Immune Responses Against Bacterial Infection in Freshwater Fishes and their Modulation By Natural Immunostimulants

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Abstract

Fishes are the earliest vertebrates with well developed immune system. The immune system of aquatic vertebrates like those of higher animals are sensitive to immune challenges by environmental stresses. Fishes are the oldest animal group with an immune system showing clear similarities with the defense systems of mammals and birds. Fish because of its earliest evolution mostly depends upon Non-specific Immunity and also it's immune system is poorly developed and studied. Fishes are in intimate contact with their environment which contains very high concentration of disease causing pathogens. Under Normal conditions the fish maintains a healthy state by defending itself against potential invaders by a complex system of Non Specific and Specific defence mechanisms. Non Specific immune system in fishes acts as a first line of defense against various invading pathogens.

Introduction:

Fishes are the earliest vertebrates with well developed immune system. The immune system of aquatic vertebrates like those of higher animals are sensitive to immune challenges by environmental stresses. Fishes are the oldest animal group with an immune system showing clear similarities with the defense systems of mammals and birds. Fish because of its earliest evolution mostly depends upon Non-specific Immunity and also it's immune system is poorly developed and studied. Fishes are in intimate contact with their environment which contains very high concentration of disease causing pathogens. Under Normal conditions the fish maintains a healthy state by defending itself against potential invaders by a complex system of Non Specific and Specific defence mechanisms. Non Specific immune system in fishes acts as a first line of defense against various invading pathogens. Their importance is three fold: Firstly, the protection is Non specific and does not depend upon recognition of the distinctive molecular structure of the invading species as specific mechanisms do. Secondly, there is no or only little time lag for them to act , even the inducible defences like inflammation , are relatively quick to respond and thus give pathogens little time to establish themselves. Thirdly, they are relatively temperature independent.

Any disease or infection that is naturally transmissible from vertebrate animals to humans and vice-versa is classified as a Zoonosis and the diseases caused are known as Zoonotic diseases. They can be caused by germs including viruses, bacteria, parasites, and fungi. Zoonosis can cause many different types of illnesses in people and animals ranging from mild to serious illness and even death. It is important to know that animals do not always appear sick when carrying a zoonotic disease. Many animals can appear healthy, but still be carrying germs that can make people sick.

Fish borne Zoonoses are rare; recently they have received attention due to an overall focus on zoonotic diseases like avian influenza, tick borne illness, bovine spongifoem encephalopathy (BSE). Fortunately, the diversity of fish borne zoonotic pathogens is restricted to a small number of opportunistic bacterial pathogens.

Bacterial infection in freshwater fishes

Fish are susceptible to a wide variety of bacterial pathogens. Many of these bacteria capable of causing disease are considered by some to be saprophytic in nature. This bacteria only become pathogens when fishes are physiologically unbalanced, nutritionally deficient, or there are other stressors, i.e., poor water quality, overstocking, which allow opportunistic bacterial infections to proceed. Some of these bacterial pathogens of fishes are fastidious and require special growth media for laboratory culture. Others grow at different temperatures, dependent upon the aquatic environmental temperature of the fish. Bacteria are the main fish borne zoonotic agents that should concern the aqua culturists. Infection is typically acquired through the abrasions, cuts or penetrating wounds in the skin when handling infected fish or water. Recirculating systems have high organic loads that are an excellent growth media for many bacteria. Most of the bacteria are often present even when clinical symptoms are absent in fish, so personal protection equipment (PPE) such as gloves should always be worn. Majority of these bacterial pathogens are gram negative, although certain gram positive bacteria are medically relevant. Some of the more common bacterial pathogens are listed below.

Mycobacterium:

The most important fish borne zoonotic bacterial agent is atypical Mycobacterosis. This group of gram positive, aerobic, non spore forming, acid fast positive, nonmotile rods are present in freshwater environments. Atypical mycobacteria are different from their more familiar cousins (*Mycobacterium tuberculosis, M.bovis, M.avium)* because they are slow growing. Psychrophilic to mesophilic environmental organisms that typically cause infections in the extremities of humans where temperatures are cooler than 33degree centigrade. There are nearly 120 species of *Mycobacteria* with *M. marinum*, *M. fortuitum* and *M.ulcerans* are most common causes of fish borne zoonoses.(kattari *et al*., 2006; Jacobs *et al*., 2009; Aubry et al., 2002; Jernigan *et al*., 2000)

Streptococcus iniae

It is gram positive fish borne zoonotic bacterium which was described in a captive Amazon river dolphin. It has been found to infect several species of commercially valuable fish including *Tilapia (Oreochromis spp* and *Sarotherodon* spp.) channel catfish *Ictalurus punctatus* and hybrid sea bass *Morone* spp.(Perera *et al*., 1994; Shoemaker *et al*., 2001; Bowser *et al.,* 1998)Clinical signs in fish include the loss of orientation, petechial haemorrhages, exophthalmia and corneal hypopyon.

In humans handling of live or infected fish can produce cellulitis of the hand or endocarditis, meningitis and arthritis in severe systemic infections.(Angew *et al.,* 2007; Weinstein *et al.,* 1997; Fackalm *et al.,* 2005) Culture of *S.iniae* is best achieved from brain tissue of affected fish while most other bacterial pathogens are best isolated from kidney cultures.

Erysipelothrix rhusiopathiae

It is a gram positive bacterium associated with fish borne zoonoses. These are commonly found in cutaneous mucus of fishes. Infection occurs when exposed wounds encounter bacteria in fish mucus usually during handling of live or dead fish. Clinical symptoms include localized infections of the extremities, diffuse cutaneous infections, and systemic disease including endocarditis.(Gorby *et al*., 1988; Klauder *et al.,* 1993) This bacteria is resistant to freezing so handlers of frozen fish as food should also wear appropriate gloves.

