Biological Forum – An International Journal **13(1): 798-805(2021)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Effect of Probiotic on the Growth Status of Rohu (*Labeo rohita*)

N. Das[1*], S. Das[2] and B.K. Khuntia[1]
[1]College of Fisheries (OUAT), Rangailunda, Berhampur (Odisha), India.
[2]Krishi Vigyan Kendra, Jagatsinghpur (Odisha), India.

(Corresponding author: N. Das)*
(Received 11 March 2021, Accepted 21 May, 2021)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: The yearlings of Rohu (*Labeo rohita*) was fed with commercial pellated feed as T_1(Control), feed incorporated with *Lactobacillus sporogenes* @ 4% as T_2, *Saccharomyces cerevisiae* @ 4% as T_3 and both *Lactobacillus sporogenes* @2% and *Saccharomyces cerevisiae* @ 2% as T_4. The experiment was designed for 120 days in the cement tanks. Feeding was done with probiotics and without probiotics at alternate 15 days. Sampling was done at an interval of 15 days. The samples were analysed to determine the weight gain %, specific growth rate %, FCR, FER of fish. The average initial weight of fish in all treatment was about 44 g. After feeding with probiotic incorporated feed, the weight increased to 150.78±0.68 gm, 176.13±0.75g and 183±0.91g in T_2, T_3 and T_4 respectively as against 102.05±0.99g in T_1 (control). The growth in T_4 was more due to may be more colony formation of microbe in the gut of fish after 15 days.

Keywords : Yearlings, Probiotic, Feed, Growth, Rohu.

INTRODUCTION

Aquaculture has made significant advance in recent years in the production of a wide range of aquatic organisms. Indian fisheries and aquaculture is an important sector of food production, providing nutritional security to the food basket, contributing to the agricultural exports and engaging about fourteen million people in different activities. The total fish production in India is 10.07 million metric tonnes presently with nearly 65% contribution from the inland culture fisheries. Over the years aquaculture sector has gone a vast change in order to meet the increasing demand but are still facing lots of problems (Anon, 2014). Some of the problems like quality seed, feed and fertilizer, environmental problems with growing different kinds of diseases, environmental issues, introduction of new species, use of different chemicals and antibiotics etc are facing challenges in front of aqua farming sectors. The traditional aqua farming has been revolutionized into aqua farming industries. The production is maximized through intensification with addition of commercial diets, growth promoters, antibiotics and several other additives. Aquaculture is facing heavy production loss both in hatcheries and grows out systems due to disease outbreak. Fish disease, especially bacterial infection is a major problem in fish farming industry. The use of antibiotics in aqua farms in enormous quantities over the years has attracted varied criticism worldwide, which has resulted in imposition of ban on use of antibiotics in farms. Therefore research is going on in search of alternative mode to prevent disease attack in aqua farms. One promising approach in this regard is to devise various strategies to modulate the composition of the gut micro-biota for better growth, digestion, immunity and disease resistance of the host that have been demonstrated in various fishes. This indiscriminate use of antibiotics has led to bacterial resistance (Cabello, 2006), toxicity and bioaccumulation in fish and environment (Vine *et al.*, 2004). Recently probiotics and immunostimulants have become a useful alternative to chemotherapy and antibiotics in controlling fish diseases.

Probiotics are live microbial feed supplements that beneficially affect the host by producing inhibitory compounds, competing for chemicals and adhesion sites, and modulating and stimulating immune function (Giri *et al.*, 2012). Probiotics are also known to enhance the specific and non specific immune responses (Nayak, 2010). In the aquaculture industry, probiotics species of *Bacillus* (Keysami *et al.*, 2007), *Lactobacillus* (Abraham *et al.*, 2007) and *Saccharomyces* (Rumsey *et al.*, 2007) singly or mixed culture (Mohapatra *et al.*, 2012a, 2012b), are most commonly used. Bacteria are considered to be the most common cause of fish mortality in aquaculture. *Aeromonas hydrophila* affects a wide variety of fresh water as well as marine fish species (Zhou *et al.*, 2010). Probiotics are known to

reduce the disease caused by *A. hydrophila*.

