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Enrichment of rotifer *Brachionus rotundiformis* with calcium

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Abstract
Possibility of enrichment of rotifer *(Brachionus rotondiformis)* with calcium (Ca) for feeding the fish fry was investigated. Rotifer was kept for 24 h with aeration in normal seawater (Treatment 1), seawater with 400 mg/l supplemental Ca from Ca-lactate (Treatment 2) and seawater with 400 mg/l supplemental Ca from Ca-chloride (Treatment 3). After the experimental period, Ca contents of rotifer were 0.20, 0.29 and 0.39% of dry weight in T-1, T-2 and T-3, respectively. Ca content of media did not affect phosphorus, zinc and manganese contents of rotifer. Results revealed that rotifer can be enriched with Ca for feeding -fish fry and Ca-chloride might be a better source for Ca enrichment.

Key words : Rotifer, Calcium, enrichment

Introduction

Rotifer, *Brachionus spp.*, have a number of features that make them useful for aquaculture, including rapid reproduction, slow swimming speed, high nutritional quality and easy culture method. The biological composition of rotifer closely reflects what they eat. Therefore, rotifer can be fed specific nutrients and antibiotics that in turn can be transferred to larval fish. Gastesoupe (1982) observed that in fish hatcheries rotifers are considered living food that transmit macro and micronutrients, vitamins and even antibodies to fish larvae. Recently, rearing procedures for various kinds of fish and method for the mass production of live feeds have been markedly progressed (Stottrup and Attramdal 1992). As a consequence, the number of cultured fish species is increasing year by year. Among zooplanktons, the rotifers have been extensively used for the feeding of larva of marine and freshwater fishes. Larval rearing of marine fishes would be virtually impossible without the mass culture of rotifers (Watanabe *et al.* 1983a). It is a widely accepted opinion in Japan that the number of fish and crustacean larvae produced is limited by the amount of rotifers available (Hirata 1979, Hirata *et al.* 1983). However, the nutritional values of live feeds have not been widely reported.

Watanabe *et al.* (1978) reported the proximate and mineral composition of rotifers, where culture media such as backer's yeast and freshwater and marine chlorella greatly affected the proximate but not the mineral composition. The digestibility of protein in rotifers was high as 89-94%, irrespective of culture organism used. They observed it important to 'enrich' or 'pack' rotifers with essential fats, vitamins and other nutrients just before feeding to larval fish.

The nutritional quality of rotifers as a live food for fish larvae has been investigated mainly from the viewpoints of essential fatty acid (EFA) required for fish (Watanabe *et al.* 1983b,c). The fatty acid composition of rotifer is readily affected by the fatty acid composition of the organisms in culture media. So, direct and indirect methods have been developed for enrichment of rotifers with n3-HUFA for feeding fish larvae (Yoshimura *et al.* 2003).

A high incidence of lordosis (bone deformity) has been reported in several cultured marine fishes and many authors have suggested possible counter measures (Kitajima *et al.* 1977 & 1994, Isheda *et al.* 1982, Hayashida *et al* 1984), which mainly considered the shortage of EFA as the main cause for lordosis. Inadequacy of Ca as a possible cause for lordosis is yet to be investigated. There is a general consideration that as seawater contains adequate Ca, which is available to marine fish through absorption, marine fish may not need a dietary Ca supplement (Love 1980). However, dietary Ca supplement has been found necessary for some marine fishes (Hossain and Furuichi 1998, 2000a,b). At the growing stage of fish, Ca is needed for building the hard tissues and for other physiological functions. Lordosis in fish may be due to shortage of dietary Ca, as it is a major component of bone. Therefore, the supplement of an increased level of Ca to larval diets might fulfill the Ca requirement of larval fish and might prevent lordosis. Rotifer is the widely used live food for larval stage of fish and could be an important source of feeding Ca to fish larvae. In order to feed Ca to larval fish through rotifer, it is necessary to enrich rotifer with Ca. However, reports on the enrichment of rotifer with Ca and on the feeding of Ca-enriched rotifers to fish larvae are scarcely available. In the present study, the possibility of enrichment of rotifers with Ca has been investigated.