Aeromonas

These gram negative bacteria are most common microbial pathogens of fish. The Aeromonads are ubiquitous gram negative, usually freshwater, motile rods that can cause opportunistic infection in numerous fish species. *Aeromonas hydrophila* is the most commonly reported species that possesses zoonotic potential. Fish lesions are consistent with sepsis including petechia in the skin and fins, ulcerations and erythema of skin, anorexia, exophthalmia and ascites. Waters and high nutrient levels can create *Aeromonas* spp blooms that could be infectious to humans through wounds or ingestion; infections in humans are rare and typically involve immune suppression. *Aeromonas salmonicida* , a non motile facultative aerobic rod, is an obligate fish pathogen in both freshwater and marine fish infection is often described as Fish furunculosis and is a well known disease among salmonid aquaculture and cyprinid hobbyists.

Aeromonas salmonicida is the most common bacterial pathogen of fishes worldwide. This bacteria can cause the following diseases:

Goldfish Ulcer Disease - this disease is typically localized to the skin and only becomes systemic late in the disease. The skin lesions range from whitish discolorations to shallow hemorrhagic ulcers to deep lesions which may expose underlying muscle or bone. These lesions can become secondarily infected with fungi, protozoa, or other bacterial agents. Fish may exhibit hemorrhage on the body as well as the base of the fins. Large numbers of bacterial microcolonies are observed in many of the lesions.

Additionally, several other species of *Aeromonas*, including: *A. hydrophila, A. formicans, A. liquefaciens,* and *A. hydrophila* complex are capable of causing a disease known as "Motile Aeromonas Septicemia" or "Bacterial Hemorrhagic Septicemia" Clinical signs of motile aeromonas septicemia range from sudden death with high morbidity in peracute cases to superficial to deep skin lesions. Skin lesions include variously sized areas of hemorrhage and necrosis and the base of the fins. These lesions may progress to reddish to gray ulcerations with necrosis of the underlying musculature. Ulcers may be observed in conjunction with a hemorrhagic septicemia which can produce non-specific lesions and clinical signs of exophthalmos, ascites, visceral petechiation, and a hemorrhagic and swollen lower intestine and vent. Anorexia and cutaneous discoloration are also observed with the septicaemia,With the septicemia, there may be depletion and necrosis of the renal and splenic hematopoietic tissue, as well as necrosis in the intestinal mucosa, heart, liver, pancreas and gonad. (*Lowry et al.,* 2007)

Vibrio spp

They are considered the marine analog of the Aeromonads, but both bacterial genera have marine and freshwater members. *vibrio* spp infection can remain asymptomatic until environmental stressors produce disease. Clinical signs in fish are consistent with sepsis including anorexia, ulcerations, exophthalmia and petechia/ erythema. (Weise et al., 2001; Austin 2010; Lee *et al*., 2003)

Edwardsiella

E. tarda is a gram negative motile, facultative anaerobic rod that is a zoonotic pathogen found in numerous species of freshwater fish. *E. ictaluri*, *E. tarda* causes clinical signs including myonecrosis, organ necrosis, hepatic abcesses, coelomitis, hypopyon, hemorrhage, ascites, ulceration / hemorrhage and necrosis of lateral line system. Human infection mostly occurs due to the consumption of infected tissue / water resulting in gastroenteritis although exposure to open woumds with immune suppression can cause significant cellulitis and sepsis. (Janda *et al.*, 1993; Slaven *et al*., 2001; Wilson *et al*., 1989)

Salmonella and others

These bacteria are zoonotic, gram negative rods classically associated with freshwater aquatic turtles and amphibians. Piscine francisellosis is a recently reported fish disease with the potential for zoonosis due to close homology of fish isolates to the deadly mammalian pathogen, *Francisella tularensis*, although no human infections have been reported. Nile tilapia, *Oreochromis niloticus*, with francisellosis show signs including erratic swimming, exophthalmia and anorexia. Other bacteria associated with water that may be remotely associated with fish borne zoonoses are *Citrobacter* spp, *Serratia* spp, *Pseudomonas* spp, *Shigella* spp, *Staphlococcus* spp, *Listeria* spp and *Clostridium* spp. (Soto *et al*., 2009; Maquel *et al.,* 2007)

Yersinia ruckeri

Yersiniosis is also known by the following synonyms: *Enteric* Redmouth Disease, Redmouth, and Blood Spot Disease. This disease is caused by the pathogen *Yersinia ruckeri*. This is an important pathogen of salmonids, particularly rainbow trout. Outbreaks of this disease usually begin with chronic, low mortality which slowly escalates. The early stages of this disease may resemble MAS with petechial hemorrhages observed around the fin and on the skin. Additionally, there is discoloration of the dorsum of the fish, as well as anorexia and lethargy. With chronic disease, there is ascitic fluid and unilateral or bilateral exophthalmos and hyphema (hence the term, “blood spot disease”). The characteristic gross lesions of this disease include hemorrhage of the oral cavity and skin erosions of the mouth. Histopathology includes bacterial colonization of well-vascularized tissues and hemorrhage of the gills, kidney, liver, spleen, and heart, as well as muscle. Definitive diagnosis of this disease involves culture of the target organ (kidney) as well as attendent clinical signs and lesions.

