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Application of immunostimulants as an alternative to vaccines for health management in aquaculture

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Abstract
Aquaculture, one of the fastest growing food producing sectors, is gaining momentum in several parts of the world. Diseases in fish constitute one of the most important problems and challenges for aquaculturists. Hence, aquaculturists undertake good management practices to ensure the production of healthy fish. Intensification has become a common practice in both finfish and shellfish culture to optimize the returns. High stocking densities, artificial feeding and fertilization have become common husbandry practices in carp culture systems. Due to intensification of culture practices, diseases in rearing of fishes have become major threat to the sustainability of aquaculture industry. Use of synthetic chemicals and antibiotics has been partially successful in preventing or treating fish diseases. Vaccination is an important tool in preventing infectious disease in humans and animals and both passive and active vaccinations are extensively employed in fish. A vaccine targets the specific immune response. It requires primary challenge with antigen and is dependent upon the clonally derived lymphocytes subsets to be implemented. Vaccines against some specific pathogens have been developed recently against some particular diseases. Thus, an alternative approach to boost or stimulate the innate immune system of farmed fish is an application of immunostimulants. Immunostimulants are considered as attractive and promising agents for prevention of diseases in fish and shellfish. In recent years, the proven beneficial effects of immunostimulants in many living systems promote their application for disease management in aquaculture practices.

Keywords: Immunostimulants; Immune system; Aquaculture, Vaccine, fish

1. Introduction
Aquaculture has been growing rapidly for food production in the last few decades. Several commercial fish species have been cultured intensively in narrow or enclosed spaces such as ponds, cages or tanks under overcrowding or high density conditions, thereby causing adverse effect on their health with a potentially stressful environment and infectious diseases [1]. The outbreaks of infectious disease in cultured fish have emerged as constraints for the development of aquaculture. These occurrences have spread through the uncontrolled movement of live aquatic animals resulting in the transfer of pathogenic organisms among countries [2]. Antibiotics and chemotherapeutics have been used to prevent or control bacterial infections in aquaculture for about 20 years [3]. Unfortunately, antibiotics treatment is not successful and sustainable due to increase antibiotic-resistant in bacteria, negative effects on the indigenous microflora of juveniles or adult fish [4], accumulation of antibiotic residues in fish tissue and environment causing human and animal health issues. Vaccination is an effective prophylactic treatment for infectious diseases in fish culture, but it may be very expensive and stressful to the fishes. A single vaccine is effective against only one specific type of pathogen, but limits the effectiveness for wide range of pathogens due to the complex antigenic structure [5]. Therefore, eco-friendly disease-preventive alternative techniques have to be taken into account. One such promising alternative technique to strengthen fish immune systems is the application of immunostimulants in aquaculture [1].

1.1 Concept of Immunostimulant
Immunostimulants, also known as immunostimulators, are substances comprising of drugs and nutrients that activate the immune system by increasing activity of any of its components.

During the last two decades very intensive investigations have been carried out for the production in producing a novel category of biologically active substances, the immunostimulants these are the products derived from natural sources or synthetically made with different chemical characteristics and varied modes and mechanism of action [6]. Immunostimulants activate different components and mechanisms of the immune system of humans and animals reinforce the body's natural resistance in order to successfully and amiably cope with various viral and bacterial infections on help in the treatment of disastrous and chronic ailments and severe immune suppression [7]. Thus, an immunostimulant is a chemical, drug, or stressor that enhances the innate (non-specific) immune response by interacting directly with cells of the system activating them. Immunostimulants can be grouped as chemical agents, bacterial preparations, polysaccharides, animal or plant extracts, nutritional factors and cytokines [3]. Immunostimulants promote synthesis of specific antibodies and cytokines and represent an emerging class of drugs for treatment of infectious disorders. The two main categories of immunostimulants include: (i) Specific immunostimulants, which provide antigenic specificity in immune response such as vaccines or any antigen. (ii) Non-specific immunostimulants, which act irrespective of antigenic specificity to augment immune response of other antigen or stimulate components of the immune system without antigenic specificity, such as adjuvants and non-specific immunostimulators. Pathogens successfully controlled by using immunostimulants in fish are bacteria such as *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Edwardsiella tarda*, *Edwardsiella ictaluri*, *Vibrio anguillarum*, *Vibrio vulnificus*, *Vibrio salmonicida*, *Yersinia ruckeri*, *Streptococcus* spp.; virus such as infectious hematopoietic necrosis, yellow head virus, viral hemorrhagic septicemia and parasite *Ichthyophthirius multifiliis*. Immunostimulants are dietary additives that enhance the innate (non-specific) defense mechanisms and increase resistance to specific pathogens [3].

