ejpmr, 2018,5(1), 280-286

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH
www.ejpmr.com

Research Article
ISSN 2394-3211
EJPMR

EFFECT OF WATER PROBIOTIC IN WATER QUALITY MAINTENANCE AND GROWTH OF ROHU (*LABEO ROHITA*) FINGERLINGS

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Article Received on 09/11/2017 Article Revised on 29/11/2017 Article Accepted on 19/12/2017

ABSTRACT
The accumulation of organic wastes deteriorates water quality and in turn decreases the growth of fingerlings (*Labeo rohita*) in fresh water ponds. To avoid this problem a supplementation trail was conducted for 60 days to determine the effect of a commercial water probiotic 'Eco-Pro' in the water quality maintenance and growth of Rohu (*Labeo rohita*) fingerlings in fresh water plastic troughs. Probiotic bacteria are known to improve water quality in many ways. Eco-Pro contains a combination of *Rhodopseudomonas palustris* and some basic mediums. Fresh water plastic troughs were treated with control and experimental diets which contains water probiotic for 60 days. The factors responsible for the improved water quality and significant growth of finger lings Rohu (*Labeo rohita*) under the influence of water probiotic was analyzed and discussed in the present study.

KEYWORDS: *Rhodomonas palustris*, Probiotic, *Labeo rohita*, water quality maintenance, growth performance.

1. INTRODUCTION
Aquaculture which is now the fastest growing food producing sector in the world is moving in new direction with the increasing in the intensification and commercialization of aquaculture production (Tran N.T et al., 2013). In aquaculture to improve water quality fish raisers may really on remove of toxic material from water. The mechanism of actions to improve on water quality is still needed. There is a considerable interest in use of probiotics to improve conditions in pond aquaculture (Mai D. Ibrahem 2015). Moreover the availability of feed for aquaculture is another significant challenge in the intensifying aquaculture industry (Muzinic et al., 2004). Feed quality and feeding methods therefore need to be thoroughly considered in order to growth performance and water quality management. Li et al., 1997 performed a study to configure the possible role of probiotic bacteria in improving shrimp water culture, they found that the addition of photosynthetic bacteria in to the water resulted in elimination of a number of toxic metabolic and toxic products thus enhance water quality. Due to the high mortality and contagious nature of disease large amount of antibiotics or often used for therapy. However, in discriminate use of antibiotics has led to development of drug resistant bacteria that are becoming increasing difficult to control and eradicate (Zhou et al., 2010). Therefore the need for alternative techniques is increasing and the contribution of probiotics may be considerable.

The term probiotic was introduced by Parker (1974). Moriarty (1998) proposed to extend the definition of probiotics in aquaculture to microbial water additives. According to his original definition probiotics are "organisms and substances which contribute to intestinal microbial balance". Fuller (1989) proposed that a good probiotic has following characteristics.

1. Effectiveness in application
2. Nonpathogenic and nontoxic
3. Existing as viable cells, preferably in large numbers
4. Surviving and being actively involved in the metabolism of the gut environment and
5. Being stabilized and remaining viable during long periods of storage and under field conditions.

The commercial water probiotic 'Eco-Pro' contains a combination of *Rhodopseudomonas palustris* and some basic mediums. Therefore this study was attempted to investigate the effect of probiotics, which are responsible for the improvement in water quality and significant growth of finger lings Rohu (*Labeo rohita*) in fresh water pond.

2. MATERIALS AND METHODS
2.1 Source of fish
Fingerlings of *Labeo rohita* (Hamilton) (**Figure 1**) collected from government fish form at Tirupati, Chittoor district, Andhra Pradesh, were brought and acclimatized to the laboratory conditions.

Fig. 1: Fingerlings of *Labeo rohita* (Hamilton).

2.2 Acclimatization

Fingerlings of fish were collected from local fisheries department as per the standard piscicultural procedures and were kept in cement tanks for a week with sufficient aeration and dechlorinated water to acclimatize them to laboratory conditions (**Figure 2**).

Fig. 2: Fingerlings of fish *Labeo rohita* acclimatize to laboratory conditions.

