid scallop—our results for hybrid grouper (3.8 mg/L) are substantially lower, reflecting species-specific responses. For some marine fish like gilthead seabream (*Sparus aurata*), adequate carbohydrate metabolism is essential for normal embryonic development, and insufficient carbohydrate metabolism can arrest development and reduce hatch rates. Further analysis of lipid metabolism characteristics during hybrid grouper embryonic and yolk-sac larval development is warranted.

High glucose concentrations (8 mg/L) reduced hatch rate and increased abnormality rate, indicating negative effects at elevated levels. Similar detrimental effects have been reported in zebrafish and gibel carp. Possible mechanisms include increased osmotic pressure disrupting physiological metabolism in early developmental stages with immature osmoregulatory mechanisms, and glucose-induced damage through reactive oxygen species generated via multiple metabolic pathways. Therefore, regression analysis suggests 3.8 mg/L glucose as the optimal concentration for maximum hatch rate in hybrid grouper artificial breeding.

3.2 Characteristics of ACC and FAS Activity Changes During Yolk-Sac Larval Development Lipid and fatty acid consumption and conversion in fish eggs directly affect larval survival. ACC and FAS are closely associated with lipid metabolism, and their activities influence lipid utilization and transformation. Jiang et al. (2014) reported increasing ACC and FAS activities during yolk-sac larval development in gibel carp, while Desrosiers et al. (2008) observed varying degrees of increase in substrate metabolic enzyme systems during early development of spotted wolffish (*Anarhichas minor*). In this study, ACC and FAS activities increased during 1–3 day yolk-sac larval development in hybrid grouper, likely because 1–2 day larvae rely on endogenous nutrition during rapid organ formation with high energy demands, while 3-day larvae begin feeding, further developing feeding and digestive organs and enhancing metabolic capacities. These findings align with Jiang et al. (2014) and Desrosiers et al. (2008), indicating that metabolic enzyme activities increase with embryonic and larval development and metabolic activity. Some studies suggest metabolic enzyme expression during early development correlates with egg quality—for example,

acid phosphatase and adenylate kinase serve as reliable egg quality parameters in gilthead seabream, and glucose-6-phosphate dehydrogenase activity can indicate egg quality in common dentex (*Dentex dentex*). Further research is needed to explore whether metabolic enzyme pathways can assess egg development and larval survival in hybrid grouper.

3.3 Effects of Glucose on ACC and FAS Activities During Yolk-Sac Larval Development As a key enzyme in de novo fatty acid synthesis, ACC catalyzes the first committed step and its activity directly affects lipid synthesis rates, playing an important role in lipid metabolism. Wang et al. (2015) reported that appropriate dietary glucose levels increased glucokinase activity in large yellow croaker, while studies on cobia juveniles and rainbow trout (*Oncorhynchus mykiss*) confirmed that suitable glucose supplementation promoted glucose-6-phosphate dehydrogenase activity. These findings indicate that dietary glucose increases lipid metabolism rates and enhances lipogenesis. Our study using glucose immersion during hybrid grouper yolk-sac larval development revealed significantly higher ACC and FAS activities in glucose-supplemented groups compared to the control, suggesting that exogenous glucose enters larvae, activates ACC and FAS, and accelerates fatty acid synthesis to support lipid deposition. This aligns with results from gibel carp studies.

Conclusion

1. Regression analysis determined that the optimal glucose concentration in hatching water for maximum fertilized egg hatch rate in hybrid grouper is 3.8 mg/L.
2. Immersion in 6 mg/L glucose solution significantly enhanced ACC and FAS activities during yolk-sac larval development in hybrid grouper.

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