Materials and methods

The experiment was conducted in 9 round, polycarbonate tanks of 50L size. Each tank was filled with 40L sand-filtered seawater. There were 3 treatments each with 3 replications. Treatment 1 was without supplemental calcium (Ca), treatment 2 was with 400 mg/l Ca supplement from Ca-lactate and treatment 3 was with 400 mg/l Ca supplement from Ca-chloride. Necessary amount of chemicals was measured and dissolved in the tank water to obtain required level of supplemental Ca. Tanks were monitored every 4 hours to observe salinity, dissolved oxygen, pH, ammonia, rotifer density and Ca-content. Marine rotifer *(B. rotundiformis)* were cultured in a 500-l tank following the method of Yoshimura *et al.* (1996). Rotifer were cultured at around 20,000 individuals/ml by feeding them exclusively on condensed marine chlorella *(Nannochloropsis sp.)* Culture media were supplied with constant oxygen, pH was adjusted to 7 by addition of hydrochloric acid to avoid an increase of free ammonia in the culture system. The excess suspended organic matter was removed by a nylon filtration mat. Rotifer cultured in the 500-l tank were harvested with a plankton net and distributed to the experimental tanks to obtain a concentration of 5000 rotifer/ml seawater. Tanks were provided with aeration. After a 24 h experimental period, rotifers were harvested and three samples (each sample contained approximately 10 g of wet rotifers) from each tank were collected for Ca determination. Samples were washed several times with distilled water and dried at 105^{0}C for 12 h. Dried rotifer samples were digested with wet digestion method with a nitric acid-perchloric acid mixture. Ca, phosphorus (P), zinc (Zn) and manganese (Mn) in the digested sample (insert Ref for analytical methods followed (Hossain and Furuichi 2000a)) were determined with an Atomic Absorption Spectrophotometer (Perkin-Elmer 3300, Perkin Elmer, USA). Data were analyzed for significant difference with Fisher PLSD (full Protected Least Significant Difference) test.

Results

The average salinity, DO, pH, rotifer density, ammonia and Ca-content of the culture media are shown in Table 1. The average values of salinity, dissolved oxygen, pH, rotifer density, ammonia, were similar in all the treatments. The ranges of all those water quality parameters were optimum for rotifer culture (Yoshimura *et al.* 1996). Ca contents of culture media of rotifer were 396 mg/l, 780 mg/l and 770 mg/l in treatment-1, treatment-2 and treatment-3, respectively. High calcium contents in treatment 2 and treatment 3 were due to the addition of Ca-lactate and Ca-chloride, respectively. Ca content of seawater was 396 mg/l. The Ca contents of rotifer after the experimental period with different treatments are presented in Fig. 1. The Ca content of rotifer was 0.23% of dry matter in the control group (Treatment 1). Calcium content of rotifer increased when reared in Ca supplemented seawater for 24 hours, which were 0.33% in Treatment 2 and 0.42% in Treatment 3. The P, Zn and Mn contents of rotifers are shown in Fig. 2. Concentrations of these minerals in rotifers were independent of Ca supplement to the rearing media.

Table 1. Water quality parameters of different culture media of rotifer *(Brachionus plicatilis)*

Parameters	Treatments*		
	T-1	T-2	T-3
Salinity (ppt)	35.4±0.3	34.5±0.5	36.3±34.9
DO (mg/l)	5.6±0.4	5.8±0.2	5.6±0.3
pH	7.8±0.2	7.8±0.3	8.0±0.4
Rotifer density (ind./ml)	5000±50	4900±60	5100±65
Ammonia (mg/l)	45.4±3.2	33.3±2.5	37.4±7.0
Ca contents (mg/l)	396±32	780±34	770±20

* T-1, Control; T-2, Ca-lactate; T-3, Ca-chloride.

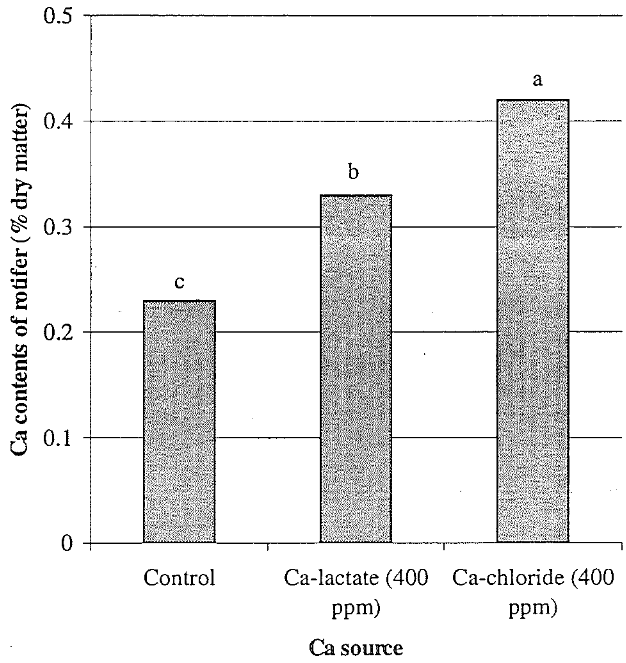

Fig. 1. Ca contents of rotifer after 24 hours enrichment with Ca. Analyses were made of three samples from each of three replicates (N=9 samples per treatment). Different letters indicate significant differences among each other ($p<0.05$, Fisher PLSD test).

