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EFFECT OF WATER PROBIOTICS ON THE GROWTH PERFORMANCE OF INDIAN MAJOR CARP LABEO ROHITA FINGERLINGS

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ABSTRACT

The present study is aimed to determine the growth performance of Indian major carp *Labeo rohita* fingerlings for a period of 60 days. 15 days aged fingerlings divided into two experimental groups E1, E2 and control (C). Two experimental groups incorporated with supplements like water probiotic Eco-Pro with Vitamin-C as E1, water probiotic Eco-Pro with groundnut oil cake and Vitamin-C as E2 compared with control. The maximum increase in length, weight and width of *Labeo rohita* fingerlings 6cm, 23 grams and 4cm respectively was observed in E2, 4% concentration of water probiotic Eco-Pro food supplement has given maximum Food Conversion Ratio (FCR) (0.01) and P.E.R (0.62) S.G.R (0.85). The food conversion ratio is strongly significant with P.E.R and S.G.R. The supplementation of probiotic in combination with Vitamin-C have proved remarkable effect on the amount of protein, lipid and carbohydrates, when compared to alone probiotic supplementation and also controlled *Labeo rohita* fingerlings. Hence it is concluded that the supplementation of probiotics in combination with vitamin-C and groundnut oil cake will be most preferable feed to obtain healthy with significant specific growth rate of fish to improve economic status of fish farmers as well as consumers.

KEYWORDS: water probiotic (Eco-Pro) specific growth rate (SGR), food conversion ratio (FCR), Protein Efficiency Ratio (PER), growth performance, *Labeo rohita*, vitamin-C, Groundnut oil cake.

1. INTRODUCTION

Aquaculture is now recognized as the fastest growing food producing sector ensuring both nutritional and livelihood security of millions (Mohammad Abdul Hassan et al, 2015).

According to food and agriculture organization (FAO) the world harvest in 2012 consisted of 91.3 millions tones captured by commercial fishing in wild fisheries, 158 millions tones produced by aquaculture (FAO 2014). Probiotics have already become a significant direction as an alternative to antibiotic treatment for aquaculture have been commercially available as feed or water additives in water (Wang et al 2005). The term of probiotic, originating from the Greek word "pro and bio's" means (for life), and was firstly created by limey and still well in 1965. The term probiotic defined as "live microbial feed supplements which when administered in adequate amount beneficially affect the host by improving microbial balance" (fuller 1989). The use of probiotics in aquaculture has not only resulted the reduction of use of harmful antimicrobial compounds, particularly the antibiotics, but also improved the bio grow performance of the farmed species in an eco-friendly and sustainable manner (Gatesoupe; 1999). In aquaculture to improve water quality, fish raisers really concentration on remove of toxic material from water. The mechanisms of actions to improve on water quality is still needed. There is considerable interest in use of probiotics to improve conditions in pond aquaculture (Mai D. Ibrahim 2015). The high mortality and contagious nature of disease in large amount of antibiotics one often used for therapy. 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).

Labeo rohita is an important fresh water fish species usually cultured in Asia mainly in the Indian sub continent (Khan et al 2004). Majority of the probiotics are non pathogenic and non-toxic and can survive in the gut and remain potent for long period under storage and field condition (Ramakrishna et al; 2008). 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 suspicious for the enhancement in water quality and significant growth of fingerlings of Rohu *(Labeo rohita)* in fresh water pond.

2. MATERIAL AND METHODS

2.1 Source of fish

The fingerlings for this experiment were collected from the government fishery department near Manchineelakunta, Tirupati in chittoor district, Andhra Pradesh, India, were brought and acclimatized to the laboratory environment (Figure-1).

