n-3高度不飽和脂肪酸強化クロレラChlorella vulgarisで培養したシオミズツボワムシによるマダイPagrus major仔魚の飼育

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Short Paper

Rotifers Fed with n-3 Highly Unsaturated Fatty Acid-enriched *Chlorella vulgaris* are Suitable for the Rearing of Larval Red Sea Bream *Pagrus major*

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Abstract: Rearing of larval red sea bream *Pagrus major* was performed by feeding with rotifers cultured in *Chlorella vulgaris* K-22 enriched with n-3HUFAs (mainly DHA). Results for larval growth, survival rate, activity, and air-bladder opening rate suggested the successful rearing of the red sea bream. The total content of n-3HUFAs in the larvae at the end of the rearing was even higher than the level immediately after hatching. It can therefore be concluded that the enhanced n-3HUFAs in *C. vulgaris* were effective via rotifer for the rearing of red sea bream larvae. The n-3HUFA-enriched *C. vulgaris* makes it possible to realize the rearing of red sea bream without a secondary rotifer-culture process for enhancement of n-3HUFAs.

Key words: *Chlorella vulgaris*; Red sea bream; Rotifer; n-3 highly unsaturated fatty acid

Chlorella vulgaris fortified with vitamin B_{12} has been widely used as a food for rotifer production because of the high dietary value for rotifer growth (Maruyama et al. 1997). However, rotifers fed vitamin B_{12}-fortified *Chlorella* cells did not contain a sufficient amount of essential fatty acids for the growth of marine fish because *Chlorella* cells do not contain n-3HUFAs (Maruyama et al. 1988). We recently achieved the uptake of exogenous n-3HUFAs (mainly docosahexaenoic acid (DHA, C22 : 6 n-3)) by the *Chlorella* cells, using hydrolysate of fish oil (Hayashi et al. 2001). Moreover, we have reported that DHA-enriched *C. vulgaris* is suitable for rotifer production, and that the produced rotifers stably contained a sufficient level of DHA (Yukino et al. 2004). These results suggest that rotifers produced with DHA-enriched *C. vulgaris* have a high nutritive value for marine fish. Generally, a secondary culture of rotifers has been performed for the enhancement of n-3HUFAs (mainly DHA), which is an essential nutrient of marine fish larvae (Watanabe et al. 1989; Takeuchi et al. 1990), because usual rotifer food such as *Chlorella*, *Nannnochloropsis*, and baker's yeast do not contain DHA (Maruyama et al. 1988). We show in this report that n-3HUFA-enriched and vitamin B_{12}-fortified *C. vulgaris* make it possible to realize successful sea bream rearing without the conventionally required secondary rotifer-culture process. This means that the rotifer-culture process will become simple and that the labor required for rotifer culture can be reduced.

The condensed cell suspension of the n-3HUFA-enriched and vitamin B_{12}-fortified *C. vulgaris*, brand name of Super Fresh Chlorella V-12 (SFCV-12), was used in this study. The cell density of SFCV-12 was 135 g dry weight/*l*. Contents of n-3HUFAs in the Chlorella cells of SFCV-12 were DHA (C22 : 6 n-3) as the major component, and docosapentaenoic acid (DPA, C22 : 5 n-3) and eicosapentaenoic acid (EPA, C20 : 5 n-3) as minor components; these fatty acids are not contained in *Chlorella* cells naturally (Table 1). Rotifer culture and rearing of larval red sea bream were conducted in the Fisheries Laboratory of Kinki University. *Brachionus rotundiformis*, so-called S-type rotifers (Segers 1995), was cultured with SFCV-12 in a 200 *l* tank at 27℃. The density of the rotifer was 300－800 individuals/*ml* throughout the culture period. SFCV-12 was given twice per day, 200 *ml* per tank at each feeding. Rotifers were given to larval fish after being filtered and washed with seawater. For the analysis of total lipid and fatty acid composition, rotifers were filtered, washed with running tap water and kept at －20℃ until use. The rearing of larval red sea bream was conducted for 20 days after hatching. Two replicate 0.5 *kl* water tanks containing 7,400 eggs each were used. Rotifers were given to each tank at the density of 5－10 individuals/*ml* twice per day beginning 2 days after hatching. The aeration rate was approximately 300 *ml*/min. Sea water was supplied to the culture tanks at the rate of 500 *l*/day beginning 5 days after hatching. The water temperature was 21.2－22.8℃. Total lengths of 30 individual fish were measured at the end of rearing. The survival rate was calculated by the numbers of eggs at the start and surviving fish at

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the end of rearing. The activity of larval fish at the end of rearing was estimated by the survival rate of 30 fish in 24 hours after exposure to air for five seconds. Fish within 24 hours after hatching and fish remaining at the end were stored in a freezer at −20℃, and analyzed for total lipid and fatty acid compositions by a previously described procedure (Yukino et al. 2004).