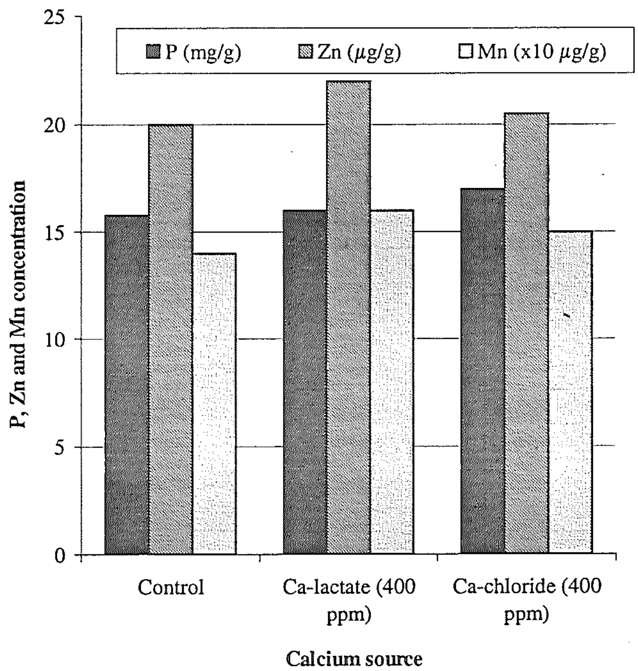

Fig. 2. P, Zn and Mn contents of rotifer after 24 hours enrichment with Ca. Analysis was made of three samples from each three replicate groups (N=9 samples per treatment). No significant difference for a mineral within 3 treatments ($p>0.01$, Fisher PLSD test).

Discussion

The Ca content of rotifer was 0.23% of dry matter in the control group (Treatment 1). The Ca content of rotifers in a dry matter basis was 0.12-0.22%, 0.09-0.24%, and 0.08-0.17% when cultured on backer's yeast, backer's yeast and marine chlorella, and marine chlorella, respectively (Watanabe *et al.* 1978). Calcium content of rotifer increased when reared in Ca supplemented seawater for 24 hours, which were 0.33% in treatment 2 and 0.42% in treatment 3 (Fig. 1). Although dietary Ca sometimes affect the P, Zn and Mn contents in fish (Hossain and Furuichi 2000a), Ca in media did not affect the contents of these minerals in rotifers in the present study (Fig 2). It indicated that rotifer can be enriched with Ca just before feeding to larval fish to fulfill the dietary Ca requirement in the larval stage of fishes without affecting other important minerals in rotifer. Reports on the enrichment of rotifer with Ca are scarcely available. However, it has been reported that rotifer can take HUFA and vitamins directly from medium. Rotifer are harvested, concentrated and then soaked in HUFA rich emulsion for 2-3 hours, thus becoming enriched with all the HUFAs required by larval fish (Watanabe *et al.* 1983c). Marine fishes need Ca for growth and bone mineralization. In the initial stage of growth, the process of bone mineralization is very rapid, hence the requirement of Ca may be even high. Generally, marine fish absorb calcium from seawater. However, Ca absorption from seawater is not sufficient for some of the marine fishes and they require dietary Ca supplementation (Hossain and Furuichi 1998, 2000a,b). Besides the investigations that considered the shortage of n3 HUFA is the possible cause of lordosis (Kitajima *et al.* 1977 & 1994, Isheda *et al.* 1982, Hayashida *et al.* 1984), the lordosis problem in larval marine fishes may be due to the shortage of Ca, because the major fraction of the body Ca (99%) are present in skeleton in the form of ca-hydroxyapatite. From the present study, it is evident that rotifer can be enriched with Ca. As rotifer is the major food of larval fishes, the enrichment of rotifer with Ca before feeding fish larvae may be effective when dietary Ca supplementation become necessary. Calcium contents of rotifer was higher in Treatment 3 than in Treatment 2, where Ca contents of the media were the same but sources were Ca-chloride and Ca-lactate, respectively. It is evident from the present study that rotifers may be enriched with Ca addition to culture media, and that Ca-chloride seems better as a source. Therefore, enrichment of rotifer with Ca may be an appropriate way to supply Ca to larval fish. Further studies are necessary to find out the effects of feeding Ca-enriched rotifers to fish larvae.

