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Effect of Dietary Probiotic on Growth Performance and Disease Resistance in *Labeo rohita* (Ham.) Fingerlings

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ABSTRACT
The effects of a commercially available probiotic product containing *Bacillus* spp, *Lactobacillus* spp. and *Arthrobacter* spp. on the growth performance, and protection against *Aeromonas hydrophila* infection in rohu was studied. Fish were fed diets containing five graded levels of probiotic (0.0, 2.0, 3.0, 4.0, and 5.0 g/kg diet) for 60 days. Dietary probiotic significantly increased the specific growth rate (SGR) as compared to the control diet. Moreover, feeding of supplemented diets containing probiotic resulted in significantly lower mortality (10-30%) against the pathogens *Aeromonas hydrophila* compared with the control group (90%).

Keywords: *Labeo rohita*; Growth, Immunity, Probiotic, *Aeromonas hydrophila*

1. Introduction
Fish is a principal animal source of protein for over half of the global population. In India, the major carps are the most preferred farm fishes because of their fast growth and higher acceptability to consumers. The rapid expansion and intensification of carp farming had led to the outbreaks of infectious diseases caused by viruses, bacteria and parasites, inflicting severe loss on fish production. The widespread use of broad-spectrum chemotherapeutants to control these diseases has led to the development of antibiotic-resistant bacterial strains and may cause water pollution in the aquaculture environment [1]. In order to rectify this situation, greater emphasis has been placed on improved husbandry and water quality, better nutrition, lower stocking densities and the use of vaccines and immunostimulants in the last decade [2, 3]. Recently, attention has focused on the use of probiotics with the demand for environmentally friendly aquaculture.

Nowadays, probiotics are becoming an integral part of the aquaculture practices to obtain high production. Probiotics are live microorganisms thought to be beneficial to the host organism. The involvement of probiotics in nutrition, disease resistance and other beneficial activities in fish has proven beyond doubt. Among the numerous health benefits attributed to probiotics, modulation of the immune system is one of the most commonly purported benefits of the probiotics and their potency to stimulate the systemic and local immunity. Though the exact mode of action of probiotics is yet to be established in any animal including fish. Probiotics may stimulate appetite and improve nutrition by the production of vitamins, detoxification of compounds in the diet and by the breakdown of indigestible components. Therefore, the present research was conducted in order to evaluate the growth performance, and disease resistance of a commercially available probiotic product enriched diets in *Labeo rohita* (Ham.) fingerlings.

2. Materials and Methods
2.1 Experimental Design
The present experiment was conducted in fifteen cemented tanks (3 m^3) for 60 days. Healthy rohu fingerlings were obtained from a fish seed production unit of MUPAT (Udaipur-India). Prior to the start of the experiment, the fish were fed the control diet for 7days in cemented tank (3.0 x 3.0 x 1.0 m) to make the fish acclimate the experimental diets and environment. The healthy fingerlings of uniform size (12.24±0.29 g) were randomly distributed in five experimental groups with each of three replicates following a CRD "complete randomized design". Each cemented tank was stocked with 100 fish fingerlings. Fish were fed twice daily

for 60 days. The ration was divided equally between two feedings, based upon total body weight, initially starting at 4% body weight per day.

2.2 Water quality analysis

Water quality parameter such as temperature, pH, dissolved oxygen, EC, alkalinity and hardness were analyzed on initial day and subsequently on 15^{th}, 30^{th}, 45^{th} and 60^{th} day of experimental period following APHA [4].

2.3 Preparation of experimental diet

A commercially available pelleted floating carp feed (with 24% proteins, 3% fat) was used as a basal diet for the experimental fish. The basal diet was used as the control diet (Dite.1). Of the control diet, four different experimental diets containing four levels of a commercially available probiotic product (2.0, 3.0, 4.0, and 5.0 g/kg) were prepared for the experimental trial. The commercially available probiotic product contains *Bacillus subtilis* (7.0×10^9 CFU/ g), *Bacillus licheniformis* (3.0×10^9 CFU/ g), *Lactobacillus* spp. (5.0×10^8 CFU/ g) and Arthrobacters spp. (1.0 x 108 CFU/ g). To add the probiotic in fish diet, powdered cassava (*Manihot esculenta*) was boiled with water to make paste, this paste was cooled down and the probiotics was mixed in different quantity (2, 3, 4 & 5 g/kg diet).The feed was coated with this paste, air dried and stored in deep freeze at – 20 ^{0}C.

