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RESEARCH ARTICLE

INFLUENCE OF PROBIOTICS ON GROWTH PERFORMANCE AND DIGESTIVE ENZYME ACTIVITY OF COMMON CARP (*Cyprinus carpio*)

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ABSTRACT

The present study was carried out to evaluate the influence of dietary supplementation of probiotic bacteria (*Lactobacillus* sp.) on growth performance, enzyme activity and water quality parameters of common carp. The Probiotic was isolated from the intestine of common carp. The feeding trail was conducted for 60 days, to determine the effect of dietary probiotic on the growth and health status of fish. The fish with a similar body weight (24±1 gm) were distributed randomly into five treatment groups, which fed a feed containing *Lactobacillus* sp. in four concentrations viz., 1.0 (T1), 1.5 (T2), 2.0 (T3) and 2.5 (T4) X 10^7 CFU g^{-1} feed. The control group (T5) was fed without *Lactobacillus* sp. for the same period. Blood samples were collected at the intervals of 15, 30, 45, 60 days. The digestive enzymes such as protease, amylase and lipase activity were analyzed. Water quality parameters such as Temperature, p^H, Dissolve oxygen, Alkalinity, Hardness, and Ammonia were examined. The *Lactobacillus* sp treated fish (T3, 2.0 X 10^7 CFU g^{-1} feed) showed maximum percentage of growth performance and better enzyme activity than in other groups. The results suggest that *Lactobacillus* sp. Could be used effectively as a probiotics for the use in aquaculture.

INTRODUCTION

Aquaculture has become an important economic activity in many countries. In large scale production facilities, aquatic animals are exposed to stressful conditions. The increased intensify of aquaculture has led to a high number of disease outbreak with an increasing range of pathogens as a result in serious economic losses. Prevention and control of diseases have led during recent decades to substantial increases in the use of veterinary medicines include vaccines and antibiotics or chemotherapeutics, but they cannot be used alone as a universal disease control measures in aquaculture. Although the excessive use of broad spectrum antibiotic in aquaculture has led to the development of antibiotic resistance among pathogenic bacteria (Villamil *et al.,* 2002; Sakai *et al.,* 1995). This concern has also been raised in aquaculture industry and has led to suggestions for other disease controls including non-specific immuostimulants, use of non pathogenic bacterial probiotics such as Lactic acid bacteria (LAB) (Ringo and Gatesoupe 1998; Kim and Austin 2006). The use of probiotics in aquaculture is thus anticipated to be an excellent strategy for the prevention of infectious microbial diseases and to replace antibiotics and chemotherapeutic (Joseluis balcozar *et al.,* 2006).

Probiotics are defined as "Live microbial feed supplements which when administered in adequate amount beneficially affect the host by improving its microbial balance (FAO/WHO 2002). Lactic acid bacteria (LAB) have been used as probiotics due to their properties of antibacterial activity against pathogens (Tatsuro hagi and Takayuki hoshino 2009; Byun *et al.,* 1997; Garrga *et al.,* 1998). In fish, it has been reported that many LAB is present in the intestine and comprise part of the intestinal flora and there have been several reports on the use of probiotics in aquaculture (Irianto and Austine 2002). The use of commercial probiotics in fish is relatively ineffective, most commercial preparations are based on strains isolated from non fish sources that are unable to survive are remain viable at high cell density in the intestinal environment of fish during the active growth phase of fish. Hence, there is elegant logic in isolating putative probiotics from the host in which the probiotic is intended for use, such strains should perform better because they have already adhered to the gut wall of the fish and thus are well adapted to compete with pathogens for nutrients. Presumably, strains that develop dominant colonies in the fish intestine are good candidates for preventing the adhesion of pathogens to the gut wall.

In recent years, the role of probiotics in nutrition and health of certain aquaculture species has also been investigated. It appears that probiotics provide benefits by establishing favorable microbial communities such as lactic acid bacteria. In the gastrointestinal track which may alter gut morphology and produce certain enzymes and inhibitory compounds causing improved digestion and absorption of nutrients as well as enhanced immune response (Verschuere *et al.,* 2000). The effects of probiotics have been linked to modulation of gut microbiota and establishment of the beneficial microorganisms, higher specific and total digestive enzyme activities in the brush-border membrane which increases the nutrient digestibility and feed utilization (Balcázar *et al*., 2006; Kesarcodi-Watson *et al*., 2008). The digestive enzymes in fish have been studied by several workers (kawai and ikeda, 1972: Das and Tripathi 1991). However, information regarding the enzyme producing intestinal bacteria, their source and their effect on fish digestion and metabolism is scarce. So, the present study was designed to evaluate the effect of different dietary probiotics *Lactobacillu* sp on growth performance and activity of digestive enzymes of *Cyprinous carp.*

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MATERIAL AND METHODS

Fish sampling

The fingerlings of common carps (*Cyprinus carpio*) were collected at regular intervals from the National fish seed farm B.R. Project, Karnataka INDIA.

