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STUDIES ON GROWTH AND IMMUNE RESPONSE OF *LABEO ROHITA* AFTER FEEDING *LACTOBACILLUS ACIDOPHILLUS* AND *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

In this study, the individual as well as combined effect of commercially available probiotics containing *Lactobacillus acidophillus* and *Sacchromyces cerevisiae* was studied on the growth performance. The study was also extended to protection against infectious bacteria *Aeromonas hydrophila* which is the cause of abdominal dropsy in fish. Three different concentrations of probiotics (2, 3 and 4%) were fed to fish fingerlings (*Labeo rohita*) in 30% commercial artificial feed for 60 days. In combination group, dietary probiotics at 4% dose showed significant average increase in growth i.e. 80% and survival rate was 99% when challenged with *Aeromonas hydrophila*, followed by 3% and then 2% doses. *Sacchromyces cerevisiae* and *Lactobacillus acidophillus* individually showed their significant effect on the growth of fish which was very close to each other, as the % age increase in weight for *Sacchromyces cerevisiae* was 67.7% and for *Lactobacillus acidophillus* it was 67% at 4% dose. Feeding of supplemented diets showed reduced mortality in combination group (1%) followed by *Sacchromyces cerevisiae* (3%) and then *Lactobacillus acidophillus* (6%) in comparison to control group where 93.33% mortality was observed.

Keywords: *Aeromonas hydrophila*, *Labeo rohita*, probiotic, immunity, growth.

INTRODUCTION

In Pakistan major carps, particularly *Labeo rohita* is the most preferred farmed fish because of the rapid growth and higher adequacy to consumers. The rapid development and intensification of carp farming to bridge up the gap of animal protein demand had led to the epidemic of contagious diseases caused by bacteria, viruses and parasites, inflicting rigorous loss on production of fish. The extensive utilization of wide-ranged chemotherapeutants to combat these diseases has led to the enhancement of antibiotic-resistant bacterial strains, in addition to this; could cause water contamination in the aquatic environment (Lunden *et al*., 2002). In order to resolve this condition, better emphasis is being placed on improved water quality, better nutrition and the application of immunostimulants and vaccines in the last decade. In recent times, the use of probiotics has been focused with the requirement for environmentally friendly aquaculture (Bandyopadhyay and Mohapatra, 2009).

Currently, probiotics are becoming an essential part of the aquaculture practices to achieve high production. Probiotics are live microorganisms contemplated to be useful to the host organism.

The World Health Organization (WHO) has defined probiotic bacteria as "live microorganisms which when administrated in adequate amounts confer a health benefit on the host" (FAO/WHO, 2001). In fish, the contribution of probiotics as nutrition, in disease resistance and other advantageous activities has been studied. Among the various health benefits attributed to probiotics, inflection of the immune system is one of the major benefits of the probiotics and their effectiveness to stimulate the local and systemic immunity. Probiotics may possibly improve nutrition by the manufacture of vitamins; stimulate appetite and detoxification of compounds in the diet (Harikrishnan *et al*., 2010).

The use of probiotics as dietary live microbial supplements and biological control agents in commercial fish culture is possible to improve growth as well as immune function of the fish (Irianto and Austin, 2002; Kesarcodi-Watson *et al*., 2008).

Earlier studies reported that the positive effect of using viable microorganisms in probiotic mixtures into fish diets (Li and Gatlin, 2004; Brunt and Austin, 2005; Pangrahi *et al*., 2005; Barnes *et al*., 2006; AboState *et al*., 2009). The aim of this study was to analyze and check the

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individual as well as combined effect of *Lactobacillus acidophillus* and *Sacchromyces cerevisiae* on the growth and immune response of *Labeo rohita*.

MATERIALS AND METHODS

Experimental design

The present study was conducted at Fisheries Research and Training Institute, Manawan Lahore. Six hundred Rohu fingerlings of size 5-8gm were procured from Fish Seed Hatchery, Chenawan, District Gujranwala. Before the start of experiment, the fish were fed to the control diet (commercially available feed with 30% protein) for 10 days in aquaria for acclimatization. Total 30 glass aquaria (50 L) filled with fresh water were used for the experiment, each aquarium was stocked with 20 *Labeo rohita* fingerlings. Aquaria were divided into four groups with each of three replicates, one group served as control and the other groups were used as experimental.