The feed probiotics is defined as live microbial feed supplements that improve health of man, terrestrial livestock and aquatic animal. The gastrointestinal micro biota of fish and shellfish are peculiarly dependent on the external environment, due to the water flow passing through the digestive tract. Most bacterial cells are transient in the gut, with continuous intrusion of microbes coming from water and food. Some commercial products are referred to as probiotics, though they were designed to treat the rearing medium, not to supplement the diet. This extension of the probiotic concept is pertinent when the administered microbes survive in the gastrointestinal tract. Otherwise, more general terms are suggested, like bio control when the treatment is antagonistic to pathogens or bioremediation when water quality is improved. However, the first probiotics tested in fish were commercial preparations devised for land animals. Though some effects were observed with such preparations, the survival of these bacteria was uncertain in aquatic environment. Most attempts to propose probiotics have been undertaken by isolating and selecting strains from aquatic environment. These microbes were Vibrionaceae, pseudomonades, lactic acid bacteria, *Bacillus* spp. and yeasts. Probiotic in the form of single or mixed cultures of selected bacteria with feed are used to modify or manipulate the microbial communities in the gut. The feed probiotics micro flora in the gut play a major role in the digestion of food, helping in the breakdown of complex substances into simpler forms, which can be easily absorbed by the body. Many other beneficial effects may be expected from probiotics, *e.g*., competition with pathogens for nutrients or for adhesion sites, and stimulation of the immune system to improve the health, growth and survival of the host species. The most promising prospects are sketched out, but considerable efforts of research will be necessary to develop the applications to aquaculture. The research of probiotics for aquatic animals is increasing with the demand for environment friendly aquaculture. Among the different species fishes, rohu was selected for the present research work as it is the most popular species among the carp.

From several researches it is proved that probiotics are of immense important in aquaculture in terms of increasing growth rate and disease resistance of fish. Therefore, to meet the increasing demand of animal protein so as to fulfill the requirement of growing population, it is advised to apply probiotics in aquaculture. Now a days, probiotics are used to a greater extent to increase production. But probiotics which are available in the market are too costly. Large farmers are able to utilise probiotics, but it is hardly possible for a marginal farmer to use it in fish culture. Moreover, continuous use of probiotics in fish culture increases the cost of cultivation which increases the expenditure. Keeping in view the above aspects, this research is based on the objective to reduce the cost in probiotic application which would reduce the cost of cultivation and increase the profit of the farmer. In this research Sporolac powder available in the local pharmacy was used as a source of *Lactobacillus sporogenes* and Backers yeast available in the bakery shop was used as a source of *Saccharomyces cerevisiae*. These probiotics were used by incorporating with commercial fish feed. These bacteria and yeast are major contents in commercially available probiotics which are proven very effective in carp culture, especially in rohu culture. Our research is to find out the growth and the time period required for the colonization of that particular bacteria and yeast in the gut of rohu (*Labeo rohita)* which are used as probiotics after application with feed and in the time period without probiotic application.

Although Indian fresh water aquaculture has expanded rapidly over the last three decades, production remains limited to a few fresh water fish species. The three Indian major carps *viz*., catla (*Catla Catla*), rohu (*Labeo rohita)* and mrigala (*Cirrhinus mrigala*) contributes the bulk of the production while the three exotics carps, *viz*., common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idella*) and silver carp (*Hypophthalmichthys molitrix*) formed the second important group. As a result, India is being referred as a carp country, with carps contributing to over 85% of the total aquaculture production in the country (Ayyappan *et al*., 2011). Among all major carps, rohu is the most preferable and most produced one with high flesh to bone ratio. So for our research the selection of species is rohu (*Labeo rohita*) only.

Lactobacillus soporogenes is a lactic acid-forming bacterial species. The organism was first isolated and described as *Bacillus coagulans* in 1915 by B.W. Hammer at the Iowa Agricultural Experiment Station as a cause of an outbreak of coagulation in evaporated milk packed by an Iowa condensary. Separately isolated in 1935 and described as *Lactobacillus sporogenes* in the fifth edition of *Bergey's Manual*, it exhibits characteristics typical of both genera *Lactobacillus* and *Bacillus*, its taxonomic position between the families Lactobacillaceae and Bacillaceae was often debated. However, in the 8th edition of Bergey's Manual of Determinative Bacteriology, spore-bearing rods producing lactic acid, facultative or aerobic and catalase positive are to be classified within the genus Bacillus. *B. coagulans* is a Gram-positive rod (0.9 by 3.0 to 5.0 μm in size), catalase positive, spore-forming, motile, and a facultative anaerobe. It may appear Gram-negative when entering the stationary phase of growth. The optimum temperature for growth is 50°C (122°F); range of temperatures tolerated are 30-55°C (86-131°F). IMViC tests VP and MR (methyl-red) tests are positive.

Lactic acid bacteria (LAB) are Gram positive, non-spore forming, catalase negative cocci or fermentative lactobacilli which produce lactic acid from fermentation of carbohydrates. These bacteria are the major component of the starters used in fermentation, especially for dairy products, and some of them are also natural components of the gastrointestinal microflora. Lactobacillus is one of the most important genera of LAB .Lactobacilli were the first genus of bacteria proved to have beneficial health effects. They have been shown to be present in the gastrointestinal tract of most animals and birds. It is one of many friendly species of intestinal microflora considered as beneficial bacteria in its ability to aid in breakdown of proteins, carbohydrates and fats in food and help absorption of necessary elements and nutrients such as minerals, amino acids and vitamins by the host. They quickly colonized in the gut epithelium to deprive the sites for attachment of pathogens. They were also referred to as "live enzyme factory" as they produce wide range of enzymes, which can breakdown even complex carbohydrates, hence beneficial to the host.