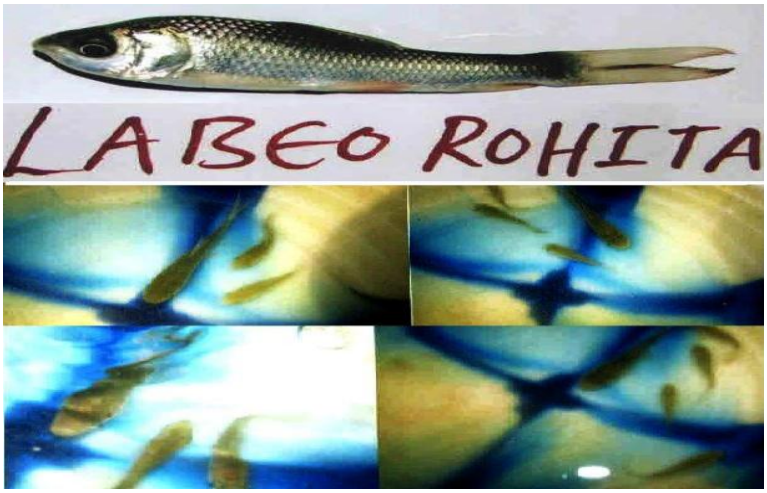

Fig: 1. Fingerlings of *Labeo rohita* (Hamilton).

2.2 Acclimatization

Fingerlings of fish were collected from local fisheries department as for the standard piscicultural procedures and were kept in plastic troughs for a week with sufficient aeration and dechlorinated water to acclimatize them to laboratory (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, Andhrapradesh, 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 used for treatment-1 (Diet-1 control diet); another is supplementary diet prepared by us 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) + soya bean meal (100g) +fish meal (40).
Diet 2: Experiemental diet -1 is prepared by using the following ingredients, control diet (10g) +Water probiotic " Eco-Pro" (2g) + Ground oil cake (3g) + vitamin –C (2g).
Diet-3: Experimental diet is prepared by using the following ingredients (Control diet (10) + water probiotic " Eco-Pro" (2g) + Ground nut oil cake (3g) + vitamin-C (2g).

Experimental duration**:** 60 days Fish size: fingerlings (6gm) No. of Replicates: 6 NO's, Number of fish in each replicate: 10 No's.

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

Diet 2: Experimental Diet1 Figs. 3 Diet 1: Control Diet.

2.5 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.

Experimental Animal and design

Fingerlings of *Labeo rohita* were procured from the government fishery department near Manchineelakunta Tirupati in chittoor district, Andhra Pradesh, India, and were brought to the laboratory in polythene bags filled with oxygen. The fingerlings were very carefully released into the plastic troughs (25 it capacities) from polythene bags for acclimatization of the fish fingerlings (fish troughs were washed to avoid fungal contamination and then sundried). *Labeo rohita* fingerlings weighing were grouped into three each with control, water probiotic 'Eco-Pro' with vitamin-C as E1, water probiotic ' Eco-Pro' with groundnut oil cake, and vitamin-c as E2. Growth performances were maintained with FCR and SGR, PER. The fishes were fed twice at 9.00 hrs and 16 hrs daily according to body weight.

3.1 Preparation and packaging of feeds

The collected feed ingredients were grounded thoroughly and served to pass through 0.5 mm Mesh. The ingredients were mixed according to the formulation control only feed. Water probiotic with vitamin-C as E1 and water probiotic with groundnut oil cake and vitamin-C as E2 the ingredients were put into the manually operated pellet machine for the preparation of pelleted feed of size 1 mm.

3.2 Feeding and sampling

The experimental feeds were supplied daily morning at 9.00 AM and evening at 5.00 PM at a rate of 3% of the body weight sampling was done at an interval of 15 days to regulate the feeding rate and the weight of the fish was measured with portable electronic balance.

3.3 Morpho metric measurement of the fingerlings

At every 15 days the fishes were measured for wet body weight. After obtaining the data five fish per treatment were sampled, wet weight gain was calculated.

Wet weight gain (g) = final weight (g)/ weight gain (g) feed conversion Ratio (FCR) was calculated

FCR= Total feed consumption (g)

SGR= specific growth rate.