References

Gastesoupe, F.J., 1982. Nutritional and anti-bacterial treatments of live food organisms: the influences of survival, growth rate and weaning success of turbot *(Scophthalmus maximus) Ann. Zootech.* 31: 353-368.

Hayashida, G., Y. Tsukashima, K. Matsukiyo and C. Kitajima, 1984. Relationship between swim bladder uniflation and lodotic deformity in hatchery-reared Japanese sea bass *Lateolabrax japonicus*. *Bull. Nagasaki Pref. Inst. Fish.*, 10: 35-40.

Hirata, H., 1979. Rotifer Culture in Japan. In: Cultivation of Fish Fry and its Live Food. E. Styczynska-Jurewicz, T. Bachiel, E. Jaspers and G. Personne (eds.) European Mariculture Society. Special Publication. 4: 361-375.

Hirata, H. S. Yamasaki, T. Kawaguchi and M. Ogawa, 1983. Continuous culture of rotifer *Brachionus plicatilis* feed recycled algal diets. *Hydrobiologia,* 104: 71-75.

Hossain, M.A. and M. Furuichi, 1998. Availability of environmental and dietary calcium in tiger puffer. *Aquaculture Int.,* 6: 121-132.

Hossain, M.A. and M. Furuichi, 2000a. Essentiality of dietary calcium supplement in fingerling scorpion fish *(Sebastiscus marmoratus). Aquaculture,* 189: 155-163.

Hossain, M.A. and M. Furuichi, 2000b. Essentiality of dietary calcium supplement in redlip mullet *Liza haematocheila. Aquacult. Nutr.,* 6: 33-38.

Isheda, H., M. Ishihara, S. Sumida, M. Owaki and S. Tabata, 1982. Prevention of lordosis in the juvenile of red sea bream, *Pagrus major* reared in pond-II. Relationship between the initial conditions of rearing environment and gas content. *Bull. Kumamoto Pref. Fish. Exp. Sta.,* 2: 25-45.

Kitajima, C., H., Iwamoto and S. Fujita. 1977. Relationship between curvature of vertebral column and undeveloped swim bladder in hatchery-reared red sea bream *Pagrus major. Bull. Nagasaki Pref. Inst. Fish.,* 5: 27-32.

Kitajima, C., T. Watanabe, Y. Tsukushima and S. Fujita, 1994. Lordotic deformation and abnormal development of swim bladders in hatchery-bred marine physoclistous fish in Japan. *J. World Aquacult. Soc.,* 25: 64-67.

Love, R. M., 1980. The Chemical Biology of Fishes. Academic Press, N.Y. 943p.

Stottrup, J. G. and A. Attramdal, 1992. The influence of different rotifer and *Artemia* enrichment diets on growth, survival and pigmentation in turbot (*Scophthalmus maximus* L.) larvae. *J. World Aquacult. Soc.* 23: 307-316.

Watanabe, T, T. Arakawa, C. Kitajima, F. Fukusho and S. Fujita, 1978. Proximate and mineral composition of living feeds used in seed production of fish. *Bull. Japan. Soc. Sci. Fish.,* 44: 973-984.

Watanabe, T, T. Arakawa, K. Fukusho and S. Fujita, 1983a. Living feeds used in seed production of fish. *Bull. Japan. Soc. Sci. Fish.,* 47: 79-87.

Watanabe, T. C. Kitajima and S. Fujita, 1983b. Nutritional value of live food organisms used in Japan for mass culture of fish: a review. *Aquaculture,* 34: 115-143.

Watanabe, T., T. Tomiyama, A. Oka, M. Hirata, C. Kitajima and S. Fujita, 1983c. Improvement of dietary value of live foods for fish larvae by feeding them on Ω-3 highly unsaturated fatty acids and fat-soluble vitamins. *Bull. Japan. Soc. Sci. Fish.,* 49: 471-479.

Yoshimura, K., K. Tanaka and T. Yoshimatsu, 2003. A novel culture system for the ultra-high-density production of the rotifer, *Brachionus rotundiformis*-a preliminary report. *Aquaculture,* 227(1-4): 165-172.

Yoshimura, K., A. Hagiwara, T. Yoshimatsu and C. Kitajima, 1996. Culture technology of marine rotifers and the implications for intensive culture of marine fish in Japan. *Marine Freshwat. Res.,* 47(2): 217-222.

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