The effect of different feeds on the growth, survival and reproduction of rotifer, *Brachionus plicatilis*

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Abstract

Aim: This study compared the effects of four types of feed, namely yeast, palm kernel cake (PKC), chicken manure (CM), and *Nannochloropsis oculata* on the growth, survival and reproductive performance (offspring production, life span, pre-oviposition, oviposition, post-oviposition, egg produced per female and production time of egg) of rotifer *Brachionus plicatilis*

Methodology: Microalgae were used as the control diet and they were compared to CM, PKC, yeast and *N. oculata* at different concentration of 0.004 g l⁻¹, 0.008 g l⁻¹ and 0.012 g l⁻¹ respectively. Feeding treatments were assessed in triplicate and experiments were performed for 30 days. The growth, survival and reproductive performance (e.g. offspring production) of *B. plicatilis* were evaluated to assess its response to different feed types at different concentrations.

Results: The population growth and the survival of rotifers were significantly affected ($P > 0.05$) when fed with diets of different concentrations. The highest population growth of 795.67 individual ml⁻¹ and survival rate of 399.01 individual ml⁻¹ were recorded with the CM and yeast at a concentration of 0.012 g l⁻¹. The reproductive performance (offspring production) of rotifers depended on the type of food. The rotifers recorded higher offspring production (1.52 eggs/female/day) when fed with 0.012 g l⁻¹ of *Nannochloropsis* sp.

Interpretation: The result indicates that different types of feed at varying concentrations are an effective strategy to replace the common food used for rotifer culture.

Key words: *Brachionus plicatilis*, Growth performance, Reproductive performance, Rotifers

Used with different concentrations for reproduction of rotifer

0.004 g l⁻¹
0.008 g l⁻¹
0.012 g l⁻¹

The highest production and survival of rotifer, *B. plicatilis* was found in the highest concentration of feeds

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Introduction

Rotifer are usually used in aquaculture because it has high reproduction rate, high survival rate, and low cost of production that work to sustain its availability (Sahandi and Jafaryan, 2014). The most important factor to ensure the culture of marine fish thrives in various parts of the world is a constant, reliable, and sustainable supply of nutrition that can be provided by rotifers (Zmora et al., 2013). Most fish larvae feed on zooplanktons (Das et al., 2012). Murillo and Villamil (2011) demonstrated that rotifers are commonly used as starter feed for a variety of fish species. *Brachionus plicatilis* (Meuller, 1786) is one of the most effective larvae feeds. Besides, rotifers are of different sizes, among them two common ones are S-size and L-size. However, Varghese (2003) found that rotifers also have a much tinier strain (SS-size) of 70–168 microns. This strain is significantly smaller than *B. rotundiformis*, which is 98–210 microns in size. Furthermore, rotifers are also called 'living capsules', as they can deliver the nutrients required for healthy development of larvae fish (Sandeep et al., 2015). Hence, various fish species in natural water bodies have specific dietary habits and require protein for growth, effective breeding, and survival (Mandal et al., 2009). Since the discovery of this new rotifer strain, the rotifer is now leading the market as a significant feed for various species, including yellowtail (*Seriola quinqueradiata*) (Matsunari et al., 2013), red sea bream (*Pagrus major*) (Takaoka et al., 2011), Asian sea bass (*Lates calcarifer*) (Thépot et al., 2016), pompano (*Trachinotus carolinus*) (Cavalin and Weirich, 2009), mullet (*Mugil cephalus*) (Kailasam et al., 2015), gilthead seabream (*Sparus aurata*) (Khairy and El-Sayed, 2012), and the European sea bass (*Dicentrarchus labrax*) (Kraul, 2006). Currently, rotifers are also used as food for culturing penaeid shrimp (Samocha et al., 1989) and mud crab (*Scylla serrata*) (Dan and Hamasaki, 2011).

Initially, because of its low market price, commercial baker yeast has been used in live feed culture. Moreover, baker's yeast has a high lipid to protein ratio; which can stimulate the rate of population growth and the ratio of eggs produced by rotifers (Radhakrishnan et al., 2017). In addition, yeast is also used to improve aquatic animal nutrition and as a probiotic (Das et al., 2012). Palm kernel cake is an ordinary fertilizer for poultry by-product industry that provides a low price and is a widely available source of poultry feed component (Ng et al., 2013) as it has high concentration of amino acids (Fasuyi et al., 2014), which supplies a good valuable dietary source of protein, energy, and fibre for the fish development. Recent studies have successfully tested palm kernel cake as poultry feed at low concentration levels (Keong, 2004). The promising use of palm kernel cake in poultry farming has triggered the researchers to apply palm kernel cake in the aquaculture sector.

