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Research Article

INFLUENCE OF PROBIOTICS ON GROWTH, IMMUNOLOGICAL PARAMETERS AND HISTOLOGICAL OBSERVATIONS OF *CATLA CATLA, LABEO ROHITA AND CTENOPHYRGODON IDELLA*

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ABSTRACT

Probiotics are often employed to bacterial pathogens control in the aquaculture systems. Beneficial probiotics such as *Lactobacillus, Nitrosomonas, Nitrobacter, Bacillus* and *Vibirio* sp. are used commercially. In the present investigation, two types of commercial probiotics were used i.e., Aqua gut (fish) as feed probiotic-treatment-2 and Nitro-PS+ Micro-Pro (fish) as soil and water probiotics treatment-3 (manufactured by Asian Bio Tech, Hyderabad, Andhra Pradesh, India). The fishponds stocked with catla (*Catla catla*), rohu (*Labeo rohita*) and grass carp (*Ctenopharyngodon idella*) were selected and are designated as control pond (T1), Treatment-2 and Treatment-3. Treatment 2 and 3 were treated with probiotics control pond without probiotic. In Experiments was conducted for the period of one year from August 2012 to July 2013. The review is therefore, aiming to highlight the serum IgM levels of experimental fish were higher than that of control fish. The fish with probiotics (T2 and T3) showed in the height of the epithelial layer of the villi of the middle portion of the intestine, then in control treatments.

INTRODUCTION

Probiotics are usually live microorganisms which administered in adequate amount confer a health benefits on host. Nowadays, probiotics are also becoming an important part of the aquaculture practices to obtain high production. In aquaculture, probiotics have been used to control diseases, enhance non-specific and specific immunity, provide nutrients and enzymatic functions and improve water quality (Schaperclaus *et al.,* 1992 and Balcázar *et al.,* 2006). The immune system of aquatic organisms, such as fish, is continuously affected by unexpected or periodic changes of their environment. Adverse environmental situations may chronically or acutely stress the health of fish, altering some of their biochemical and suppressing their adaptive and innate immune responses (Giro´n-Pe´rez *et al.,* 2007).

The study of the immune system which is the main defense mechanism of the body protecting it from various disease causing agents. Particularly early stages fish fry are very sensitive, they are prone to be affected by many disease causing agents. Immunity mainly involves the antigen and antibody interactions. In aquaculture, diseases outbreaks are major obstacles for successful aquaculture. Immunostimulation is one of the important areas to be concentrated for successful aquaculture. *Achyranthes aspera* has immune stimulating property and is used to enhance the immunity of *Catla catla* (Rao and Chakrabarti, 2005). The architectural dynamics of a tissue is essential for maintaining the structural integrity and for effective biochemical, physiological and metabolic functions. The cellular and sub-cellular constituents of tissue in terms of size, shape, position and number play an important role in the physiological and metabolic functions. Therefore, the histological structure of tissue in an animal has a profound effect on its function. The study of histology in micro anatomy of specific tissues has been successfully employed as a diagnostic tool in medical and veterinary sciences since the first cellular study carried out in the 19th century (Virchow, 1859). Hence, it is useful to have an insight into the histological analysis as they act as biological markers to assess the fish condition (Tilak *et al*., 2001).

MATERIALS AND METHODS

The fish ponds located in a farm at Machilipatnam in Krishna district, Andhra Pradesh, India were chosen for the present study. All the control and experimental ponds (triplicates) goes to rectangular in shape and size goes 2.5ha. The Experiments was conducted for the period of one year from August 2012 to July 2013. The fishponds stocked with catla (*Catla catla*), rohu (*Labeo rohita)* and grass carp (*Ctenopharyngodon idella*) were selected and are designated as control pond (T1), Treatment-2 and Treatment-3. Treatment 2 and 3 were treated with

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probiotics control pond without probiotic. In the present investigation, two types of commercial probiotics were used i.e., Aqua gut (fish) as feed probiotic-treatment-2 and Nitro-PS+ Micro-Pro (fish) as soil and water probiotics treatment-3 (manufactured by Asian Bio Tech, Hyderabad, Andhra Pradesh, India).