2.3 Probiotic

In this study, commercially available probiotic with *Rhodopseudomonas palustris* species manufactured by symbiosis animal feeds, A.D.B road, Peddapuram, East Godavari district, Andhra Pradesh, India were used. Water probiotic 'Eco pro' was dissolved in plastic troughs daily 2g/25 liters capacity.

2.4 Control and experimental diets

Two supplementary diets were compared for the growth study. One is commercial diet (**Figure 3**) used for treatment 1 (Diet 1- control diet); another is the supplementary diet prepared by us (**Figure 4 and 5**) used for treatment 2 (Diet 2- Experimental diet-1; Diet 3- Experimental diet-2).

Diet 1: Control diet was prepared by using the following ingredients,

(Rice bran (60g) + Ground nut oil cake (100g) + Soybean cake (100g) + Fish meal (40g)

Diet 2: Experimental diet-1 is prepared by using the following ingredients, (Control diet (10g) + Water probiotic "Eco pro" (2g) + Vitamin C (2g)

Diet 3: Experimental diet-2 is prepared by using the following ingredients, (Control diet (10g) + Water probiotic "Eco pro" (2g) + Ground nut oil cake (3g) + Vitamin C (2g)

Experimental duration: 60days Fish size: Fingerlings (6gm) No. of Replicates: 6 No's

Number of fish in each replicate: 10 No's

Fig 3: Diet 1- Control diet.

Fig. 4: Diet 2- Experimental diet-1.

Fig. 5: Diet 3- Experimental diet-2.

2.4 Physico-chemical parameters of water samples

In the present study, physico-chemical parameters of water and probiotic bacterial loads were studied at fortnight intervals by collecting water samples in between 8 and 10 a.m. The physico- chemical parameters such as temperature, transparency, dissolved oxygen, pH, total alkalinity, total hardness, nitrite, nitrate, ammonia of water were estimated by following the methods suggested by Golterman and Clymo (1969); Wetzel and Likens (1979); APHA (1999).

2.5 Bacterial analysis

Two fish as initial sample prior to commencement of the experiment and two fish from each trough at the termination of the experiment were starved for 24 hours in order to clean their intestinal tract before being sacrificed. The gut was aseptically dissected out both prior to commencement and at the termination of the experiment. Then the gut was homogenized with sterilized and chilled 0.1M, PBS, pH 7.4 (10:1 volume: weight). The homogenate, after five serial 1:10 dilutions, was plated on pseudomonas base medium (Hi-medium Mumbai). From the diluents, 0.1 mL of the sample was inoculated in to the medium and incubated at 37^0c, for 24 to 48 hours. All the determinations were carried out in triplicates following incubation, plates containing viable colonies were used to calculate bacterial population results. The bacterial load of fish gut was expressed as number of colony forming units per 'g' gut tissue CFU/g (Hassan M.A and Saha S., 2014).

2.6 Growth performance analysis

Specific growth rate (SGR, % day-1), feed conversion ratio (FCR) and protein efficiency ratio (PER) was calculated according to standard methods using the following formulae: FCR = dry weight of feed consumed/increase in wet weight of fish; PER = wet weight gain of fish/ protein consumed and SGR = [(In final weight – In initial weight)/days on trial] × 100 (Hassan M.A and Saha S., 2014).

2.7 Statistical analysis

Statistical analysis was done by one way analysis of variance (ANOVA) using MS-EXCEL and SPSS 16.0 soft ware. Mean difference between different treatments was tested for significance at $P<0.05$ and comparisons was made by Duncan's multiple range test to find out significant difference between different treatments in respect of growth.

3. EXPERIMENTAL DESIGN

Experimental study was conducted under laboratory conditions. The experimental design consisted of 6 round shaped plastic troughs of 25 liter capacity, 40cm diameter and 25cm depth, each filled with fresh water. For each treatment 6 replicates were maintained. The bottom of the rearing troughs was specifically made milky white to facilitate easy recognition and collection of faeces. The water in the troughs was periodically aerated to provide enough oxygen to fish throughout the experimental period. Water in the system was replaced daily with fresh water quality and to avoid accumulation of faeces and uneaten feed or any excretory products. The faeces were collected carefully after 3 hours from the morning feeding (maximum shedding of faeces was observed 3 hours after the feeding) with pipette and discarded. A constant photo period of 12 h light/12 h dark was maintained. Body mass-water volume ratio was maintained at 1g/liter.