L. sporogenes in the form of Sporlac Sachet is used for Diarrohea in young children, Irritable bowel syndrome, Whitish or yellowish discharge of mucus from the vagina, Vaginal infection and other conditions. Sporlac Sachet may also be used for purposes not listed in this medication guide. Sporlac Sachet contains *Lacto bacillus sporegens* (150 million spores/gm) as an active ingredient. Sporlac Sachet works by maintaining a healthy balance of microflora in the intestine. The medicine is manufactured by the following companies Uni-Sankyo.

Saccharomyces is a genus of fungi that includes many species of yeasts. *Saccharomyces* is from Greek word and means *sugar fungus*. Many members of this genus are considered very important in food production. It is known as the brewer's yeast or baker's yeast. They are unicellular and saprophytic fungi. One example is *Saccharomyces cerevisiae*, which is used in making wine, bread, beer, and for human and animal health. Other members of this genus include the wild yeast *Saccharomyces paradoxus* that is the closest relative to *Sacccharomyces cerevisiae*, *Saccharomyces bayanus*, used in making wine, and *Saccharomyces cerevisiae* var *boulardii*, used in medicine.

Baker's yeast is the common name for the strains of yeast commonly used as a leavening agent in baking bread and bakery products, where it converts the fermentable sugars present in the dough into carbon dioxide and ethanol. Baker's yeast is of the species *Saccharomyces cerevisiae*, which is the same species (but a different strain) commonly used in alcoholic fermentation, which is called brewer's yeast. Baker's yeast is also a single-cell microorganism found on and around the human body. The Backer's yeast (Angel) were used as a live source of *Saccharomyces cerevisiae* with 15 billion viable cells per g.

The use of probiotics as growth promoters of edible fishes has been reported. Diet of Nile tilapia (*Oreochromis niloticus*) was amended with a probiotic Streptococcus strain, increasing significantly the content of crude protein and crude lipid in the fish, also weight has increased from 0.154g to 6.164g in 9 weeks of culture. Due to the commercial importance of this species, the effect of supplementing diet with probiotics produced an increase of 115.3% when commercial formulation was used at a concentration of 2%.Examples of growth improvement of ornamental fishes include swordtail (*Xiphophorus helleri, X. maculatus*) and guppy, (*Poecilia reticulate, P.sphenops*), their feed was supplemented with *Bacillus subtilis* and *Streptomyces*, finding significant increases in growth and survival of *Xiphophorus* and *Poecilia* after 90 and 50 days of administration, respectively .

The gut is the major organ, where probiotics establish and execute their functions. Therefore, the discussion between probiotics and gut environment warrants high consideration. Enhancement of colonization resistance and/or direct inhibitory effects against pathogens are important factors where probiotics have reduced the incidence and duration of diseases. Probiotic strains have been shown to inhibit pathogenic bacteria both in vitro and in vivo through several different mechanisms. Factors known to influence the colonization of microorganisms can be grouped as follows: (i) host related factors: body temperature, redox potential levels, enzymes, and genetic resistance. For example, bacteria may enter through the mouth, either with water or food particles, and pass down the alimentary tract, at which point some of them are retained as part of a resident microflora. Others are destroyed by the digestive process or pass through the gut, and are eliminated via the faeces. In addition, bacterial growth may be inhibited by any antimicrobial compound produced by the host. (ii) Microbe-related factors: effects of antagonistic microorganims, proteases, bacteriocins, lysozymes, hydrogen peroxide, formation ofammonia, diacetyl, and alteration of pH values by the production of organic acids (Gullian *et al.*, 2004). Divya *et al.* (2012) studied the colonization of probiotic bacteria and its impact on ornamental fish *Puntius conchonius.*

Marzouk *et al*. (2008) studied the influence of some probiotics on the growth performance and intestinal microbial flora of *O. niloticus*. He studied the effect of feeding Lactobacillus based probiotics on the gut microflora, growth and survival of post larvae of *Macrobrachium rosenbergii* (de Man). Probiotics are live microbial feed supplements that beneficially affect the host by producing inhibitory compounds, competing for chemicals and adhesion sites, and modulating and stimulating immune function (Giri *et al*., 2012). As less study has been undertaken in the colonisation of microbe in the gut of fish, this study is an attempt to use

the two probiotic microorganisms, namely *Lactococcus sporogenes and Saccharomyces cerevisiae* in single and combined manner in the diet of *Labeo rohita* and to evaluate the growth, gastrointestinal colonization of the supplemented probiotics along with the commercial feed.

Gobinath and Ramanibai (2012) studied the effect of probiotic bacteria culture on pathogenic bacteria from fresh water fish *Oreochromis mossambicus.* According to him *Lactobacillus* sps. produced indole, methyl red ,VP, nitrate reduction, catalase and oxidase –ve, but +ve for citrate utilization, urease, fructose , glucose and lactose. Dhanasekaran *et al*. (2010) studied the effect of Lactobacillus isolates against bacterial pathogens in fresh water fish. They observed different biochemical characteristics of Lactobacillus isolates.