Isolation of Lactic acid bacteria

Healthy fishes were selected for the isolation of Lactobacilli, fishes were brought to laboratory alive and sacrificed. The ventral surface was sterilized using 70% ethanol and aseptically dissected to remove the intestines. The intestines were opened by a longitudinal incision and thoroughly flushed with a sterilized normal saline solution (NSS) to remove the feed materials, dirt and other impurities. Excess moisture was blotted with filter paper and the intestines were weighed, macerated with sterile glass rod and homogenized in sterile NSS (1:10: wt: vol) using a vortex mixer. These samples were serially diluted in NSS and aseptically plated by the spread plate technique on MRS media (Hi media, India) (Gohs *et al.,* 2007). The inoculated agar plates were incubated at 30-40^{0}C for 5-7 days. MRS agar was used for enumeration and cultivation of LAB (De man *et al.,* 1960). Well isolated colonies with typical characteristics namely pure white, small (2-3 mm diameter) with entire margins were picked from each plate for further identification.

Identification of the lactic acid bacteria

The cultures were identified according to their morphological, cultural, physiological and biochemical characteristics based on Gram reaction, Motility, Spore formation, Catalase and Oxidase activity, Nitrate reduction, Hydrogen sulfide production. Casein and urea hydrolysis, Gelatin liquefaction and IMVIC test were done. Phonotypical identification of Lactic acid bacteria was done by using a carbohydrate fermentation test kit (Hi media) (Kandler and Weiss 1986).

Experimental diets

The formulation of the experimental diet is given in (Table I). Feed diet was prepared containing similar ingredient composition (soya bean meal 25%, ground nut oil cake 25%, rice bran, 38%, wheat flour 10%, vitamin and mineral mixture 2%). Soya bean meal was used as sources of protein, ground nut oil cake was used as lipid sources, wheat and rice bran were used as the carbohydrate source. Bacterial strain of *Lactobacillus* sp at five different levels (1.0 (T1), 1.5 (T2), 2.0 (T3) and 2.5 (T4) X 10^7 CFU g^{-1} were mixed with feed supplements. The control diet (T5) was not supplemented with bacterial cells.

Table I. Ingredient composition (g kg^{-1} dry weight) of the experimental diets

Ingredient	Composition
Soya bean meal	25%
Ground nut oil cake	25%
Rice bran	38%
Wheat flour	10%
Vitamin and mineral mixture	2%

Experimental design

The experiment was conducted in laboratory condition for 60 days. Common carps were obtained from National fish seed farm B.R. Project, Karnataka. The collected fish was transferred alive in polyethylene bags and brought to the laboratory and acclimated for two days feeding on mixed plankton. One hundred acclimated common carp of similar size (average weight 24±1gm) were randomly distributed in plastic containers filled with unchlorinated water. Constant aeration was provided for each container using an air compressor.

Physico-chemical parameters of water

Water quality parameters like temperature, pH, Dissolved oxygen, Alkalinity, Hardness and Ammonia were recorded at weekly intervals during the entire experimental period by using standard methods of APHA (2005). pH was measured by a digital pH meter (Lab India).

Collection of blood sample

During the experimental period 15, 30, 45, 60 days intervals, blood samples were collected randomly, Blood was drawn from both probiotic fed fishes and control fishes by cardiac puncture using 2ml syringes and gauge hypodermic needles. The point of insertion for heart puncture is ventral, midway between the anterior bases of the pectoral fins. The syringe is flushed with EDTA (Anticoagulant) about 150 to 200µl of anticoagulant were retained in the needle and then the blood was drawn to avoid coagulation. The collected blood was transferred in to eppendrofs of 1.5 ml capacity and stored in refrigerator for further analysis.

Digestive enzyme activity

The digestive enzymes such as protease activities were assayed according to Anson (1938) and Folin (1928). Amylase activities were determined based on the method of Smith and Roe (1949). Lipase activities were determined by the titrimetric method (Teitz and Fiereck, 1966: Borlongan, 1990) measuring the fatty acids liberated.

Statistical analysis

The results are presented as means± SD, difference between parameters were analyzed by one way analysis of variance(ANOVA) and statistical significance was tested at $p< 0.05$ and $p< 0.001$ level. Statistical assessment of result was carried out using SSPS software.

RESULT

Isolation of lactic acid bacteria

The LAB was isolated from fish intestine. After isolation the isolated organism was identified up a genes level based on their morphological, cultural, physiological and biochemical characteristics (Sharpe *et al.,* 1979). After Incubation on MRS agar for 24 to72 hr, isolate formed round, creamy white colony, grown at 30 to 40^0 C, the optimum pH was 5.5-6.5. The isolate was tested for biochemical and other physiological characteristics. Their distinguishing feature is shown in (Table II and Table III). The isolate was hetero fermentative *Lactobacillu* sp, with negative patter of H_2S formation, nitrate reduction, catalase activity and urease activity. Fermentation test was shown in (Table III).

