

Nutrition and Temperature Regulate the Expression of Heart-Type Fatty Acid Binding Protein Gene in Golden Pompano Larvae (*Trachinotus ovata*, Linnaeus, 1758)

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ABSTRACT

This study examined the regulation of nutrition and temperature on the expression of heart-type Fatty acid binding protein (H-FABP) gene in golden pompano to understand importance of protein development in fish larvae. Firstly, H-FABPs expression during ontogeny of larvae in the first 18 days after hatching was tested, and then the responses of H-FABPs to temperature (23, 26 and 29°C) on 12-days post hatching (DPH) and 18 DPH were compared. On 18 DPH, the response of H-FABPs to the manipulation of nutrients was evaluated. The expression of H-FABPs increased after transition to endogenous feeding, and was significantly affected by temperature. The expression of H-FABP at 23 and 26°C was higher than at 29°C on 12 DPH, and the expression of H-FABP at 29°C was higher than at other temperatures on 18 DPH, suggesting that H-FABP expression is determined by both temperature and fish age. The expression of H-FABP on 18 DPH was highest when Algamac 3080 enriched *Artemia* nauplii were fed to fish and lowest when fish were fed with *Nannochloropsis* sp. enriched *Artemia* nauplii. The correlation analysis between the expression of H-FABP and fatty acids shows that the ratio of fatty acids is more important than the relative quantity of single fatty acid in regulating the expression of H-FABP at the early stage of fish larvae. This study indicates that changes in environmental temperature and feed type influence H-FABP expression, growth and survival of golden pompano in early life. FABPs subfamilies play various roles in fatty acid metabolism [13]. The heart-type FABPs (H-FABPs) subfamily contains the muscle and heart FABPs that have a high binding affinity for C16-C20 fatty acids [14]. Despite wide distribution in the heart, skeletal and smooth muscle, mammary epithelial cells, aorta, distal tubules of the kidney, lung, brain, placenta and ovary, H-FABP is mainly expressed in the brain and heart with a predominant expression in muscle cells [15]. H-FABPs in mammals have a role to play with the intramuscular fat accretion [16]. However, in previous studies on shark, catfish and lamprey, FABPs in the liver are clearly related to H-FABPs but not to liver-FABPs (L-FABPs) [17- 19]. H-FABPs are mainly expressed in the liver of aquatic organisms and play a role in fatty acid mitochondrial β -oxidation [12].

Materials and Methods : These fish specimens were obtained from a previous feeding trial [33]. In brief, fertilized eggs of golden pompano were obtained from Lingshui, Hainan Province, and transported to the Tropical Fisheries Research and Development Center, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Science, Xincun Town. Eggs hatched in 500-L fiberglass incubators at 26.5°C with a hatching rate of $97.5 \pm 1.5\%$ (mean \pm SD). On 2 DPH, larvae were stocked into three 1000 L larval rearing tanks. Larval rearing tanks were supplied with filtered seawater (5 μ m pore size) from the bottom of each tank through upwelling with a daily exchange rate of 200% tank volume. Two air stones were used in each tank to maintain dissolved oxygen close to saturation. Light intensity was maintained at 2400 lux and the light regime was controlled at 14 h light and 10 h dark. The salinity was maintained at $33 \pm 0.8\%$ and water temperature was $26.5 \pm 1.0^\circ\text{C}$ throughout the experiment. Rotifers (*Brachionus rotundiformis*) were fed to fish larvae from 2 DPH to 10 DPH at a density of 10-20 ind/mL. *Artemia* nauplii were added into the rearing tank from 10 DPH until the experiment was completed. Both rotifers and *Artemia* nauplii were enriched with docosahexaenoic acid (DHA) Protein Selco (INVE Aquaculture, Salt Lake City, USA) according to the manufacturer's instruction. On 0, 1, 2, 3, 4, 5, 12 and 18 DPH, approximately 300 mg (wet weight) fish larvae were sampled from each rearing tank in triplicate to study the ontogenetic expression of the H-FABP. On 18 DPH, a total of 100 individuals were collected in triplicate and examined under a dissecting microscope for tissue expression analysis. Response of H-FABP gene to rearing temperature. Fertilized eggs of the same batch were obtained from Lingshui, Hainan Province. Upon arrival, all eggs were transferred into 500 L incubators and hatched at 26.5°C. The experimental design included three constant temperatures 23, 26 and 29°C with three replicates each. In this study, 26°C was used as the control temperature. On 2 DPH, yolk-sac larvae were acclimatized at each desired temperature for 5 h and then stocked in 500 L fiberglass tanks at a density of 60 fish L⁻¹. Except for the rearing temperature, all the feeding protocols and rearing conditions were the same as described in the previous section. Approximately 50 individuals were collected in triplicate on 12 DPH and 18 DPH to analyze the response of H-FABP gene to rearing temperature.

Keywords: FABP; Nutrition; Temperature; Gene expression; Fish larvae