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Research Article

Population Growth and Growth Performance of Freshwater Rotifer (*Filinia longiseta*) Fed with Different Supplemented Microalgae Diets

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ABSTRACT

The lack of size-suitable live food for small-gaped fish larvae is a major impediment to their growth and survival. Rotifers are viable live food that can bridge the gap between the dependence of such fish larvae from their endogenous yolk to larger-sized live feeds. The present study focused on developing *Filinia longiseta*, a very small local rotifer, as an important live food for fish larviculture. The study specifically investigated the effects of four different supplements (yeast, commercial fish fry booster, rice bran and fish waste) in the microalgae (*Chlorella vulgaris*) diet of *Filinia longiseta* to its population growth and individual growth. Yeast, fry booster and fish waste significantly ($p<0.05$) improved the population growth of *F. longiseta* better than rice bran and *Chlorella* only diet after 6 days of culture. Individual growth did not significantly vary among different diet treatments and control ($p>0.05$). Performance of these different supplements have been investigated in the past and showed that their effectivity in improving rotifer population and growth parameters were attributed to proper preparation, their digestibility, efficient absorption and interaction with microflora in the culture media.

Key words: *Filinia longiseta*, yeast, rice bran, fry booster, fish waste

INTRODUCTION

Aquaculture, provide a vital source of high quality and inexpensive animal protein crucial to balanced diets in marginally food secure communities. In developing countries, aquaculture development has become a necessity because of rapid growth of populations and deterioration of natural fisheries (Akpaniteaku *et al*., 2005). The development of aquaculture has been hampered by several constraints, including the shortage of quality fish seed, leading to scarcity of enough seed to sustain production (Charo & Oirere, 2000). One major limiting factor in fish seed production in aquaculture is the availability of suitable initial food for the developing larvae (Buchalla *et al*., 2023). This impediment in successful larviculture ensues in the transition period between endogenous (yolk) to exogenous feeding (live feed), which is marked by high mortality rates and low production efficiency due to great histomorphological changes in the digestive tract (Jin *et* al., 2023; He *et al*., 2012; Papandroulakis *et al*., 2005). The size of the prey eaten by the fish larvae is a function of the larval mouth width, wherein the mean diameter of the prey consumed by fish larvae is only 38% of their gape width (Hunter, 1980). Fish larvae are mostly smaller in size, and swim very slowly and thus prefer slow swimming live food like rotifers as a food (Arimoro, 2006).

Rotifers can be viable live food to bridge the gap between the dependence of the fish larvae with small gape to their endogenous yolk and larger-sized live feeds like *Artemia*. Rotifers are the most dominant zooplankton in all freshwater aquatic ecosystems (Tingson & Zafaralla, 2018; Mendoza *et al*., 2019) and considered as valuable live food for the culture of larvae of most fish species due to its small size ranging in 105-160 µm in width (Udit *et al*., 2020). Their slow swimming motion makes them easy prey to fish larvae which usually have underdeveloped locomotory organs (Valentin *et al*., 2013). They also reproduce rapidly and can be cultured in high density, making them available in large quantities within a relatively short period. Rotifers can also be enriched with antibiotics, fatty acids, and other nutrients very easily, making them effective vehicle for these substances that beneficial to survival and growth of fish larvae (Louvado *et al*., 2023; Ma *et al*., 2023; Udit *et al*., 2020). Aside from the potential benefits of rotifers to culture of key food fish species, it can also be applied to the larviculture of threatened/ endangered fish species which are in dire need of restocking in the wild. While restocking is widely used as a fisheries management tool, it has also been used for the conservation and management of threatened species (Flagg *et al*., 1995; Brown & Day, 2002). Restocking programs, both for fisheries and conservation purposes should consider improving fish culture methods (including appropriate live feed production) a priority in the future. Since small improvements in the proportion of fish surviving to adulthood could result in substantial gains in absolute numbers of individuals entering the fishery (Brown & Day, 2002).