Flexibacter columnaris

It causes Columnaris Disease, which is a common bacterial disease that affects the skin or gills of freshwater fish. This disease is also known by a wide variety of synonyms including the following: *mxyobacterial disease, peduncle disease, saddleback, fin rot, cotton wool disease,* and *black patch necrosis*. This bacteria is usually pathogenic at temperatures greater than 59 °F. Both mortality and acuteness of the disease will increase at higher water temperatures. Virulence mechanisms associated with this disease are not well understood, however, the mineral content of the water is thought to be important, since this bacteria has been shown to be less pathogenic in soft water as compared to hard water. Other risk factors include physical injury, low dissolved oxygen, organic pollution and high nitrite levels.

This is primarily an epithelial disease, i.e., it causes erosions and necrosis of the skin and gills which may become systemic. It often presents as whitish plaques that may have a red peripheral zone on the head or back (hence the name *saddleback*) and/or the fins (hence, *fin rot)* and especially the caudal fin (hence, *peduncle disease).* Fragments of the fin rays may remain after the epithelium has sloughed, leaving a ragged appearance. Lesions rapidly progress to ulcers, which may be yellow or orange due to masses of pigmented bacteria. Ulcerations spread by radial expansion and may penetrate into deeper tissues, producing a septicemia. Gill infections are less common but more serious. Columnaris begins at the tips of the lamellae and causes a progressive necrosis that may extend to the base of the gill arch. Definitive diagnosis is dependent upon the isolation of the bacterial agent in the presence of attending clinical lesions. It should be noted that a presumptive identification of F*lexibacter columnaris* can be made by examination of wet mounts and observation of long thin bacterial rods which a characteristic flexing or gliding motion. In addition to *Flexibacter columnaris,* other bacterial agents which have been implicated in this disease include: *Flexibacter psychrophilia* as well as *Cytophaga* and *Flavobacterium branchiophila.*

Prevention of Disease

Nearly every zoonotic bacteria gains access through disruption of the innate immune system, primarily through puncture, cuts, scrapes, abrasions or sores in the skin. Aquaculture systems range in design from home hobbyist aquaria, to flow through raceways, to huge acre ponds, but they have all have nutrient rich waters that benefit bacterial proliferation. Numerous studies have been conducted on proper chemical disinfection of contaminated surfaces; contact time, proper safe handling of disinfectants and accurate dosing of disinfectants should be emphasized. One of the best disinfection methods exposed for surfaces is simple dessication or drying.

Significant exposure to drying environments like direct sunlight when given enough time will completely kill aquatic pathogens including the resistant atypical mycobacteria.

Immune response against Bacterial infection

In innate defence mechanisms of fish against bacteria include production of broad- spectrum anti microbial substances and acute phage proteins, non classical complement activation, release of cytokines, inflammation and phagocytosis. While these mechanisms provide potent defences against invasion against saprophytic environmental bacteria, pathogenic bacteria have evolved means of avoiding many of them. Disease outbreaks and mortalities often result from the fish being stressed and these non specific disease mechanisms are compromised.

A. Integumental innate defence mechanism:

Previous researches have clearly suggested the importance of innate defences in the integument of fish.

(i) Mucus:

The protective role of skin mucus has been demonstrated carefully by removing mucus with swab and then challenging turbot with *Vibrio anguillarum* and induced increased mortality. Mucus is continually being produced and sloughed from the integumental surface thus physically trapping and preventing bacteria from attaching to the epithelium and having an opportunity to invade the fish's tissues. Mucus also contains many substances with antibacterial properties.

(ii) Anti bacterial peptides

These substances are low molecular weight peptides with the ability to disrupt bacterial membranes. They have been identified from mucus secretions of a number of fish species but very less information is available about their ability to kill fish pathogenic bacteria. Pathogenic strains of *Aeromonas salmonicida* are less susceptible to cecropin C1 (an antibacterial peptide) than non pathogenic strains lacking the A- layer, but they are, nevertheless, killed by higher concentrations. These peptides may provide an important line of defence before development of the specific immune response in larval fish.

(iii) Proteases

Trypsin like proteases and cathepsin L and B proteases have been found in skin mucus of a number of a number of fish species. The ability of these enzymes to lyse formalin killed *Vibrio anguillarum* has led to the suggestion that they may play role in defence against bacteria but their action on live bacteria has not been studied yet.

(iv) Lectins

Once bacteria have made contact with their fish host, many pathogenic species can adhere to the mucus and the epithelial cells by surface molecules known as adhesions. These interactions involve binding of carbohydrates. Once the bacterium has attached to the host cell, the latter is induced to endocytose the bacterium that can then grow and spread in the host to produce disease for e.g. vibriosis.

Lectins are group of proteins with different specificities for binding carbohydrates. They have been found in salmon eggs, serum and mucus.

These lectins are Ca^{++} dependent and can agglutinate a number of fish bacterial pathogens. It is possible that an important role of lectins in fish mucus is to bind to the carbohydrates on the surface of bacteria, which are involved in attachment to the integumental cells. This blocks attachment and subsequent invasion of the host but such experiments have not been done yet.

(v) Lysozyme

This enzyme can attack the peptidoglycan layer of bacterial cell walls causing them to lyse. Lysozyme has been found in fish mucus, serum and ova. Peritoneal macrophages and blood neutrophils contain lysozyme and are thought to be the major source of serum lysozyme. Fish lysozyme occurs in two forms and one of these appears to be much more bactericidal than lysozyme of higher vertebrates. There are several reports of lysozyme isolated from fish serum and ova, being bactericidal even for important fish pathogens like *Aeromonas salmonicida* and *A. hydrophila*.

