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Feeding effect of selenium enriched rotifers on larval growth and development in red sea bream *Pagrus major*

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ABSTRACT

Feeding trials were conducted to investigate the effect of selenium (Se)-enriched rotifers on growth and development of red sea bream *Pagrus major* larvae. Fish were reared from fertilized eggs (98% hatch rate) to 20 days post hatch (dph) at 19 °C with two different food sources; non-enriched S-type rotifers (0.0 μg Se/g D.W., control diet) or Se-enriched rotifers (2.2 μg Se/g D.W., Se-enriched diet) at 10 rotifers/mL, respectively. On the last day of larviculture, the Se-enriched diet accelerated growth and developmental stage of fish larvae. The larvae fed Se-enriched rotifers were advanced in the following parameters compared to those fed control diet: total length (6.06 vs 5.53 mm), standard length (5.74 vs 5.26 mm), head length (1.46 vs 1.28 mm), eye diameter (0.57 vs 0.50 mm), the number of caudal fin rays (5.8 vs 1.9), and the proportion of individuals undergoing notochord flexion (55 vs 3%). Fish larvae of 20 dph showed higher Se concentration (9.5 ± 0.2 μg/g DW) with the Se-enriched diet than with the control diet (1.3 ± 0.3 μg/g DW), but there were no significant differences in the composition of polyunsaturated fatty acids which significantly affect larval growth and development. Therefore, the feeding of Se enriched rotifers enhanced growth and development of the red sea bream *P. major* larvae.

1. Introduction

The rotifer is widely used as an initial food source for marine fish larvae with small mouth size in aquaculture, but in the wild copepods are main food source for larvae. The nutrient profiles of rotifer and copepods had been analyzed, and it was found that the rotifer showed considerably lower level of minerals than copepods (Hamre et al., 2008a), also than fish requirements (NRC, 1993). Among deficient minerals, selenium (Se) concentration of rotifers (0.08–0.09 mg/kg dry weight, DW) is about 30-fold lower than the level of copepod (2–5 mg/kg DW) and 3 to 8-fold lower than the Se requirements for juvenile fish (Hamre et al., 2008b; Penglase et al., 2011; Ribeiro et al., 2011). Se is the component of the enzyme glutathione peroxidase which has the function of protecting cells from oxidative damage (Rotruck et al., 1973) and is an essential trace element for health of vertebrates including fishes (Doucha et al., 2009). Although Se is the most deficient mineral of rotifers (Penglase et al., 2010), it can be enriched up to copepod levels by fortification of the diet (Bell and Cowey, 1989; Penglase et al., 2011). It has been confirmed that Se-enriched rotifer *Brachionus* sp. by feeding of Se-fortified *Chlorella vulgaris* showed active reproduction such as higher population growth rate and resting egg production (Kim et al., 2014).

Se supplementation of artificial diets is known to enhance growth and development of rainbow trout *Oncorhynchus mykiss* (Hilton et al., 1980) and grouper *Epinephelus malabaricus* (Lin and Shiau, 2005). Selenomethionine (organic Se) is a natural food source of selenium and has higher bioavailability than the sodium selenite (inorganic Se) for Atlantic salmon *Salmo salar* (Lorentzen et al., 1994) and channel catfish *Ictalurus punctatus* (Wang and Lovell, 1997). In addition, it was reported that simultaneous supplementation of Se and I affected the larval fatty acid compositions which are significantly related to growth and development of Atlantic cod *Gadus morhua* larvae (Hamre et al., 2008a). To investigate the effects of supplemented Se associated with fatty acid composition, the present study used rotifers fed with Se-fortified *Chlorella* diet as feed for fish larvae. The red sea bream *Pagrus major* was chosen as experimental organism for Se-enriched rotifers since it is a major finfish species cultured in Japan and effects of fatty acid on growth, survival and viability of larvae were reported (Izquierdo et al., 1989). The final goal of this study was to investigate effects of Se on larval growth and development to promote more effective larviculture.

2. Materials and methods

2.1. Rotifer preparation

We employed the euryhaline rotifer *Brachionus rotundiformis* (S-type) as larval feed. Rotifers were cultured with the following

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two-types of HUFA enriched *C. vulgaris* (Super Fresh Chlorella-V12, Chlorella Industry Co. Ltd., Fukuoka, Japan): 1) non-fortified *Chlorella* (0.0 μg Se/g DW), and 2) selenium (Se)-fortified *Chlorella* (3.2 μg Se/g DW) by adding sodium selenite (Na_2SeO_3) into the phytoplankton culture medium. Each feeding regime was applied to 30–40 L of batch cultures at 17 ppt (artificial sea water) and 25 °C with aeration. The daily amount of *Chlorella* for rotifers was adjusted as 40.5 g DW/10^8 rotifers. On the last day of fish larviculture, remaining rotifers in each tank were sampled by plankton net (45-μm mesh size), rinsed with Milli-Q water (Millipore 0.22 μm) to remove salt, dried from beneath the net using filter paper and were transferred into brown glass screw-capped bottles (20 mL) for chemical analysis. Sampled rotifers were stored at −80 °C until chemical analyses.