Now-a-days farmers are applying probiotics regularly, but they should know which microbe is necessary for the growth of fish . Therefore this research may be useful for them for the application of right dose and right species of microbe as probiotic for the growth of fish rohu.

MATERIALS AND METHODS

The experiment was conducted over a period of 120 days in the cement tanks of College of Fisheries, Rangailunda, Ganjam, Odisha. Experiment was conducted in 16 numbers of rectangular cement tanks. Each cement tank of size 7m × 3m × 3m was divided into two tanks by putting a partition in the middle. The tanks are with inlet and outlet facilities and having water supply from bore well. Each tank was washed and cleaned properly and tank preparation was made as per CIFA technology. About 20 numbers of fishes were taken per tank. For each treatment 4 tanks were used. The treatments are as follows.

Treatment 1 (T_1): Feeding with commercially available pellated floating feed @ 2% of total body weight of stocked fish.

Treatment 2(T_2) : Feeding with commercially available pellated floating feed @ 2% of total body weight of stocked fish with *Lactobacillus sporogenes* @ 4% in the applied feed.

Treatment 3(T_3): Feeding with commercially available pellated floating feed @ 2% of total body wt. of stocked fish with *Saccharomyces cerevisiae* @ 4% in the applied feed.

Treatment 4(T_4) : Feeding with commercially available pellated floating feed @ 2% of total body wt. of stocked fish with *Lactobacillus sporogenes* @ 2% in the applied feed and *Saccharomyces cerevisiae* @ 2% in the applied feed.

The probiotics for the experimental study, *viz.*, the Backers yeast(Angel), were used as a live source of *Saccharomyces cerevisiae* with 15 billion viable cells/g, the Sporolac powder was used as a live source of *Lactobacillus sporogenese* and having not less than 150 million spores of Lactic Acid *Bacillus* (*Lactobacillus sporogenese)*/gm.

The yearlings of rohu (*Labeo rohita*) were procured from a private fish seed farm of Chatrapur, Odisha weighing around 44.93 ±2gm and the average length of about 14.06 ±2 cm and used as experimental animal in the present study. Acclimatization of the fish was done in cement tank for 15 days only. The uniform size of fish was collected to stock in each tank. They were released @ 20 numbers per tank containing 200 lt non - chlorinated bore well water. They were reared for 135 days (15 days for acclimatization purpose and 120 days for experiment). The fishes were fed with commercial feed @ 2% of their body weight twice daily. Samplings was done in every 15 days interval and analysis work done for growth parameters and one fish was sacrificed for microbial colony observation, biochemical test and molecular test.

After 15 days of acclimatisation the sampling was done to know the initial growth parameters, presence of the probiotic microbe as *Lactobacillus sporogenes* and *Saccharomyces cerevisiae* and the presence of fish pathogen such as *Aeromonas hydrophila*. Then next 15 days the fishes were fed with commercial feed with probiotics as *Lactobacillus sporogenes* @ 4% of total applied feed in T_2 tanks and *Saccharomyces cerevisiae* @ 4% of total applied feed in T_3 tanks and *Lactobacillus sporogenes* @ 2% and *Saccharomyces cerevisiae* @ 2% of the total applied feed in T_4 tanks. In T_1 tanks the fishes were fed with normal feed. Next 15 days the fishes were fed with commercial without any probiotics feed and sampling was done. In the next 15 days the fishes were again fed with again the probiotic incorporated feed and sampling was done. Likewise the fishes were fed with commercial feed for 15 days and probiotic- incorporated feed for next 15 days and sampling was done up to 120 days.

Experimental feed were incorporated with probiotic in 3 ways as *Lactobacillus sporogenase* @ 4% of the total applied feed, *Saccharomyces cerevisiae* @ 4% of the total applied feed and *Lactobacillus sporogenase* @ 2% of total applied feed and *Saccharomyces cerevisiae* @ 2% of total applied feed by using commercially available binder carboxymethyl cellulose (CMC) .