4. RESULTS

4.1 Physico-chemical parameters of water

The different physico-chemical parameters of water were studied and their values of Mean ± S.D (n=6) and ranges during the supplementation of control diet and supplementary diets were shown in **Table 1.**

4.2 Bacterial analysis

Water probiotic 'Eco pro' contain *Rhodopseudomonas palustris* and some basic mediums were added to water of the plastic troughs supplemented with experimental diet 1 and diet 2, whereas no probiotic added to control troughs. Heterotrophic bacterial count (CFU/g gut tissue) in *Labeo rohita* fingerlings fed with control diet and experimental diets were shown in **Table 2.**

The total heterotrophic bacteria (THB) were observed to be high in experimental diets received fingerlings when compared with control. The loads recorded were 3.80×10^3 cfu/mL in experimental diet 1; 4.02×10^3 cfu/mL in diet 2, 1.01×10^3 cfu/mL in control diet received fingerlings. The relative bacterial loads during the culture period in different petriplates were shown in the **Figure 6.**

The differences in growth and survival of number of bacterial colonies from the gut of fingerlings *Labeo rohita* between control and treated groups could be attributed to the quality of diets.

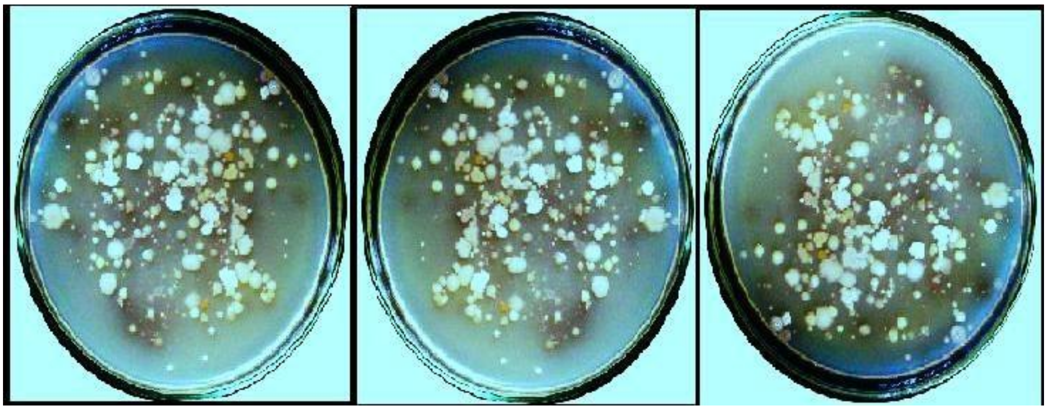

Fig 6: The relative bacterial loads during the culture period in different petriplates.

Table 1: Physico-chemical parameters of water in plastic troughs contain *Labeo Rohita* fingerlings received control diet and supplementary diets.

Physico- Chemical parameters	Feed 1: Control diet			Feed 3: Experimental diet-2		
	Mean± S.D	R	Mean± S.D	Range	Mean± S.D	Range
Water temperature (⁰C)	28.06±0.81	2	29.12±0.66	25.20-32.70	29.33±1.32	27.60-32.50
Secchi disc transparency (cm)	26.33±1.43	2	27.03±1.05	24.50-31.20	28.06±0.09	26.50-34.20
Dissolved Oxygen (mg/mL)	5.20±0.05	3	5.05±1.33	2.40-6.40	4.55±1.30	2.00-5.20
pH	7.31±0.04	7	7.42±0.36	7.50-8.56	7.63±0.40	7.44-8.65
Total alkalinity (mg/L as CaCO3)	114.0±4.66	1	120.0±8.33	120-180	89.6±9.36	100-184
Total hardness (mg/L as CaCO3)	106.3±0.05	1	132.3±6.32	130-195	108.6±6.39	105-190
Nitrite-N (mg/L)	0.06±0.03	0	0.04±0.06	0.09-0.07	0.05±0.03	0.02-0.11
Nitrate-N (mg/L)	0.18±0.06	0	0.22±0.08	0.12-0.46	0.20±0.05	0.09-0.41
Ammonia-N (mg/L)	0.28±0.04	0	0.26±0.06	0.27-0.50	0.27±0.08	0.38-0.73

Table 2: Heterotrophic bacterial count (CFU/g gut tissue) in Labeo rohita fingerlings fed with control and experimental diets for 60 days.

