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Submission date: 20-Mar-2019 02:56PM (UTC+0700)
Submission ID: 1096515152
File name: A_simple_and_lowcost.pdf (155.66K)
Word count: 1587
Character count: 8177

LARVI '17 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
C.I. Hendry (Ed)
European Aquaculture Society, Special Publication No. XX, Oostende, Belgium, 2017

A SIMPLE AND LOW-COST TECHNIQUE FOR CULTURING ROTIFERA WITHOUT MICROALGAE

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Introduction

So far, microalgae are the first choice diet for feeding rotifers (Maruyama et al., 1997), thanks to their excellent influence on rotifer growth rate (Hagiwara et al., 1997; Dhert et al., 2001; Yoshimatsu and Hossain, 2014), rotifer nutrition (Watanabe, 1983; Brown, 2002) and for the nutrition of fish larvae that feeds on the rotifers (Reitan et al., 1997). However, culturing microalgae require high investment and running expenses (Borowitzka, 1997), with difficulties in production (Lee, 2001), harvesting (Baros et al., 2015), and storage/preservation (Camacho-Rodrigues et al., 2015). For this reason, baker's yeast and other products such as condensed algae and Selco (Inve.Co.Ltd) were introduced. The former is cheap but cultures are unstable while the latter is costly. The present study investigated the use of microalgal replacement diet (MRD) based on fish-wastes as a simple and low-cost diet for culturing the rotifers without microalgae for aquaculture.

Materials and methods

The fish waste MRD preparation protocol and its use as dietary source for rotifers are extensively described in a patent Registration Number: P00201609066 in Indonesia. The suitability of the MRD as dietary source for rotifer was investigated using the rotifer, *Brachionus rotundiformis*. The rotifers were batch cultured in fifteen 25-ml flasks containing sterilized seawater of salinity 22ppt. The effects of MRD on growth of rotifer were tested under different MRD weights (i.e. 0.05, 0.1, 0.2, and 0.4gl^{-1}) and *Nannochloropsis oculata* at density of 1.2×10^{7}cells.ml^{-1} was used as experimental control. About 10 rotifers ml^{-1} were transferred to each flask followed by the addition of MRD treatment in triplicates. All MRD treatments were added only once at the beginning while *N. oculata* was maintained at1.2×10^{7}cells.ml^{-1} daily.

Growth responses of the MRD-fed rotifers at different culture containers were investigated. Four different glass containers, i.e. small-tall (ST; volume: 25ml, length: 65mm and width: 30mm), small-short (SS; volume: 75ml, length: 95mm and width: 55mm), medium-tall (MT; volume: 750ml, length: 181mm and width: 95mm), and large-short polycarbonate tanks (LS; volume: 30 l, length: 320cm and width: 350cm) were used in triplicates. About 0.2gl^{-1} MRD was added to each culture container following the initial inoculation of 10 rotifers.ml^{-1}.

Responses of the MRD-fed rotifers were also investigated under different initial densities of rotifer. Three initial densities i.e. 10, 50, and 200 rotifers.ml^{-1} were introduced into 30-l transparent polycarbonate cylindrical tanks after the addition of 0.2g.l^{-1} of MRD.

Growth responses of the rotifer in the mass cultures were investigated using semi-continuous culture method performed in two fiberglass tanks (750 l) placed outdoor for a month. About 10 and 400 rotifers.ml^{-1} were introduced into each tank after the addition of 0.27g.l^{-1} of MRD into the culture medium. The cultures were harvested every 2 to 5 days depending on the density of the rotifers. The MRD was added to each culture after every harvesting. There was no aeration or water exchange during the experiment. The effect of MRD-cultured rotifers on growth and survival of fish larvae were assessed using larvae of *Sillago japonica* Temminck et Schlegel 1843. About 10 fertilized eggs.l^{-1} of *S. japonica* were transferred into six transparent polycarbonate tanks containing 100 l artificial seawater (32ppt). First feeding was done on day 2 post hatching (dph) with two feeding regimes i.e. MRD-fed rotifers (test diet) and rotifers cultured using enriched super fresh *Chlorella* V12 ® (control diet). The fish larvae were fed at 10 rotifers.ml^{-1} in each triplicate tank until 10dph.