Growth parameters. Sampling was done at 15 days interval till 120 days to assess the weight gain the fishes. All the fishes in a tank were caught and bulk weighed without water was noted by the help of an electronic balance .The initial weight and final weight was used to calculate the following growth parameters using the standard formulae (Samantaray and Mohanty 1997).

$$\text{Increment in weight} = \text{Mean final weight of fish} - \text{Mean initial weight of fish}$$

$$\text{Percentage weight gain} = \frac{\text{Final weight of fish} - \text{Initial weight of fish}}{\text{Initial weight of fish}} \times 100$$

$$\text{Daily weight gain (g)} = \frac{\text{Final weight of fish} - \text{Initial weight of fish}}{\text{Total no. of experimental days}}$$

$$\text{Specific growth rate (\%)} = \frac{(\log_e \text{Final body weight} - \log_e \text{Initial body weight})}{\text{Total no. experimental days}} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Dry feed fed in gm}}{\text{Wet weight gain in gm}}$$

$$\text{Feed efficiency ratio (FER)} = \frac{\text{Wet weight gain in gm}}{\text{Dry feed fed in gm}}$$

Statistical methodology. The recorded values were evaluated statistically through DMRT (Duncan's multiple range tests)by statistical package SPSS version 19.0 (SPSS Incorporation, Chicago, USA). A 5% level of possibility ($p<0.05$) was taken to decide the statistically significant responses between the treatments means. Results are represented as mean ± S.E. (standard error). Moreover, the data arrangements and graphs were performed by using MS excel sheet 2007.

RESULT AND DISCUSSION

The body weight of rohu yearlings at different days of observation in T1, T2, T3 and T4 are depicted in Table-4 and Table-5. The Table 4 shows that on the first day , the body weight in Treatment 1, 2, 3, 4 were 44.37 ± 0.86, 44.78 ± 0.63, 45.00 ± 0.91, 44.40 ± 0.90 g respectively. It shows that all the yearlings are near about same in weight when they are ready for experimental work. In each 15 days interval the sampling was done up to 120 days. After 15 days of starting the probiotic incorporated feed the growth increased. The growth was increased to 54.66 ± 0.83, 60.97 ± 0.95, 61.07 ± 1.09 g in T2, T3 and T4 respectively . The final weight in T1, T2, T3 and T4 are also presented in Table 4 and 5 as 102.05 ±0.99, 150.78 ±0.68, 176. 00 ± 0.91 and 183.00 ± 0.91g respectively. It shows that the growth of fish is more in the Treatment-4. This shows the growth of fish in T4 is more than other treatments. The growth of fish in weight was found to be very significant ($P < 0.05$) among the different treatment group at the end of the experimental period.

The body weight gain and specific growth rate are represented in the Table 5. The weight gain was lowest in T1 as 57.68±0.83 g (130.02 ± 3.45%) and highest in T4 as 138.35±0.67g (.311.25 ± 7.2%). Likewise daily wt. gain was lowest in T1 as 0.48± 0.01 g and highest in T4 as 1.15 ± 0.005 g. Similar trend was also found for SGR. Highest SGR was recorded in T4 (1.18±0.0.01%) and the lowest in T1 (0.70±0.02%. Fig. 2-4 and shows that weight gain (g), weight gain (%) and specific growth rate (%) in different treatment respectively. This also shows that in all the cases T4 is the best.

The FCR and FER values of the different experimental treatments were shown in the Table 5. All the treatments showed better FCR values are ranging from 1.705±0.01 to 2.72±0.04. In the treatment 4 the FCR value is the best as 1.705±0.01. Fig. 5 shows the different FCR values in different treatments which also shows the best FCR in case of T4.Similarly FER was observed and it was near about similar in all treatments with the value of 0.57±0.005 in case of T4 and 0.57±0.01in T3 and 0.53±0.02 in T2 and 0.37 ±0.005 in T1.

Table 1: Weight of yearlings.

Treatment	Replication	0days	15days	30days	45days	60days	75days	90days
T1	R1	65.45	67.23	68.64	70.43	71.67	73.48	75.86
	R2	63.24	64.51	66.86	67.32	68..59	70.87	71.65
	R3	61.56	62.47	64.96	66.78	67.46	68.98	69.78
	Average	63.42±0.95	50.00±0.91	57±0.91	63.03±0.95	69±0.91	72.00±0.91	79.05±0.99
T2	R1	66.34	71.68	74.72	80.78	83.44	88.90	91.10
	R2	67.45	73.24	75.65	80.53	82.96	88.53	90.65
	R3	65.67	70.43	73.58	79.74	81.65	87.89	89.58
	Average	44.775±0.63	54.665±0.83	60.75±0.65	74.775±1.78	85.75±0.65	103.85±1.54	118.75±0.65
T3	R1	64.48	71.51	76.45	84.67	89.93	97.35	103.69
	R2	63.56	69.89	75.62	82.72	88.24	95.61	102.45
	R3	62.34	69.91	74.98	82.13	87.69	95.16	102.57
	Average	45±0.91	60.975±0.95	69±0.91	90.025±0.95	95.025±0.95	117.05±0.97	133.025±0.95
T4	R1	67.34	75.42	82.46	91.62	98.47	109.25	117.37
	R2	64.27	73.25	81.89	90.56	97.18	107.67	115.43
	R3	63.58	72.56	80.32	90.15	96.73	106.38	114.12
	Average	44.4±0.90	57.7±0.91	69.55±0.95	87.575±0.94	103.025±0.95	125.55±0.99	141.9±0.91

Table 2: Growth parameters of Rohu yearlings.

