

Use of Immunostimulants to Improve the Water Quality in Aquaculture Systems: Histopathological studies on the Effect of Immunex Ds and H-Treat Treated Fish, Labeo Rohita During Aeromoniasis

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Abstract

Immunostimulatory effect of commercially available supplementary diets, Immunex Ds and H-Treat in disease against *Aeromonas liquefaciens* was investigated in *Labeo rohita*. In the present study, 6 groups were employed. Group A (treated with Immunex Ds @ 40mg/100g. of feed), Group B (infected with *A. liquefaciens* @ 10^5 CFU/fish), Group C (untreated and uninfected controls), Group a (feed with H- Treat @ 40mg/100gm of feed), Group b (infected with *A. liquefaciens* @ 10^5 CFU/fish) and Group c (untreated and uninfected controls) of gill, intestine & thymus samples from fish of each group studied histopathologically on day 5 of experiment. Marked changes were observed in all the tested organs of experimented groups compared with controls.

Keywords

Immunostimulations, Immunex-Ds, Liquefaciens

I. Introduction

Aeromoniasis is one of the most common bacterial diseases of fresh water fish. This disease has been associated with several members of the genus *Aeromonas*, including *A. liquefaciens* and *A. hydrophila*. *A. liquefaciens* is ubiquitous in nature and is even found in the intestinal tract of the fish. In natural situations, infection of fish with *Aeromonas* is probably a minor problem. However, in intensive fish farming systems, like outdoor ponds, aquaria or tanks, some abnormal factors other than pathogens play a role in the onset of disease when fish are feed with unbalanced diet, nutritionally deficient diet, or exposed to stress i.e. poor water quality, overstocking etc. *A. hydrophila* causes disease in fish known as Mobile *Aeromonas* Septisemia (MAS), Halmorrhagic Septicemia, ulcer disease or Red sore disease. The clinical symptoms of this disease related to the lesions caused by this bacterium which include septicemia where the bacterial or bacterial toxics are present within numerous organs of the fish, and ulcers on the skin. The clinical symptoms include swimming abnormalities, pale gills, bloated appearance and skin ulcerations. The skin ulcers may occur at any site on the fish and often are surrounded by a bright rim of red tissue. Other organs commonly affected with aeromoniasis include gill, kidney, liver, spleen, pancreas and skeletal muscle. The onset of disease symptoms are dependent upon a number of factors including the virulence of the organism, the resistance of the fish to infection, the presence or absence of bacteria or septicemia and stress factors associated with the fish. This may be economically disastrous to the fish produces.

These fish diseases can be controlled with the help of antibiotics and other drugs. However, the emergence of antibiotic resistant microorganisms is an important obstacle to their extensive use. Recently the use of immunostimulants was introduced as a

prophylactic measure (Anderson, 1992). An immunostimulant is substance that elevates non specific defense mechanism and specific immune response if the treatment is followed by vaccination or infection (Anderson, 1992). Traditional use of medicinal herbs in India and China to control fish diseases has been reviewed.

Immunex Ds is a special formulation to include response and to protect fish against pathogens. It contains β carotins, lysine, DL methionine, fatty acids, livamisol hydrochloride, vitamins A, D3, E, C, B12, minerals and probiotics. H-Treat is an herbal mixture of Arjuna, Aswaganitha, Vitis vinifera, Harada and Neem. These herbal plants are well known for hepatoprotective, antistress, antioxidant, astringent, expectorant etc. The objective of the present study is to investigate the histopathological studies in gill, intestine and thymus (on day 5 of experiment) in experimental and controls.

II. Material and Methods

A. Experimental Fish

Experiments were performed on the common fresh water carp, *Labeo rohita* (Indian Major carp) which is extensively cultured in India and is valued as an important food fish.

B. Procurement and Maintenance of fish:

Healthy fish with an average weight of 50-60 gm. were obtained from Jalipudi fish farm, Jalipudi Mandal, West Godavari District, Andhra Pradesh and kept in the laboratory for acclimatization for about 4-5 days before they were used for experimentation. Dechlorinated ground water was used during acclimation and experimental period. The water in acclimation tanks were frequently oxygenated with electrical aerators.

C. Bacterial strain and cultivation:

Aeromonas liquefaciens strain, MTCC 2654 (virulent strain) was obtained from MTCC, Chandigarh, India. From this parent culture, sub cultures of *A. liquefaciens* were prepared and doses were made under aseptic condition.