1.2 Immunological system in fish

The immune system continuously fights against pathogens and gives proper protection to the body. The two types of immune system are innate immune system or non-specific immune system and acquired immune system or adaptive immune system. The essence of immunological system of vertebrates is to react and to protect against the infections. Immune system of a fish involves different cells and organs. There are different inherent factors like health and age, and extrinsic factors like temperature or changes in abiotic parameters that affect the immunological response of fishes. Such changes occasionally cause stress, which can generate an immunological system to collapse if at very low levels [8].

Cells of the immune system are leucocytes or white blood cells. These are found in blood stream or in tissues. Lymphoid tissues on fishes are thymus, spleen, anterior kidney and lymphoid tissues associated with mucus and intestine [9]. Classification of leucocytes as in the vertebrates has been done following morphological criteria whereby various groups can be distinguished such as lymphocytes, granulocytes and macrophages [10]. A short explanation of each group is given below in order to comprehend the main characteristics and functions.

1.3 Lymphocytes

Lymphocytes are highly differentiated cells responsible for immune responses. The most common are mature lymphocytes with an irregular surface or border [9]. Previous studies have defined lymphocytes as highly metabolic due to the presence of organelles in the cytoplasm such as Golgi apparatus, mitochondria, ribosomes, and endoplasmic reticule. They are found all over the body circulating in the blood stream and gathered in lymphoid organs with varied quantity. The main function is to produce antibodies, immunological memory, and regulatory factors such as lymphokines in response to humoral and cell specific immunity [11]. Lymphocytes B are bone marrow derived while T lymphocytes are thymus derived. T cells are responsible for cell mediated immunity as well as providing assistant to B lymphocytes; which are responsible to produce antibodies against antigen [12].

1.4 Granulocytes

Occurrence and functions of granulocytes vary within species of fish, which usually originates in the kidney tissues. Based on morphology there are three types of granulocytes in teleosts; Neutrophil, eosinophil and basophil, the first being the most common [13]. Granulocytes recognize foreign materials invading the body but not specific antigens. This kind of defense is called non-specific defense mechanism, explained later in this review. When foreign materials invade these cells migrate and destroy the estranged particles by phagocytosis or by killing via a cytotoxic response [14].

1.5 Macrophages

Macrophages can be used as indicators to evaluate the health of a fish. These cells play an important role in killing pathogens as in immune response. The pathogens are killed either by releasing toxic substances or by phagocytosis. It involves producing ROS-reactive oxygen species or microbicidal oxygen radicals [9]. Lymphokines can regulate macrophage functions like macrophage activating factor (MAF) [14]. The immunological system of fish can be divided into two branches depending on the functionality such as natural or nonspecific and acquired or specific.

1.6 Immunostimulation of non-specific defense mechanisms

Most immune-stimulatory compounds examined in fish and shellfish have been shown to have immune enhancing potential due to increase of nonspecific immune responses of the organism. The nonspecific immune responses in contrast to specific response does not require prior exposure to an antigen and consists of barriers such as skin, scales, lytic enzymes and phagocytic cells [15]. The nonspecific immune response is also considered to be the first line of defense against invading pathogenic microorganisms and is the sole immunological mechanism by which invertebrates protect themselves from diseases [16]. It has been hypothesized that fish and shellfish are more reliant on nonspecific immune response [17]. For these reasons, a large portion of research on immunostimulation has focused on up-regulating the nonspecific immune response of fishes [18]. Nonspecific immune responses such as phagocytosis and production of oxidative radicals are quickly activated by immunostimulants and help to protect the host against a broad spectrum of pathogens [19].

