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Artificial Microparticle Diets for Culturing Rotifer, *Brachionus plicatilis*

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Rotifer culture fed on five types of artificial microparticle diets were evaluated to substitute the natural diets such as *Chlorella* or ω-yeast. These microparticle diets including solidified blood using squid oil (SBSO), solidified blood using soybean oil (SBSB), nylon protein walled particle (NPW) simple coacervation oil capsule (SCO), complex coacervation oil capsule (CCO), were tested for the evaluation of feeding efficiency. The prepared microparticle diets had diameters ranging from 3 to 30 μm. Rotifer culturing experiments were carried out in 3-liter beakers for 13~16 days. The initial inoculum density of rotifers was 10 ind./mℓ. The rotifers fed on *Chlorella* or ω-yeast showed maximal densities of 2,000 ind./mℓ in 9 days or 500 ind./mℓ in 7 days, respectively. Those fed on SBSO, SBSB or NPW showed maximal densities of 1568 ind./mℓ, 586 ind./mℓ or 503 ind./mℓ, respectively and the reproductive rates for those diets were equivalent to or better than ω-yeast. However, the coacervated oil capsule showed lower maximal densities of 400 ind./mℓ for SCO and less than 100 ind./mℓ for CCO due to the unbalanced diet formulation and indigestibility.

Key words: artificial diet, microparticle, microcapsule, rotifer

Introduction

The culture of fish at a small planktonic larval stage requires a suitable food source, such as live rotifers. The Rotifer is a suitable diet for those larval staged fish because of its size and easy culturing (De La Cruz and Millares, 1974; Watanabe et al., 1983). The rotifer, *Brachionus plicatilis*, is widely used today as the primary food organism offered to a large variety of fish species during their early developmental stages (Luzens et al., 1995). Rotifers were mass-cultured by using marine *Chlorella* or baker's yeast, *Saccharomyces cerevisiae*, as feed organisms (Nozawa et al., 1972; Ohara et al., 1974). However, the seasonal variations in the fatty acid composition of the algal biomass lower the diet quality of rotifers (Luzens et al., 1995), and yeast-fed rotifers lack the essential fatty acids required for the proper development and survival of marine fish larva (Watanabe 1979, 1993; Luzens et al., 1989). Algal culture is also technically demanding and expensive and may represent between 30% and 50% of hatchery operating costs (Jeffrey and Garland, 1987). Therefore, it has long been the aim of the aquaculture industry to replace natural foods with artificial diets. Artificial food particles are known to be acceptable to a wide range of filter feeders. There is an obvious need for the development of a successful artificial diet for filter feeder and fish larva (Jones et al., 1974).

We have carried out the studies on the development of artificial microparticle diets to substitute natural diets such as *Chlorella or* ω-yeast (Lee and Kim, 1996; Lee et al., 1996) Therefore, in this study we evaluated the efficiency of those artificial diets by feeding them to rotifer.

Materials and Methods

Reagents

Concentrated *Chlorella*, ω-yeast and squid oil were obtained from Ewha oil and fat Ind., Ltd. (Pusan, Korea). Soybean oil was purchased from local-market. Dried bovine blood was purchased from Harimex B.V. Co. (Netherland). Rotifer (*Brachionus plicatilis*) was obtained from the Institute of Fisheries Science, Pukyong National University. For coacervation, gelatin (175 bloom), polyvinyl maleic anhydride (PVMMA), carboxymethyl cellulose (CMC, low viscosity : 4% solution at 25℃, 50~200 cps)

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Table 1. Proximate nutritional composition of artificial and natural diets for rotifer culture

Microparticle feeds	Moisture	Protein	Fat	Ash
Chlorella	75.8%	12.2% (61.3)*	5.4% (21.1)	2.3% (11.6)
ω-Yeast	62.1%	14.6% (49.5)	12.7% (43.1)	2.2% (7.4)
NPW Particle	13.7%	73.1% (84.8)	12.6% (14.8)	0.5% (0.3)
SBSO or SBSB Particle	16.4%	69.6% (85.3)	11.0% (12.9)	1.0% (1.1)
Simple or Complex Coacervation Oil Capsule	63%	5.0% (14.8)	27.5% (81.6)	1.2% (3.6)

( )*percentage calculated by dry weight.

were purchased from Sigma Co. (St. Louis, USA).

Rotifer culture

Three-liter beakers were used to grow the rotifer with 7 different diets (SBSO particle, SBSB particle, CCO capsule, SCO capsule, NPW capsule, *Chlorella*, ω-yeast) and 0.02 g of diets in dry weight was fed to 10,000 rotifers in exponential feeding mode. The temperature for the culture was maintained at 26℃ in water bath. Constant illumination of 2000 lux was provided by two fluorescence lights above the water bath. Sea water was filterred using 0.49 μm nylon mesh and used for rotifer culture. Air was supplied at the speed of 360 mℓ/min. The initial inoculum rotifer density was 10 ind./mℓ. The number of rotifer was counted by grid slide. The samples were fixed with Lugol's iodine reagent.

