

Comparative analysis of immune response in respect of different diet condition of *Catla catla*

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Abstract

Immunoglobulin's fill in as an essential arm of the versatile safe framework against hindering pathogenic dangers in transports. Be that as it may, regardless of whether the novel Ig isotope IgZ is available in the Indian significant carp, catla has not yet been clarified. The present examination reports the nearness of IgZ ortholog in C. catla (CcIgZ) and further exhibits its relative tissue explicit articulation with IgM (CcIgM) in light of bacterial and parasitic incitement. The putative 139 amino corrosive grouping of IgZ substantial chain cDNA of C. catla demonstrated homology with IgZ steady spaces of different transports. Phylogenetic investigation of the anticipated IgZ transcript succession bunched with recently distinguished IgZ overwhelming chain arrangements of Cyprinidae relatives.

Keywords: *Catla catla*, IgZ, pathogen, tissue expression

Introduction

Immunoglobulins are the significant effectors atoms of the versatile insusceptible framework and their development in the paraphyletic gathering of fish is a trademark in the advancement of vertebrates. The guideline of prototypic versatile safe frameworks is inferable from age of express neutralizer receptor assorted variety through different lymphocyte proclivity development procedures, for example, VDJ recombination, physical hypermutation and class switch recombination in completely jawed vertebrates (1–3). The steady overwhelming chain space of various Ig isotopes directs its most significant effectors capacities, for example, activating of supplement course, commencement of phagocytosis and organization of mucosal discharges (4, 5). As opposed to warm blooded animals, who have five distinctive Ig isotopes—IgG, IgM, IgA, IgD and IgE, until 2005 the most crude vertebrate class, fish, were accepted to have just two Ig isotopes-IgM and IgD (6–9).



Figure: 1 Catla Fish

Indian significant carps are broadly refined across Bangladesh, Myanmar, Nepal and Pakistan. Both rogue and catla have been brought as colorful species into nine nonnative nations and marital into seven. Until the nineteenth century, carp culture was kept to terrace lakes in the eastern Indian conditions of West Bengal, Orissa and Bihar. At that point, the seed was collected from the nearby waterways. In 1957, incubation facility and hypophysation reproducing technologies were created, and this gave the driving force to another time of carp culture in the nation. Somewhere in the range of 1963 and 1984, the fruitful exhibit of polyculture frameworks dependent on Indian and Chinese carps by the Focal Inland Fisheries Exploration Organization in West Bengal, and an effective show

program by the Fish Rancher Improvement Offices brought about the commercialization of the technologies.

Infectious diseases are a significant issue in aquaculture making overwhelming misfortune the fish farmers. The ongoing development of serious aquaculture rehearses has prompted high enthusiasm for understanding the different fish diseases, with the goal that they can be dealt with or forestalled. It is generally shown that the event of diseases in fish ranch is because of a few variables worried about the raising techniques, ecological conditions and varieties. Thusly, developed fish can turn out to be progressively vulnerable not exclusively to pathogenic yet in addition to artful microscopic organisms (Charm and Bruno, 1998). Stimulant is an interesting and incredible mix of safe stimulatory particles that have been gotten from characteristic sources and have the solid capacity to enact the vague protection instrument in fish and shrimps.

Cynodon dactylon (L.) Pers. (Gramineae, Poaceae) is a natural plant normally known as 'Arugampul' in Tamil Nadu, India, which is treated as a gift plant. This grass is broadly circulated in India and in practically all pieces of the world. Generally, the juice of this plant is regularly expended as a wellbeing drink during early morning in south India. It plays a significant job in Ayurvedic medication. *C. dactylon* extricate is utilized to treat madness, epilepsy and craziness. Customary healers utilized *C. dactylon* for cleansing blood, anuria, biliousness, conjunctivitis, looseness of the bowels, gonorrhea, tingles and stomachache (Muthu et al., 2006). The present examination assesses the impacts of dietary organization of *C. dactylon* ethanolic extricate on the potential recuperation in *C. catla* against *A. hydrophila*, which is related with hematological, biochemical and histological changes.

Aquaculture is one of the rapidly creating frameworks on the planet, which has ascended as an industry possible to supply protein rich nourishment all through the world. Fish is a fundamental dietary creature protein source in human sustenance. Creation of maritime species through freshwater fisheries and aquaculture for protein supply is being invigorated all through the world. As demonstrated by nutritionists, fish is a bewildering substitute of protein for red meat. Fish tissue contains all the essential amino destructive and minerals viz., iodine, phosphorus, potassium, iron, copper and nutrient an and D in charming fixations. Breaker of Duckweed in diets of catla can be considered as a cost ground-breaking approach reducing the usage of generally exorbitant soybean.

