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Use of Immunostimulants in aquaculture disease management

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Abstract
A number microbial components and plant products such as polysaccharides, lentinan, levamisole, schizophyllan, oligosaccharides, muramyl dipeptide and yeast derivatives have been used as immunostimulants in aquaculture to stimulate the innate immune system of fish and shrimp. Among the all the immunostimulants examined, Beta- glucans were found to be most promising immunostimulants, which are used in aquaculture to control diseases.

Keywords: Immunostimulants**,** Aquaculture, Diseases Management

1. Introduction
Intensification of aquaculture has become an important practice in recent years to optimize the returns. High density stocking, providing artificial feeds and fertilization of the pond water have become common husbandry practices in both fish and shrimp culture systems. Due to intensification of culture practices in aquaculture, diseases caused by microbes have surfaced significantly in culture systems. Several drugs, synthetic chemicals and vaccination programmes have been in practice to prevent and control the diseases, but partial success has been achieved. An alternative approach has been the application of various compounds to boost or stimulate the immune system of fishes and shrimp. Such compounds are known as the immunostimulants. It has been established that the beneficial role of these compounds for disease management in aquaculture systems.

For the last twenty years, the problem of microbial diseases has emerged as a major constraint to aquaculture industry. Increased disease occurrences have resulted due to the transfer of pathogenic organisms among cultured species of fish and shrimp, between different countries without proper quarantine measures. Due to this, the shrimp industry in India as well as other Southeast Asian countries has suffered significant economic losses. As there is no effective remedies against these viral diseases, use of immunostimulants in culture systems has become powerful measure to control diseases in aquaculture ponds.

An immunostimulant is defined as a substance which enhances the innate or non-specific immune response by interacting directly with cells of the immune system and activating them. Immunostimulants can be grouped under different agents based on the source, such as bacterial preparations, polysaccharides, animal or plant extracts, nutritional factors and cytokines [15]. The pathogens that were controlled successfully by using immunostimulants in fish/shrimp, include bacteria *Aeromonas salmonicida, A.hydrophila, Vibrio anguillarum, V. vulnificus, V. salmonicida, Yersinia ruckeri, Streptococcus* spp.; viruses causing infectious such as hematopoietic necrosis, yellow head virus, viral hemorrhagic septicemia and the parasite like *Ichthyophthirius multifiliis* [4].

Immunostimulants are dietary additives that enhance the innate (non-specific) defense mechanisms and increase resistance to specific pathogens [15]. There is no memory component developed and duration of the immune response is over a short period. Immunostimulants are chemical substances which activate leukocytes [10]. Adjuvant (FCA) is one of the first immunostimulants used in animals to enhance the specific immune response, and it has also been successfully used in conjunction with injection of fish bacterins [1]. So far glucans, which are polymers of glucose, found in the cell walls of plants, fungi and bacteria appear to be most promising of all the immunostimulants investigated, in fish and shrimp culture ponds through oral application, which was found to be the route of choice [11].

Use of these different types of immunostimulants is an effective means to increase the immune competency and disease resistance of fish and shellfish. The present review reports the use of immunostimulants in aquaculture disease management.

2. Types of Immunostimulants used in Aquaculture

2.1 Muramyl dipeptide

Muramyl dipeptide is a simple glycoprotein, also a purified form of mycobacteria. It can enhance the antibody activity, stimulation of polyclonal activation of lymphocytes and activation of macrophages.

2.2 Chitin and Chitosan: Both chitin and chitosan have a major role in aquaculture. They are non-specific immunostimulators which are effective on a short term basis. Chitin is a polysaccharide which constitutes the principal component of exoskeletons of crustaceans and insects and cell walls of few fungi [15]. It can stimulate macrophage activity and give resistance from certain bacteria [9]. Anderson and Swicki [2] administered chitosan to brook trout (*Salvenus fontinalis*) by injection and immersion and found that high levels of protection occurred 1, 2, 3 days afterwards, but protection was greatly reduced by day [14]. Injection of chitosan was also more effective than simple immersion.

