International Journal of Science, Environment and Technology, Vol. 5, No 5, 2016, 3038 – 3047

ISSN 2278-3687 (O)
2277-663X (P)

GROWTH PROMOTING AND IMMUNOSTIMULATORY EFFECTS OF FEED PROBIOTIC (PROTEUS) IN *LABEOROHITA* (HAMILTON) FINGERLINGS

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Abstract: The present study deals with the "Growth Promoting and Immunostimulatory Effects of Feed Probiotic (Proteus) in *Labeo rohita* (Hamilton) Fingerlings". The experiment was conducted in triplicate for each test diet. During experiment of 60 days, the fingerlings of rohu (*Labeo rohita*) were fed on four different probiotic (Proteus), mixed in basal diet containing rice bran, groundnut cake, fish meal and mineral mixture. The four doses of probiotic (Proteus) have been mixed with conventional carp diet at T_1 2g/kg, $T_2$3g/kg, T_3 4 g/kg,$T_4$5g/kg and Control 0g/kg of Probiotics were used. The control diet containing rice bran, groundnut cake, fish meal and mineral mixture was also run separately. These experimental feeds were given to fishes @4% of body weight per day. The growth performance of test fishes was observed at every fortnight and accordingly the diet of fishes was readjusted. During experimental period, the water quality parameters were found congenial for fish growth.

Keywords: Growth, Immunostimulatory, Probiotic, *Labeo rohita,* feed.

Introduction

In aquaculture feed accounts for bulk of the total cost and therefore hold key of success of fish farming. These growth point of view fish nutrition has great relevance to fish farming of intensive and semi intensive types. For achieving good growth of fish the feed must be nutritiously balanced with moderate amounts of protein, carbohydrate and minerals as deficiency for these ingredients in the supplementary diets may result ailment in the body of fish for growth. Considering this, search for ideal fish feed is growing process wherein, several attempts have been made so far upgrading the quality of supplementary fish diet for culturable fish.

Nowadays, probiotics are becoming an integral part of the aquaculture practices to obtain highproduction. Probiotics are live microorganisms thought to be beneficial to the host organism. The involvement of probiotics in nutrition, disease resistance and other beneficial

*Received Aug 13, 2016 * Published Oct 2, 2016 * www.ijset.net*

activities infish has proven beyond doubt. Indian freshwater aquaculture constitutes mainly culture of Indian Major Carp'sviz. (*Catla catla, Labeo rohita, Cirrhinus mrigala*). Carp culture in India constitutes 87% of total aquaculture production (Ayyappan and Jena, 2003). *Labeo rohita*is the most commercial fish with maximum market demand and acceptability as food by the consumers due to its taste and flesh quality in India.

Proteus is an optimum blend of feed probiotic of colony forming unit's counts of selected strains of Proteusspp. and entire spectrum of beneficial microorganisms for maintaining an optimal fish culture practices. They also help in decomposition of excess waste organic matter and suppress the growth of undesirable microbes in the pond and on the hosts, besides having an activity inside the gut. For pursuing such type of research probiotic (Proteus)have been taken as growth promoting ingredients in the traditional carp feed i.e. rice bran, groundnut cake, fish meal and mineral mixture for the rohu (*Labeo rohita*) fingerlings. These above four probiotic doses have been mixed with conventional carp diet at T_1 2g/kg, $T_2$3g/kg, T_3 4 g/kg,$T_4$5g/kg and Control 0g/kg of feed levels during the experimental period of 60 days, the fingerlings of rohu (*Labeo rohita*) were fed @ 4 % of their body weight per day.

Materials and Methods

This experiment was conducted in rectangular plastic tanks with 200 liters of bore-well water. In each tanks ten rohu (*Labeo rohita*) fingerlings were introduced from a homogenous stock with an average body weight of 4.637±0.15g. These fishes were acclimatized for 7 days prior to commencement of feeding trial with basal diet. Thereafter, the initial weight of fish was noted and food was given @ 4 % of their body weight per day. The quantity of feed required was adjusted accordingly with the increase in their body weight, which was recorded at every 15^{th} day intervals. The growth parameters of fishes were monitored periodically, but haematological parameters were observed initially and at the end of experiments.