Furthermore, chicken manure is a poultry-originated organic matter and indeed is an acceptable replacement diet for

zooplanktons. In an earlier experiment between different subspecies of copepod (*Thermocyclops decipiens* and *Mesocyclops aspericornis*), the development of two copepods was successfully improved (Hyder et al., 2014). Furthermore, chicken manure can be used in aquaculture as pond fertilizer as well. This study evaluated the growth, survival, and reproduction of *B. plicatilis* when fed with microalgae (*N. oculata*), bakery yeast (*S. cerevisiae*), palm kernel cake, and chicken manure at three different concentrations of 0.004 g l⁻¹, 0.008 g l⁻¹, and 0.012 g l⁻¹ respectively.

Currently, phytoplankton is used as the primary diet for rotifers. Recent studies have concentrated on assessing the use of yeast and phytoplankton as rotifer diet (Ortega-Salas et al., 2013; Ferreira et al., 2009). For this research, variety types of feed are applied to the rotifer to find a similar substitute for phytoplankton (*N. oculata*), yeast (*Saccharomyces cerevisiae*), chicken manure, and palm kernel cake. This study examines the consciousness disparity by evaluating whether palm kernel cake and chicken manure will be able to substitute microalgae as a rotifer diet. These feed types are categorized as by-products, so their use as a rotifer diet could reduce aquaculture cost and ensure that fertiliser sources are used more productively.

Materials and Methods

The rotifer, *B. plicatilis* was collected from Setiu, Terengganu, Malaysia and brought back to the hatchery of Universiti Malaysia Terengganu (UMT), Malaysia for stock culture. Yeast (*S. cerevisiae*), palm kernel cake (PKC), chicken manure (CM), and *Nannochloropsis oculata* at different concentrations of 0.004 g l⁻¹, 0.008 g l⁻¹ and 0.012 g l⁻¹, respectively were used (Ortega-Salas et al., 2013) for the experiments with three replicates. All feeds were measured in the same concentration to identify the different types of feeds and different types of concentrations that could replace *N. oculata*. The types of feed and different levels of concentrations were manipulated to determine the best growth, survival and reproductive performance of rotifers (Li and Olsen, 2015).

Experimental design: Rotifers were first cultured in a 100 ml flask and were periodically upscaled to 2000 litre tanks for mass culture once density reached 10 individuals per millilitre before transferring to the feeding treatments at concentrations of 0.004 g l⁻¹, 0.008 g l⁻¹ and 0.012 g l⁻¹ (Ortega-Salas et al., 2013). Every treatment was conducted in a 5 l water with aerated tank and exposed to sunlight. Twice a day, the feeding system for rotifers was developed. For each individual count, aliquot of 1ml from each treatment was collected. Each replicate of rotifer from the different treatments (Yeast, PKC, CM and *N. oculata*) was taken using the Neubauer Haemocytometer in triplicate. The samples were observed under a compound microscope.

The growth and survival of rotifer as a result of each treatment was calculated using the equation below (Ortego-Salas et al., 2013):

$$Ke = (\ln N_t - \ln N_0) / T$$

Where, N_0 is the number of organisms at the start of period T days; N_t is the number of organisms after T days and T is the days.

Hence, growth was measured daily until the end of the experiment to obtain associated rotifer growth rate data. The survival rate was determined based on the length of the experiment for each procedure in order to determine the efficiency of the rotator. The culture variables were periodically calculated, and the density was calculated three times per sample.

Life table observation: Offspring production for the rotifer in this experiment was calculated by using the equation given below (Edmonson, 1965):

$$B = E/D$$

Where, E is the number of eggs/females with eggs, D is the day of the experiment.

The offspring production of the rotifer was observed daily for collecting the performance data of each feeding treatment.

Life span and life stage of rotifers: Rotifer specimen was screened through a different mesh size of 300 μm and a 100 μm mesh before retaining a mesh of 50 μm consecutively. Two neonates were each placed on a cultivation plate with different feed concentrations. The life span and life process of the rotifer was observed every 12 hours using compound microscope at 10X magnification. Each egg produced was extracted out and transferred to the new cultivated plate. In this study, the feeding experiment was done in triplicates.