Growth parameters

1. Percentage Net weight gain

Variation in Net weight gain was calculated by the method of (sambhu and Jayaprakash 2001) initial and final average Net weights were recorded using a standard balance. The net weight gain (g) was calculated as the difference between initial and final average wet weights (m).

$$\text{Net weight gain} = \frac{\text{final weight} - \text{intial weight}}{\text{intial weight}} \times 100$$

2. Specific growth rate (SGR)

Specific growth rate was determined by the method of (Ravi et al; 1998). After getting the initial and final weights, the specific growth rate was calculated by using the following formula.

$$\text{Specific growth rate} = \frac{\text{Ln (final weight} - \text{Ln (intial weight)}}{\text{experimental periods in days}} \times 100$$

3. Food conversion Ratio

FCR because feed is expensive, feed conversion Ratio (FCR) or feed efficiency (FE) is important calculations for the grower. They can be used to determine. If feed is being used as competently as possible. FCR is calculated as the weight of the feed fed to the fish separated by the weight of fish growth (Mustafa and Ridzwan 2000).

$$\textbf{Formula:}\ \text{Food conversion Ratio (FCR)} = \frac{\text{Feed given(dry weight)}}{\text{Body weight gain(wet weight)}}$$

4. Length

Variations in length were measured by the method (Ravi et al; 1998). Initial and final lengths were recorded using standard scale (1 mm gradation Gain in length (cm) was calculated by subtracting values of initial length from those of final length.

Formula: Length (L) = Final length – initial length

5. Protein Efficiency Ratio: (PER) this was calculated by using the relationship. The increase in the body weight of fish and consumed according to the methods of Zeitoun et al. (1976).

$$\text{Protein Efficiency Ratio(PER)}: = \frac{\text{Total weight gain (g)}}{\text{Dry weight of protein fed (g)}}$$

Table 1: proximate composition (%) of the experimental Diets.

Ingredients	Control Diet (grams)	Diet E1 Pro +Vit -C	Diet E2 Pro+ Vit-C + Groundnut oil cake
Fish meal	25 g	25 g	25 g
Soya bean meal	15 g	15 g	15 g
Groundnut oil cake	- - -	- - -	16.5 g
Rice bran	12.0 g	12.0 g	12.0 g
Probiotic	- - -	0.5 ml	0.5 m1
Vit -C	0.2 g	0.2 g	0.2 g

FCR (Feed conversion Ratio) when feed conversion Ratio and specific growth rate were worked out the fishes of E1 showed significantly high body weight at control fishes, while total feed input for both the experimental groups showed significant increase over the control values at accomplishment of experiment SGR of E2.

3.4 Growth performance

The growth of *Labeo rohita* was comparatively high in all the treatments than control. The highest net weight gain of was observed in E2 followed by control and E1. However, the lowest being in control the survival rate was cent percent in all the treatments and control. The data regarding growth performance and feed utilization by Labeo rohita fingerlings fed experimental diets were presented in Table (2) and Table (4). Dietary supplementation of ‘ Eco-Pro’ probiotic resulted in better growth performance and feed utilization efficiencies over the control diet fingerlings. Live weight gain percent was highest for the experimental diet-2 fingerlings which differed significantly ($p<0.05$) than that of experimental diet-1 and control Specific growth rate (SGR) showed the similar trend. Feed conversion ratio(FCR) and Protein efficiency ratio (PER) was best for experimental diet-2 fish group which showed significance difference ($p<0.05$) than that of experimental diet-2 and control. Survival was 100% in experimental diet fed groups.

RESULT

In our study, we fed the fishes with combinations of probiotic and various growth parameters i.e. weight gain, SGR and FCR, PER were determined by the effect of water probiotic feed on

growth performance of Labeorohita fingerlings. The control feed, water probiotic with vitamin-C as E1, water probiotic + vitamin- C + Groundnut oil cake E2 with formulated probiotic for 60 days of the fixed diets. The one in corporating probiotic + vitamin-C + Groundnut oil cake E2 produced the highest growth rate 3.46 mg/ day followed by E1 and control and E1 (2.46 mg/ day) SGR (3.16 ± 1.14) and FCR (1.77 ± 0.24) of E2 were significantly (P<0.05) better than the control diet. A positive relationship was noticed between SGR and FCR i.e. maximum SGR corresponded with minimum FCR[Table(2)] feed consumption and feeding rates were also significantly increases in E2 compared to the E1 and control (Table 3). The highest PER observed in E2 followed by control and E1 (Table-4). The highest protein in was observed in E2 and lowest protein in E1 (Table -5).