2.4 Proximate composition of experimental fish

To evaluate the impact of probiotics feeding in the biochemical configuration of fish initial and final samples of whole fish were processed for the analysis of moisture, protein, fat, and ash content [5].

2.5 Preparation of Antigen for challenge study

Pathogenic strain of *Aeromonas hydrophila* was received from the Fish Pathology and Microbiology Division, CIFE, Mumbai. *A. hydrophila* was grown on nutrient broth (Hi Media Ltd, India) for 24 h at 37 ^{0}C. The culture broth was centrifuged at 3000 x g for 10 min. The supernatant was discarded and the pellet was re-suspended in sterile phosphate buffer saline (PBS, pH 7.4) and the OD of the solution was adjusted to 0.5 at 450 nm, which corresponded to 1×10^7 cells ml^{-1}. After feeding fish with different doses of probiotic for 60 days, 10 fish from each experimental tank were injected intraperitoneally with 100µl of bacterial suspension and the mortality was observed for 15 days. Sampling of the survivors was carried out on the 15^{th} day of *A. hydrophila* infection.

2.6 Growth parameters

The growth (net weight gain) and survival percentage were studied following standard methods. The following variables were calculated:

Specific growth rate (SGR) = (Ln Wt- Ln W_0) x 100/t

Survival rate = Ntx 100/N_0

Where: **Wt** and **W_0** were final and initial weight of rohu, respectively; **Nt** and **N_0** were final and initial number of rohu, respectively; **t** is duration of experimental days.

2.7 Statistical Analysis

The results were expressed as mean ± SEM (standard error of the mean) and were analyzed by using the one-way ANOVA in SPSS software. A second order polynomial regression analysis method [6] was conducted to analyze the SGR and cumulative mortality of the rohu in response to dietary probiotic supplementation level.

3. Result

3.1 Water Quality

The water quality parameters such as temperature, pH, dissolved oxygen (DO), electrical conductivity (EC), alkalinity and hardness remained more or less same in different treatments (Table 1). In general all the water quality parameters were congenial for the growth and survival of *Labeo rohita*.

Table 1: Range and mean values of selected water quality parameters.

S. No	Water Quality parameters					
	pH	Temperature ^{0}C	Dissolved oxygen (ppm)	EC (mMho/cm)	Alkalinity (ppm)	Hardness (ppm)
Minimum	7.18	24.92	6.27	1.79	132.0	494.0
Maximum	8.09	26.78	7.72	1.95	163.0	515.1
Mean ±SEM	7.81±0.38	26.8±0.06	6.77±0.61	1.80±0.06	149±1.89	538±3.78

3.2 Growth Performance

The growth of *Labeo rohita* was comparatively high in all the treatments than control (Table 2). The highest net weight gain of 19.8±0.15 g was observed in Diet4 followed by diet5 (15.60±0.09 g), diet3 (15.3±0.13 g) and diet2 (14.60±0.08 g). However, the lowest (11.3±0.06 g) being in control (diet1). Moreover, the survival rate was cent percent in all the treatments and control. The net weight gain in different treatments was significant at 5% level of probability ($p<0.05$) except in diet 2 & 3. The overall SGR values were higher in all treatments than control (Table 2). The statistical analysis showed that the values of SGR among different treatments were significant at 5% ($p<0.05$) level of probability. However, the values of SGR between diet2 and diet3 were statistically insignificant. A second order polynomial regression analysis for SGR indicated that a growth optimum occurred at 3.92 g/kg supplementation of probiotic (Fig. 1)

3.3 Proximate Composition

It would be seen from Table 3 that the highest (23.98±0.764%), lowest (20.12±0.79%) level of protein was in diet4 and diet1 (control) respectively. The respective lowest (6.30±0.37%) and highest (6.87±0.29%) fat content was recorded in diet5 and diet1. The contents of ash ranged between 2.43±0.12 to 2.97±0.08 percent with minimum in diet3 and maximum in diet2 (Table 3). The moisture content ranges between 65.44±1.77 to 66.88±1.93 per cent. The maximum and minimum values of moisture were recorded in diet3 and diet5 respectively.