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MATERIALS AND METHODS

Collection of Filinia longiseta

Wild samples of *F. longiseta* were collected in Laguna de Bay, mainly in its limnetic areas where rotifer populations are expected to be dominant against other major zooplankton group (Tingson & Tamayo-Zafaralla, 2018). Approximately 400 liters of lake water was be filtered through a 60 µm mesh-sized plankton net by the buckets. Vertical hauling of the plankton net through the lake's water column was not employed due to shallow water depth (~1.5m) at the collection sites. Collected water in the sample receptacle bottle of the plankton net were further concentrated down to 50 mL by filtration through a 60µm sieve for ease of isolation. Viable *F. longiseta* individuals were isolated by handpicking through a fine-tip glass Pasteur pipette under a digital dissecting microscope.

Culture media and diet preparation

Base culture media for *F. longiseta* was prepared by mixing 1L of nutrient stock (1L water steeped with 50 grams of oven-dried chicken manure in a pouch for 24 hours), 1L of water and 50 mL of green water containing cultured *Chlorella vulgaris* with an estimated average density of 1,290,870 cells/mL. Isolated *F. longiseta* from Laguna de Bay were acclimated and sub-cultured in the base culture media for 1 month prior to diet experiments. Four different supplemented microalgae treatment diets were prepared by mixing 375 mL of green water containing *C. vulgaris* at the same estimated density as above with the following four different feed supplements, (a) 0.94 g of yeast, (b) 0.75 g of commercial fry booster, (c) 3.75 g D1 rice bran and (d) 3.75 mL fish waste. Fish waste was prepared by soaking blended gills and abdominal organs of round scad (*Decapterus spp.*) in water until extracts leeched out. All prepared diets were filtered using 20µm plankton net before given to the rotifers.

Population growth and growth performance

To determine the increase of *F. longiseta* population over culture time under different supplemented diet treatments, a total of 45 identical culture containers with capacity of 1L were filled with 655 mL aged water and 45 mL of chicken manure nutrient stock. Thirty-six culture containers were seeded with 10 adult individual *F. longiseta* and fed with 7mL of supplemented microalgae diet based on the treatments assigned experimental feeding (there were 9 culture containers per treatment) once a day after seeding. The remaining 9 containers were assigned as the control, wherein only *Chlorella* was provided as feed and also were also stocked with 10 adult individual *F. longiseta.* Three (3) culture containers from each treatment and control were collected at intervals of 2, 4 and 6 days after seeding. Rotifer samples were collected using a 20 µm filter, preserved in 10% buffered formalin and counted using a gridded Sedgewick rafter under a compound microscope. The population growth rate (r) was calculated according to Yin *et al*. (2013), using the following equation: $r = (\ln N_t - \ln N_0)/t$ where N_0 and N_t are the initial and final population densities, and t is the culture time in days.

Individual growth performance of *F. longiseta* was assessed by preparing 60 plastic cups divided among 4 diet treatments and with capacity of 50 mL each. Each cup was filled with 5mL of chicken manure nutrient stock diluted in 100 mL aged tap water and seeded with nine (9) *F. longiseta* neonates were seeded in each cup. Neonates had initial mean body length of 99.6 µm and body depth of 65 µm and fed once a day after seeding with 3.75mL of supplemented microalgae diets based on the assigned treatment. Samples were harvested in triplicates (n=9 per replicate) from each treatment at 10-, 20-, 30-, 40- and 50-hours after seeding using the same filter and preserved in 10% buffered formalin. Samples were then stained with rose Bengal for better visualization prior to measurement of body length of each collected individuals using a calibrated digital microscope with on-screen measurement feature.

Welch Test ($p \leq 0.05$) was employed to assess the difference in the population growth and individual growth performance among diet treatments. Statistical analysis of the data was carried out using SPSS ver. 22 for Windows.

RESULTS

Overall population growth of *F. longiseta* was significantly higher after 6 days of culture when its

Table 1. Mean population and population growth rate of *Filinia longiseta* fed with different supplemented microalgae diets through 6 days of culture.