Treatment	T1					T2					T3					T4				
Parameter	1	2	3	4	Average	1	2	3	4	Average	1	2	3	4	Average	1	2	3	4	Average
Initial Weight	43.48	44	45.5	44.5	44.37±0.86	45.5	44	45	44.6	44.78±0.63	44.5	44	45.5	46	45.00±0.91	43.5	44	44.5	45.6	44.4±0.89
Final Weight	101.5	101	102.5	103.2	102.05±0.99	150.5	151	151.6	150	150.78±0.68	175.5	175	176.5	177	176.00±0.75	182	182.5	183.5	184	183±0.91
Weight gain	58.02	57	57	58.7	57.68±0.83	105	107	106.6	105.4	106.00±0.95	131	131	131	131	131.00±0.50	138.5	138.5	139	137.4	138.35±0.67
Weight gain(%)	133.4	129.5	125.27	131.91	130.02±3.55	230	243	236	236	236.25±5.32	294	297	287	284	290.5±6.39	318	314	312	301	311.25±7.27
Daily weight gain	0.48	0.47	0.47	0.48	0.48±0.01	0.87	0.89	0.88	0.87	0.88±0.01	1.09	1.09	1.09	1.09	1.09±0.00	1.15	1.15	1.15	1.14	1.1475±0.00
Specific growth rate	0.71	0.69	0.67	0.71	0.70±0.02	0.99	1.03	1.01	1.01	1.01±0.02	1.15	1.16	1.12	1.12	1.14±0.02	1.19	1.19	1.18	1.16	1.18±0.01
Total feed fed	155.25	156	158.1	158.5	156.96±1.58	199.65	200.7	199.2	199.8	219.3±1.26	219.3	218.85	220.35	222.9	220.35±1.81	234.9	235.5	237.3	237.9	236.4±1.42
Food conversion ratio	2.67	2.73	2.77	2.7	2.72±0.04	1.9	1.86	1.86	1.89	1.88±0.02	1.67	1.67	1.68	1.7	1.73±0.01	1.69	1.7	1.7	1.73	1.705±0.01
Food efficiency ratio	0.37	0.36	0.36	0.37	0.37±0.00	0.52	0.53	0.53	0.52	0.53±0.01	0.59	0.59	0.59	0.58	0.59±0.01	0.58	0.58	0.58	0.57	0.5775±0.00

The initial and final weight of fish are represented in the Table 1 and 2. The final wt. of fish in T4 was the highest as 183±0.91 g and weight gain was 138.35±0.67% where the initial wt. of fish was 44.4±0.90g. The growth in T1 was less *i.e.*102.5±0.99g, without the application of probiotic. This shows that growth of fish is increased due to the application of probiotic only. The Table 2 also shows that the specific growth rate is more in T4 *i.e.*1.18 ± 0.01% and FCR is also less in the treatment 4 *i.e.* 1.705±0.01. Again the feed efficiency ratio is more in T4 *i.e.* 0.57.This is due to mainly interaction of two probiotic bacteria as *Lactobacillus sporogenes* and *Saccharomyces cerevisiae* in T4.

In the above experiment the feed was applied in @2% of the total body wt. of fish and probiotic as *Lactobacillus sporogenes* was applied @ 4% of total applied feed in T2 and *Saccharomyces cerevisiae* was applied @ 4% of total applied feed in T3 and in T4. *Lactobacillus sporogenes* @ 2% and *Saccharomyces cerevisiae* @ 2% of the total applied feed. Here the experiment is designed in such a way that the normal feed is incorporated with probiotic bacteria and fed to the fishes for 15 days and next 15 days the fishes are fed with normal feed. Likewise it was continued for 120 days. The Table 1 and 2 shows that when fishes are fed with probiotic feed there is more growth than the application of normal feed. Again in Treatment 4, the growth is more as there is an interaction between the two species.So the growth in T4 is more than T3 than T2 than T1.Because there is interaction between two species in T4, more colonization of *Saccharomyces cerevisiae* in T3 , non colonisation of *Lactobacillus sporogenes* in T2 and application of normal feed in T1(control)..An investigation was carried out by them Similar application was done by Gupta and Dhawan (2012) to determine the effect of adding probiotic 'Improval', containing bacteria *Lactobacillus sporogenes* and the yeast *Saccharomyces cerevisiae* as growth promoters, in the diet of freshwater prawn (*Macrobrachium rosenbergii*) during the post larval stage of growth. Post larvae (n=450; 0.38±0.02 g mean weight) were divided into five experimental groups each with three replicates.