Table: (2) growth performance of Labeo rohita fingerlings fed different probiotic diets (values are given in means of three replicates ± standard deviation).

Diets	Initial weight (g)	Final weight (g)	Growth (g)	Growth rate (mg/ g) (fish/day)	Specific growth rate (SGR)	Food conversion rate (FCR %)	Protein efficiency ratio (PER %)
Diet–I control DietE1 probiotic + vit-C DietE2 probiotic +vit-C +Groundnut oil cake	0.269a±0.07 0.282a±0.01 0.286a±0.01	0.456a±0.02 0.568a±0.001 0.586a±0.001	0.186a±0.01 0.290a±0.001 0.312a± 0.01	2.350a±0.01 2.46a± 0.02 3.46a± 0. 01	1.46a±0.01 1.98b±0.01 3.16b±1.14	1.76b±0.02 1.88a±0.01 1.77a±0.04	1.02±0.01a 1.33±0.02b 1.56±0.01c

Means with in columns with different letter are significantly different (P<0.05)

Table 3: over all energy budgets of Labeo rohita fingerlings fed different probiotic diets (values are given in means of three replicates ± standard deviation).

Feed types	Total food consumption (g) (FC)	Feeding rate mg/g Fish /day	G.E.C%(Gross Efficiency Conversion)
Control	72.40a ± 1.10	22.00a ± 1.10	22.23a ± 1.59
Diet E1	72.15a ± 1.41	24.28a ± 1.02	34.00a ± 1.17
Diet E2	78.16b ± 1.58	28.00a ± 0.74	36.00b± 0.77

Means with in columns with different letter are significantly different (P<0.05).

Table 4: Protein budget of Labeo rohita fingerlings fed different probiotic diets values are given in means of three replicates ±standard deviation.

Types of feed	Protein intake (%) mg/day	Protein efficiency ratio (per %)	Average net protein Utilization (Ampu %)
Control	40.00b ± 1.63	0.39a ± 0.02	24.50ab ± 1.28
Diet E1	38.23a ± 1.66	0.48a± 0.02	25.04a ± 1.45
Diet E2	36.5a ± 1.27	0.62a ± 0.01	27.20b± 1.6

Means with in columns with different letter are significantly different (P<0.05).

Table 5: Different feed composition of Labeo rohita fingerlings feed probiotics diets (values are given means of three replicates ± standard deviation).

Feed types	Protein (%)	Carbohydrates (%)	Lipid (%)
Initial (control)	36.00 ± 1.52	14.10 ± 1.52	8.11 ± 1.53
Diet E1	39.00 ± 1.44	18.00± 1.2	11.00 ± 1.08
Diet E2	40.50± 1.96	20.46 ± 1.93	14.00± 1.52

DISCUSSION

Probiotics are bio-preparations containing living microbial cells that optimize the colonization and composition of the growth and gut micro flora in animals and stimulate digestive process and immunity (Bomba et al; 2002). The present study confirms the result from other studies that the incorporation of probiotics in the diets can improve growth performance in terms of SGR, FCR and FR (Gate Soupe 1991) reported and vitamin-C and Groundnut oil cake. The result of this growth promoting effects on Labeo rohita fingerlings which is an accordance with reports of (wtto et al; 1994, Chaudhari and Razi 2007). There are various other benefits of using probiotics for fish aquaculture Indian major carps (swain et al; 1996). Result of present study on *Labeo rohita* fingerlings revealed that vitamin-C supplement resulted in significant increase in length, weight, Food conversion ratio (FCR) and Specific growth rate (SGR), when fed on diet with combination of Vitamin- C (Ashraf et al; 2008). Reported that administration of Vitamin-C enhanced growth survived and immune resistance in *Labeo rohita* fingerlings and hence use of Vitamin-C supplementation in the diet has manifold benefits like growth promotion, survival and enhancement of immunity. Hence diet supplementation of needs to be considered for successful and sustainable aquaculture of economically important Indian major carps Vitamin-C is essential for normal physiological functions in animals including fishes (Wilson and Poe 1973 lim and Lovell 1978). Vitamin-C is a biological reducing agent and is involved in many intra and extra cellular processes, including assembly of collagen by hydroxylation of tryptophan, tyrosine and proline for use in cartilage synthesis collagen which is a principal constituent of shrink, scales, mucous,