Nutritional composition of natural diets and artificial diets

Protein content was analyzed by the Micro-Kjeldahl method and fat was analyzed by the Soxhlet method (Meloan and Pomeranz, 1973). Moisture was analyzed by dry oven at 110℃ for 4 hrs. Ash was analyzed by electric furnace at 600℃ for 4 hrs.

Reproductive Rates

Reproductive rates were calculated according to the following equation (Luzens et al., 1995).

$$G=\frac{1}{T}\ln(N_T-N_0)$$

Where; T=duration of culture in days.
N_0=the initial number of rotifers and the eggs that they carry in the culture.
N_t=the final number of rotifers and the eggs that they carry at time T of the experiment.

Preparation of Artificial Microparticle Diets

· Solidified blood particles (SBSO, SBSB)

Twenty milliliters of 40% bovine blood meal was added to 200 mℓ of soybean oil or squid oil with 2% soy lecithin and emulsified by homogenizer. The emulsion was heated in hot water bath for 20 min at 80℃. After heating, the capsule were allowed to settle out from the suspension for 24 hours. The settled particles were then washed twice by cyclohexane. Cyclohexane was removed from by vacuum evaporator. The particles had the size range of 3~30 μm (Lee et al., 1996).

· Nylon protein walled capsule (NPW)

NPW capsule was prepared with 40% bovine blood meal dissolved in 0.5M ethylenediamine. Hundred milliliters of bovine blood powder solution was added to the 100mℓ of chloroform with 2% soy lecithin and homogenized for 1 min at room temperature. The emulsion was agitated with magnetic stirrer and 20 mℓ of cyclohexane with 2% soy lecithin was added to the emulsion. The mixture was added to 400 μℓ BTC and agitated for 20 min (Langdon, 1989). The capsule suspension was washed three times with cyclohexane. The capsule had the size range of 3~10 μm.

· Oil capsules

Simple and complex coacervation capsules using squid oil were prepared according to the procedure of Lee and Kim (1996). The capsules had diameter of 10~30 μm

Results

Nutritional composition of diets

In order to provide the diets in dry weight base to rotifer and to know how much protein and fat are provided, the proximate protein and fat contents as well as moisture content were determined and shown in Table. 1.

The growth of rotifer and nutritional composition of rotifer are affected by diets. *Chlorella* had 12.2% protein and 5.4% fat which provide protein and fat balance for rotifer culture. However, it was reported that the seasonal variations in the fatty acid composition of the *Chlorella* affected the

nutritional value of rotifers (Scott and Middleton, 1979; Watanabe, 1993). ω-Yeast had 14.6% protein and 12.7% fat which have higher fat composition than *Chlorella*.

The artificial diets prepared with proteins and oils showed quite different nutritional compositions from those of *Chlorella* and ω-yeast. NPW particle, SBSB, SBSO particles showed similar composition, however, the protein content was much higher than *Chlorella* and ω-yeast. Coacervation oil capsule was composed of mainly oil (81.6%) due to the preparation method using oil in water emulsion. Therefore, these artificial diets were nutritionally unbalanced comparing to *Chlorella*, the well balanced diet for rotifer culture.

Rotifer culture fed on artificial diets

The initial inoculum rotifer density was 10 ind./mℓ. Rotifer fed on *Chlorella showed* maximal density of 2,000 ind./mℓ in 9 days of culture as shown in Fig. 1. The growth rate and maximum rotifer density indicated that *Chlorella* was the best diet and the nutiritional components were well balanced for the rotifer culture. In order to enhance the diet quality of rotifer, ω-yeast has been often used as a diet for rotifer. The maximum rotifer density reached to 500 ind./mℓ in 7 days of the culture when ω-yeast was used as a diet for the culture. The artificial diets were also fed to rotifer in same method. The rotifer fed on solidified blood prepared in squid oil (SBSO) showed the maximal rotifer density of 1600 ind./mℓ in 9 days of the culture. The rotifer culture fed on SBSO showed the pattern similar to that of *Chlorella* except for the growth initial growth rate. That fed on solidified blood prepared with soybean oil (SBSB) showed the maximal rotifer density of 600 ind./mℓ in 10 days of the culture, which was equivalent to that fed on ω-yeast. Rotifer fed on nylon protein walled particles showed maximal rotifer density of 500 ind./mℓ in 13 days of the culture. Overall, the quality of these three artificial diets were equivalent to ω-yeast or *Chlorella* in the growth of rotifer. However, the time to reach to the maximal rotifer density of the culture fed on artificial diets was delayed, compared to the natural diets.