2.2. Larviculture

Fertilized fish eggs of red sea bream *P. major* were obtained from a local fish farmer in this study. Eggs were transferred into 100-L polycarbonate tanks at 10 eggs/L following the procedure of Ruttanapornvareesakul et al., 2010. In each feeding regime, four polycarbonate tanks containing 100 L of 34-ppt artificial sea water with each type of *Chlorella* (non- or Se-fortified one) at 5×10^5 cells/mL, were prepared with aeration at a rate of 50 mL/min. Fish were reared at 19 °C with 12-h diurnal photoperiod (900–2100) for 20 days. Larvae were fed on two-type rotifers: rotifers fed on non-fortified *Chlorella* (control diet) or those fed on Se-fortified *Chlorella* (enriched diet), at 10 ind/mL from 2 days post hatch (dph) at mouth opening. Every 5 days (1, 5, 10, 15 and 20 dph), 10 fish were randomly sampled from each tank and were anesthetized with MS 222 followed by 5% formalin fixation. Total and standard lengths were measured for all sampled larvae using a microscopic measurement system including stereomicroscope (Discovery V8, Zeiss, Germany) equipped with a digital camera (AxioCam, HSm) and an image-analysis software (AxioVision 4.8). Additional measurements such as body depth, head length, eye diameter, notochord flexion and the number of caudal fin rays (Fig. 1) were made on 20-dph samples. On the last day of larviculture (20 dph), the viability and survival rate were estimated. The viability of fish larvae was conducted with air exposure test; the rate of surviving individuals after 10 min from 5-s air exposure. The survival rate of larvae was calculated from the average number of surviving larvae in four aquaria and these larvae were collected by the same method as rotifer preparation for chemical analyses. To evaluate the quality of employed fish eggs and hatched larvae, hatching rate and survival activity index (SAI, Shimma and Tsujigado, 1981) of hatched larvae were calculated. We placed 30 fertilized eggs in a 500-mL beaker containing 500-mL same saline water as the larviculture at 19 °C in total darkness without aeration. Dead larvae were counted and removed every 24 h until total larval mortality to estimate survival and resistance to starvation. Triplicate observation was used to calculate SAI using the following equation:

$$SAI = \frac{1}{N}\sum_{i=1}^{K}(N - hi) \times i$$

where N is the total number of examined larvae, h*i* is the cumulated mortality by *i*-th day, and K is the number of days elapsed until all larvae died due to starvation.

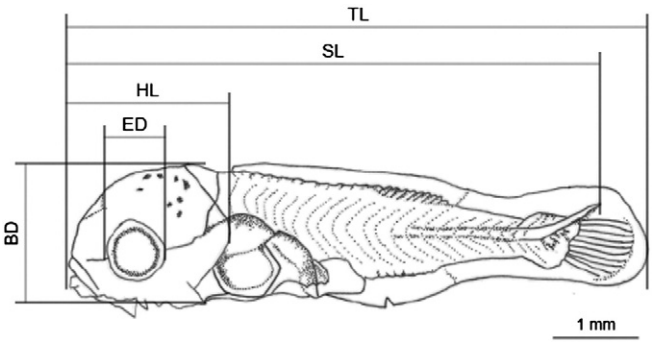

Fig. 1. Five morphological characteristics to estimate larval growth and development. Abbreviations are defined as follows: TL, total length; SL, standard length; HL, head length; ED, eye diameter; BD, body depth.

2.3. Selenium and fatty acid analysis

Se and lipid compositions of cultured rotifers and fish larvae were performed by Chlorella Industry Co., Fukuoka, Japan. To analyze Se concentration, four freeze-dried samples (each 100 mg of rotifers or 20 mg of fish larvae) were digested with 60% HNO_3 (0.5 mL for rotifers or 1 mL for fish larvae) at 190 W for 4 min using microwave oven followed by one-minute cooling (Homma-Takeda et al., 2013). This procedure was repeated six times. The digested samples were diluted by ultrapure water and were analyzed for Se by Agilent technologies 7700x series ICP-MS system (Agilent Technologies, Tokyo, Japan) with 0.05 (for rotifers) or 0.125 (fish larvae) μg/g of detection limit.

Total lipid and fatty acid composition were analyzed after the extraction following Folch et al. (1957). The sample methanolysates were prepared at 100 °C for 2 h after the addition of 2 M hydrogen chloride methanol. Fatty acid methyl esters (FAME) were extracted by n-hexane. Gas chromatography analysis was performed using a GC-2010 (Shimadzu Scientific Instruments, Inc.) equipped with a HR-SS-10 column (Shinwa Chemical Industries, Ltd.). The column temperature was regulated at 150 to 220 °C. Individual fatty acids were quantified by means of the response factor to 15:0 fatty acid as the internal standard.

2.4. Statistical analysis

The effect of Se enrichment on larval growth, development, and fatty acid composition was analyzed by *t*-test. Tukey–Kramer *post hoc* test was performed after repeated measures ANOVA to test dietary effect on the growth of fish larvae associated with age. All of the statistical analysis was carried out using StatView version 5.0 software (SAS Institute, Inc., USA).

3. Results

3.1. Nutritional level of rotifers

Se enriched rotifers contained 2.2 μg/g DW of Se, whereas Se was not detected in non-fortified *C. vulgaris*. The fatty acid composition of rotifers from the two dietary regimes was similar (Table 3), except for 22:1 (*t*-test, $P = 0.0152$) and the sum of unknown fatty acid ($P = 0.0368$).

3.2. Larviculture

Red sea bream eggs showed 98.9 ± 1.9% of hatching rate and hatched larvae from these eggs survived 9 days of starvation. Calculated survival activity index (SAI) of employed larvae was 13.9 ± 0.5. After 20 days of rearing, the fish larvae showed no significant differences in survival rate (87.7 ± 7.8–93.2 ± 7.0%) or viability (70.2 ± 19.4–71.6 ± 20.1%) between two different diet regimes; non (control)- or Se-enriched diet (Table 1). There was no significant difference in dry weight (0.15 ± 0.05–0.18 ± 0.05 mg DW/ind., Table 1). Total length and standard length of collected larvae were not significantly different until 15 dph (Fig. 2), but on 20 dph, these parameters and developmental stage (notochord flexion, Fig. 3) were more advanced with Se