B. Systemic innate humoral defences

If bacteria are successful in crossing the integumental defences there are a number of plasma proteins, which may prevent further spread of infection. Most of the proteins mentioned above are also present in higher concentrations in serum, e.g. lysozyme and lectins. The most important of the serum defence factors is the complement system because of its activating effects on the cellular defences.

(i) Complement

The complement of teleost fish can be activated directly by lipopolysac charide(LPS), which is a major constituent of the cell wall of gram negative bacteria. This is also called as alternative complement pathway (ACP) and results in the lysis of the cell membrane of many non virulent bacteria. The species of the bacteria that cause disease in fish are resistant to being killed by this mechanism, though some can be killed when the complement is activated by classical (antibody -mediated) pathway. Complement has another important innate defence function. During its activation on the bacterial cell wall, two components are important for recruiting phagocytes. The C5a component is released from the complement complex and is a potent chemotactic agent for macrophages and neutrophils. These cells have receptors for the C3b component, which remains attached to the bacteria and are then more readily phagocytosed. The ACP activity is very high in fish serum as compared with mammals suggesting its importance in the defence mechanisms in fish.

(ii) Lectins

They have been isolated from the number of fish but evidence for their role in defence is only recently coming to light. An N - acetyl galactosamine binding lectin has been isolated from the serum of blue gourami. This lectin is shown to have opsonising activity and lectin treated virulent *Aeromonas hydrophila* cells are killed in the presence of complement. Supernatants obtained macrophage cultures exhibit significant bacterial killing activities.

(iii) Pentaraxins: C reactive protein (CRP) and Serum amyloid protein (SAP)

These serum proteins are usually acute phage proteins in mammals but in fish they appear to be constitutively expressed and may show only a slight decrease or increase in concentration during inflammatory responses. Pentaraxins are capable of binding to a number of polysaccharide structures in the presence of Ca^{++} ions.

C. Bacterial growth inhibitors

(i) Transferrin and the hypoferraemic response

Bacteria like other cells require iron as a cofactor for many enzyme systems but in the host the availability of iron is highly restricted by being bound to the high affinity iron binding protein, transferring, in the plasma. Most bacteria are thereby unable to grow in the host tissues. Pathogenic bacteria have evolved several ways of overcoming this defence by producing high affinity iron sequestering mechanisms of their own. To reduce the availability of iron to pathogenic bacteria, vertebrates show a hypoferraemic response.

A novel role of transferrin in fish has recently been reported. In mixed lymphocyte reactions or mitogenic stimulation of gold fish kidney leucocytes, proteolytic fragments of transferring are released and these fragments, but not the full length transferring, are able to induce the production of nitric oxide by LPS stimulated goldfish macrophage cultures. Thus, as monocytes infiltrate inflammatory sites, transferrin derived peptides may initiate the differentiation of these cells into mature tissue macrophages with enhanced bacterial properties.

(ii) Anti proteases

For bacteria to invade and grow in the fish tissues they need to digest host proteins as a source of amino acids. Fish plasma contains a number of protease inhibitors, principally anti protease, anti plasmin and á 2 macroglobulin that may play role in restricting the ability of bacteria to invade and grow *in vivo.*

(D) Systemic innate cellular defences

(i) Inflammatory response and phagocytes

If bacteria gain entry into the tissues of the fish an inflammatory response is induced with the ultimate influx of phagocytes, which have potent bactericidal properties.

(ii) Control of inflammation

The initiation of inflammation is highly complex and multifactorial. A number of blood enzyme systems, including the clotting system, the kinin system and the compkement system play a major role and while little is known of the details in Fish.

(iii)Phagocytosis

The Phagocytosis of bacteria by fish macrophages and neutrophils first requires attachment of the bacteria to the surface of phagocytes. This may involve hydrophobic interactions 0r sugar/ lectin interactions.

(iv) Phagocyte bactericidal mechanisms

Fish macrophages contain many hydrolytic enzymes i. E lysozymes which can digest engulfed microorganisms and importantly these cells produce bactericidal reactive oxygen species (ROS) during the respiratory burst on contact with or during phagocytosis of bacteria. Neutrophils contain large amounts of myeloperoxidase, which in mammals is involved in the production of bactericidal enzymes.

(E) Specific defence against bacteria

(i) Specific Humoral defence

This is mediated by the production of specific immunoglobulins (antibodies) by the B lymphocytes following stimulation with specific antigens and antibodies are important in immunity to bacterial pathogen in variety of ways

Anti adhesins

Bacteria must first adhere to the surface epithelium of the fish host and they possess a variety of structures in the cell wall.Antibodies, which react with these adhesions may help to prevent the bacteria in gaining access. Fish produce antibodies in mucosal tissue like gut and gill.

Anti-toxins

Many bacterial pathogens produce potent toxins and antibodies, that neutralise them, may have a protective role. Atypical strains of *Aeromonas salmonicida* produce a potent letal metalloprotease and antibody responses against this toxin confer good protection against experimental challenge.

Anti invasins, activation of classical component pathway, activated macrophages and opsonising antibodies are major pathways of specific immune system.

Immunomodulation by Natural immunostimulants

They are group of natural, biological or synthetic substances, which enhance the non specific defence mechanisms as well as the specific immune response when administered as adjuvants with a vaccine. It can be defined as an chemical, drug or stressor that either elevates the non specific defence mechanisms as well as the specific immune response (Sakai, 1999).They are used to prevent the organisms from disease attacks by enhancing the immune system and also to combat immunosuppressive conditions, can also be used as prophylactic treatment in anticipation of expected seasonal outbreaks of known endemic species. Current method of disease prevention include antibiotics and chemotherapeutics which have several disadvantages - risk of generating resistant pathogens, drug residues accumulating in fish, detrimental effect on environment.