Time and number of eggs produced: The rotifer samples were screened with 300 μm and 100 μm mesh. Each sample had two individuals and set on a culture plate with eggs. The quantity and time period of egg produced were measured every 30 minutes

under a 10x magnification compound microscope.

Statistical analysis: The data were analysed by two-way analysis of variance (ANOVA) to test the relationship between the various feed forms used and different concentration rates. The data were expressed as mean $\pm$ SD. This step was taken, where appropriate with several comparison tests performed by Tukey to assess major media variations for each parameter. All the data were confirmed with descriptive statistics and homogeneity testing to satisfy the ANOVA at the level of significant differences ($P < 0.05$).

Results and Discussion

The highest daily population growth rates were observed with CM at 0.012 g l^{-1} (795.67 ind ml^{-1}). Furthermore, the highest population growth of *B. plicatilis* was also found at 0.012 g l^{-1} feed with yeast (731.33 ind ml^{-1}), PKC (728.67 ind ml^{-1}) and *Nannochloropsis* (638.33 ind ml^{-1}), respectively (Table 1). As the concentration level of feeds was increased from 0.004 g l^{-1} , 0.008 g l^{-1} , to 0.012 g l^{-1} , the daily population growth of *B. plicatilis* among the yeast, PKC, CM and *N. oculata* showed a significant difference at all concentration's levels ($P < 0.05$). This finding is similar to that of Byod (1982), who found almost double the rotifer population growth after being added with chicken manure. According to Ogello and Hagiwara (2015), chicken manure is an optimal feed for enhancing the rotifer population density and hence, it can be used in aquaculture. Similarly, Sulehria et al. (2015) found that the rotifer growth is rapidly responsive to changes in food type and different rates. This could be due to the fact that rotifers are opportunists that react to changes in food types and concentrations quite quickly.

The highest survival rate of the rotifer was recorded when fed with 0.012 g l^{-1} of yeast (399.01 ind ml^{-1}), followed by 0.012 g l^{-1} of chicken manure (369.53 ind ml^{-1}), while the lowest survival rate was observed when fed at 0.008 g l^{-1} of PKC (343.87 ind ml^{-1}) and 0.012 g l^{-1} of *N. oculata* (317.61 ind ml^{-1}) (Table 2). The survival rate of *B. plicatilis* fed with yeast, PKC, CM and *N. oculata* were significantly different with various concentration levels ($P < 0.05$). It is revealed that the highest survival rate of rotifers was observed when fed with 0.012 g l^{-1} of yeast. The outcome contrasted with

Table 1 : The growth rate of *B. plicatilis* on different types of diets and different level of concentrations. All values are mean $\pm$ standard deviation ($n=3$). The different capital letters indicate significant different between diets and different small letters indicate significant different between concentrations ($P < 0.05$)

Diets	The growth rate (ind ml^{-1})		
	0.004 g l^{-1}	0.008 g l^{-1}	0.012 g l^{-1}
Yeast	584.67 $\pm$ 9.29 ^{B,c}	668.67 $\pm$ 27.73 ^{B,b}	731.33 $\pm$ 28.85 ^{B,a}
Palm kernel cake	588.67 $\pm$ 6.11 ^{B,c}	674.00 $\pm$ 23.58 ^{B,b}	728.67 $\pm$ 23.46 ^{B,a}
Chicken manure	635.00 $\pm$ 9.64 ^{A,b}	748.67 $\pm$ 0.57 ^{A,a}	795.67 $\pm$ 9.45 ^{A,a}
<i>N. oculata</i>	504.00 $\pm$ 6.25 ^{C,c}	612.33 $\pm$ 13.429 ^{C,b}	638.33 $\pm$ 2.08 ^{C,b}

Table 2: Survival rate of *B. plicatilis* on different types of diets and different level of concentrations.