[a]CFU/g gut	Control diet	Experimental diet 1	Experimental diet 2
Bacterial count on pseudomonas base medium (PBM)	1.01×10^3cfu/mL	3.80×10^3cfu/mL	4.02×10^3cfu/mL

[a]Colony forming units per gram gut tissue.

4.3 Growth performance

The data regarding growth performance and feed utilization by *Labeo rohita* fingerlings fed experimental diets were presented in **Table 3.** Dietary supplementation of 'Eco pro' probiotic resulted in better growth performance and feed utilization efficiencies over the control diet fed fingerlings. Live weight gain (%) was highest for the experimental diet 2 fingerlings which differed significantly ($P<0.05$) than that of experimental diet 1 and control. SGR showed the similar trend. FCR and PER was best for experimental diet 2 fish group which showed significant difference ($P<0.05$) than that of experimental diet 1 and control. Survival was 100% in experimental diet fed groups.

Table 3: Growth and feed utilization efficiency in *Labeo rohita* fingerlings fed with control and experimental diets for 60 days.

Parameter	Control diet	Experimental diet 1	Experimental diet 2
Initial average weight	6.30 ± 0.01^{a}	7.35 ± 0.02^{b}	9.26 ± 0.01^{c}
Final average weight	8.93± 0.01^{a}	12.33±0.02^{b}	18.63±0.03^{c}
Weight gain (%)	29.40± 0.02^{a}	40.38±0.02^{b}	50.29±0.02^{c}
Survival	80	100	100
Feed intake (g/kg b.w of fish/day)	10.03±0.11^{a}	10.05±0.02^{a}	10.04±0.15^{a}
FCR	2.01±0.03^{a}	1.98±0.04^{b}	1.79±0.01^{a}
PER	1.02±0.01^{a}	1.33±0.02^{b}	1.56±0.01^{c}
SGR (% day^{-1})	1.08±0.20^{a}	1.28±0.20^{b}	1.38±0.20^{c}

Data are Mean values ± S.D (n=3) Values in the same row with the same superscripts are not significantly different (P<0.05) (DMRT).

5. DISCUSSION AND CONCLUSION

Aquaculture development has been considered a very rich source of high biologic value protein diets to ever growing human population. Consequently the sector has developed strategies in various countries to improve water quality and fish growth. Among these strategies, the more promising is the use of probiotics. In this investigation the commercial diet treated fingerlings as well as the formulated probiotics treated fingerlings were analyzed for their potential growth promoting effects on rohu (*L. rohita*) fingerlings. Probiotics are micro organisms with health benefit to the host. They are used in aquaculture as means for improving water quality and as supplementary nutrients for the development of growth of fishes (Ghosh et al., 2008). In the present study, no mortality was observed in fingerlings fed with experimental diets containing the probiotic bacteria which proved that the probiotic bacteria used in the present study (*Rhodopseudomonas palustris*) is safe for *Labeo rohita* fingerlings.

In the present study, water quality parameters of the plastic troughs treated with probiotics were observed to be good which might be because of the various roles played by the microbes. During the period of observation, the amplitude of variation in water temperature was very narrow (**Table 1**) which is considered to be characteristic of tropical waters (Rahman et al., 1982; Dewan et al., 1991; Wahab et al., 1996; Ahmed et al., 2000). The water temperature in general follows with the pattern of fluctuation of air temperature. Transparency of water showed an inverse correlation with temperature and negatively correlated with dissolved oxygen. These relations are interdependent and could be explained by the fact that the high temperatures are conductive for the development of phytoplankton. With the increase in the densities of phytoplankton's a corresponding decrease in the Secchi disc transparency and increase in dissolved oxygen content was observed. pH of water has been found to be positively correlated with dissolved oxygen. It is well established that the carbonates increase the pH of water on hydrolysis. Simultaneously the photosynthetic release of oxygen increases the dissolved oxygen content in the water. The relationship of pH, free carbon dioxide, carbonates and bicarbonates has been discussed by many workers (Seenayya, 1971; Rao 1972).