Therefore, the use of natural Immunostimulants seems to be an alternative way of reducing disease in Aquaculture.Natural immunostimulants are gaining wider acceptance because they are-biocompatible, biodegradable ,safe for the environment.

To prevent loss of valuable Fish species through disease caused by pathogens in Fish culture and to add to economic benefit in fish farming, Immunostimulants are widely used in farms for health management. Fish treated with immunostimulants usually show enhanced protection against various pathogens. Various natural immunostimulants which are widely used in aquaculture are

1. Garlic - *(Allium sativum)* called Lasan in India, is a medicinal plant which has been used for years in Indian ayurvedic medicine. Many beneficial health properties of garlic are attributed to organosulphur compounds particularly to thio sulfinates(R-S-S(O)- R) (Block, 1992). Allicin is the most abundant compound representing about 70% of thiosulfinates present, or formed in Crushed Garlic (Block, 1992 ; Han *et al.,*1995). It is produced by the interaction of the non-protein amino acid allinin(=S- allyl- L-cysteine sulfoxide), with the enzyme allinase (Cavalitto *et al.,*1944). Various Studies have shown the following properties of Garlic on Immune System it has been reported to be hypolipidemic, antimicrobial (Umar and Berwal, 1998), anti hypertensive (Suetsuna, 1998), Hepatoprotective(Wang *et al.,* 1998)and insecticidal properties. Garlic extract has also been shown to reduce serum cholesterol levels (Bordia *et al.,*., 1975; Augusti, 1977) and increase blood coagulation time (Bordia *et al.,*1975). An antifungal activity of garlic bulbs (Fromthing and Bulmer, 1978) is also on record. S- allyl cysteine present in crushed garlic was found to inhibit tumor metabolism and enhance immune response (Sumiyoshi, 1997). Allium species also have immune enhancing activities that include promotion of lymphocyte synthesis, cytokine release, Phagocytosis and natural killer cell activity (Kyo *et al.,*1998). Jain *et al.,* 2013 have reported immunomodulatory effects of garlic on Indian snake head *Channa punctatus.*

2. Vitamin C

Vitamin C has been correlated with health in humans, animals and in cultured cells. In fish it is assumed that Vitamin C is an essential nutrient for optimum growth and maintenance (Dupree, 1966; Halver *et al.,*1969; Lovell,1973; Mazik *et al.,*1987). Fish are particularly sensitive to a correct level of Vitamin C because they are unable to synthesize it *de novo* (Dobrowski, 1990). Most teleosts are unable to synthesize ascorbic acid due to lack of L- gulonolactose oxidase that is responsible for synthesis of Vitamin C *de novo* (Wilson, 1973; Fracalossi *et al.,*2001). The effects of it's absence have been described in several fish species and defeciencies have been correlated to reduced growth rate, skeletal deformation, capillary fragility, slow wound repair and depression of the immune system, specimens being more susceptible to bacterial diseases

(Lovell, 1973; Lim and Lovell, 1978). Vitamin C has been found to be one of the nutrients correlating with fish immunity (Roberts *et al.,*1995; Anbarasu and Chandran, 2001). A number of studies have shown beneficial effects of Viatmin C on immunological parameters such as lysozyme activity, Phagocytic activity, respiratory burst (Li and Lovell, 1985; Navarre and Halver,1989; Verlhac *et al.,*1998; Ortuno *et al.,*1999, 2001; Anabarasu and Chandran,2001) and enhanced resistance to stress and diseases (Durve and Lovell,1982; Navarre and Halver,1989; Montero *et al.,*1999). Effects of Vitamin C on a variety of nonspecific resistance mechanisms and the specific immune responses have been reported in fish (Hardie *et al.,*1991; Verlhac and Gabaudan, 1994; Ortuno *et al.,*1999). Jain *et al.,* 2014 have reported immunomodulatory effects of Vitamin C on Indian snake head *Channa punctatus*.

3. Turmeric (*Curcuma longa*)

Haldi *(Curcuma longa* Linn.), a medicinal plant, has been used for thousand of years in Indian ayurvedic medicine. Components of turmeric are collectively termed as curcuminoids, which mainly include curcumin, demethoxy curcumin and bisdemethoxy curcumin. But the major biologically active component of turmeric is curcumin, which is yellow phytochemical, hydrophobic and polyphenolic compound. Although curcumin has recently gained much attention for its therapeutic potentials in traditional Indian medicine for human uses due to its low toxicity and large biological activities but its pharmacological potential is still under investigation. Several research findings indicate that curcumin can act as a potent immunomodulatory agent that can modulate the activation of T cells, B cells, Macrophages, Neutrophils, natural killer cells, dendritic cells, transcription factors, cell cycle proteins and signal transducing kinases. It also has a strong effect on cytokine production, humoral and cell mediated immunity. In this Way, Curcumin regulates multiple targets, which is needed for treatment of most diseases. Moreover, it is inexpensive, extremely safe even at very high doses and used as an immunomodulator in various animal models including human beings. Jain *et al.,* 2016 have reported immunomodulatory effects of curcumin on Indian snake head *Channa punctatus.*

4. Beta Glucan-

Yeast extracted â glucans have been examined with great interest in fish disease prevention (Anderson, 1992). Additionally these substances have been shown to enhance the disease resistance of several fish species, such as carp, *Cyprinus carpio* L. (Yano *et al.,*. 1991), Atlantic salmon, *Salmo salar*(Robertsen *et al.,* 1990), Yellowtail, *Seriola quinqueradiata* (Matsuyama *et al.,*, 1992), rainbow trout, *Oncorhynchus mykiss* (Jeney & Anderson 1993), brook trout, *Salvelinus fontinalis* (Anderson & Siwicki, 1994) and African catfish, *Clarias gariepinus* (Yoshida *et al.,* 1995), against various major bacterial pathogens including *Vibrio sps*.(Robertsen *et al.,* 1990) and *Aeromonas hydrophila* (Yoshida *et al.,* 1995). Jain *et al.,* 2012 have reported immunomodulatory effects of beta glucan on Indian snake head *Channa punctatus*.