Diets	Survival rate (ind ml ⁻¹)		
	0.004g l ⁻¹	0.008g l ⁻¹	0.012g l ⁻¹
Yeast	278.92 ± 2.62 ^{A,c}	345.54 ± 8.93 ^{A,b}	399.01 ± 33.21 ^{A,a}
Palm kernel cake	290.42 ± 15.85 ^{B,c}	343.87 ± 10.38 ^{B,b}	337.78 ± 6.74 ^{B,b}
Chicken manure	317.48 ± 2.93 ^{A,b}	358.13 ± 12.87 ^{A,b}	369.53 ± 10.37 ^{A,a}
<i>N. oculata</i>	251.84 ± 1.52 ^{C,c}	305.98 ± 10.24 ^{C,c}	317.61 ± 2.25 ^{C,b}

All values are mean ± S.D. (n=3). The different capital letters indicate significant different between diets and different small letters indicate significant different between concentrations (P<0.05)

Table 3 : Offspring production (egg/female/day) of *B. plicatilis* with different types of diets and different level of concentrations

Diets	Offspring production (egg/female/day)		
	0.004g l ⁻¹	0.008g l ⁻¹	0.012g l ⁻¹
Yeast	1.44 ± 0.24 ^{AB,a}	1.47 ± 0.37 ^{AB,a}	1.39 ± 0.44 ^{AB,b}
Palm kernel cake	1.18 ± 0.17 ^{C,c}	1.25 ± 0.26 ^{C,c}	1.38 ± 0.36 ^{C,c}
Chicken manure	1.29 ± 0.44 ^{BC,c}	1.43 ± 0.28 ^{BC,b}	1.36 ± 0.30 ^{BC,b}
<i>N. oculata</i>	1.49 ± 0.26 ^{A,b}	1.5 ± 0.25 ^{A,a}	1.52 ± 0.25 ^{A,a}

All values are mean ± S.D. (n=3). The different capital letters indicate significant different between diets and different small letters indicate significant different between concentrations (P < 0.05)

Table 4: Life table parameters of *B. plicatilis*

Diets	Concentration (g l ⁻¹)	Lifespan (days)	Pre-oviposition (days)	Oviposition (days)	Post-oviposition (days)	Number of eggs per female (ind)	Egg reproduction (hours)
Yeast	0.004	7.86 ± 0.15 ^{A,a}	2.83 ± 0.15 ^{A,a}	4.56 ± 0.11 ^{B,a}	1.13 ± 0.15 ^{A,a}	5.67 ± 0.5 ^{A,a}	5.33 ± 0.15 ^{A,a}
	0.008	7.73 ± 0.05 ^{A,a}	2.83 ± 0.05 ^{A,a}	4.76 ± 0.05 ^{B,b}	1.13 ± 0.15 ^{A,a}	5.33 ± 1.1 ^{A,a}	5.06 ± 0.1 ^{A,a}
	0.012	7.93 ± 0.05 ^{A,a}	2.93 ± 0.05 ^{B,a}	4.90 ± 0.10 ^{B,b}	1.26 ± 0.05 ^{B,b}	5.33 ± 0.5 ^{A,a}	4.96 ± 0.05 ^{A,a}
	0.004	7.80 ± 0.20 ^{A,a}	2.86 ± 0.15 ^{A,a}	3.26 ± 0.15 ^{A,a}	1.90 ± 0.10 ^{A,a}	5.33 ± 0.5 ^{A,a}	6.33 ± 0.05 ^{B,b}
Palm kernel cake	0.008	7.93 ± 0.11 ^{A,a}	2.76 ± 0.05 ^{A,a}	3.53 ± 0.05 ^{A,a}	2.00 ± 0.00 ^{B,a}	5.33 ± 1.15 ^{A,a}	6.23 ± 0.05 ^{B,b}
	0.012	7.86 ± 0.05 ^{A,a}	2.76 ± 0.05 ^{A,a}	3.33 ± 0.05 ^{B,a}	1.93 ± 0.0 ^{B,a}	5.0 ± 1.0 ^{A,a}	6.26 ± 0.05 ^{B,b}
	0.004	7.70 ± 0.10 ^{A,a}	2.86 ± 0.11 ^{A,a}	3.13 ± 0.11 ^{A,a}	1.76 ± 0.05 ^{A,a}	4.0 ± 1.0 ^{B,a}	6.13 ± 0.11 ^{B,b}
Chicken manure	0.008	7.66 ± 0.05 ^{A,a}	2.96 ± 0.05 ^{B,b}	3.13 ± 0.15 ^{A,a}	1.83 ± 0.05 ^{B,b}	4.33 ± 0.5 ^{B,a}	5.83 ± 0.05 ^{B,b}
	0.012	7.56 ± 0.05 ^{A,a}	2.93 ± 0.05 ^{B,a}	2.96 ± 0.05 ^{A,a}	1.73 ± 0.05 ^{B,b}	3.67 ± 1.15 ^{B,a}	5.13 ± 0.11 ^{A,a}
	0.004	7.96 ± 0.05 ^{A,a}	2.80 ± 0.20 ^{A,a}	5.60 ± 0.10 ^{B,b}	1.08 ± 0.02 ^{A,a}	5.67 ± 0.5 ^{A,a}	5.0 ± 0.0 ^{A,a}
<i>N. oculata</i>	0.008	7.86 ± 0.05 ^{A,a}	2.83 ± 0.05 ^{A,a}	5.66 ± 0.05 ^{B,b}	1.13 ± 0.02 ^{A,a}	5.33 ± 0.5 ^{A,a}	4.93 ± 0.05 ^{A,a}
	0.012	7.92 ± 0.11 ^{A,a}	2.70 ± 0.10 ^{A,a}	5.83 ± 0.15 ^{B,a}	1.07 ± 0.01 ^{A,a}	5.0 ± 1.0 ^{A,a}	4.96 ± 0.11 ^{A,a}