Preparation of Diet

Carp feed (with 24% protein) was formulated using groundnut cake, rice bran, fish meal and mineral mixture and a commercially available probiotic (Proteus). The treatment tank (T_0) considered as a control which was without probiotic, while groups T_1, T_2, T_3 and T_4 was included with probiotic and added at @ 2g/kg, 3g/kg, 4g/kg and5g/kgof feed levels respectively.

Table: 1 Proximate composition of diet

S. No.	Parameter	Control	T_1	T_2	T_3	T_4
1	Moisture (%)	7.32	6.620	6.380	7.28	7.41
2	Crude protein (%)	23.50	23.66	23.85	23.78	23.48
3	Fat (%)	7.20	7.08	7.15	7.25	7.23
4	Ash (%)	9.45	10.08	9.75	9.12	9.29

Growth parameters of Fish

Growth measurement: Following growth parameters were studied using standard methods.

Growth:

Growth was measured as gain in body weight and also per cent gain in body weight.

Weight Gain

Weight gain was determined between the final weight and initial weight of experimental fish.

Weight gain (g) = Final weight (g) - Initial weight (g)

$$\%\ \text{gain weight in g} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Specific Growth Rate

$$\text{SGR}\ (\%) = \frac{(\ln \text{Wt} - \ln \text{Wo})}{\text{D}} \times 100$$

Where,

Wo = Initial weight of live fish (gm), Wt = Final weight of live fish (gm), D= Duration of feeding (days)

Feed Conversion Ratio (FCR):

$$\text{FCR}\ (\text{g}) = \frac{\text{Weight of food given (g)}}{\text{Weight gain of fish (g)}}$$

Gross conversion efficiency (GCE):

$$\text{GCE} = \frac{\text{Weight gained (g)}}{\text{Food given (g)}}$$

Proximate Analysis:

The proximate composition for experimental fish carcass was measured according to AOAC [1970] methods.

STATISTICAL ANALYSIS

The statistical analysis of the measured parameters was carried out using standard statistical methods to draw meaningful conclusion.

Results:

The experimental results of water quality parameters, fish growth parameters and haematological parameters carried out during the experimental period are presented in (Tables 2 to 8). It is apprehended from the results of the present study that the fish growth parameters and haematological parameters were significantly different in all the treatments and control.

Growth parameters

Different doses of Proteus - a probiotic supplementation, revealed significant differences in growth rates among various treatments.

Weight gain

The diet T_3 bearing 4 g/kg gave the highest weight gain of (4.902 g), whereas, the lowest (3.090 g) being in (T_0) control (Table 2). The weight gain of fishes was noticed in the following ranking order T_3 (4.902) > T_4 (4.100) > T_2 (3.965) > T_1 (3.618 g) > T_0 (3.090). The total weight gain in per cent was highest (105.419 %) in T_3 as compared to (69.283 %) in Control. Whereas, the weight gain in T_4, T_2 and T_1 were 86.19 %, 82.43 % and 80.26 % respectively.

Statistical analysis of variance indicated that weight gain was significantly higher in all the treatments as compared to control. Moreover, the test of critical difference (CD) also indicated significantly difference CD = 0.188 between all the treatments in 0-60 days (Appendix-I). Similarly, the per cent weight gain in 60 days of feeding trial has also shown a statistically significant (CD = 6.619) increase in weight gain as compared to control).

Increase in length

The diet T_3 resulted in the highest increase in length of fish i.e. 5.531 cm, whereas, the lowest increase (3.104 cm) was recorded in T_0 i.e. control (Table 3). The diet (T_3) containing 4g/kg 'Probiotic (Proteus)' showed highest increase in length among the treatments followed by T_4 (4.017 cm), T_2 (3.981 cm) and T_1 (3.532 cm). Similarly, the total length increase in per cent was also higher in T_3 (71.893 %) followed by T_4 (53.041 %), T_2 (52.382 %), T_1 (49.746 %) and lowest in T_0 (41.204 %) i.e. control (Table 3).