Table 2 shows the results of total length, survival rate, activity test, and air-bladder opening rate of larval fish at the end of rearing. Growth, survival rate and activity of larval fish, which were reported to decrease because of the insufficiency of n-3HUFA content of rotifers reached a satisfactory level compared with other experiments (Izquierdo et al. 1989; Watanabe et al. 1989). The air-bladder opening rate, examined as a health index of larval fish, was also relatively high. These results suggest the successful rearing of red sea bream without the insufficiency of n-3HUFA content of rotifers. As shown in Table 3, the major fatty acids of rotifers produced with *C. vulgaris* were linolenic acid (C18 : 2), palmitic acid (C16 : 0), linolenic acid (C18 : 3n-3), and DHA. Minor components of DPA and EPA were also contained as n-3HUFAs. The fatty acid composition and n-3HUFA level of rotifers were similar to those of the *C. vulgaris* fed on the rotifers (Tables 1, 3). The total n-3-HUFAs of the larvae at the end of the rearing that were approximately 40% in total fatty acids, even higher than the level immediately after hatching (Table 3). It can therefore be concluded that the enriched n-3HUFAs in *C. vulgaris* were effectively transferred to the larvae of red sea bream via the rotifer. In this experiment, the total n-3HUFA content of the rotifers fed to the larvae was approximately 2.4% in dry bases estimated by lipid content and fatty acid composition. That content was lower than 3.5%, which is the level reportedly required in the rotifer-feeding period for red sea bream (Izquierdo et al. 1989). Nevertheless, a highly positive effect was achieved, presumably because of the predominant use of DHA, which has an excellent feeding value for fish larva (Watanabe et al. 1989; Takeuchi et al. 1990). One future research theme is to explore the possible improvement of the activity, growth and survival rate of fish larvae by increasing the level of n-3HUFA enrichment of *C. vulgaris*.

Table 1. Lipid analysis of *Chlorella vugaris*

	HUFA-enrichment[1]	Without enrichment
Lipid (% in dry weight)	17.8 ± 1.3	14.3
Fatty acid (% in total fatty acids)		
C16	13.6 ± 0.7	18.6
C16:1	1.5 ± 0.1	2.9
C16:2	8.1 ± 0.3	10.4
C18	7.3 ± 1.5	11.0
C18:1	4.0 ± 2.6	3.4
C18:2	17.8 ± 0.1	25.9
C18:3 (n-3)	11.8 ± 1.8	15.5
C20:5 (n-3)	1.1 ± 0.2	0
C22:5 (n-3)	5.1 ± 0.6	0
C22:6 (n-3)	14.8 ± 2.0	0
Total n-3HUFA	21.0 ± 2.5	0

[1] $n = 3$, Mean ± SD.

Table 2. Total length, survival rate, vitality test, and air-bladder opening rate of larval red sea bream at the end of rearing

Total length (mm)		Survival rate (%)	Activity[1] (%)	Air-bladder opening rate (%)
Tank 1	7.59 ± 0.40	75.4	70.0	87.5
Tank 2	7.53 ± 0.31	83.3	66.6	96.7

[1] Survival rate in 24 hours after exposure to air for five seconds.

Table 3. Lipid analysis of rotifer and larval red sea bream

	Rotifer[1]	Larval red sea bream		
		Start of rearing	End of rearing	
			Tank 1	Tank 2
Lipid (% in dry weight)	13.8 ± 0.4	ne[2]	18.4	17.6
Fatty acid (% in total fatty acids)				
C16	16.4 ± 1.2	15.7	26.8	28.5
C16:1	1.0 ± 0.7	3.3	nd[3]	nd[3]
C16:2	4.7 ± 2.8	0.4	0.9	nd[3]
C18	2.0 ± 0.9	2.4	7.7	8.3
C18:1	4.9 ± 4.4	16.1	1.5	1.5
C18:2	23.9 ± 5.5	14.6	12.6	12.7
C18:3 (n-3)	10.8 ± 3.7	1.3	1.7	1.8
C20:5 (n-3)	2.4 ± 0.3	7.8	5.2	4.8
C22:5 (n-3)	5.6 ± 1.5	2.4	8.2	7.6
C22:6 (n-3)	9.7 ± 0.7	18.2	26.0	28.2
Total n-3HUFA	17.7 ± 2.0	28.4	39.4	40.6

[1] $n = 3$, Mean ± SD.
[2] No data.
[3] Not detected.

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