	Mean population and mean population growth rate				
Culture Duration (days)	Yeast	Fry Booster	Rice Bran	Fish Waste	Control
2	40 ± 1.70 (0.69% ± 0.03)	68 ± 5.58 (0.95% ± 0.05)	107 ± 14.52 (1.17% ± 0.09)	76 ± 9.94 (1.00 ± 0.08)	44 ± 0.98 (0.74% ± 0.01)
4	1555 ± 482.23 (1.76% ± 0.18)	3837 ± 715.83 (1.99% ± 0.17)	830 ± 467.15 (0.74% ± 0.33)	1392 ± 130.13 (1.46% ± 0.06)	482 ± 173.23 (0.96% ± 0.41)
6	7500 ± 820.99[a] (0.85% ± 0.25)	7622 ± 317.71[a] (0.37% ± 0.16)	2120 ± 676.27[b] (0.66% ± 0.42)	6981 ± 1134.55[a] (0.79% ± 0.05)	2053 ± 473.70[b] (0.90% ± 0.54)

mean population ± SE (mean % population growth ± SE), initial n=10); in a row, means superscripted by a common letter are not significantly different at 5% level.

microalgae diet was supplemented with yeast, fry booster and fish waste ($p<0.05$) compared to rice bran and without supplementation (Table 1; Figure 1 & 2). Rice bran supplemented diet and control diet influenced the population growth and population rate of *F. longiseta* the least at day 4 and 6 of culture.

Individual growth of *F. longiseta* was not significantly different across different feed supplements after 50 hours of culture time. Fry booster supplemented microalgae diet produced *F. longiseta* with the longest mean body length at the initial two observation periods of 10- and 20-hours after seeding with 120.74±6.11 µm and 122.96±3.84 µm, respectively. Eventually, at the last two observation periods of 40- and 50-hours after seeding, yeast supplementation yielded *F. longiseta* with the longest mean body length of 134.82±5.76 µm and 133.33±5.01 µm, respectively (Figure 3).