cartilaginous and conjunctive tissue formation is heavily dependent on body storage of this vitamin-c and its supply from outside sources. It is involved in carnitine and adrenal steroids synthesis and detoxifies pesticides and other toxicants using cytochrome P-450 system. (De Silva and Anderson 1995) vitamin-c also plays a critical role in its repair and wound healing (Halver 2002). The capacity of biosynthesis of vitamin-c due to absence of an essential enzyme gluco - lactono oxidizes (Fracalossi et al; 2001). Deficiency of vitamin-c result in impaired collagen formation, spinal reformation, hemorrhage and growth retardation (Halver et al; 1969, Al amaudi et al; 1992 coustans et al; 1998)[probiotic bacteria are known to improve water quality in many ways, Heterotrophic bacteria necessitating some organic source of carbon in addition to inorganic forms for growth have a significant role in the decomposition of organic matter and production of particulate food materials from dissolved organics.[Jana & De 1990].

The probiotic supplement fish[E2] are mainly the outcome of stimulation by probiotic itself enzyme produced to synthesize endogenous digestive enzyme which might have improved nutrient digestibility leading to better growth performance and efficiency in fish. Similar observations have also been reported for other fishes in which the nutrient digestibility increased considerably with use of probiotic supplemented diet (Lara Flores et al, 2003 Yanbo & Zirong 2006). Groundnut oil cake is alternative protein source. It is deficient in some vital amino acids such as lysine and methionine that are present in fish meal (Eyo olatinde, 1998). Groundnut oil cake is a valuable source of vitamin E/k and B (FAO 2000). 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; 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 (Labeo 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). study of revealed that the commercial water probiotic ‘Eco-Pro’ has beneficial effect on growth of the experimental fingerlings. The beneficial effect activity of ‘ Eco-Pro’ is ascribed to the beneficial activity of its component bacteria Rhodopseudomonas palustris is one of the phototrophic purple non –sulfur bacteria (PNSB) that the belong to the class Alpha proteobacteria (Imhoff J.F., 2006). This bacterium is widely distributed in various aquatic ecosystems as well as in sediments, moist soils, natural water

lands and paddy fields. (Hiraishi et Sal., 1984; oda et al., 2002; Roper et al., 1995). Rhodopseudomonas palustris is able to grow under photoautotrophic, 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). Proximate composition (%) of the experimental diets it divided into three groups that are control diet, diet E1 and diet E2 it shows (Table -1). The growth effect of commercial and formulated feed, significantly higher ($p<0.05$) levels of growth asseing parameters found for the fingerlings fed the experimental fees as compared to the control groups clearly demonstrate the potential role of probiotics. The dietary supplementation 'Eco-Pro' also influenced digestion and assimilation of nutrients in *Labeo rohita* fingerlings, measured in terms of FCR and PER compared to group fed with control diet. The end of the experimental period (60 days), the group of fingerlings fed the control diet. The finally body weight of the fish groups fed on diets 1 and 2 had significantly higher compared to control diet fed fingerlings the SGR and FCR, PER values calculated and are depicted in (Table-2). Feed consumption feeding rates were also significantly increased in E2 and compare to the E1 and control (Table-3). The highest PER observed in E2 compared to the E1 and control (Table -4). The highest protein was observed in E2 and lowest protein in E1 (Table-5).

CONCLUSION

It can be concluded that the prepared experimental feed with probiotic 'Eco- Pro' improve the water quality as well as enhance the growth of fingerlings rohu *(Labeo 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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