Rotifer fed on simple coacervation oil (SCO) capsule showed maximal density of 400 ind./mℓ in 10 days of the culture as shown in Fig. 2. However, initial growth rate was low until 9 days of the culture. Rotifer fed on complex coacervation oil (CCO) capsule did not show any growth of rotifer. This was probably due to the fact that CCO capsule was hard to digest in the gut of rotifer. Therefore, the rotifer could not use the nutrients from CCO capsules.

Reproductive rates of the rotifer cultured with various diets were determined as shown in Table. 2. Reproductive rates of the cultures fed on SBSO, SBSB and NPW particles were almost equivalent to those of culture fed on ω-yeast. This indicates that these diets can be used as a substitute of ω-yeast considering reproductive rates and maximal rotifer densities. However, SCO and CCO capsules showed

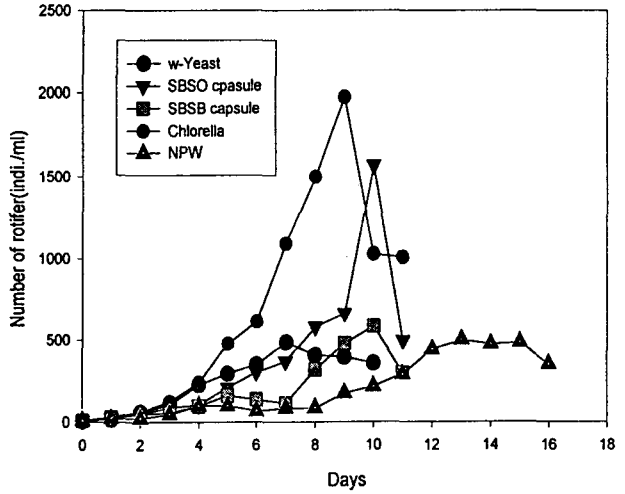

Fig. 1. The growth of rotifer fed on the natural or protein based artificial diets for 16 days (S1 BSO : solidified blood using squid oil, SBSB : solidified blood using soybean oil, NPW : nylon protein walled particle).

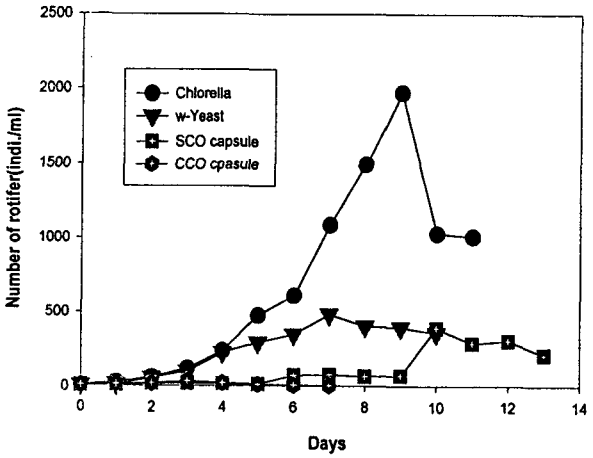

Fig. 2. Growth of rotifer fed on the natural or oil based artificial diets (SCO : Simple coacervation oil capsule, CCO : Complex coacervation oil capsule).