The experiment was conducted for 60 days. Control diet (CD) had no Improval, diet 1 (D1), 2 (D2), 3 (D3) and 4 (D4) contained 2, 4, 6 and 8% Improval, respectively. Significantly ($P<0.05$) higher growth for final body weight (16.14±3.57 g), net body weight gain (15.76±3.24 g) and specific growth rate (7.50±0.38 % body weight day-1) was recorded in groups of prawn fed diet containing 4% Improval (D2). In addition, the feed conversion ratio and protein efficiency ratio in reatments receiving 'Improval' as growth promoters were significantly ($P<0.05$) better than those fed the control diet. The protein content of carcass showed the highest value for prawn fed diet D2 (65.3±2.21%) and the lowest was observed in D4 (58.9±2.5%). No significant differences were observed in lipid content among groups of prawn fed diet D1, D4 and CD, while the best and lowest values of lipid carcass were recorded for those fed on D2 (7.14±0.98%) and D3 (7.63±0.77%). The result suggests that the addition of probiotic 'Improval' as growth promoter in the diet @ 4% improved the growth performance of *Macrobrachium rosenbergii* post larvae.Similar type of experiment was designed by Mohapatra *et al*. (2012). They studied the different microbial probiotic in the feed of rohu fingerlings on the growth, nutrient digestibility, digestive enzyme activities and intestinal microflora. Lara-Flores *et al.* (2003) also used the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus* and the yeast *Saccharomyces cerevisiae* as growth promoter in nile tilapia (*Oreochromis niloticus*). Sreenivasan *et al.* (2014) studied the effect of *Lactobacillus sporogenes* on survival, growth, biochemical constituents and energy utilization of fresh water prawn Macrobrachium rosenbergii post larvae. They used the probiotic in different level and he observed that @ 4% level the growth is more than 1%, 2% and 3% and observed that weight gain in gm was 1.22 and specific growth rate was 0.989% for the culture period 90 days in cement tank. Likewise Ademola *et al.* (2011) used baker's yeast *Saccharomyces cerevisiae* in the feed of juvenile of catfish (*Clarius gariepinus*). He applied probiotic @0%, 2%,4% , 6% and 8% and observed the growth is more in 4%.The wt. gain in gm was 174.80, specific

growth rate was 3.75% and FCR was 0.56. Marzouk *et al*. (2008) studied the influence of some probiotic on the growth performance and intestinal microflora of *Oreochromis niloticus*. He used *Bacillus subtilis* and *Saccharomyces cerevisiae* and observed that the weight gain was 12.3% when feed was applied @3% of total biomass and probiotic applied 1.5 g/kg of feed.FCR was 4.73 and specific growth rate g/day was .0096.

The main objective of the present study is to study the periodic changes in the gut micro flora after feeding the fish rohu (*Labeo rohita*) with probiotic incorporated feed, to determine the time requirement for the individual species to colonize in the gut and to study the growth of fish fed with probiotic incorporated feed. Among the culturable fish in India, rohu is the best species . Because it has more growth, the meat content of fish more, tasty, it has good appearance and demand in the market. Therefore for this study rohu was selected.

Generally Fish farmers are using probiotic daily in the culture period of fish. They do not know which species of probiotic is the best for the growth of fish and what is the dose of this species with the feed. Therefore this study is an attempt to fulfill the need of the fish farmer. In this study the yearlings are stocked in cement tanks and fed with the commercial feed in control tanks and probiotic incorporated feed in treatment tanks. Feed applied in the tank *i.e*. @2% of total body wt. of fish. But probiotic applied @4% of the total applied feed in T2, T3 and in T4 *Lactobacillus sporogenes* applied @2% and *Saccharomyces cerevisiae* applied @ 2% of the applied feed. From this treatment T4 is the best.

CONCLUSIONS

At last it can be concluded that the fish farmer can benefit from this study as they are not applying the probiotic in a systematic way. The probiotic as *Lactobacillus sporogenes* and *Saccharomyces cerevisiae* should be applied @2% each of the applied feed. But only *Saccharomyces cerevisiae* incorporated feed application @4% of total feed for 15 days may be sufficient to get more production, but not as the both species. This study has been done for only 120 days (4 months). So further research is necessary for the entire culture period. Further study may also be done for multiple species of probiotic microbes application in Aquaculture to meet the cost effective aquafarming.

Acknowledgement. The research work has been done as a Master degree research work. The author of this paper is working as Guide for the student and he is very much thankful to the Dean, College of Fisheries (OUAT), Rangailunda for allowing this work and he is also very much thankful to ICAR and State Govt. for helping in the Research work.

REFERENCES

Abraham, T. J., Babu, C. H. S., Mondal, S. and Banerjee, T. (2007). Effects of dietary supplementation of commercial human probiotic and antibiotic on the growth rate and content of intestinal microflora in ornamental fishes. *Bangladesh Journal of Fisheries Research*, *11*(1), 57–63.

Ademola, A. Z., Lawal, M. O., Ali, T. O. and Aarode, O. O. (2011). Utilization of Baker's Yeast (*S. cerevisiae)* in the diet of juvenile, African Catfish (*Clarius garipinus). Journal of Sci*., *13*, 19-27.

