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Dietary administration of the commercially available probiotics enhanced the survival, growth, and innate immune responses in Mori (*Cirrhinus mrigala*) in a natural earthen polyculture system

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A B S T R A C T

The use of probiotics is considered effective for survival, growth and enhanced immune response in aquaculture. In the current study, effects of commercially available probiotic (Magic Plus) was investigated on survival, growth and immune response of Mori (*Cirrhinus mrigala*) in a polyculture system. The experiment was conducted for 90 days on 1200 fingerlings in two groups i.e. control and probiotic supplemented groups each having 600 fingerlings. Control group was fed with 35% protein basal diet without any supplements and the other group was supplemented with commercially available probiotic at the rate of (10^{12} CFU kg^{-1} diet). After 90 days, probiotic supplemented group was characterized with significant increase ($p < 0.05$) in growth parameters like, total weight, total length, %weight gain, specific growth rate and survival growth rate. Immunological indices like, lysozyme activity, white blood cells, total plasma protein level and immunoglobulin (IgM) of supplemented group were also significantly ($p < 0.05$) enhanced. Moreover, digestive enzymes i.e. cellulase, protease and amylase were also found to be significantly ($p < 0.05$) hyper-active in probiotic supplemented groups. Haematological parameters like, RBCs, Hb, Hct, MCH and MCHC were also significantly ($p < 0.05$) increased. Thus, the current study strongly suggests that a commercially available probiotic Magic plus may serve as a healthy and immunostimulating feed additive in *C. mrigala* culture.

1. Introduction

Fish is an excellent substitute for red meat and contains all the essential amino acids and minerals in desirable concentrations, hence can make a valuable contribution to a healthy diet [70]. World aquaculture has grown tremendously during the last decades (with the greatest potential to meet the growing demand for aquatic food [26,76]. Asia share is prominent in global aquaculture [21] and Pakistan also export 10% of the total catch. In 2002-03 Pakistan had exported fish and fishery products of 117 million US$ [27]. In Pakistan, per capita fish consumption is increased from 1.0 kg in 1961 to 2.3 kg in 2001, however still low, compared to 9.0–16.3 kg per capita consumption worldwide [25].

C. mrigala is a natural inhabitant of the Indus and Ganges river systems, however aquaculture has successfully distributed it among other countries of Asia (including Pakistan) and Europe [15,29]. High aquaculture potential and more consumer demand have greatly increased the specie's commercial value. *C. mrigala* is an important component of a polyculture system where it is cultivated with other Indian major carps and effectively utilize the bottom niche. It is reported that 573,627 million tons of *C. mrigala* is produced annually through aquaculture that contributes 1.6% to global fish production [25]. Thus, this specie represents a huge commercially valued group of fresh water fishes. Owing to ease of cultivation, taste and size, it is highly accepted in Pakistani markets also. .

Antibiobiotics are proven to show harmful effects on the environment and health of the fishes, thus, are prohibited in many countries. Carcinogenic effects of the antibiotics have been reported in many teleosts [31,44,64]. Development of antibiotic resistance by pathogens is another side effect of the frequent use of antibiotics. The probiotics are considered as the safe alternatives of the antibiotics and are successfully used by the fish farmers as feed additives and biological control agents. It is reported that growth performance and immune function of the fish are greatly enhanced with the use of probiotics [31].

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Thus, the probiotics in aquaculture not only control diseases but also increased the feed efficiency and husbandry parameters.

The use of probiotics, therefore, lead to new health management strategies ranging from their increasing use as growth supplements to be effectively used as therapeutic agents in fish/shellfish [56]. Different modes of actions are attributed to probiotics by several authors that may include an improvement of water quality, enhancement of host immune response, production of supplemental digestive enzymes to improve nutrition of host species and production of inhibitory compounds to eliminate pathogenic bacteria [81,84].

Lactobacillus acidophilus, L. bulgaricus, L. plantarium, Streptococcus latis and Saccharomyces cerevisiae are some of the common probiotic strains used in aquaculture [25]. Probiotics are sometimes expected to have direct growth promoting effects on fish, either by directly involving in nutrients uptake or by providing nutrients or vitamins [64]. Yeast was used as a probiotic by Refs. [8,16,42]. Positive effects of yeast (*Saccharomyces cerevisiae*) as a probiotic on growth parameters, survival and carcass quality in fry rainbow trout (*Oncorhynchus mykiss*) have been reported [60].

Yeast is found to be a resistant probiotic against many antibiotics like sulfatides and other antibacterial agents. These probiotics effectively act at a broad range of aquaculture [38,40,45,82] and freshwater ornamental fish culture [3–5] *via* increasing survival rate [85], promoting growth [14], enhancing immune system [31], developing resistance against stress [67] etc. According to Ref. [55], use of yeast as a probiotic increases the growth rate of lactic acid bacteria and also causes the competitive exclusion of pathogenic bacteria and its products, especially the cell wall constituents.

Among bacteria, *Bacillus* species have the proved ability to develop probiotics. *B. subtilis*, being the most investigated bacterial specie as a probiotic is harmless to human as well as other mammals [53]. It produces a diverse amount of secondary metabolites including antigens and vaccines, antibiotics, fine chemicals and enzymes and also heterologous proteins [12,28,54,74,75,83,87].