Literature Review

Akhil Abhishek, Dr. Akhilesh Kumar (2018), An extensive preliminary was attempted to survey the impact of different kinds of feed fixings on the biomass change rate in a 12-week encouraging preliminaries to assess the utilization of agro - based items, as locally accessible feed fixing materials for fish (catla) fingerling development exhibitions. Aquaculture is one of the speediest creating sustenance divisions of the world and records for directly around half of the world fish creation. Functional creation is affected principally by components, for instance, poor water quality administration, healthfully imbalanced valuable reinforce and the infection rate. To control of these diseases, unusual use of antimicrobial medications in fish developing lead to the ascent of against contamination safe tiny living beings. Probiotics expect a basic part in development expansion and stress control in fish.

Bhakti Patel, Rajanya Banerjee, Madhubanti Basu, Saswati Lenka, Mrinal Samanta and Surajit Das(2016), Immunoglobulin's fill in as a vital arm of the versatile resistant framework against inconvenient pathogenic dangers in teleports. Notwithstanding, regardless of whether the novel Ig isotope IgZ is available in the Indian significant carp, catla, has not yet been explained. The present examination reports the nearness of IgZ ortholog in *C. catla* (CcIgZ) and further exhibits its relative tissue explicit articulation with IgM (CcIgM) because of bacterial and parasitic incitement. The putative 139 amino corrosive succession of IgZ overwhelming chain cDNA of *C. catla* indicated homology with IgZ steady areas of different teleports. Phylogenetic examination of the anticipated

IgZ transcript succession bunched with recently distinguished IgZ overwhelming chain groupings of Cyprinidae relatives.

R. Ramakrishna, Thomas A. Shipton (2013), This examination surveys the aquaculture of Indian significant carps, rohu (*Labeo rohita*), (catla) and mrigal (*Cirrhinus cirrhosus*) with extraordinary reference to current bolstering and feed the board rehearses in Andhra Pradesh, India. The investigation depends on an overview of 106 farmers from four areas in Andhra Pradesh (Kolleru, Krishna, West Godavari, and Nellore). Kolleru and the encompassing regions of Krishna and West Godavari are the essential culture regions. In Nellore locale, Indian significant carp culture is rehearsed at a lower power to that rehearsed in Kolleru. In East Godavari area, Indian significant carps are fundamentally refined in polyculture frameworks with either dark tiger shrimp (*Penaeus monodon*) or goliath stream prawns (*Macrobrachium rosenbergii*). While the investigation fundamentally centered on the feed the board rehearses related with Indian significant carp creation, the board rehearses that are utilized under polyculture conditions with different species-bunches were likewise surveyed.

H.A.C.C. PERERA and ASOKA PATHIRATNE (2008), Impacts of momentary organization of levamisole by submersion on resistant reactions of the refined nourishment fish, catla were explored. Sub-grown-ups of *C. catla* were given two hr shower in 1.25 or 2.5 mg/L levamisole arrangements and the invulnerable reactions were evaluated on 14, 21, 28, 42 and 56 days after the treatment in contrast with the controls. Results uncovered that leucocrit levels, all out leucocyte tallies, plenitude of leucocytes, all out phagocytic movement, phagocytic list, myeloperoxidase action and oxygen radical creation by phagocytes were expanded fundamentally in levamisole offered fish in correlation the controls. No noteworthy contrasts in the level of immuno-incitement were seen between the fish bunches presented to the two convergences of levamisole tried. For both introduction levels, the majority of the parameters tried were extraordinarily raised on 42 days present presentation on levamisole.

C.Kandeepan(2014), A characteristic element of fish is the wide physiological scope of blood parameters and furthermore the huge individual varieties. The point of this investigation was to analyze the hematological profile, Blood glucose and Blood protein levels of four teleost fish species (*Channa striatus*, *Cyprinus carpio*, catla and *Labeo rohita*) and to build up the likenesses and contrasts between these species which are broadly present in the Palar-porunthalaru dam Condition. The blood parameters viz., absolute WBC and RBC count, DLC,ESR, Hb, PCV, MCV, MCH and MCHC values were broke down utilizing standard strategies. Measurable investigation affirmed factual contrasts in blood parameters among the four species. Our discoveries show a more significant level of Glucose, Protein, Red platelets, White platelet, Easnophils, Hematocrit and hemoglobin in Catla catla with regard to different species.

Research Methodology

Fish

Catla sub-grown-ups and fingerlings were gotten from the Udawalawa Fish Rearing Station, NAQDA, Sri Lanka. Fish were kept up in outside concrete tanks loaded up with matured faucet water with constant air circulation under the common photoperiod for 30 days. During the acclimation time frame, fish were nourished day by day with business fish nourishment pellets (Prima, Colombo, Sri Lanka). Sub-grown-up phase of the fish was utilized in the hematological and serological tests though the fingerling stage was utilized in the trial challenge tests. The fish utilized right now liberated from net injuries or parasitic diseases remotely and considered as evidently solid people. The incubation center reproduced produce of catla, after acclimatization, were sustained with research facility made egg-custard feed (Table 1). The sound fish were isolated to direct sustaining test.