Statistical analysis of variance indicated that length increase is significantly higher in all the treatments as compared to control. Moreover, the test of critical difference (CD) also indicated significant difference (CD= 0.315) between all the treatments. Similarly, the per cent length increase in 60 days of feeding trial has also shown a statistically significant (CD= 4.179) increase in length as compared to control.

Specific growth rate (SGR)

The value of specific growth rate is used to compare growth on a daily basis. The significantly higher growth rate and specific growth rate indicate the effective role of probiotics in the growth performance. The highest SGR (0.521%) was recorded in treatment T_3 (diet with 4g/kg Proteus supplement) after 60 days of experiment which was significantly different from T_0,T_1, T_2, and T_4($P = 0.05$). The treatments mean revealed that the best SGR was recorded in T_3 (0.521%), followed by T_4 (0.450), T_2 (0.435), T_1 (0.427) and lowest (0.381) in Control (Table 4) Thus, the treatment T_3 showed best SGR which was significantly ($P = 0.05$) high as compared to control T_0as well as all other treatments.

Feed given

The amount of feed given to the fishes in 0-60 days, duration of investigation as per requirements. The maximum amount of food was given to the fishes in T_3 i.e., 14.5 g followed by T_4 (14.0 g), T_2 (13.9 g), T_1 (13.0 g) and T_0 (12.7 g) respectively.

The statistical analysis of variance indicates the amount of feed given was significantly higher from control to all other treatments.

Food conversion ratio (FCR)

The results on FCR in different treatment groups have been depicted in Table 5. The lowest FCR was found with T_3(diet with 4g/kg. Proteus powder) which was significantly different from T_0,T_1, T_2, and T_4 ($P = 0.05$). The mean values of treatments revealed that the best FCR was recorded with T_3 (3.207), followed by T_4 (4.207), T_2 (4.653) and T_1 (4.765) and T_0 control (5.195). Thus, treatment T_3 showed best FCR which was significantly ($P = 0.05$, CD= 0.369) high as compared to control T_0as well as all other treatments during 0-60 days of feeding experiment.

Gross conversion efficiency (GCE)

The results on gross conversion efficiency in different treatment groups have been depicted in Table 6.The best GCE (0.330) was recorded in treatment T_3 (diet with 4g/kgProteus)followed by T_4 (0.282), T_2 (0.273), T_1 (0.267) and lowest in T_0 (0.236).The statistical analysis of variance also indicated significant deference ($P = 0.05$, CD= 0.022) of GCE between treatments and control during 0-60 days of experimental period.

Proximate Composition of Fish Carcass

The proximate composition of fish carcass has been assessed after the completion of experimental period, which has been shown in Table 7. The results have shown significant changes in the proximate composition of fingerlings fed with probiotic (Proteus) diets. The

protein, contents increased significantly in T_1, T_2, T_3 and T_4 as compared to control. The highest protein contents were recorded in T_3 (24.06%) followed by T_4, T_2, T_1 and Control, 22.82%, 22.75%, 20.64% and 20.22%, respectively. It is noteworthy that the protein values of fingerlings fed with basal diet was the lowest as compared to that of fingerlings fed with probiotic (Proteus) diets. That fat content was also found higher in the fingerlings fed with Probiotic (Proteus) diets as compared to that of control diet.

However, fat content of Control was higher (6.90 %) and lowest in T_3 (6.61%) followed by T_1, T_2, and T_4 i.e., 6.76, 6.74%, and 6.72, respectively. The carbohydrate values of Control, T_1, T_4, T_2 and T_3, were 3.71%, 3.34%, 2.25%, 1.26%, and 0.64% respectively. The moisture contents of whole fish were found in the order of $T_2 > T_0 > T_1 > T_3 > T_4$ where the moisture values of 66.77%, 66.45% 66.26%, 66.08% and 65.50%, respectively were observed. The highest Ash contents recorded in treatment T_1, (3.00%), followed by Control, (2.72%), T_4, (2.71%), T_3 (2.61%) and lowest in treatment T_2 (2.48%) respectively.