Probiotics are supplemented to a diverse group of animals throughout the world. Thus, they can be also used upon fish with intentions of improving the growth and immune responses of the fish. With the increasing demand of fish meat production, probiotic can be among the best choices to gain high fish production in short time.

Therefore, the current study was designed with the aims to investigate the role of probiotics on survival, growth, digestive enzymes activity and immunological indices of *C. mrigala* in a natural polyculture system. This study may lead us to the better decision regarding use of probiotics in a feed of *C. mrigala* for better production, survival and enhanced immunity of the fish.

2. Materials and methods

2.1. Experimental design

Three months' experiment was conducted in semistatic condition, earthen ponds (area: 0.065 ha each) at Fisheries and Aquaculture research station, Quaid-i-Azam University, Islamabad. Before stocking, all 6 ponds were sun dried and their outlets were closed while inlets were screened with gauze of fine mesh for avoiding any unwanted fish. Before filling with stream water, each pond was prepared by adding calcium carbonate at the rate of 125 kg/ha [6] for disinfection and stabilization of pH and fertilized with Animal manure; cow dung at the rate of 3333.33 kg/ha [35] to enhance pond productivity. All ponds were filled with water at a level of 1.5 m and this level was maintained throughout the feeding trial. Semi-intensive culture system was adopted and during experiment fortnightly pond productivity was checked with sacchi disc, if reading was greater than 30 cm then animal manure was added.

Twelve hundred active fingerlings (having no sign of infection) of *C. mrigala* with average body weight and length 10 ± 2.34 g and 9.50 ± 5.50 cm respectively were purchased from Himalaya fish hatchery and transferred to the earthen fish ponds of Fisheries and Aquaculture research station. On the pond site, bags were opened and tempering of fish was done by gradually adding pond water in bags. At random, 6–10 fingerlings from each bag were collected and their lengths and weights were noted. Fingerlings were equally distributed in 6 ponds (200 fingerlings/pond), already having 400 rohu and 200 silver carp fingerlings. Experiment was designed in a randomized manner consisting of two groups, i.e., control (C) group fed with basal diet without any supplement while experimental group (P) fed with probiotic supplemented diet.

2.2. Experimental feed

Locally available probiotic product Magic Plus contains 1×10^6 CFU/g *Bacillus subtilis* and 1×10^6 CFU/g yeast, *Saccharomyces Cerevisiae* with final concentration of 1×10^{12} CFU/g used for experimental purpose. The viability of bacteria and yeast in Magic Plus before and after coating on feed was investigated using plate count. *Bacillus subtilis* was grown on Tryptic soya agar (TSA) at 37 °C while *Saccharomyces Cerevisiae* was grown on Oxytetracycline glucose agar (OGA) for 24–48 h. Standard method was used to estimate the colony forming unit [49]. The dry pelleted 35% CP feed, 2 mm size was purchased from Oryza organic PVT LTD and used as basal and probiotic supplemented diet for the control and experimental groups respectively. Before starting feeding trial, all fish were fed with basal diet for 5 days in order to wean them on this diet. To add probiotic in experimental diet, 1 g Magic Plus powder was dissolved in 10 ml phosphate buffer (pH, 7.2), mixed well to make homogenous solution and sprayed on 1 kg pelleted feed. Feed was kept overnight at ambient temperature. It was then dried and stored in ziplock bag at low temperature for further use. At every second or third day, new batch of experimental diet was prepared.

2.3. Experimental setup

The experiments were performed starting on 25th May and ended on 28th August. Initially fish were fed with their respective diet twice a day (09:00 a.m. and 4:00 p.m.) and then feeding rate was adjusted fortnightly after checking the weight of fish. During the experimental period, water quality parameters i.e. temperature and (DO) were checked at early 09:00 a.m. and 03:00 p.m. on daily basis, while the total ammonia and pH were checked on weekly basis. Initially, water temperature of all ponds was 23.43 ± 0.48 °C. It increased gradually and reached to 30.86 ± 0.43 °C in July, remained same up to 5th Aug with slight fluctuation and then dropped to 29.07 ± 1.10 on 28th August. Furthermore, DO level fluctuated but remained within a range 5.0–7.0 mg L^{-1} suitable for growth of *C. mrigala*. However, total ammonia remained less than 0.25 mg L^{-} while pH showed slight fluctuation and remained within 7.5–8.0. The experiment was conducted in similar environmental conditions, at same stocking density and all ponds were in same vicinity, therefore water quality parameters of control and experimental groups did not showed noticeable differences.

2.4. Survival and growth

The total number of fish and biomass of each pond was noted at the time of stocking. Moreover, before stocking at least 6 fish from each bag were collected and weighed individually. To monitor growth and to adjust feeding, fortnightly, 6–8 fish from every pond were collected and weighed individually. At the end of feeding trial (90 days) or before harvesting, fish were starved for 24 h, and all fish from each pond were collected. After harvesting, *C. mrigala* were separated from other fish, weighed collectively and noted the total number of fish for calculating average weight of fish. The increase in biomass, average increase in weight, specific growth rate (SGR), percent survival and percent weight