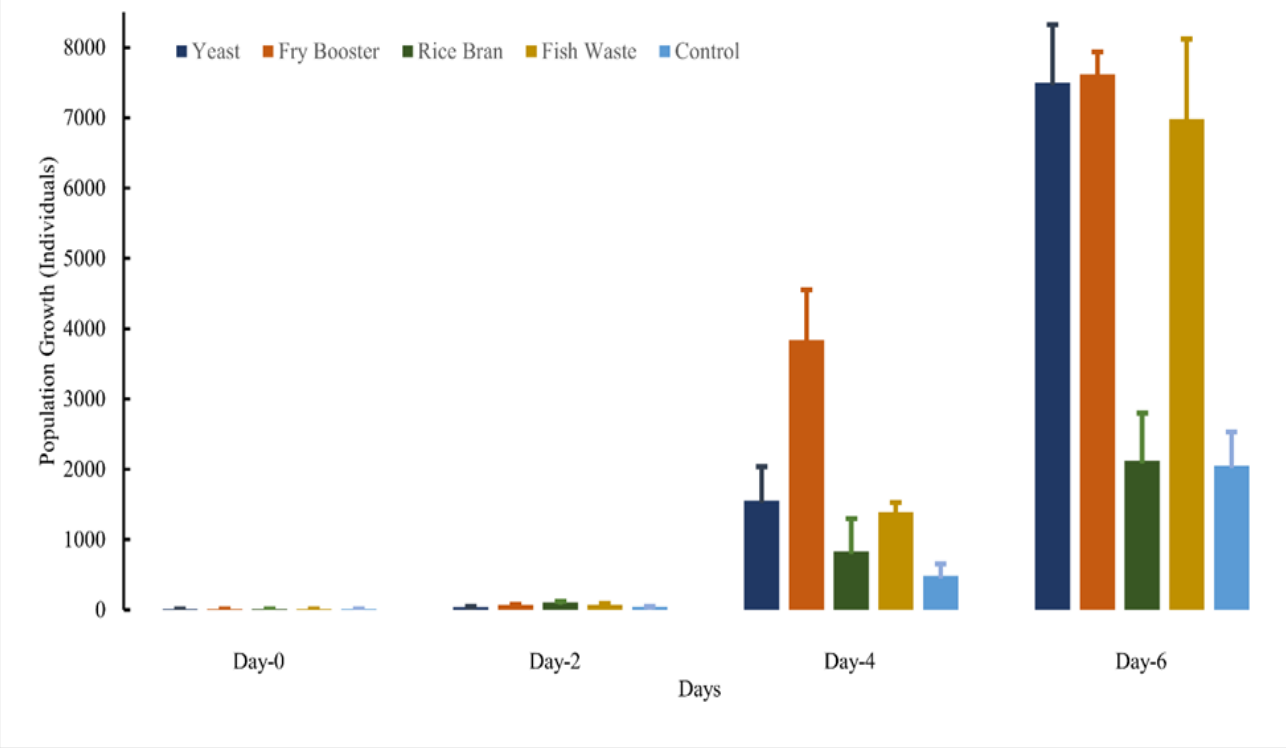

Figure 1. Mean population of *Filinia longiseta* fed with different supplemented microalgae diets through 6 days of culture (bars = standard error).

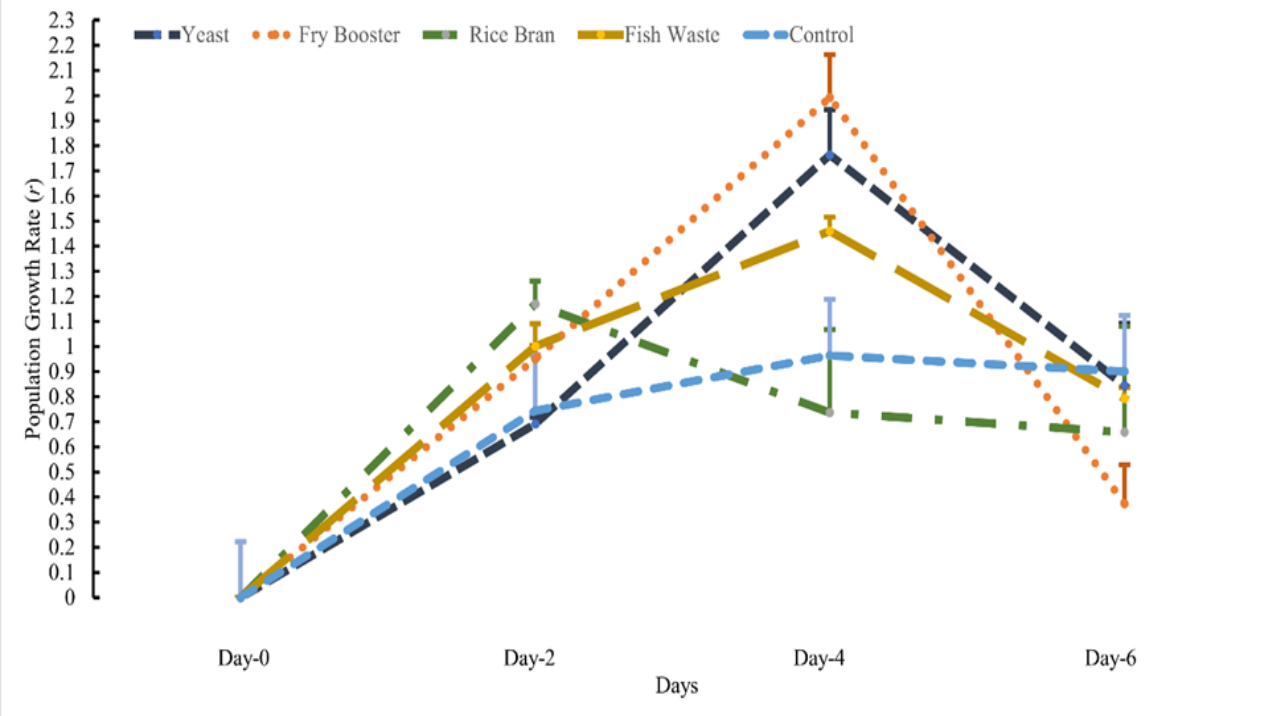

Figure 2. Mean population growth rate of *Filinia longiseta* fed with different supplemented microalgae diets through 6 days of culture (bars = standard error).