Collection of blood samples

For bleeding, each fishes were individually caught using a dip net and were bled from common cardinal vein using 1 ml tuberculin syringe fitted with 24 gauge needle (Michael *et al*., 1994). In order to collect the blood for serum separation, 200 µl of blood was drawn and whole bleeding procedure was completed within 1 min. The blood was collected in a serological tubes and clot was stored at 20^{o}C overnight. The clot was then spun down at 400 g for 10 min. The serum collected by aspiration was stored in sterile Eppendorf tubes at 20^{o}C for further use.

Preparation of serum samples

Blood samples were collected into a 1 ml sterile syringe (24 gauge). The blood was transferred to the Eppendrof tube and centrifuged for about 6 min. at 3000 g and 4^{o}C. The supernatant liquid was used as serum.

***Measurement of concentration of immunoglobulin-like substances*, (Ig-G-like, Ig-A-like, and Ig-M) in the serum**

The Immunoglobulin like substances in the serum was measured with the help of kit (Biosystems S.A Costa Brava 30, Barcelona, Spain) and was expressed as g/l. The estimation of Immunoglobulins was done in collaboration with **Medcis Pathlabs India Pvt. Ltd.,** Secunderabad.

Histological parameters

Fish samples were stored in formalin solution (4%) and then eviscerated to remove their digestive tract. Paraffined blocks of fish intestine were prepared and then sliced by microtom to give sections of 4 to 5 µ. Sections were stained by the coloration methods of hematoxilin and eosin and then studied under microscope (Mumford, 2004).

RESULTS

Immune Parameters

The use of probiotics in feed has showed positively good results in *Catla catla, Labeo rohita* and *Ctenopharyngodon idella*. In the present study, it was observed that the immune response regarding the serum Ig levels were significantly more in probiotic included control pond and Experimental ponds (Table 1- 3)

Ig Levels

The IgA, IgG and IgM levels were estimated in the present study. The Ig levels were observed to be control pond (T1), Experimental ponds (T2) and (T3).

Table 1 IgA, IgG and IgM levels in *Catla catla,* Values expressed as (g/l)

T1			T2			T3		
IgA	IgG	IgM	IgA	IgG	IgM	IgA	IgG	IgM
0.16	1.55	0.26	0.39	01.99	0.48	0.40	1.95	0.50

Table 2 IgA, IgG and IgM levels in *Labeo rohita* Values expressed as (g/l)

T1			T2			T3		
IgA	IgG	IgM	IgA	IgG	IgM	IgA	IgG	IgM
0.35	2.02	0.29	0.59	2.42	0.43	0.60	2.48	0.46

Table 3 IgA, IgG and IgM levels in *Ctenopharyngodon idella* Values expressed as (g/l)

T1			T2			T3		
IgA	IgG	IgM	IgA	IgG	IgM	IgA	IgG	IgM
0.29	1.91	0.38	0.53	2.57	0.33	0.56	3.00	0.36

Histopathological studies

The histology section of the experimental ponds revealed that the disease resistances was enhanced in *Catla catla, Labeo rohita and Ctenopharyngodon idella* control pond (T1), feed probiotic (T2) and water and soil probiotics (T3). Among the feed probiotic (T2) Catla *catla, Labeo rohita and Ctenopharyngodon idella* water and soil probiotics (T3) showed good structure and functional differentiation of selective tissues, but was less when compared with control pond. The controlled pond fishes showed diseases area in their tissues. There were many bacterial infections found among *Catla catla, Labeo rohita* and *Ctenopharyngodon idella* control pond (T1). The feed probiotics (T2) showed highest efficiency than water and soil probiotics. This show that *Lactobacillus* and *Bacillus* sp. showed not only good growth and survival but are also very effective against disease especially bacterial infections.

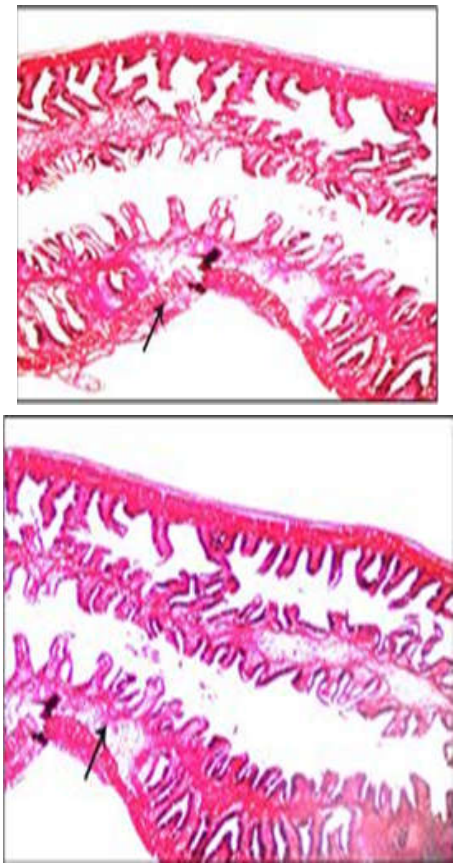

Fig 1 Micrographsphoto of the epithilium layer of the villi of the middle intestine of *Labeo rohita* Expermental fish (T2) and (T3): Epithilium: (EP): stain (H&E)

