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A Comparison of Yield and Quality of the Rotifer (*Brachionus plicatilis* – L-strain) Fed Different Diets Under Aquaculture Conditions, Vietnam

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Abstract

Effects of different diets on growth and quality of rotifer of the L-strain were investigated in this study. Rotifers were cultured in triplicate in 200 l tanks with water maintained at 20 ppt, and fed four different diets: (1) bakers' yeast (*Saccharomyces cerevisiae*) in wet form plus 10% squid liver oil (by dry weight), (2) *S. cerevisiae* in dry form plus 10% squid liver oil, (3) microalgae *Nannochloropsis oculata* and (4) microalgae *Chaetoceros muelleri*. The results showed that there were significant differences in rotifer growth rate, viability, size and ciliate contamination between the four dietary treatments ($P < 0.05$). Rotifers fed on microalgae showed better viability, larger size and low ciliate contamination compared to those fed on yeast. However, no differences in the protein and lipid contents of rotifers were evident. In fact, higher density and production of rotifers were found with the use of yeast, but there was a problem in controlling the ciliate contamination and maintaining the cultures.

Introduction

The rotifer *Brachionus plicatilis* has been extensively used as live food for various types of small larvae, particularly for marine species (Watanabe et al. 1983). Rotifer size varies in response to different environmental conditions (Walker 1981). The larvae select the rotifers according to their size, generally preferring larger sized rotifers as the larvae grow, and this enables the latter to maintain a high growth rate, and reduce mortality (Hunter 1980).

Diet is regarded as the most important criterion that could affect growth and quality of rotifers (Oie et al. 1994; Lubzens 1987). Equally, in rotifer culture, contamination is a major problem which affects the stability of cultures (Pourriot 1980; Reguera 1984; Hino 1993), production (Watanabe et al. 1983) and chemical composition (Watanabe et al. 1983; Olsen et al. 1993a; Oie et al. 1994). Marine microalgae were first found to be an excellent food that could provide best rotifer quality for marine fish larvae. However, the use of algae is expensive because of high labor cost and associated difficulties in increasing rotifer production (Watanabe et al. 1983; Lubzens 1987). Rotifer quality is often enhanced by different enrichment techniques. For example, it has been suggested that the replacement of baker's yeast supplemented with oils rich in n-3 HUFA can be used as an alternative food for rotifer culture (Watanabe et al. 1983). Using yeast however, could deteriorate water quality.

Vietnam has great potential for mariculture. Unfortunately, mariculture has not developed yet and there is limited information about rotifer cultures. Research on live food for marine larvae is an essential step for successful propagation of marine fish species. The aim of the present research was to investigate the suitability of two algal and two yeast cultures, with the latter appropriately enriched with squid liver oil for production of the rotifer *B. plicatilis*. In addition, the quality of the rotifers produced was also evaluated.

Materials and Methods

The experiments were based on the L-strain of *B. plicatilis* imported from Norway in February 1998 and carried out at the University of Fisheries, Nha Trang, Vietnam from October 1998 to February 1999. The algal cultures of *Nannochloropsis oculata* and *Chatoceros muelleri* were obtained from the Research Institute for Aquaculture No. 3, Vietnam, while the yeast *Saccharomyces cerevisiae* was obtained commercially.

Two marine microalgae *N. oculata* and *C. muelleri* were cultivated semi-continuously in 1 m^3 outdoor tanks in THO4 medium (Hoang 1993) and NBBS medium (Le 1996), with 20-30% and 50-60% daily dilution of culture medium, respectively. The quantities of algal supplies were determined based on algal density (cell•ml), using a haemocytometer. Algal volume supplied to rotifer tanks were calculated by using the following equation:

$$V_1 = V\frac{N - N_2}{N_1 - N_2}$$

where, V is the volume of rotifer culture; V_1 is the volume of algal supplied; N is the target density of algae; N_1 and N_2 refer to the density of algae supplied and before feeding, respectively.

Squid liver oil (Step-Forward, Thailand) was blended for 25 sec with commercial baker's yeast (*S. cerevisiae*) in wet form (Song-ma, Vietnam) and

dry form (Mauripan, France) in the ratio of 10: 1 by wet weight (Olsen et al. 1993; Oie et al. 1994). The specific food ration given to rotifers in the early growth phase was adjusted to 1.5 µg•ind•day by wet weight.