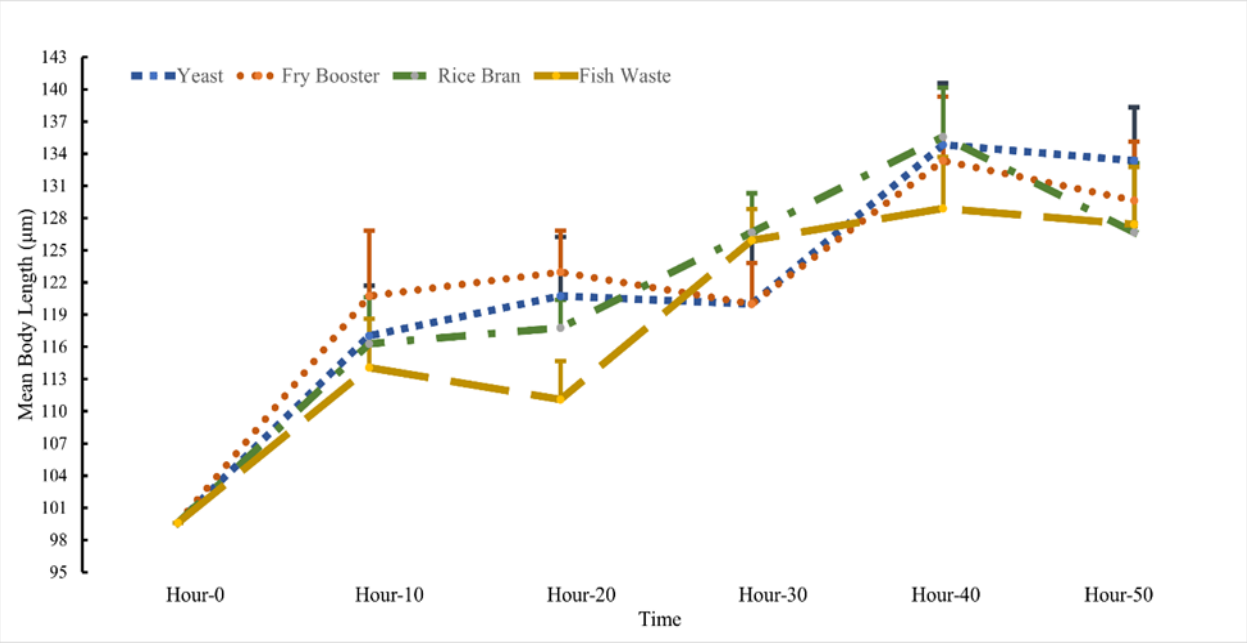

Figure 3. Mean body length (µm) of *Filinia longiseta* fed with different supplemented microalgae diets through 50 -hours of culture (n = 9 per replicate; bars = standard error).

DISCUSSION

The suitability of yeast as replacement or supplement for live microalga in larviculture has been investigated for years (Kagali *et al*., 2022). Studies using yeast as diet for rotifer have demonstrated its significant positive effect in rotifer densities, however, survival rate of fish larvae reared with yeast fed rotifers was significantly lower compared to those produced with rotifers fed on *Chlorella* and has been attributed to low nutritional value of yeast (Nagata & Whyte, 1992; Hamre, 2016). Mixed diets of microalgae (i.e. *Chlorella*, *Scenedesmus*) and yeast produce higher population growth of rotifer (*Brachionus calyciflorus*, *Brachionus rubens*, *Euchlanis dilatata*) and other zooplanktons such as cladocerans (*Ceriodaphnia*, *Moina*) and *Artemia* (Sarma *et al*., 2002; Peña-Aguado *et al*., 2005; Farhadian *et al*., 2013) by providing an extra source of required nutrients compared to a monospecific diet. Kagali *et al*., (2022) suggested that yeast in live food cultures promotes growth of beneficial microflora (bacteria) which could be a supplementary source of nutrients and improve the digestibility of yeast cells (Coutteau *et al*., 1990; Lim e *et al*., 2003). The exoenzymes secreted by the bacteria aid in the degradation of the thick yeast cell envelope that impedes its digestibility by the live foods (Coutteau *et al*., 1990). Additionally, yeast-supplemented live food cultures allow the growth of facultative anaerobic bacteria that can produce cobalamin or vitamin B12 (Hirayama, 1987), a nutrient that has been shown to enhance sexual reproduction in rotifers (Hayashi *et al*., 2007; Le *et al*., 2017; Park *et al*., 2017).