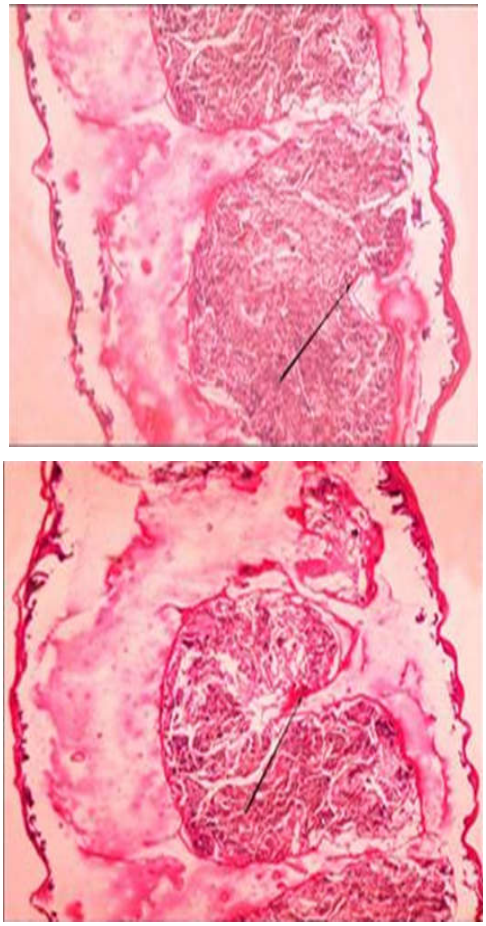

Fig 2 Micrographsphoto of the epithilium layer of the villi of the middle intestine of Catla catla Expermental fish (T2) and (T3): Epithilium: (EP): stain (H&E)

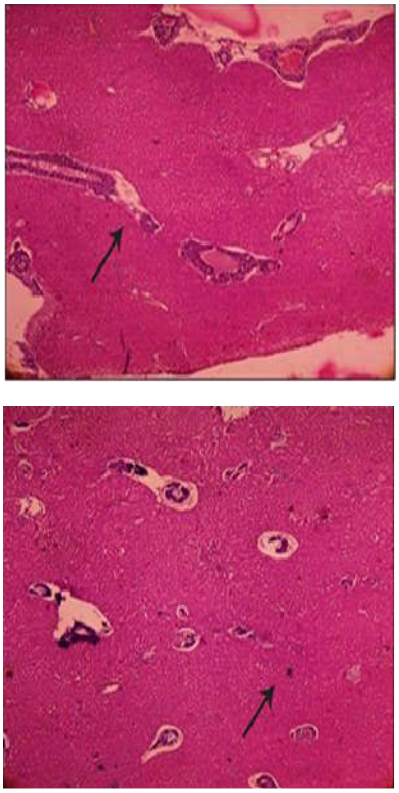

Fig 3 Micrographsphoto of the Liver of *Labeo rohita* Expermental fish (T2) and (T3): Epithilium: (EP): stain (H&E)