Table 1: Feed compositions used during rearing of *C. catla* spawn

Ingredients	Percentage
Hen egg white	28.0
Lactogen powder	60.0
Fishmeal powder	10.0
Vitamin & Mineral Mix*	2.0

Levamisole treatment

Tests of sub-grown-ups of Catla (18-26 cm in all out length and 160-210 g in body weight) were washed in glass aquaria containing 1.25 mg/L or 2.5 mg/L levamisole (Sigma, MO, USA) in matured faucet water for two hr. Tantamount size fish which were acquainted with glass aquaria containing just matured faucet water at same natural burdens, filled in as controls. After two hr of introduction, the control fish (matured faucet water) and the fish presented to levamisole (1.25 mg/L or 2.5 mg/L) were moved to open air concrete tanks loaded up with persistently circulated air through matured faucet water. As the two levamisole medications couldn't be tried simultaneously because of viable limitations, the two fixations were tried at two phases. Consequently two practically identical benchmark groups were utilized for the two medications. Fish were given business nourishment pellets every day at 2% of the body weight. Half of the water in every one of the tanks was traded with new matured faucet water at regular intervals. At pre-decided time focuses (14, 28, 42 and 56 days) after the treatment, levamisole treated and control sub-grown-ups of Catla were killed by pithing to evaluate the immunomodulatory impacts of the medicines dependent on hematological and serological tests.

Haematocrit and leucocyte counts

For detraction of the haematocrit and leucocrit levels, blood tests were taken into heparin zed narrow cylinders and centrifuged in the haematocrit rotator. Heamatocrit estimation of each example was estimated utilizing the haematocrit measure. Tallness of the leucocyte section was estimated under a light magnifying instrument utilizing a micrometer scale so as to decide the leucocrit level. All out leucocyte tally was deterred utilizing Shaw's answers as weakening liquids following the strategy for Hesser (1960). Blood smears of the fish were readied, fixed in 100% methanol and were recolored with Wright-Giemsa recolor. Various kinds of leucocytes were recognized as portrayed by Hibiya (1982).

Phagocytosis assays

Phagocytic cells were identified utilizing *Staphylococcus aureus* (Sigma, MO, USA) as portrayed by Anderson and Siwicki (1995). An example (0.1 mL) of blood was put in a microtiter plate well, 0.1 mL of *Staphylococcus aureus* 1×10^7 cells suspended in phosphate cushioned saline pH 7.2, was included and afterward blended well. The microscopic organism's blood arrangement was brooded for 20 at room temperature. Five μ L of this arrangement was taken on to a perfect glass slide and a smear was readied. The smear was air dried, at that point fixed with ethanol (95%) for 5 min and air dried. At that point the smear was recolored with Giemsa recolor for 10 min. The two smears were produced using each fish. The aggregate of 100 neutrophils and monocytes from each smear were seen under the light magnifying lens and the quantity of phagocytizing cells and the quantity of microbes overwhelmed by the phagocyte were tallied.

Lysozyme assay

Lysozyme movement of blood serum was deterred as depicted by Anderson and Siwicki (1995) with certain changes. Blood serum was set up by centrifuging the blood at 3000g for 5 min. Serum (0.1 mL) was put in test tubes and 0.9 mL of a 0.75 mg/mL *Micrococcus lysodeikticus* (Sigma, MO, USA) suspension in phosphate supported saline, pH 6.2 was included and blended well. The absorbance was estimated at 450 nm by a spectrophotometer at 1 min interims for 10 min in the wake of blending in

with microscopic organisms and pace of progress of absorbance determined. Lysozyme exercises were determined utilizing hen egg white lysozyme (Sigma, MO, USA) as a standard.

Total protein and total immunoglobulin in plasma

All out protein content in blood plasma was resolved utilizing Peterson's changes of the miniaturized scale Lowry strategy utilizing a protein test pack (Sigma Diagnostics, P 5656, Sigma, MO, USA). The protein focuses were resolved utilizing an alignment bend arranged utilizing cow-like serum egg whites as the standard. For the assurance of the immunoglobulin in the plasma, immunoglobulins were isolated from the plasma by precipitation with polyethylene glycol as portrayed by Anderson and Siwicki, (1995). Plasma (0.1 mL) was put in plastic serum vial and 0.1 mL of 12% polyethylene glycol was included and brooded at room temperature for 2 hr under consistent blending. After brooding, the arrangements were centrifuged at 7000 g for 10 min. The protein content in the supernatant was deterred utilizing protein measure pack.