Water quality parameters

Water quality parameters in all the treated tanks were observed to be normal and remained within ranges allowing for high growth rate of *Cyprinus carpio.*

Growth performance

The growth performance including IBW, FBW, SGR and survival rate of *Cyprinus carpio* shown in (Figure I). No significant difference was observed in IBW among all treatments. Fish fed the experimental diets T1, T2, T3 and T4 exhibited higher FBW and SGR compared to control diet. The higher FBW and SGR were observed in T3 at 45 days (56.05±2.18) and (1.38±0.07) compared to control diet. Results showed that fish fed diets containing different probiotic groups had significantly better growth performance than the control diet.

Table II. Morphological, cultural and physiological characteristics of the isolated organism

Test	Result
Growth temperature	30-40^0 C
Colony color	pure white
Colony size	small (2-3mm)
Colony margin	entire
Gram stain	positive
Shape	rod
Motility test	negative
Catalase test	negative
Oxidase test	negative
Indole test	negative
Citrate utilization	negative
Nitrate reduction	negative
Gelatin liquefaction	negative
H2s production	negative
Methyl red test	negative
Voges –proskaur test	negative
Casein hydrolysis	negative

Table III. Biochemical characteristics of the tested isolate by utilization of Carbon sources

Carbohydrate Source	Reactions
Arabinose	+
Cellobiose	+
D -Fructose	+
Galactose	+
D -Glucose	+
Lactose	+
Maltose	+
Mannitol	+
Mannose	+
Melibiose	+
Raffinose	+
Rhamnose	-
Ribose	+
Salicin	+
Sorbitaol	+
Sucrose	+
Terhaldose	+

Symbols: + Positive, - Nagative

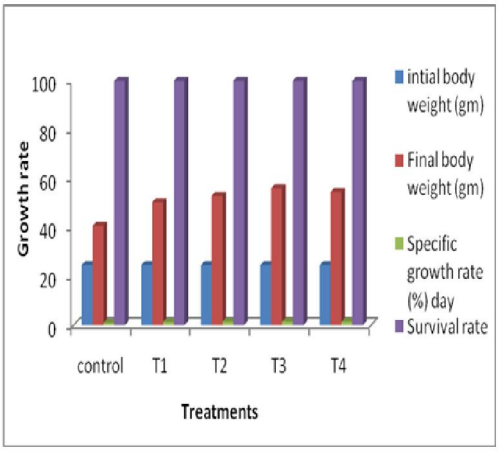

Figure 1. Growth performance of *C. Carpio* fed with different probiotic groups.

Digestive enzyme activity

The total Amylase activities of *Cyprinus carpio* fed different dietary probiotics were significantly higher than the control diet showed in (Figure II). The higher total amylase activities were recorded for fish fed T3 for 45 days (43.98±0.01) than the control diet (19.20±1.161). The total Protease activities in all experimental treatments was shown in (Figure III). The highest protease activity was recorded for fish fed the T3 at 45day (6.60±0.01) than compared control diet (3.16±0.58). The Lipase activity was illustrated in (Figure IV). The supplementation of probiotic isolate to the diet improved the total lipase activity of *Cyprinus carpio* compared to the control diet. The highest total lipase activity was observed for fish fed T3 at 45 days (5.16±0.09) than compared to control diet (2.26±0.34).