According to Boyd (1982), the total hardness is usually related to total alkalinity as the cations of hardness and anions of alkalinity are normally derived from the solution of carbonate minerals. Arce and Boyd (1980) also observed a high positive correlation between total alkalinity and total hardness in pond waters. Conductivity and total dissolved solids represent the mineral content of water and hence they exhibit significant positive relationship with pond waters. Higher values of conductivity were noticed in the month corresponding to the organic fertilizer application in the ponds. This could be explained by the fact that poultry manure has high soluble inorganic salts and is responsible for the increase of conductivity in the water (Ray and David, 1969).

The nutrients, nitrate-N, nitrite-N and ammonia-N in the pond water did not follow the same pattern of distribution and the variations may be due to biological or chemical reactions or combination of these two. The application of probiotics, fertilizers, supplementary feeds and metabolites released by the fish might also be responsible for such variations. During the study period, the levels of ammonia and nitrites were relatively low in experimental diet fed troughs than in control. This might be because of the use of nitrifying bacteria in the form of probiotics. As these bacteria are known to convert ammonia to nitrite and then to nitrate, low levels of ammonia and nitrite were observed.

After 60 days of feeding trail, fish growth and conversion efficiency of both feed and protein were assessed to understand the impact of incorporating of probiotics in the experimental diets for *L. rohita* fingerlings. The differences in growth and survival of number of bacterial colonies from the gut of fingerlings *Labeo rohita* between control and treated groups (**Table 2**) could be attributed to the quality of diets. The study revealed that the commercial water probiotic 'Eco pro' has beneficial effect on growth of the experimental fingerlings. The beneficial activity of 'Eco pro' is ascribed to the beneficial activity of its component bacteria *Rhodopseudomonas palustris*. *Rhodopseudomonas palustris* is one of the phototrophic purple non-sulfur bacteria (PNSB) that belong to the class α-proteobacteria (Imhoff J.F., 2006). This bacterium is widely distributed in various aquatic ecosystems as well as in sediments, moist soils, natural wetlands, and paddy fields (Hiraishi et al., 1984; Oda et al., 2002; Roper et al., 1995). *R. palustris* is able to grow under photoautotrophic, photo heterotrophic, chemoautotrophic, and chemo heterotrophic conditions and may play an important role in the nutrient cycles of natural environments (Hunter et al., 2008; Larimer et al., 2004; Sasikala et al., 1998). Due to its extraordinary metabolic versatility, *R. palustris* has been extensively used in industries for bioremediation and sewage treatment (Harrison et al., 2005; Harwood et al., 1988; Karpinets et al., 2009).

Regarding the growth effects of commercial and formulated probiotic feed, significantly higher ($P<0.05$) levels of growth assessing parameters found for the fingerlings fed the experimental feeds as compared to the control groups clearly demonstrate the potential role of probiotics. The dietary supplementation of 'Eco pro' also influenced digestion and assimilation of nutrients in *L. rohita* fingerlings, measured in terms of FCR and PER compared to group fed with control diet. At the end of the experimental period (60 days), the group of fingerlings fed the experimental diets grew as well or better than the group of fingerlings fed the control diet. Whereas, the final body weight of the fish groups fed on diets 2 and 3 had significantly higher compared to diet 1, control diet fed fingerlings. The specific growth rate (SGR % day^{-1}) values are calculated and are depicted in **Table 3.**

It can be concluded that the prepared experimental feed with probiotic 'Eco pro' improves the water quality as well as enhances the growth of fingerlings rohu (*L. rohita*). The prepared experimental diet used in the present study incorporated with Vitamin C (2g/day) is useful to optimize the growth of *Labeo rohita* fingerlings.

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