Rotifers were grown in a semi-continuous system in batches of three, at an initial density of 30 ind•ml, each batch being fed either one of the algae or the enriched yeast types. The daily feeding rates employed were $5x10^6$ cells•ml, $2x10^6$ cells•ml, and 200 g•m^3 of *N. oculata, C. muelerii* and the two yeast types, respectively. Daily, 5 random samples of 5 ml were taken from each treatment to estimate ciliate contamination level, rotifer density and egg ratio. When rotifers in each of the treatments reached an equilibrium, 10% of the culture medium was removed daily and replenished with new water of 20 ppt. In addition, random samples were also taken at these times for determination of lorical length and rotifer motility.

Viability test on rotifers were conducted by direct introduction to freshwater for 5 sec to determine the motility, and the recovery rate determined at intervals of 1, 2, 4, 8, 16, 30, and 60 min after introduction into 20 ppt sea water.

Ten percent of rotifer samples removed daily from each batch was filtered and frozen at –20ºC. A number of such samples (approximately 2 g) from each treatment was thawed and dried at 70ºC to constant weight and dry weight determined. The dry samples were then divided and used for estimation of protein content following the Kjeldahl method and the other for total lipid using a Soxlet apparatus.

Results and Discussion

Growth and yield of the rotifers

Density and production of rotifers are dependent on food availability and quality. In this trial, four different food types were used and these resulted in differences in maximum density and production of the rotifer. Maximum density was observed in the culture fed the wet form of yeast (MTT) followed by the dried form (MKT). The growth rate however, was higher in the ones fed the algae *N. oculata* (NAN) followed by that on *C. muelleri* (CHA) (Table 1). Besides, egg ratio was positively related to the growth rate.

Table 1. Growth and production of rotifers fed four different diets.

	Diets			
	MKT	MTT	NAN	CHA
Maximum growth rate:	0.35	0.41	0.55	0.43
Maximum density (ind•ml):	248	298	145	94
Production ($x10^6$ ind):	72.64	94.46	43.16	28.24

MKT- cultures fed dry yeast; MTT- cultures fed wet yeast; NAN- cultures fed algae *N. oculata*; CHA- cultures fed algae *C. mueleri*

Growth rate of rotifers depends on feeds (Yufera and Navarro 1995). The present results illustrate that algae are excellent food for rotifers. Besides, *N. oculata* provided the best growth rate, and *C. muelleri* also can result in producing very good quality rotifers. The B_2 content of 100 µg•g and vitamin C content of 15 µg•g in *C. muelleri* is higher than that of *N. oculata and* other marine microalgae (Brown et al. 1989; 1997). *C. muelleri* also contains high levels of n-3 HUFA that are very essential for marine fish larvae.

The wet form of yeast provided better biomass than the dry form probably as a result of the leaching of vitamins during drying process. The nutrition value of dry yeast can also readily decrease when introduced into water due to changes in biochemical composition and loss of inorganic matter contributing to a deterioration of water quality (Yufera and Navarro 1995).

Ciliates contamination

Ciliate contamination appears from day five from inoculation in the yeast fed cultures. The occurrence of the ciliate blooms in the dry yeast fed culture coincides with a decrease in egg ratio, rotifer density and water quality. In contrast, ciliate contaminations were found to be low and water quality more stable in the algae fed cultures.

Presence of ciliates in the culture medium is not necessarily harmful except at high ciliate concentrations (Dhert 1997). High level of ciliates in mass cultures lead to considerable reduction in yield because of their activity which brings about an aggregation of food and thereby reduce the availability to rotifers; high concentrations of ciliates have been related to low concentration, low fertility and mass mortality of rotifers (Reguera 1984). Ciliate blooms in cultures fed with dry yeast could result in pollution of the medium; dry powders in general, easily lose nutrients due to the leaching of dissolved organic compounds increasing water quality deterioration. Low ciliate contamination in all algae based diets compared to the yeast based diets are in agreement with earlier reports (Reguera 1984; Dhert 1997) and confirmed the important role of algae in controlling water quality.

Quality of cultured rotifers

Rotifer size is a very important factor for fish larvae. According to Lubzens (1987), mean of lorical length was found to be largest in rotifers fed on *C. muelleri* and lowest in the ones fed dry yeast. There was a significant difference between the two groups fed on algae and yeast but no significant difference was found between the rotifers fed on *N. oculata* and *C. muelleri* or dry yeast and wet yeast (t-test, $P < 0.05$) (Table 2).

Variation of rotifer lorical length is well documented (Iwamoto 1980; Walker 1981; Fukusho et al. 1983; Fukusho 1983; Snell and Carillo 1984; Lubzens 1987) and up to 15% modification in size was possible by changing diets or salinity (Lubzens 1987). In the present trial, salinity was maintained at 20 ppt and it is therefore most likely that rotifer size was mainly affected

by the diets. Trend of the lorical variation agreed with the result of Oie et al. (1994) but the significant difference (t-test, $P < 0.05$) between rotifers fed algal diets and yeast diets was contrary.