1.7 Method of administration

Immunostimulants potentiate the immunity of the host itself, enabling it to defend more strongly against pathogens. Several immunostimulants also activate natural killer cells, complement cells, lysozyme and antibody response of fishes [15]. There are mainly three ways to deliver immunostimulants: *injection*, *immersion* and *oral uptake*. Injection of immunostimulants can produce strong non-specific response, but it is time consuming and labour intensive. It is applicable for large size weighing more than 10-15 g. It has been reported that injection has wide protection against a range of pathogens. It has been reported that injection has wide protection against a range of pathogens. For instance, intra-peritoneal injection with glucan injected to channel catfish shows increased phagocytic activity thereby reducing fish mortality challenge with *Edwardsiella ictaluri* [20]. For small fishes vaccination is impractical. Immersion produces less non-specific immune response, but is more cost effective than injection. It increases more stress to fish while handling, which makes it applicable in intensive culture system. Immersion method is very effective during acclimation of juveniles to ponds in field condition. Using immersion of levamisole showed increase in circulating leukocytes, phagocytic rate and increased protection against *P. damselae* in European Seabass [21]. Oral ingestion produces good nonspecific immune response and can be the most economically viable method. It is mostly suited for extensive aquaculture system [22]. Immunostimulant powders are mixed with food using a fish oil coating. Now a day, bioencapsulation method is also followed to immunize fish larvae during their early larval stages.

1.8 Timing of administration

It is necessary to apply immunostimulants at the right time. Anderson (1992) proposed that the application of immunostimulants should be implemented before the outbreak of disease to reduce disease-related losses. Effective dosage and exposure time is more complicated based on different culture systems with feeding regime [23]. In Atlantic salmon, injection with high dose of glucans @ 100 mg kg^{-1} led to absence of protection for 1 week, but maximum benefits occurred after 3-4 weeks. Also, a low dose of injection @ 2-10 mg kg^{-1}, gives only a week long protection [24].

1.9 Mode of action

The mode of action of immunostimulants is to enhance the immunity level of organisms against invading pathogens. The approach is very diverse in nature depending factors such as the type of immunostimulants, dose, route of administration, time and length of exposure. In general immunostimulants activate the phagocytosis and bacterial killing ability of macrophages, complement cells, lymphocytes and nonspecific cytotoxic cells, resulting in resistance and protection to various diseases and invading microorganisms [9].

1.10 Immunostimulants in aquaculture health management

Immunostimulants have been extensively studied in fish and shellfish both at whole organism and on a cellular level. It has been used as prophylactics to control infectious disease in animals and as indication molecules that activate the immune system [25]. Fish and shrimp depend more heavily on nonspecific defense mechanisms than mammals and therefore immunostimulants play a vital role in health management strategies of these aquatic organisms. There are at least 20 different compounds, including levamisole, lipopolysaccharides, glucan, vitamin C and E etc. that are used as immunostimulants, adjuvant and vaccine carriers in fish [16]. Among these compounds, glucan is one of the most promising stimulants for nonspecific defense mechanism and also the most studied immunostimulant in aquatic species. Glucan has been reported to enhance resistance against bacterial pathogens such as *Vibrio anguillarum, Aeromonas salmonicida, A. hydrophila* and *Yersinia ruckeri* in several species in fish such as the carp *Cyprinus carpio*, Atlantic salmon *Salmo salar*, rainbow trout *Onchorhynchus mykiss*, yellow tail *Seriola quinqueradiata* and African catfish *Clarias gariepinus* [26].

2. Conclusion

Immunostimulants appear to be the most promising and useful tool for prophylactic treatment of cultured fish and shrimp. It is safer than chemotherapeutics and more efficient than vaccines. However, these compounds will not replace vaccines, proper nutrition or good management techniques. The strength of these compounds appears to lie in their ability to enhance larval culture before the specific immune system matures. Animals can be vaccinated to improve nonspecific immune function against a broad spectrum of pathogens. Thus, application of immunostimulants to aquatic health management in aquaculture has immense potential. In order to capitalize on the issue many scientific research are necessary. Additional research is needed to define the specific dosage rates and efficacy of various compounds for a variety of aquatic species and their pathogens and to decrease costs of the immunostimulants. Hence, it is expected that in the near future immunostimulants may be an effective tool for controlling infectious diseases in aquaculture.

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