Preparation of Experimental Diet

A commercially available feed (with 30% protein) at the rate of 3% body weight was used as a basal diet for the experimental fish. Fish were fed twice daily in 60 days. The basal diet was used as the control diet. Nine experimental diets were prepared from basal diet adding 2.0, 3.0, and 4.0% of *Lactobacillus acidophillus* and *Sacchromyces cerevisiae* and a combination of above two probiotics by using the water spraying method. The feed was dried in oven at 50ºC overnight. The growth rate in fish for each experimental diet was noted every 15 days and the feeding was adjusted according to the body weight of fish in each aquarium.

Water Quality Analysis

Important water quality parameter such as pH, temperature and dissolved oxygen, were recorded daily and kept within the range throughout the experimental period (Table 1).

Preparation of Antigen for Challenge Study

Pathogenic strain of *Aeromonas hydrophila* was collected from commercial source (Microbiologist USA, Exon Scientific Corporation, 4 Leek Road Chuburji Chowk, Lahore). *Aeromonas hydrophila* was grown in lab on sheep blood agar for 24 h at 37°C. In order to prepare the bacterial suspension, the growth of bacteria was mixed in sterile phosphate buffer saline (PBS, pH 7.4). The turbidity of solution was checked by turbidity meter to get same concentration of bacterial culture. After feeding fish with experimental doses of probiotics for 60 days, 10 fish from each aquarium were injected intraperitoneally with 100µl of bacterial suspension and the mortality was observed for 15 days.

Growth Parameters

The percentage increase in weight in *Lactobacillus acidophillus*, *Sacchromyces cerevisiae* and in combination was checked by applying formula:

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

RESULTS

Water Quality

Water quality parameter such as pH, temperature and dissolved oxygen was monitored with their minimum and maximum range for the growth and survival of *Labeo rohita* (Table 1). All the faecal matter was removed daily by siphoning method.

Growth Performance

The growth of *Labeo rohita* was increase in all the treatments as compared to control. The highest %age weight gain of fish was observed in combination group (*Sacchromyces cerevisiae and Lactobacillus acidophillus*) at 4% dose, followed by 3% and then 2% (Table 4). *Sacchromyces cerevisiae* and *Lactobacillus acidophillus* individually showed their significant effect on the growth of fish which was very close to each other, as the %age increase in weight for *Sacchromyces cerevisiae* was

Table 1. Range of selected water quality parameters.

S. No.	pH	Temperature ºC	Dissolved oxygen (ppm)
Minimum	7.18	19.3	5.5
Maximum	8.09	28.8	6.2

Table 2. Average % age increases in weight of fish for probiotic *Lactobacillus acidophillus.*

Treatments	Probiotic *Lactobacillus* %	Initial body weight (g)	Final weight (g)	Percentage increase in weight %
Control	0.0	5.0	6.2	24
T1D1	2.0	5.4	8.3	53
T1D2	3.0	5.3	8.5	60
T1D3	4.0	5.2	8.7	67

67.7% and for *Lactobacillus acidophillus* it was 67% at 4% dose (Table 2, 3).

Table 3. Average % age weight gain of fish for probiotic *Sacchromyces cerevisiae*.

Treatments	Probiotic *Sacchromyces cerevisiae* %	Initial body weight (g)	Final weight (g)	Percentage increase in weight %
Control	0.0	6.1	7.6	24.5
T2D1	2.0	6.3	9.8	55
T2D2	3.0	6.1	10.0	63
T2D3	4.0	6.2	10.4	67.7

Table 4. Average %age weight gain of fish for probiotics *Lactobacillus* and *Sacchromyces cerevisiae* in combination.

Treatments	Combination of two Probiotics (L.B+Yeast) %	Initial body weight (g)	Final weight (g)	Percentage increase in weight %
Control	0.0	7.1	8.8	23.9
T3D1	2.0	7.2	12.5	73
T3D2	3.0	7.2	12.8	77.7
T3D3	4.0	7.3	13.2	80

Disease Resistance

Feeding of probiotic supplemented diets after 60 days led to a considerable decrease in fish mortality after challenge with *A. hydrophila*. Therefore, highest 93.33% mortality was observed for the control when compared with probiotic fed groups (Fig. 1). Significant decline in mortality was found in combination group (1%) followed by *Sacchromyces cerevisiae* (3%) and then *Lactobacillus acidophillus* (6%). Highest survival of fish was found to be in combination group.