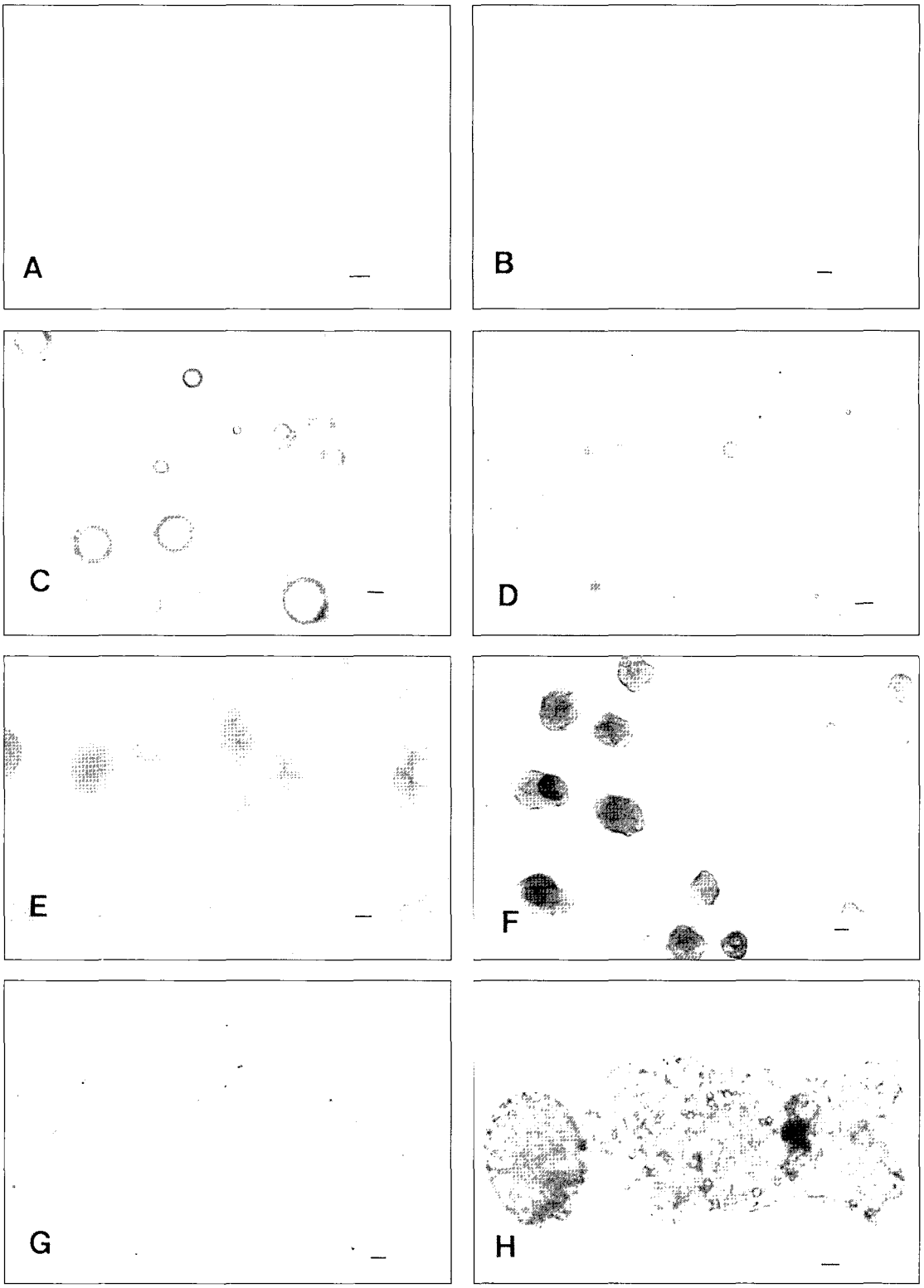

Fig. 3. Artificial microparticle diets and natural diets. (Scale bar : 10 μ) A : *Chlorella* B : ω-Yeast C : Simple coacervation oil capsule D : Complex coacervation oil capsule E : Solidified blood using soybean oil particle F : Solidified blood using squid oil particle G : Nylon protein walled particle H : Rotifer, *Brachionus plicatilis*.

Table 2. Reproductive rates of rotifer cultured with various diets

Days / Diets	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Chlorella	**1.099**	0.920	0.837	0.795	0.773	0.686	0.670	0.626	0.587	0.463	0.420					
ω-yeast	0.470	**0.896**	0.781	0.779	0.676	0.593	0.555	0.463	0.409	0.358						
SBSO particle	**0.956**	0.795	0.721	0.585	0.608	0.569	0.515	0.508	0.466	0.505	0.356					
SBSB particle	**1.099**	0.741	0.580	0.568	0.556	0.436	0.346	0.432	0.430	0.407	0.309					
NPW particle	**0.833**	0.394	0.516	**0.571**	0.459	0.322	0.306	0.269	**0.318**	0.308	0.307	0.316	0.301	0.276	0.259	0.222
SCO capsule	0.336	**0.371**	0.305	0.185	0.128	**0.338**	0.297	0.243	0.224	**0.367**	0.306	0.286	0.236			
CCO capsule	**0.642**	0.416	0.454	0.229	0.094	0.030	−0.032									

low reproductive rates probably due to the nutritional unbalance and indigestibility.

As shown in Fig. 3 the size of opening of rotifer is around 50 μm. Therefore, the size of particle or capsule should be smaller than 50 μm. The sizes of artificial diets shown in Fig. 3. C, D, E, F, G were in the range of 3~30 μm.

Discussion

The qualities of artificial diets were evaluated. Solidified blood particles and nylon protein walled capsule showed high reproductive rates and rotifer densities which were equivalent to those of ω-yeast. However, as shown in the nutritional composition (Table. 1), the nutritional unbalance of the artificial diets might cause lower reproductive rates and rotifer densities comparing to those fed on *Chlorella*. Therefore, the future research will be focused on the balance of nutritional components.

The feeding for the rotifer culture for this study was carried out in exponential feeding mode which increased the amount of feeds according to the number of rotifers. This caused a sudden deterioration of water quality which might kill the rotifer after reaching the maximal rotifer densities. The water quality was also an important factor for the rotifer culture.

The CCO capsules which had a double membrane composed of gelatin layer and PVMMA and cellulose layer were not acceptable diet for rotifer. The CCO capsule might not be digested in the gut of rotifer. This resulted in low growth of rotifer.

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