All the nonspecific immune parameters were enhanced significantly in *Channa punctatus*.

Conclusion:

Bacterial infections induces the immune system to respond variously such as increased Phagocytosis, lysozyme, bactericidal activity, superoxide anion production myeloperoxidase antiprotease etc. Immunostimulants are effective in reducing mortalities in farmed fish. The intraperitoneal injection of these immunostimulants (viz., Garlic, Vitamin C, Haldi and â- glucan) are able to modulate the non-specific immunity in *Channa punctatus* without accompanying undesirable side effects, these substances may substitute the need for certain vaccines which are too expensive to produce commercially as well as the use of antibiotics to prevent diseases. The maximum immunomodulation was observed by the use of Vitamin C as compared to other immunostimulants, it showed the highest level of survivability and disease resistance.

References

1 **Agnew., W. Barnes., A. (2007)** *Streptococcus iniae* : an aquatic pathogen of global veterinary significance and a challenging candidate for reliable vaccination. **Vet Microbiol; 122: 1-15.**

2 **Anabarasu, K. Chandran, M.R. (2001)** Effects of Ascorbic acid on the immune response of cat fish *Mystus gulio*(Ham.) to different bacterins of *Aeromonas hydrophila.* **Fish Shellfish Immunol. 11, 347-355.**

3 **Anderson, D.P. (1992)** Immunostimulants adjuvants and vaccine carriers in fish: application to aquaculture. **Annu. Rev. Fish Dis. 2, 281-307**.

4 **Anderson, R.,Smith,M.J., Joone,G.K.,Van Staden,A.M. (1990)** Vitamin c and cellular immune functions: protesction against hypochlorous acid mediated inactivation of glyceradehyde-3-phosphate dehydrogense and ATP generation in human leucocytes as a possible mechanism of ascorbate mediated immunostimulation**. Ann. N. Y. Acad. Sci. 587, 34-48.**

5 **Anumohini. (2012)**The Immunomodulatory effects of beta glucan on non specific immune parameters in Channa punctatus (Bl.). **Thesis C.C.S. University, Meerut.**

6 **Aubry., A. Chosidow., O. Caumes., E. (2002)** – Sixty three cases of *Mycobacterium marinum.*Infection. **Arch.Intern Med; 162: 1746-52.**

7 **Augusti,K.T., (1977).** Biochemical studies on combined effects of Garlic *Allium sativum* and ginger in albino rats. **Indian. J. Exp. Biol.15: 489-490.**

8 **Austin., B. (2010)** – *Vibrios* as causal agents of zoonoses. **Vet Microbiol ; 140: 310 -7.**

9 **Block, E., 1992.** The Organ sulphur Chemistryof the genus *Allium* implicationsfor the organic chemistry of Sulfur. **Angen.Chem. Int. Ed.31: 1135-1178**.

10 **Bordia, A., Bansal,H.C., Arora,S.K., Singh,S.V. (1975).** Effect of essential oils of garlic and onion on alimentary hyperlipemia. ***Atherosclerosis 21: 15-19.***

11 **Bowser., P. Wooser., G. Getchell., R. (1998)** *Streptococcus iniae* infection of Tilapia *Oreochromis niloticus* in a recirculation production facility. **J. World Aquaculture Soc; 29(3): 335-9.**

12 **Brattgjerd, S.; Evensen, O; Laure, A., (1994)** Effect of injected yeast glucan on the activity of macrophages in Atlantic salmon *Salmo salar* L., as evaluated by *in vitro* hydrogen peroxide production and phagocytic capacity. **Immunology 83, 288-294.**

13 **Cavallito,C.J., Buck, J.S., Suter,C.M. (1944)**. The antibacterial principal of the *Allium sativum* II. Determination of Chemical Structure. **J. Am. Chem. Soc.66: 1952-1954.**

14 **Dabrowski, K. (1990).** Ascorbic acid status in the early life of white fish (*Coregonus lavaretus* L). **Aquaculture 84, 61-70.**

15 **Dalmo, R.A and Seljelid, R. (1995).** The immunomodulatory effect of LPS, Laminaran and Sulphated laminaran [(1, 3) –d glucan] on Atlantic salmon, *S. Salar*, macrophages *in vitro*. **J. of Fish Diesases 18: 175-185.**

16 **Dupree, H.K. (1966).**Vitamins essential for growth of channel catfish. **U.S. Bureau of Sports Fisheries and wild life Technical paper No.7.**

17 **Durve, V.S. & Lovell, R.T.(1982).** Vitamin C and disease resistance in Channel catfish (*Ictalurus punctatus*). **Can.J. Fish. Aq. Sci.39, 948-951.**

18 Engstad, R.E., Robertson, B., Frivold, E., 1992. Yeast glucan induces increase in lysozyme and complement mediated hemolytic activity in Atlantic salmon blood. ***Fish and Shellfish Immunology* 2: 287-297.**