D. Antigen Dose

Various doses like 10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} and 10^{-9} CFU/fish were injected to the fish to include aeromoniasis and determined 10⁻⁴ dose as LD50. So 10⁻⁵ CFU/fish was selected for experimentation as optimum dose.

E. Route of Infection

A. liquefaciens bacterial suspension was injected to the fish, *L. rohita* intramuscularly near the anal region.

F. Immunostimulants

Immunex Ds and H-Treat were used in the present investigations (manufactured from PVS laboratories Ltd., Vijayawada, Krishna District, A.P, India). Test does of H-Treat was selected as per the recommended dosage given by PVS lab i.e. 5g.per kg. of pellets).

G. Culture method of *Aeromonas liquefaciens* :

Culture of *A. liquefaciens* was done following the method of pelczar et. al (1993). Healthy fishes were divided into six groups and maintained in separate tanks each with twenty fish as derailed below.

- Group A - treated with Immunex Ds @ 40mg/100g. of feed
- Group B - infected with *A. liquefaciens* 10-5CFU/fish
- Group C - untreated and uninfected controls
- Group a - feed with H- Treat @ 40mg/100gm of feed
- Group b- infected with *A. liquefaciens* @ 10-5 CFU/fish
- Group c - untreated and uninfected controls

Histopathological studies were made from gill, intestine, and thymus from experimental (on day 5 of experiment) and control groups of fish following H & E methods.

All the experimental groups (A, a, B, b) of fish were observed daily for disease symptoms and mortality up to 5 days of experimental period. Infected fish (group B and group b) exhibited lethargy reduced appetite, breathing difficulty, haemorrhages on the lateral sides of tail and scale falling and erosion at certain places in the abdomen.

III. Results

A. Histopathology

Gross external symptoms of disease (aeromoniasis) such as haemorrhagic skin, scale erosion, distended abdomen as well as fin and tail rot were observed in the experimental fish, rohu (Plate 1). The histopathological changes observed in the gill, intestine and thymus on day 5 of experiment (in all experimental groups) revealed that infective and pathogenic bacteria, *A. liquefaciens* caused marked effect in their architecture. The pathological alterations in the tissues of experimental animals would be due to the direct effect of pathogens. The organ of respiration (gill), digestion (intestine) and primary lymphoid organ (thymus) showed much pathological changes due to the ill effect of infectious pathogens.

B. Discussion

Gills which perform many functions like respiration, Osmoregulation and excretion remain in close contact with the aquatic environment are particularly sensitive for the invasion of pathogen. In group B which received infection, gills showed degenerative and necrotic changes in primary and secondary lamellae. These pathogenic changes may be due to the pathogenic action of bacteria or an adoptive response to prevent the invasion of pathogens. These observations are in agreement to those of Thophon (2003), Camargo and Martinez (2007) and Mohamed (2009) who described gills as sensitive organs contaminants and display varied pathological changes due to the exposure of toxicants. The pathological changes like currently and clubbing of secondary lamellae, partial fusion of secondary lamellae, enlargement of primary lamellae are the defense mechanisms by which the host tries to get rid of pathogens as suggested by poleksic and mirtovic Tutundzic (1994) and Fernandes and Mazon (2003) in freshwater fish. The pathological changes in the gills in the present study are similar to those observed in other fish species

exposed to different pollutions (Kakuta and mirachi, 1997, olurin et.at , 2006; camargo and Martinez, 2007; Mohmed, 2009).

The pathological findings in the intestine of all the experimental groups of fish include degeneration and necrosis in the intestine mucosa, fragmentation of villi and aggregation of fragments of villi in the intestinal lumen. Severe damage is seen only in fish infected with high dose of bacteria. These observations are in agreement with many researches about the ill effect of toxicants to intestine (Hanna et. al, 2005; Cengie and Unlu, 2006; Soufy et. al, 2007; Bhatnagar et. al, 2007).

The common alterations in the thymus of experimental fish (infected and / or treated with antibiotic) were degenerative and necrotic changes in the medulla, reduction of lymphoid tissue and lymphocytes and appearance of focal areas and cellular debris. The degeneration and necrotic charges in the lymphoid tissue may affect the hematopoiesis. Lymphocyte stimulation and production of specific antibodies. The observed changes in the histopathology of thymus are indicators of immunotoxicity as suggested by Haley et. al (2005).

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