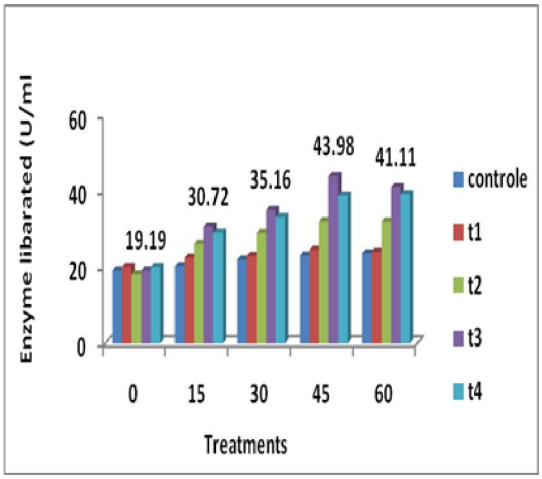

Figure 2. Amylase enzyme activity fed with Different probiotic groups

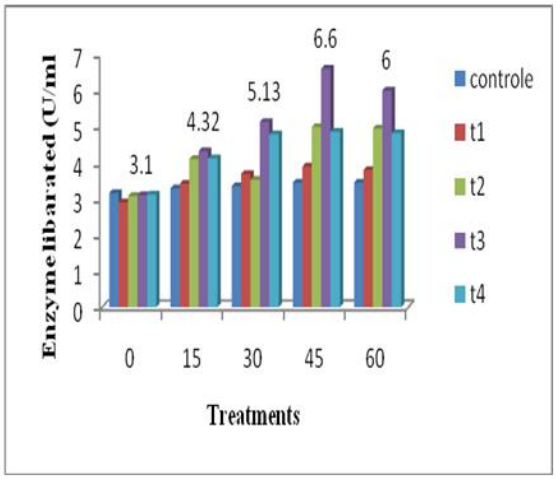

Figure 3. Protease enzyme activity fed with different probiotic groups

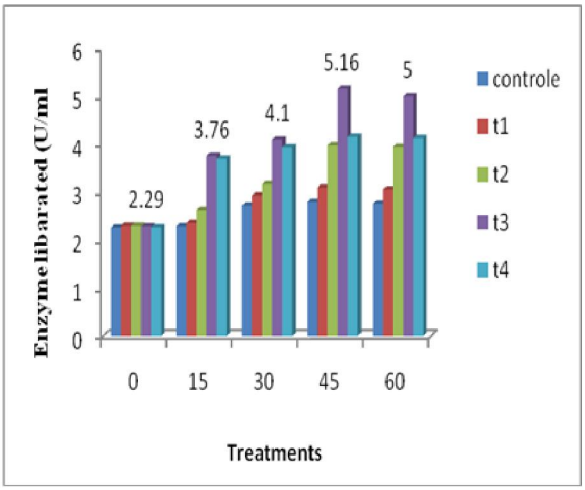

Figure 4. Lipase enzyme activity fed with different probiotic groups

DISCUSSION

The LAB are a major component of the microbial flora that developed in various animal intestines (Devriese *et al*., 1987: Mitsuoka, 1980). The exact mode of action of the probiotic has not been fully elucidated and there is continuous argue about its effect on the water quality. In the present, there is no obvious effect of the probiotics added to feeds on water quality, this agrees with the finding of (Yanbo and Zirong. 2006: Mohamed *et al.,* 2010). The probiotics supplementation of the experimental diets resulted in higher growth and feed utilization as compared with the control diet. The increase in growth of of *Cyprinus carpio* by the inclusion of *Lactobacillus* sp, may be due to that most of *Lactobacillus* sp can produce secondary metabolites which have been used industrially for the production of antibiotics, bio insecticides, and enzymes that readily hydrolyze carbohydrates, lipids and proteins into sugars, fatty acids, peptides and amino acids (Sonnenschein *et al.,* 1993; Godfrey and West, 1996; Olmos *et al*., 1998). Similar results were found for *Cyprinus carpio* (Yanboand Zirong, 2006).

The improvement of feed utilization of fish fed diet supplemented with *Lactobacillus* sp. could be due to improvement in intestinal microbial flora balance which in turn will lead to better nutrient digestibility, higher absorption quality, increase enzyme activities (Lara-Flores *et al.,* 2003; Balcázar *et al*., 2006) and also more degradation of higher molecular weight protein to lower molecular weight peptides and amino acids (De Schrijver and Ollevier, 2000). Digestive enzymes are one of the most important factors that influence the efficiency of feed utilization in the fish and characterization of these enzymes provides some information regarding the digestive capacity of the fish to hydrolyze the carbohydrate, protein and lipid of feed ingredients (Lemieux *et al*., 1999). The addition of probiotic as live supplements in the diet allows probiotic to survive passage through the intestinal tract (Fuller, 1992). Microorganisms and their enzymes have an important role in the digestion process (Munilla-Moran *et al*., 1990) by increasing the total enzyme activity of the gut (Ding *et al*., 2004). As pointed by several authors the digestive enzymes (amylase, protease and lipase) could be improved by administration of probiotics to the diet (Ziaei-Nejad *et al*., 2006; Wang, 2007; Gomez *et al.*, 2008). The present study showed that the highest levels of amylase, protease and lipase have been recorded for *Cyprinus carpio* fed *Lactobacillus* sp. This may be attributed to the higher ability of *Lactobacillus* sp to secrete a wide range of Exo enzymes or enhance the activities of endogenous digestive enzymes (Suzer *et al.,* 2008).

Conclusion

The present study suggested that the incorporation of probiotic to *C.carpio* which can stimulate the fish growth and digestion. The beneficial effects of probiotics on fish growth appears to be associated with colonization of favorable microbiota in the gut, which produce enzymes that hydrolyses complex molecules, facilitate better digestion and absorption of macronucleus resulting in higher protein and energy retention in the body. The results suggest that *Lactobacillus* sp. could be used effectively as a probiotics for the use in aquaculture.

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