The viability of rotifers evaluated by swimming speed and salinity tests (Fig. 1) is an important parameter of determining rotifer quality as it can be indicative of food availability in larval tanks. Effect of diets on viability expressed in mobile rotifer in salinity test was in agreement with the result of Oie et al. (1994). However, in the swimming speed test, the algal diets did not show clear effect in comparison to the yeast diets (Oie et al. 1994).

Dry weight of the rotifers in the treatments were similar to those reported by Watanabe et al. (1983). The protein content of rotifers in this experiment was similar to the value reported by Oie et al. (1994) but different to those of Watanabe et al (1983) and Dendrinos and Thrope (1987). According to Oie et al (1994), protein content of rotifers is stable and the different estimates reported in various studies may be due to the use different analytical methods. Lower lipid content of yeast grown rotifers is comparable to

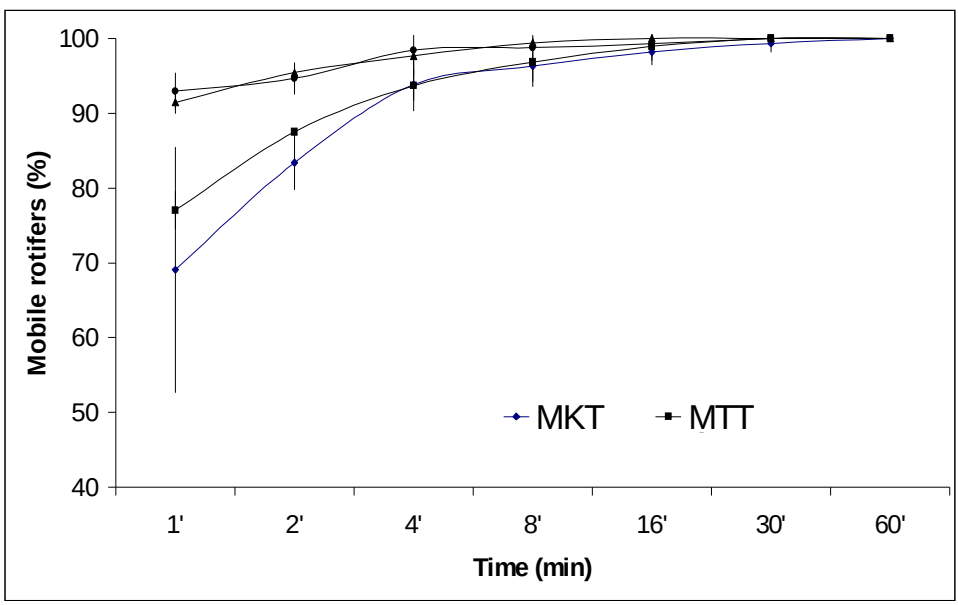

Fig. 1. Viability of rotifers fed different cultures in the salinity test. The different symbols refer to the different food types as given in table 1.

Table 2. Parameters (±se) pertaining to rotifer quality fed four different diets.

	Diets			
	MKT	MTT	NAN	CHA
Size (µm)	260±19.02[a]	261±18.29[a]	283±20.86[b]	278±19.62[b]
Swimming speed (mm/min)	51.47±5.66	49.60±3.77	53.00±3.48	55.13±4.26
Dry weight (% wet weight)	9.64±1.47[a]	11.07±0.80[a]	9.70±1.06[a]	10.28±0.62[a]
Lipid content (% dry weight)	11.45±1.01[a]	12.85±0.37[a]	11.23±1.14[a]	12.66±0.98[a]
Protein content (% dry weight)	56.82±2.82[a]	58.28±3.31[a]	61.28±1.73[a]	59.99±2.82[a]

the result reported by Oie et al. (1994). It is also reported that high temperatures could affect lipid accumulation. Lipid content of the rotifers does not necessarily reflect their overall nutritional quality. The nutritional value of rotifers needs to be considered in respect of the fatty acids (n-3 HUFA) profile, which are essential for larval growth.

Conclusions

Different diets, wet yeast, dry yeast added 10% squid livers oil by wet weight, *N. oculata* and *C. muelleri* showed significant effects on growth rate, lorical length, viability of rotifers and level of ciliate contamination but did not significantly affect dry weight and protein and lipid contents of rotifers. In general, the algal diets proved to be better for rotifer growth and viability compared to baker's yeast. The former, however, resulted in low rotifer density and production since it was difficult to increase the food ration. Use of algae can minimize ciliate contamination and increase stability of the cultures. Wet yeast plus oil seemed to be the best diet, resulting to the highest yield of the cultures.

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