Anon (2014). Fish to 2030, Prospects for Fisheries and Aquaculture. World Bank Report No 83177-GLB, *Agriculture and Environmental Service Discussion* , 03.

Ayyappan, S., Moza, U., Gopalkrishna, A., Meenakumari, B., Jena, J. K. and Pandey, A. K. (2011). Handbook of Fisheries and Aquaculture 2nd edn.Indian Council of Agriculture Research, New Delhi.

Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environmental Microbiology*, *8*, 1137–1144.

Giri, S. S., Sen, S. S. and Sukumaran, V. (2012). Effects of dietary supplementation of potential probiotic *Pseudomonas aeruginosa* VSG-2 on the innate immunity and disease resistance of tropical fresh water fish, *Labeo rohita*. *Fish and Shellfish Immunology*, *32*, 1135–114.

Gobinath, J. and Ramanibai, R. (2012). Effect of probiotic bacteria culture on pathogenic bacteria from fresh water fish *Oreochromis mossambicus. Journal of modern biotechnology, 1***(**1), 50-54

Gullian, M., Thomposon, F. and Rodriguez, J. (2004). Selection of probiotic bacteria and study of their immunostimulatory effect in *Penaeus vannamei*. *Aquaculture*, *233*, 1-14.

Gupta, A. and Dhawan, A. (2012). Effect of dietary probobioti Improval (*L. sporogenase* and *S. cerevisiae)* on growth and feed utilization of *M rosenbergai* post larvae. *Animal Nutr. Feed Tech*, *12*, 209-217.

Keysami, M. A., Saad, C. R., Sijam, K., Daud, H. M. and Alimon, A. R. (2007). Effect of Bacillus subtilis on growth development and survival of postlarvae *Macrobrachium rosenbergii* (de Man). *Aquaculture Nutrition*, *13*, 131–136.

Lara-Flores, M., Olvera-Novoa, M. A., Guzman- Mendez, B. E. and Lopez-Madrid, W. (2003). Use of the bacteria *Streptococcus faecium* and Lactobacillus acidophilus, and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile Tilapia (*Oreochromis niloticus*). *Aquaculture*, *216*(1-4), 193-201.

Marzouk, M. S., Moustafa, M. M. and Nermeen, M. M. (2008). Evaluation of immune modulatory effects of some probiotics on cultured *Oreochromis niloticus*. 8th International Symposium on Tilapia in Aquaculture 2008, Cairo, Egypt.

Mohapatra, S., Chakraborty, T., Prusty, A. K., Das, P., Pani Prasad, K. and Mohanta, K. N. (2012a). Use of different microbial probiotics in the diet of rohu, *Labeo rohita* fingerlings: effects on growth, nutrient digestibility and retention, digestive enzyme activities and intestinal microflora. *Aquaculture Nutrition*, *18*(1), 1–11.

Mohapatra, S., Chakraborty, T., Prusty, A. K., Kumar, K., Pani Prasad, K. and Mohanta, K. N. (2012b).

Fenvalerate induced stress mitigation by dietary supplementation of multispecies probiotic mixture in a tropical freshwater fish, *Labeo rohita* (Hamilton). *Pesticide Biochemistry and Physiology*, *104*, 28–37.

Nayak, S. K. (2010). Probiotics and immunity: a fish perspective, *Fish and Shellfish Immunolog, 29*, 2-14.

Rumsey, G. L., Hughes, S. G. and Kinsella, J. L. (2007). Use of dietary yeast *Saccaromyces cerevisae* nitrogen by Lake Trout. *Journal of the World Aquaculture Society, 21***(**3), 205–209.

Samantaray, K. and Mohanty, S. S. (1997). Interactions of dietary levels of protein and energy on fingerling snakehead, *Channa striata*. *Aquaculture*, *156*, 241-249.

Sreenivasan, C., Radhakrishnan, S., Santhi, R., Muralisankar, T. and Saravana, B. (2014). Effect of *L sporogenase on survival, growth, biochemical* constituents and energy utilization of freshwater prawn *M. rosenbergii* post larvae. *The journal of basic and applied zoology, 67*(2),19-24.

Vine, N. G., Leukes, W. D., Kaiser, H., Daya, S., Baxter, J. and Hecht, T. (2004). Competition for attachment of aquaculture candidate probiotic and pathogeni bacteria on fish intestinal mucus. *Journal of Fish Diseases*, *27*(6), 319–326.

Zhou, X., Wang, Y., Yao, J. and Li, W. (2010). Inhibition ability of probiotics, *Lactococcus lactis* against *A.hydrophila* and study of its immunostimulator effect in tilapia (*Oreochromis niloticus*). *International Journal of Engineering, Science and Technology*, *2*(7), 73–80.

How to cite this article: N. Das, S. Das and B.K. Khuntia (2021). Effect of Probiotic on the Growth Status of Rohu (*Labeo rohita*). *Biological Forum – An International Journal*, *13*(1): 798-805.