On the other hand, the use of commercial fish feed like fish fry booster as supplement to microalgae in rotifer culture has been scarcely studied in the past. In a similar investigation, commercial fish feed has been shown to support the population growth of three zooplankton species namely: *Moina micrura*, *Scapholeberis kingi* and *Brachionus calyciflorous* (Kar *et al*., 2017). The positive influence of fish fry booster as a supplement to the population growth performance of rotifer culture can be attributed to its high essential nutrient content and its ability to be easily broken down and absorbed by its recipient. Commercial fish fry booster used in the present study contains 48% protein and 10% fat with particle size of 0.25 mm (Tateh feed). Formulated feed products particularly for zooplankton have been produced at commercial scale previously. These products come usually containing a wide array of heterotrophic or phototrophic organisms in spray-dried form (e.g., the *Schizochytrium sp*. based Aquagrow Gold©, the *Crypthecodinium cohnii* based Aquagrow DHA©, and single cell marine organism-based Multigain©) and fish oil-based emulsions (e.g., Easy Selco© and Red Pepper©; Cavalin & Weirich, 2009; Boglino *et al*., 2012). Such diversity of composition leads to some variations in nutritional compositions, such as lipids, fatty acids, amino acids, minerals, and vitamins (Ribeiro *et al*., 2011; Hache & Plante, 2011). These supplement products have been reported to be employed in hatcheries to increase the yield and nutrients of live food for fish larvae (Boglino *et al*., 2012).

Like yeast and fry booster, fish waste also demonstrated positive influence on the population growth of *F. longiseta* as a supplement to its microalgae diet. The use of fish wastes as a diet to enhance zooplankton population has been previously reported (Mo *et al*., 2018) and are excellent sources of protein, minerals, and fats (Schneider *et al*., 2006; Rebah & Miled, 2013). A study by Ogello *et al*. (2018) revealed that *Brachionus rotundiformis* cultured on fish waste showed significantly high densities compared to those fed with microalgae only. *Proales similis* also showed higher growth peaks, specific growth rate when cultured with fish waste (Kagali *et al*., 2018). Rotifers were found to be directly feeding on minute particles of fish waste as well as on the bacteria that grew in the presence of fish water, while also simultaneously producing beneficial enzymes and vitamins like vitamin B12 (Zink *et al*., 2013) that is important to rotifer reproduction as discussed above.

Rice bran have not shown significant influence on the population of growth of *F. longiseta* and was comparable to the control set up without any supplementation.

Rice bran in other studies may have shown to have more considerable nutrients such as 19.8% protein and 20.4% lipids than yeast but the nutrient availability of cereal bran in diets might be considered low (Azani *et al*., 2023; Monteiro *et al*., 2020). Zdradek (2001) attributed this unavailability of nutrient in rice bran to the strong bond between the outer layer of the grains, proteins and other micronutrients to cellulose and hemicellulose which make it hard to digest and absorb these nutrients. To make these nutrients more accessible to rotifers, Monteiro *et al*. (2020) demonstrated that utilizing fermented rice bran resulted in comparable population performance of rotifers to yeast and better than using unfermented rice bran like that used in the present study. Employing fermentation to modify substrates during their metabolic activity is an effective mechanism to increase the availability of nutrients in raw material (Pelizer *et al*., 2007). The chemical composition of the substrate is altered by the fermenting agent through production of extracellular enzymes and proper metabolites (Monteiro *et al*., 2020). In turn the substrate may be enriched, depending on the availability of nutrients present in it, which before the microbial action were not accessible to chemical or enzymatic extractive processes (Oliveira *et al*., 2010). Rice bran presents a large amount of nutrients such as vitamins, minerals, and lipid content that should improve the reproduction process but can only be harnessed if prepared properly.

CONCLUSION

After 6 days of culture trials, *F. longiseta* fed with either yeast, fry booster and fish waste supplemented *Chlorella* diet significantly resulted to better population growth while the control microalgae diet and rice bran supplementation showed significantly lower population growth. Improvement in population growth was attributed by prior investigations to the digestibility, efficient absorption, interaction with microflora and nutrient stability during preparation of these supplements to microalgae in the culture media.

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