19 **Facklam., R. Elliot., J. Shewmaker., L. (2005)** - Identification and Characterization of Sporadic isolates of *Streptococcus iniae* isolated from humans. **J Clin Microbiol; 43: 933 -7.**

20 **FAO , 2004**. The state of world fisheries and aquaculture 2004. Rome, Italy. **pp 14-17.**

21 **Fracalossi, D.M. Allen, M.E. Yuyama, L.K., Oftedal, O.T. (2001)** Ascorbic acid Biosynthesis in Amazonian fishes. **Aquaculture. 192,321-332.**

22 **Fromthing, R.A., Bulmer,G.S. (1978).** In Vitro effect of aqueous garlic extract on the growth and viability of *Cryptococcus neoformans*. **Mycologia 70: 397-405.**

23 **Gorby., G. Peacock., J. (1988)** – *Erysipelothrix rhusiopathiae* endocarditis: microbiologic, epidemiologic and clinical features of an occupational disease. **Rev Infect Dis; 10(2): 317 -25.**

24 **Halver,J.E., Ashley,L.M. and Smith, R.E. (1969)** Ascorbic acid requirements of Coho salmon and rainbow trout. **Trans. Am. F. Soc. 98, 762-772.**

25 **Han,J., Lawson,L., Han,G., Han,P. (1995).** A spectophotometric method for Quantitative determination on allicin and total garlic thiosulfinates. **Anal.Biochem.225: 157-160.**

26 **Hardie, L.J. Fletcher, T.C. and Secombes, C.J.(1991)** The effect of Dietary Vitamin C on the immune response of the Atlantic salmon (*Salmo salar* L.) **Aquaculture. 87, 1-13.**

27 **Jacobs., J. Stine., C. Baya., A.(2009)** – A review of mycobacteriosis in Fish. **J.Fish Dis.;32: 119-30.**

28 **Jain., S. Varma., M. (2012)** Effect of Garlic on non-specific immune parameters in *Channa punctatus* (Bl.) infected with *Aeromonas hydrophila.* Published in Proceedings of 21st All India Congress of Zoology by **Narendra Publishing House, New Delhi In Management issues in Inland Fisheries and Aquaculture.2012, PP 129-143. ISBN: 978-93-80428-59-58.**

29 **Jain., S. Varma., M.(2014).** Vitamin C modulated Non specific immune responses in *Channa punctatus*. **Book published by Lambert Academic Publishing, Germany ISBN: 978-3-659-79000-3.**

30 **Jain., S. Varma., M.(2016).**Effect of *Curcuma longa* on Non specific immunological profile of *Channa punctatus* infected with *Aeromonas hydrophila* **. South Asian J. Of Exp. Biology Vol.6, No.2. pp.**

31 **Janda., J. Abbott., S.(1993)** Infections associated with the Genus Edwardsiella : the role of *Edwardsiella tarda* in human disease. **Clin Infect Dis; 17 (4) : 742 – 8.**

32 **Jeney, G.; Anderson, D.P., (1993)** Glucan injection or bath exposure given alone or in combination with a bacterin enchance the non-specific defense mechanisms in rainbow trout (*Oncorhynchus mykiss*). **Aquaculture 116, 315-329**.

33 **Jeney, G.; Galeotti, M.; Jeney, Z.; Anderson, D.P., (1997)** Prevention of stress in rainbow trout (*O. mykiss*) fed diets containing different doses of glucan. **Aquaculture 154, 1-15.**

34 **Jernigan., J. Farr., B.(2000)** – Incubation period and sources of exposure for cutaneous *Mycobacterium marinum* infection : case report and review of literature. **Clin Infect Dis ; 31: 439 -43.**

35 **Kattari., I.M. Rhodes., M.W. Kattari., S.L. (2006)** The evolving story of *Mycobacterium tuberculosis*clade members detected in fish. **J.Fish Dis.; 29: 509-20.**

36 **Klauder., J. Kramer., D. Nicholas., L (1943)** *Erysipelothrix rhusiopathiae* septicaemia: diagnosis and treatment : report of fatal case of erysipeloid. **JAMA; 122 (14); 938-43.**

37 **Li, Y. and Lovell, R.T. (1985)** Elevated levels of dietary ascorbic acid increase immune response in channel cat fish . **J. Nutri.115, 123-131**.

38 **Lovell, R.T. (1973)** Essentiality of Viatmin C in feeds for intensively fed caged Channel cat fish. **J. Nutr. 103,134-138.**

39 **Lovell,R.T.(1996).** Feed deprivation increases resistance of Channel cat fish to bacterial infections. **Aq. Managm.6, 65-67.**

40 **Lowry., T. Smith., S. (2007)** Aquatic Zoonoses associated with food, bait, ornamental and tropical fish. **JAVMA; 231(6): 876-80.**

41 **Lygren, B., Sveiver, H. and Waagbo, R. (1999b)** Examination of immunomodulatory properties and the effect on disease resistance of dietary bovine lactoferrin and Vitamin C fed to Atlantic salmon (*Salmo salar*) for a short term period. **Fish Shellfish Immunol. 9, 95-107.**

42 **Matsuyama, H.; Mangindaan, R.E.P.; Yano, T., (1992)** Protective effect of Schizophyllan and Scleroglucan against *Streptococcus* sp. infection in yellowtail (*Seriola quinqueradiata*). **Aquaculture 101, 197-203.**

43 **Mauel., M. Soto., E. Morale., M. (2007)** – Piscirickettsiosis – like syndrome in cultured Nile Tilapia in Latin America with *Francisella* spp. As the pathogenic agent. **J Aquatic Anim Health; 19 :27 34.**