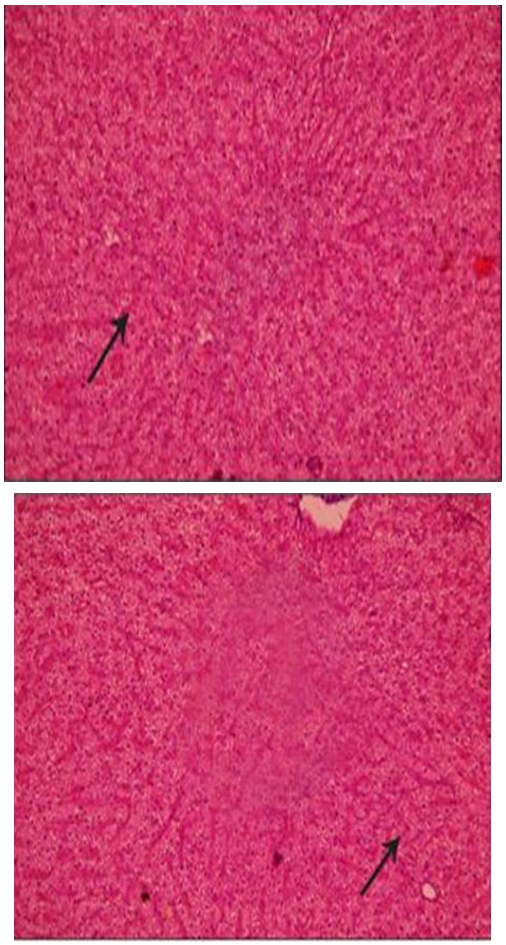

Fig 4 Micrographsphoto of the Liver of *Ctenopharyngodon idella* Expermental fish (T2) and (T3): Epithilium: (EP): stain (H&E)

DISSCUSSION

Sustainable aquaculture depends on the management of health condition of fish. The use of antibiotics and chemotherapeutics to combat fish diseases has the risk of generating resistant pathogens, bioaccumulation and environmental pollution. Commercial vaccines are very expensive for fish farmer and they are specific against particular pathogens. The use of probiotics as biological control agents or as dietary live microbial supplements in commercial fish culture to immune function and improve growth of the fish (Gatesoupe, 1999; Irianto and Austin, 2002; Kesarcodi-Watson *et al.*, 2008). Fish immunology is a key element in establishing strategies for the control of disease in fish aquaculture. There is a great deal of potential for the application of Immunostimulants. Probiotics, according to this currently adopted definition by Food and Agricultural Organisms/World Health Organization, are live microorganisms that when administered in sufficient amounts confer a health benefit on the host (FAO, 2001).

Feed probiotics are live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance (Fuller, 1989, 1991). The potential benefits include improved nutrition and growth and prevention of various disorders. Products containing probiotics are available for human nutrition as animal feed supplements and also for aquaculture (Rowland, 1999; Tournot, 1989; Rolfe, 2000 and Verschuere *et al*., 2000). Serum immunoglobulins are major part of the humoral immune system and IgM is the main

immunoglobulin present in fish (Wlison *et al.,* 1995). Previous studies revealed that probiotic bacteria could stimulate immunoglobulins production in fish (Nikoskelainen, *et al.,* 2003; Panigrahi, *et al.,* 2005; Al-Dohail *et al,* 2009 and Watts *et al.,* 2001). In the present study, the serum IgM levels of experimental fish were higher than that of control fish (Table 1-3). Therefore, concluded that the stimulation of the immunoglobulin levels was a short-term phenomenon attributable to probiotics.

The histopathologic effects in the internal organs (intestine and liver) of *Catla catla, Labeo rohita* and *Ctenopharyngodon idella* are showed in the present study (Fig 1-4). The mucosal tissue, including mucus, epithelial cells lining the gill and digestive tract of fish, provide an initial barrier the entry of disease-causing microorganism like bacteria and viruses (Press and Evensen, 1999). The fish with probiotics (T2 and T3) showed in the height of the epithelial layer of the villi of the middle portion of the intestine, when compared to the control treatments (Fig 1-2). That fact shows that the probiotic promoted an increase in the epithelial layer of the middle intestine of those fish, coinciding with the studies described by Medri *et al.,* (1999). The results of hepatic enzymes analysis which decreased in fish kept on probiotics in comparison to control treatment indicating a normal, positive and beneficial effect of both probiotics on the maintenance of the integrity of hepatocytes and their roles in improvement of liver histology (Table 3-4). Similar results observed by were supported by several authors (Jessus *et al*. 2002; Nayak *et al*. 2004 and Safinaz 2006).

CONCLUSION

The probiotics played a major role in maintaining physico-chemical parameters and bacterial load data that the *Lactobacillus, Bacillus, Nitrosomonas and Nitrobacter* species were dominated and suppressed the *and Vibrio sp* in the probiotic used ponds when compared to the control pond. The present study revealed that the Ig levels were higher in T2, T3 than T1. This showed that T2 and T3 a very good resistance to the commonly seen in carps. Hence higher yields with better growth and survival can be achieved by using probiotics in aquaculture ponds.

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