Discussions

In general, the growth (net weight gain) in the control and other treatments in the present study was in following order:T_3 (4.902 g) > T_4 (4.100 g) > T_2 (3.965 g) > T_1 (3.618 g) > control (3.090 g). The per cent weight gain increase in treatments as compared to the control was as follows:T_3 (71.893 %) > T_4 (53.041 %) > T_2 (52.382 %) > T_1 (49.746 %) > control(41.204 %)

The mean values of treatments revealed that the best SGR was recorded in T_3 (0.521%), followed by T_4 (0.450), T_2 (0.435), T_1 (0.427) and lowest (0.381) in T_0 (control). The results are in conformity with the findings of Bisht and Pandey (2013) who found higher SGRas compared to control in *Labeo rohita* fed with *Bacillus subtilis* incorporated diets for 90 days. Sahu *et al*. (2007) and Dhawan and Kaur (2002) have also found that the provision of each additional input including fertilization such as cowdung, urea and single super phosphate, and supplementary feed did affect fish growth**.**

The mean values of treatments revealed that the best FCR was recorded with T_3 (3.207), followed by T_4 (4.207), T_2 (4.653) and T_1 (4.765) and T_0 control (5.195). Ramos *et al.* (2013) have conducted an experimentto evaluate the effect of dietary supplementation of multi species (*A. bacillus sp., Pediococas sp., Enterococcus sp., Lactobacillus sp.*) and single species probiotics *(B. pediococcusacidilactici*) on growth performance and gut microbialcomposition of rainbow trout (*Onchorhynchusmykiss*). The results showed that dietary supplementation changed the gut microbial composition and improved growth in the

fish. De Silva and Davy (1992) have stated that digestibility of fish plays an important role in lowering the FCR value by efficient utilization of food. The digestibility depends on daily feeding rate, its frequency, and the type of food used (Chiu *et al*., 1989). The wastage of food might also lead to poor feed utilization and higher FCR.

In the present study the best GCE (0.330) was recorded in treatment T_3 (diet with 4g/kg. Proteus) followed by T_4 (0.282), T_2 (0.273), T_1 (0.267) and lowest in T_0 (0.236). Suzer *et al.* (2008) in his study investigated the influence of commercial probiotic supplementation on the larval stages of Gilthread Sea bream (*Sparusaurata*, L.). Both growth performance and digestive enzyme activities increased in the treatment as compared to control. This is in agreements with those of Steffens (1989). Thus, it is evident that the diet T_3 (diet with Proteus 4 g/kg) has a great potential in aquaculture as a growth promoter for *Labeo rohita* (Ham.) fingerlings. In view of the above discussion and results it may be concluded that the growth (net weight gain) and its indices such as SGR, FCR and GCE are found to be favorable in T_3 with diet having Proteus 4 g/kg was incorporated in diet of *Labeo rohita* (Ham.) fingerlings. Hence, it is inferred that this diet T_3 is optimum under agro-climatic condition of southern Rajasthan.

Acknowledgement

Authors record their sincere thanks to Dean, College of Fisheries for their encouragement and for extending facilities for conducting this research.

Table: 2 Average weight gain of Rohu, *Labeo rohita* (Ham.) fingerlings fed with varying levels of Probiotic (Proteus) mixed diet

S. No.	Treatment	Initial weight (g)	Fish weight gain (g)				Total net weight gain (g)	Percent weight gain
			0 -15th day	16th - 30th day	31th -45th day	46th- 60th day		
1.	Control	4.460	0.265	0.738	0.990	1.097	3.090	69.283
2.	T_1	4.507	0.283	0.764	1.227	1.344	3.618	80.275
3.	T_2	4.810	0.315	0.786	1.410	1.454	3.965	82.432
4.	T_3	4.650	0.595	1.177	1.428	1.702	4.902	105.419
5.	T_4	4.757	0.365	0.987	1.311	1.437	4.100	86.189
CD at 0.05 %		0.221	0.017	0.040	0.064	0.068	0.188	6.619
CV %		2.62	2.53	2.49	2.76	2.66	2.63	4.29