Results and discussion

The rotifers grew well at all MRD weights and showed a trend of sharp increasing density on day 2 or 3 (at 0.1-0.4g.$l^{-1}$of MRD), then followed by a sharp density decrease on day 4 and 5 of culture. As comparison, growth of rotifer fed daily with *N. oculata* increased slowly at beginning then increase continuously till day 5. A two-way analysis variance showed that MRD weight significantly affected population density of rotifer during 5 days of culture (ANOVA, p=0.001), where the densities at 0.4 and 0.2 g.l^{-1} of the MRD were significantly higher than those at 0.1 and 0.05g.l^{-1} (TukeyHSD test, p<0.05). Population densities of rotifers at all MRD weights were not significantly different with rotifers fed daily with *N. oculata* as control (TukeyHSD test, p>0.05).

There was significant effect of container design on the rotifer density (ANOVA test, p=0.001), where the density of those cultured in larger containers (MT and LS) was significantly higher than those cultured in smaller containers (ST and

SS) on day 2 or 3 (Tukey HSD test, $p<0.05$). Rotifer densities at larger containers increased sharply to around 797-1176 ind.ml^{-1} on day 3 or 4 of culture, while those at smaller containers increased on day 2 or 3 at densities of about 178-399 ind.ml^{-1}. All treatments showed a trend of sharp increase in rotifer growth between days 2 to 4 and, a sharp decrease between days 3 to 5.

There was significant effect of rotifer initial density on population growth during 5 days of culture (ANOVA test, $p<0.01$), where rotifer growth at higher initial density (200 ind.ml^{-1}) was significantly higher than those at lower initial density (50 ind.ml^{-1}) on day 4 of culture (Tukey HSD test, $p<0.05$). Maximum rotifer densities of about 987-1179 ind.m^{-1} were attained on day 3 in all the treatments followed by sharp density decline afterwards. The rotifer at initial densities of 200 ind.ml^{-1} showed a little decreased on day 2 then sharply increased until day 3 of culture.

Semi-continuous mass cultures of rotifer using MRD were successfully performed in two 750-l fiberglass tanks for 15 and 30 days. In the first trial, the culture was harvested 5 times at densities about 1086-2230 ind.ml^{-1} during 15 days of culture. Rotifer in the second trial was harvested 5 times at densities around 2495-3305 ind.ml^{-1} during the first 21 days while it was harvested 2 times at densities about 975-1530 ind.ml^{-1} afterward until day 30. Maximum densities of rotifers in two trials were mostly attained within 2 to 5 days before harvesting.

The larvae of *S. japonica* were fed on MRD-cultured rotifers for 10 days. The number of ingested rotifers by the larvae increased from about 2ind.larvae^{-1} (2dph) to 12ind.larvae^{-1} (10dph). Total length and dry weight of *S. japonica* larvae in this treatment increased from 2.41mm and 0.08±0.01mg.ind^{-1} (2dph) to 3.65±0.30mm and 0.26±0.03mg.ind^{-1}, respectively on 10dph. There was a significant difference in total length of the fish larvae fed with MRD-cultured rotifers compared to those larvae fed with control-rotifers (3.42±0.22 mm) on 10dph (One-way ANOVA, $F = 15.18$, $p = 0.00$). The dry weight was not statistically different among the diets. Survival rate of the larvae fed MRD-cultured rotifers and control on 10dph were 9.70±9.61 and 4.83±0.66%, respectively, and was not statistically different between the diets (Kruskal-Wallis test, $\chi^2=0.19$, df=1, $p=0.66$)

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