All values are mean ± S.D. (n=3). Different capital letters indicate significant different between different diets and different small letters indicate significant different between concentrations (P<0.05)

Sahandi and Jafaryan (2014) in their 10-day experiment of 0.008 g l⁻¹ that expressed the highest survival rate. Differences in the diet and concentrations significantly affects the growth and survival rate of rotifers in this study. In other words, the rotifer culture could be maintained by the diet used for the experiment but could not efficiently increase the population number of the rotifer.

The highest fecundity of 1.52 eggs per female was recorded with 0.012 g l⁻¹ of *N. oculata*, followed by 1.47 eggs per female at 0.008 g l⁻¹ for yeast and 1.43 eggs per female with 0.008

g l⁻¹ of CM. The lowest fecundity rate of 1.18 eggs per female was observed with PKC treatment at the concentration of 0.004 g l⁻¹ (Table 3). Significant differences in rotifers fecundity were observed among the different treatments (P < 0.05).

The highest rotifer lifespan of 7.96 ± 0.05 days was achieved when fed with 0.004 g l⁻¹ of CM, followed by 7.93 ± 0.11 days with 0.008 g l⁻¹ of PKC (Table 4). The average lifespan of *B. plicatilis* was not significantly different among the treatments (P < 0.05). Life phase period of rotifers was influenced significantly (P

< 0.05) among the treatments. Pre-oviposition phase (2.96 days) was the highest with 0.004 g l⁻¹ of CM. The highest oviposition period of 5.83 days was attained when fed with 0.012 g l⁻¹ *N. oculata* and the lowest with 0.012 g l⁻¹ of CM (Table 4). This is in agreement with Sun *et al.* (2017), they found that rotifers fed with microalgae had longer oviposition stage than both the pre and post-oviposition stages. The highest post oviposition phase of 2 days was recorded when fed with 0.000 g l⁻¹ of PKC and the lowest with 0.012 of *N. oculata*. Sarma *et al.* (2001) stated that feeding with certain food types for prolonged periods, may lead to the production of longer post oviposition stage for rotifer.

The number of eggs (5.67 eggs/female) produced was greatest when fed with 0.004 g l⁻¹ of yeast, followed by the treatments of 0.008 g l⁻¹ of PKC with produced 5.33 eggs/female, 0.008 g l⁻¹ of CM with 4.33 eggs/female, and 0.004 g l⁻¹ of *N. oculata* with 5.67 eggs/female (Table 4). Female rotifer fed with 0.004 g l⁻¹ of *N. oculata* had a shortest egg reproduction rate of 4.93/hrs while the longest were with 0.008 g l⁻¹ PKC of 6.26/hrs (Table 4). Significant differences were observed in number of eggs per female and egg reproduction period among the treatments ($P < 0.05$). The rotifer egg reproduction in the study was marginally longer after being fed with yeast (0.012 g l⁻¹), palm kernel cake (0.008 g l⁻¹) and chicken manure (0.012 g l⁻¹) than rotifer fed with *N. oculata* (0.004 g l⁻¹). However, there were substantial variations in the amount of eggs produced per female during each day when it was fed with yeast (0.004 g l⁻¹), palm kernel cake (0.008 g l⁻¹) and chicken manure (0.008 g l⁻¹) compared to *N. oculata* (0.004 g l⁻¹). It has been found that rotifer fed with microalgae at 6.61 individuals ml⁻¹ could produce higher eggs per day and reproduce in relatively shorter time (Lubzens and Zmora, 2003).

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