Table 2: Growth response of *L rohita* fed the diet with graded levels of probiotic (Data are expressed as mean ± SEM. Means in the same column sharing the same superscript letter are not significantly different determined by Duncan's test -P > 0.05).

Diet No.	Supplementation level (g/kg diet)	Initial body weight (g)	Final body weight (g)	Net weight gain (g)	SGR (%/day)
1	0.0	21.2±1.10	32.5±0.99	11.3±0.06[a]	0.32±0.017[a]
2	2.0	20.9±0.93	35.5±1.46	14.60±0.08[b]	0.37±0.016[b]
3	3.0	21.2±1.06	36.5±1.37	15.3±0.13[b]	0.39±0.009[b]
4	4.0	21.2±0.98	41.0±1.29	19.8±0.15[c]	0.47±0.013[c]
5	5.0	21.6±0.95	37.2±1.35	15.60±0.09[d]	0.39±0.021[d]

Table 3: Proximate composition of whole body of L rohita fed with graded levels of probiotic

Diet No.	Supplementation level(g/kg diet)	Moisture (%)	Fat (%)	Protein (%)	Ash (%)
1	0.0	66.55±2.01	6.87±0.29	20.12±0.79	2.69±0.22
2	2.0	66.00±1.53	6.70±0.32	20.40±0.63	2.97±0.08
3	3.0	66.88±1.93	6.65±0.19	22.55±0.59	2.43±0.12
4	4.0	66.06±1.72	6.53±0.24	23.98±0.76	2.71±0.08
5	5.0	65.44±1.77	6.30±0.31	22.78±0.81	2.66±0.14

3.4. Disease Resistance

Feeding of probiotic supplemented diets after 60 days led to a marked decline in fish mortality after challenge with *A. hydrophila*. Thus, highest 83.33% mortality was recorded for the control compared with probiotic fed groups (Fig. 2). Significantly highest ($P < 0.05$) survival of fish was recorded in the groups fed with probiotic supplemented diet. Based on a second order polynomial regression analysis, a lowest mortality occurred at 3.89 g/kg supplementation of probiotic after challenge with *A. hydrophila* (Fig.1) Survivors did show a localized disease sign in the shape of abrasion at the site of injection. This sign of injury disappeared after 1 week of the injection in fish fed treated diets.

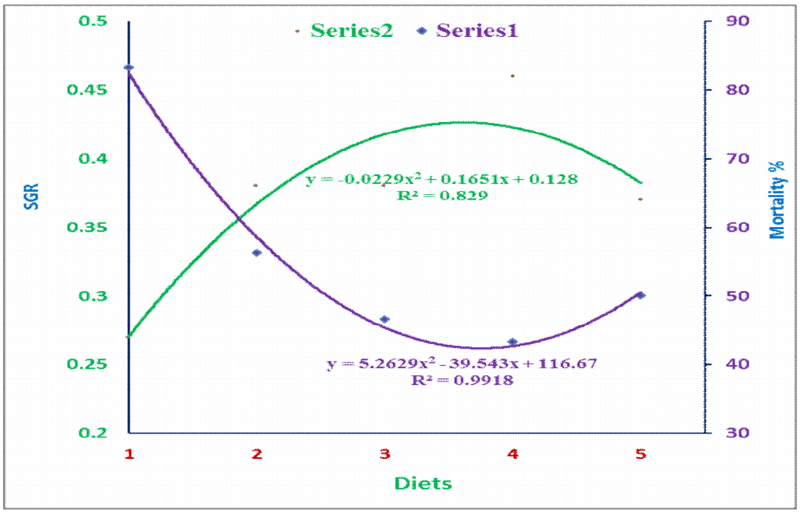

Fig 1: Relationship between probiotic level with mortality rate (Series 1) and specific growth rate (Series 2) as described by second order polynomial regression.