DISCUSSION

In the present study, *Labeo rohita* fed diets supplemented with probiotic *Lactobacillus acidophillus* and *Sacchromyces cerevisiae* along with their combination in different doses 2, 3 and 4% for 60 days were challenged with *Aeromonas hydrophila*. The mortality of fish was decreased significantly by dietary probiotic as compared with fish fed the basal diet, and the result analysis indicated that the maximum disease resistance ability of *Labeo rohita* occurred at 4% diet supplementation of probiotic i.e combination of *Sacchromyces cerevisiae* and *Lactobacillus acidophillus* (Fig. 1). It showed that combination of yeast and bacterial strain was found to be

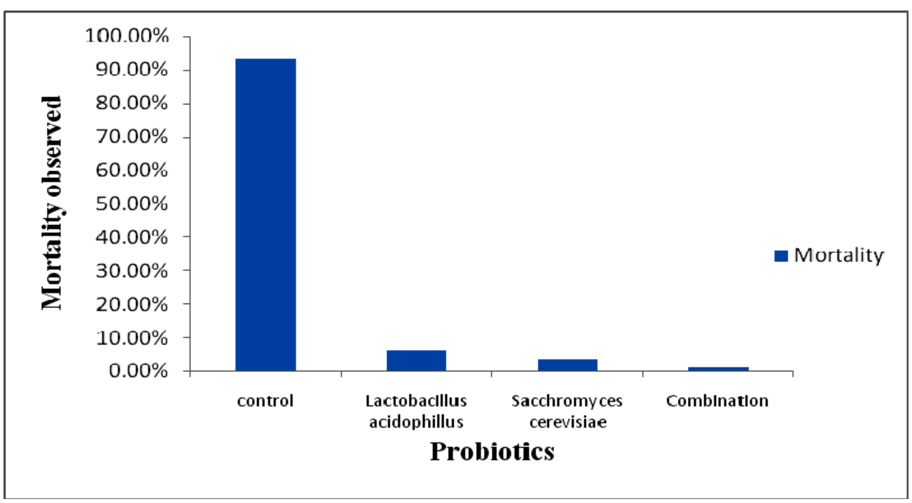

Fig. 1. Immunity in fish against *Aeromonas hydrophila* for different probiotics.

very effective and resulted in modulation of immune system of fish. In this study, the growth of *Labeo rohita* was notably increased by dietary probiotic. Enhancement in the growth of aquatic animals fed with probiotic diets resulted in increased enzymatic activity, improved digestive activity, synthesis of vitamins and weight gain (Nayak, 2010). Another study, Giri *et al*. (2013) reported that Probiotic supplementation improves growth parameters and immune responses in *Labeo rohita*.

In this study, combination group showed the highest growth rate at 4% dose followed by 3% and then 2% (Table 4). It is reported that by mixing experimental diets including *Bacillus subtilis* and *Lactobacillus acidophillus* showed significant improvement in growth of Iranian sturgeon fish (*Acipencer perscius*) for 30 days (Querroz and Boyd, 2008). Similar results were found in Tilapia by using yeast *Sacchromyces cerevisiae*, as yeast was found to be good promoter of growth and immune system (Lara-flores *et al*., 2003).

In Rainbow trout (*Oncorhynchus mykiss*), maximum growth and survival rate was observed when administrated with probiotics *Sacchromyces cerevisiae* and *Lactobacillus acidophillus* individually as compared to control when challenged with *Aeromonas hydrophila* (kesarcodi-watson *et al*., 2008). In *Cyprinus carpio*, the survival rate was 63% when supplemented with *Bacillus* NL110, whereas it was just 11% in control; not treated with probiotics when when challenged with *Aeromonas hydrophila* for two weeks (Ortuno *et al*., 2002). In the present study, average increase in growth of *Labeo rohita* was 67.7% at dose of 4% by using *Sacchromyces cerevisiae* (Table 3) and 67% in case of *Lactobacillus acidophillus* (Table 2) for 60 days. Immunity in fish was also modulated in case of yeast as compared to *Lactobacillus acidophillus*. In the control group the mortality of fish was found to be 93.33% which showed a significance of probiotics against *Aeromonas hydrophila* (Fig. 1).

CONCLUSION

Under the experimental conditions, it is concluded that probiotics are not only beneficial for the growth but it is an important immunostimulant for *Labeo rohita*. Dietary probiotics *Lactobacillus acidophillus*, *Sacchromyces cerevisiae* and its combination significantly increase growth as well as immunity against *Aeromonas hydrophila*, pathogenic bacteria that cause infection in fish. To elevate the immune resistance ability and growth in *Labeo rohita*, the dose at 4% of probiotic was found to be very effective as compared to control group which showed highest mortality. These results indicates that dietary probiotics are very advantageous for the fish and it should be taken into account by the farmers to treat their fish from different bacterial diseases as well as for the improvement of growth.

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