44 **Mazik, P. M. Brandt, T.M. and Tomasso, J.R. (1987)** Effects of dietary Vitamin C on growth , Caudal fin development and tolerance of aquaculture related stressors in Channel Cat fish. **The Progessive Fish Culturist. 49,13-16.**

45 **Montero, D., Marrerro, M., Izquierodo,M.S., Robaina, L., Vergara, J.M. and Tort, L. (1999)** Effect of Vitamin E and C dietary supplementation on some

immune parameters of gilthead seabream juveniles subjected to crowding stress. **Aquaculture.171, 269-278.**

46 **Navarre, O. and Halver, J.E. (1989).** Disease resistance and humaoral antibody production in rainbow trout fed high levels of Vitamin C. **Aquaculture. 79,207-221.**

47 **Ndong,D., Fall,J.** The effect of Garlic(*Allium sativum)* on growth and immune responses of hybrid *Tilapia*(*Oreochromis niloticus*×*oreochromis aureus).*article downloaded from Internet.

48 **Ortuno,J.,Esteban M.A., Mesguer, J.(1999)** Effect of high dietary intake of Vitamin C on non specific immune response of gilthead seabream (*Sparus aurata* L.). **Fish Shellfish Immunol. 9, 429-443.**

49 **Perera., R. Johnson., S. Collins., M. (1994)** *Streptococcus iniae* associated with mortality of Tilapia nilotica × Tilapia aurea hybrids**. J Aquat Anim Health; 6: 335 -40.**

50 **Raa, J., (1996.)** The use of immunostimulatory substances in fish and shellfish farming. **Rev. Fish. Sci. 4(3): 229-288.**

51 **Raa, J., Roerstad, G., Engstad, R., Robertsen B.(1992)**. The use of immunostimulants to increase resistance of aquatic organisms to microbial infections. **Dis. of Asian Aquaculture 1: 39-50. Vol. I Fish Health section, Asian Fisheries Society, Manila, Philipines.**

52 **Robertsen, B.; Engstad, R.E.; Jorgensen, J.B.,** (1994) â-glucans as immunistimulants in fish. In: **Modulators of Fish Immune Responses (J.S Stolen & T.C Fletcher, eds) Vol 1, pp 83-99. Fair Haven, N.J:SOS Publication.**

53 **Rumsey, G.L., (1994)** Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis. **Vet Immunol. & Immunopathol. 41, 139-159.**

54 **Sahoo, P.K.; Mukherjee, S.C. (2001):** Effect of dietary beta 1,3 glucan on immune responses and disease resistance of healthy and aflatoxin B_1 induced immunocompromised rohu (*Labeo rohita* Hamilton). **Fish & Shellfish Immunol. 11(8), 683-695.**

55 **Sahu,S., Das,B.K., Mishra,B.K., Pradhan,J., Saran,gi,N. (2007).** Effect of *Allium sativum* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila.* **J.App.Icthyol.23: 80-86.**

56 **Sakai, M. (1999**) Current research status of fish immunostimulants. **Aquaculture 172, 63-92.**

57 **Selvaraj, V.; Sampath, K.; Sekar, V.(2005):** Administration of yeast glucan enchances survival and some nonspecific and specific immune parameters in carp (*Cyprinus carpio*) infected with *Aeromonas hydrophila*. **Fish & Shellfish Immunol. 19, 293-306.**

58 **Shoemaker., C. Klesius., P. Evans., J**. **(2001)** Prevalence of *Streptococcus iniae* in tilapia hybrid striped bass and channel cat fish on commercial fish farms in the United states. **Am J Vet Res 62 (2): 174 – 7.**

59 **Slaven., E.Lopez., F. Hart., S. (2001)** Myonecrosis caused by *Edwardsiella tarda* : a case report and case series extraintestinal *E. tarda* infections**. Clin Infect Dis; 32(10): 1430 -3.**

60 **Soto., E. Hawke., J. Fernandez., D. (2009)** *Francisella sp.,* an emerging pathogen of Tilapia in Costa rica. **J Fish Dis; 32 (8): 713 -22.**

61 **Suetsuna,K. (1998)**. Isolation and Characterization of angiotensin I converting enzyme inhibitor dipeptides derived from *Allium sativum* (garlic)**. J. Nutr. Biochem. 9: 415-419.**

62 **Thomas, W.R. And Holt, P.G. (1978)** Vitamin C and immunity: an assessment of the evidence**. Cl. Exp. Immunol. 32, 370-379.**

63 **Verlhac, V., Gabaudan, J. (1994)** Influence of Vitamin C on the immune system of Salmonids. **Aq. F. Manag. 25, 21-36.**

64 **Verlhac, V., Gabaudan, J.,Obach, A., Schuep, W. and Hole, R.(1996).** Influence of dietary glucan and vitamin C on non-specific and specific immune response of rainbow trout (*Oncorhynchus mykiss*). **Aquaculture 143, 123-133.**

65 **Wahli,T., Verlhac,V., Gabaudan, J., Schuep, W., Meier, W. (1998)** Influence of combined Vitamin C and E on non specific immunity and disease resistance in rainbow trout *Oncorhyncus mykiss* (W.) **J. Fish Dis. 21, 127-137.**

66 **Weinstein., M. Litt., M. Kertesz., D. (1997)** Invasive infections due to a fish pathogen *Streptococcus iniae*. **N Engl J Med; 337:589- 94.**

67 **Welkar, T.L., Lim,C., Yildirim-Aksoy, M. and Klesius, P.H (2007).** Growth, immune function, and disease and stress resistance of juvenile Nile tilapia