Fig 2: Effects of dietary supplemented graded levels of probiotic on cumulative mortality of *L.rohita* after infection with *Aromonas hydrophila*

4. Discussion

The effects of probiotic have been studied in many aquatic animals. Improvement of the growth has been reported by feeding of *Bacillus sp.* in the tilapia [7], *Catla catla* [2], *Labeo rohita* [8], and *Penaeus monodon* [9]. In the present study, the growth of *Labeo rohita* was significantly increased by dietary probiotic. Such increase in the growth of aquatic animals fed with probiotic diets may be attributed to the improved digestive activity by enhancing the synthesis of vitamins, enzymatic activity, with a consequent improvement of the digestibility and weight gain [10]. The highest SGR was recorded in diet4 (0.47±0.013) which decreased with increasing level of probiotic. Thus suggesting that the high concentrations of probiotic supplemented in diets may not further promote the growth of *Labeo rohita* in this study. This result is in agreement with the report of Ghosh *et al* [11] which showed that the use of higher concentration of the probiotic did not always lead to better performances of growth.

The obtained data in Table 3 showed that there are no significant ($P>0.05$) differences in moisture between fish groups fed probiotic supplemented diet and the control group. These results agree with the findings of Kobeisy and Hussein [12], Oliva-Teles and Goncalves [13] and Fournier *et al* [14] who reported that the moisture was not affected by dietary yeast. Further, the effect of probiotic on ash content was discussed by Lunger *et al.* [15] who stated that the ash content of cobia (*Rachycentron canadum*) decreased significantly by dietary probiotic. Contrary, Olvera-Novoa *et al.*, [16] found insignificant decrease in ash content of *O. mossambicus* fed 25 and 35% probiotic. Also, Sealey *et al.* [17] showed no significant differences in ash content of *rainbow trout* treated with dietary probiotic. The fat decreased significantly ($P<0.05$) in fish groups fed probiotic supplemented diet as compared with the control (Table 3). This decrease in fat percentage could be attributed to the corresponded increase of protein content (Table 3). Similar results were found by Kobeisy and Hussein [12] and Hussein *et al.* [18] in the same fish (*O. niloticus*).

In the present study, *Labeo rohita* fed diets supplemented with various levels of probiotic for 60 days were challenged with *Aeromonas hydrophila*. The mortality of fish was reduced significantly by dietary probiotic, compared with fish fed the basal diet, and the result analysis indicated that the optimum disease resistance ability of *Labeo rohita* occurred at 4 g/kg diet (diet 4) supplementation of probiotic. Previous studies have demonstrated that feeding of *B. subtilis* significantly increases the resistance against disease in tilapia [8], rainbow trout [19] and shrimp [20, 21]. Other *Bacillus* spp. was also able to decrease the mortality of aquatic animals after challenged with the pathogens [2, 22]. Some other studies reported that the probiotic LAB can electively provide protection for aquatic animals against the pathogens [23]. The reason why the mortality of fish decreased may be that probiotic, are able to out-compete other bacteria for nutrients and space and can exclude other bacteria through the production of antibiotics [24, 25].

5. Conclusion

In conclusion, under the experimental conditions, the results obtained not only support the use of probiotic for better growth, but also confirm it to be an important immunostimulants in *Labeo rohita*. Dietary probiotic significantly increased immunity, as well as survival against the pathogenic *Aeromonas hydrophila* infection. To elevate the growth and immune resistance ability of *Labeo rohita*, the dietary probiotic administration at 4 g/kg diet is an optimal dose, determined by growth, SGR and mortality after challenge with *A. hydrofila.* These results suggest that dietary probiotic should be taken into account when a long-term oral administration is conducted.

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