Challenge test by cohabitating test fish with parasitic infested fish

Fingerlings of Catla which were treated with 1.25 mg/L levamisole were exposed to a parasitic test by living together the fish with an example of gold fish *Carassius auratus* invaded with *Ichthyophthirius* sp and *Dactylogyrus* sp in glass aquaria. As critical fixation explicit contrast concerning the level of immunostimulation was not unmistakably observed between the two presentation levels of levamisole, it was chosen to utilize 1.25 mg/L levamisole for challenge tests. After the presentation of parasite plagued fish, mortality and irregular indications of fish were watched day by day and parasitological review was done at as depicted by Kabata (1985) on 7, 14, 21, 28, 35, 42 and 49 days after levamisole treatment.

Challenge test with bacteria

Fingerlings of Catla which were treated with 1.25 mg/L levamisole were exposed to a bacterial test. A harmful strain of *Aeromonas hydrophila* (gave by Mr. C. Hettiarachchi, Confifi Aquaculture Pvt., Ltd., Sri Lanka), that has been secluded from characteristic injuries in a decorative fish *Trichogaster leeri* (pearl gouramy), was utilized in the test tests.

Results and Discussion

All control and levamisole treated sub-grown-ups of *C. catla* made due during the test time frame. No huge contrast was found between levamisole treated fish and individual control fish corresponding to the haematocrit levels in the blood (results not appeared, $P > 0.05$). Leucocrit levels and the absolute leucocyte checks of levamisole treated fish were essentially higher than that of the particular control fish on 14 days of post introduction onwards. Greatest increment in these parameters happened on 42 days of post presentation to levamisole (Figure 1). The leucocrit levels and leucocyte include in the blood expanded by almost two overlays on 42 days of levamisole present presentation analyzed on the separate controls. Be that as it may, no fixation explicit noteworthy contrast was distinguished at each time point ($P > 0.05$).

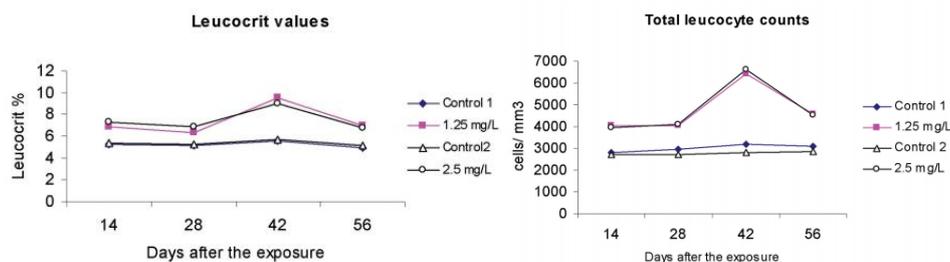


Figure 2. Time-course elevation pattern in the mean leucocrit levels and total leucocyte counts in the blood of *Catla catla* following levamisole exposure (1.25 mg/ L or 2.5 mg/L for 2 hrs) through immersion route. At each time point, the parameters in the blood of levamisole treated fish were significantly different from those of the controls (ANOVA, Tukey's test, $P < 0.05$)

The utilization of immunostimulants in fish culture is opening new chances to improve fish wellbeing and forestall misfortunes because of diseases. Levamisole has been appeared to go about as an immunostimulant in number of fish species: by oral organization or i.p. infusion for *Cyprinus carpio* (Siwicki, 1987; 1989; Sakai, 1999) by oral organization for *Sparus aurata* (Mulero et al., 1998) and for *Labeo rohita* (Wijendra and Pathiratne, 2004), by submersion for *Salmon salar* (Findlay and Munday, 2000) and by drenching and infusion for *Oncorhynchus mykiss* (Anderson et al., 1995). The present examination exhibited immunostimulatory properties of levamisole controlled through drenching course on an Indian carp, *C. catla*.

Conclusions

The result uncovered that organization of levamisole (1.25 or 2.5 mg/L for two hr) through drenching course is a potential strategy in *Catla* culture for improving the opposition of fish to diseases and stress. The two centralizations of levamisole could upgraded Leucocrit levels, complete leucocyte tallies, plenitude of neutrophils, monocytes and lymphocytes, absolute phagocytic action, phagocytic file, NBT action, myeloperoxidase movement and all out protein level in the blood on 14 - 56 days present introduction on levamisole. Time-course example of invulnerable incitement uncovered that improved resistant reactions persevere at any rate 56 days while for the greater part of the parameters tried; most extreme reaction was seen at 42 days post organization. As critical focus explicit contrast as for the level of immunostimulation was not plainly observed between the two presentation levels of levamisole, it would be monetarily increasingly favorable to utilize 1.25 mg/L levamisole for *catla* culture for potential immunostimulation.

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