Table: 3 Average increase in length of Rohu, *Labeo rohita* (Ham.) fingerlings fed with varying levels of Probiotic (Proteus) mixed diet

S. No.	Treatment	Initial length (cm)	Fish length (cm)				Total net increase in length (cm)	Percent increase in length
			0 -15th day	16th - 30th day	31th - 45th day	46th-60th day		
1.	Control	7.533	0.270	0.741	0.994	1.099	3.104	41.204
2.	T_1	7.100	0.285	0.767	1.131	1.349	3.532	49.746
3.	T_2	7.600	0.317	0.789	1.415	1.460	3.981	52.382
4.	T_3	7.693	0.597	1.791	1.434	1.709	5.531	71.893
5.	T_4	7.573	0.367	0.891	1.317	1.442	4.017	53.041
CD at 0.05 %		0.351	0.017	0.045	0.065	0.070	0.315	4.179
CV %		2.58	2.53	2.47	2.85	2.72	4.30	4.28

Table: 4 Specific growth rate (SGR) of Rohu, *Labeo rohita* (Ham.) fingerlings fed with varying levels of Probiotic (Proteus) mixed diet

S. No.	Treatment	Specific growth rate (SGR) of fingerlings (g/day) in per cent				
		0 -15th day	16th -30th day	31th -45th day	46th-60th day	Average SGR
1.	Control	0.167	0.420	0.482	0.455	0.381
2.	T_1	0.176	0.428	0.578	0.524	0.427
3.	T_2	0.184	0.413	0.619	0.525	0.435
4.	T_3	0.349	0.586	0.581	0.568	0.521
5.	T_4	0.214	0.510	0.563	0.513	0.450
CD at 0.05 %		0.010	0.022	0.029	0.025	0.035
CV %		2.54	2.51	2.78	2.66	4.32

Table: 5 Food conversion ratio (FCR) of Rohu, *Labeo rohita* (Ham.) fingerlings fed with varying levels of Probiotic (Proteus) mixed diet

S. No.	Treatment	Food conversion ratio (FCR) of fingerlings				
		0 -15th day	16th -30th day	31th -45th day	46th-60th day	Average (FCR)
1.	Control	10.098	3.841	3.311	3.529	5.195
2.	T_1	9.555	3.762	2.716	3.027	4.765
3.	T_2	9.162	3.912	2.515	3.021	4.653
4.	T_3	4.689	2.674	2.698	2.767	3.207
5.	T_4	7.820	3.114	2.796	3.098	4.207
CD at 0.05 %		0.413	0.175	0.125	0.150	0.369
CV %		2.75	2.78	2.45	2.66	4.60

Table: 6. Gross conversion efficiency (GCE) of Rohu, *Labeo rohita* (Ham.) fingerlings fed with varying levels of Probiotic (Proteus) mixed diet

S. No.	Treatment	Gross conversion efficiency (GCE)of fingerlings				Average
		0 -15th day	16th -30th day	31th -45th day	46th-60th day	
1.	Control	0.099	0.260	0.302	0.283	0.236
2.	T_1	0.105	0.266	0.368	0.330	0.267
3.	T_2	0.109	0.256	0.398	0.331	0.273
4.	T_3	0.213	0.374	0.371	0.361	0.330
5.	T_4	0.128	0.321	0.358	0.323	0.282
CD at 0.05 %		0.006	0.013	0.018	0.016	0.022
CV %		2.53	2.51	2.80	2.67	4.32

Table: 7. Proximate composition of whole body of *Labeo rohita*(Ham.) fed with graded levels of probiotic (Proteus)

S. No.	Treatments	Proximate composition of fish (in %)				
		Moisture	Fat	Protein	Ash	NFE
1.	Control	66.45	6.90	20.22	2.72	3.71
2.	T_1	66.26	6.76	20.64	3.00	3.34
3.	T_2	66.77	6.74	22.75	2.48	1.26
4.	T_3	66.08	6.61	24.06	2.61	0.64
5.	T_4	65.50	6.72	22.82	2.71	2.25

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