Chitosan is a deacetylation product of chitin. The influence of chitosan on immune response of healthy and cortisol treated *Labeo rohita* was demonstrated. After treatment with chitosan sufficiently higher responses in almost all assays of non-specific immunity were observed in comparison to their healthy control or cortisol treated counterparts respectively without chitosan treatment [15]. In aquaculture, chitosan has been used as an immunostimulant for protection against bacterial disease in fish, for controlled release of vaccines, and as a diet supplement reveal that dietary β-glucan administration increases [6].

2.3 Lentinan, Schizophyllan and Oligosaccharide

Lentinan, Schizophyllan and Oligosaccharide can increase cellular and non-cellular defence mechanisms like lysozyme activity, phagocyte activity and complement activity in fish

2.4 Levamisole

It is an anthelmintics chemical that has been shown to have some stimulating effect on the immunological reactivity of animals and humans. Activities of this agent are: enhancement of cell mediated cytotoxicity, lymphokine production and suppressor cell function, and stimulation of phagocytic activity of macrophages and neutrophils.

2.5 Yeast derivatives

Glucans- long chain polysaccharide extracted from yeast are good stimulators of non-specific defence mechanism in animals, including fish and shellfish like phagocytic activity and protection against bacterial pathogens. Several types of glucans have been investigated in fish such as yeast glucan, peptide-glucan, β-1, 3 glucan (VST). Yeast glucan (β 1-3- and β1-6-linked glucan) and β-1,3glucan (VST) is derived from cell walls of baker's yeast such as *Saccharomyces cerevisiae* and *Schizophyllum commune*, respectively [3]. β-glucans comprise diverse group of polysaccharides of D-glucose monomers linked with β-glycosidic bonds. Cellular and non-cellular defense mechanisms are increased in activity after treatment with β-glucan like lysozyme activity, phagocyte activity, complement activity and bactericidal activity of macrophages. A number of reports reveal that dietary β-glucan administration increases resistance to infection.

Selvaraj *et al*. [16] observed that the highest antibody titre against *A. hydrophila* injected with β-glucan (100-1000 µg glucans/fish). In addition, Robertsen *et al*. [13] recorded that intraperitoneal injection of β-glucan prepared from cell walls of *Saccharomyces cerelisiae* injected to Atlantic salmon showed increased resistance to *V. nguilllarm, V. salmonicidia* and *Y. ruckeri*. Yano *et al*. [22] showed that β-1, 6, branched β-1, 3 Glucans were effective in carp. Jenny and Anderson [2] reported that the use of Glucans increased activity in non-specific defence mechanism and in protection against *Yesinia ruckeri*. Glucan treatment in Atlantic salmon (*salmo salar*) induced the protection against *Vibrio salmonicidia*. Several Glucan products such as vitastim, macrogard, are marketed commercially and are used in supplementing fish feeds.

3. Plant extracts used as Immunostimulants

A number of herbal plants extract from *Ocimum sectum*, *Emblica officinalis*, *Cynodon dactylon,* and *Adathoda vasica* have been used as immunostimulants in aquaculture. They have significantly improved the immune system and reduced microbial infection in goldfish *Carassius auratus* [16]. Methanolic extracts of *Ocimum sanctum*, significantly improved the phagocytic activity, serum bactericidal activity, albumin– globulin (A/G) ratio, and leukocrit in *Epinephelus tauvina* against *Vibrio harveyi* [17]. Ardo *et al.* [3] observed that tilapia feeding with two Chinese medicinal herbal extracts (*Astragalus membranaceus and Lonicera japonica*) alone or in combination has significantly enhanced the phagocytic activity. Now days, there is a growing interest in using plant extracts as immunostimulants in aquaculture [14].

3.1 Vitamins used as Immunostimulants

Vitamin C

Vitamin C play an important role in several physiological functions including growth, resistant to infections, wound healing, response to stressors and possibly lipid metabolism through its action on carnitine synthesis while administering with feed. Vitamin C (Ascorbic acid) is a co-factor in many biological processes including collagen synthesis and cellular functions related to neuromodulation, hormone and immune systems. It has been observed by Tewary and Patra [19] that higher levels of dietary vitamin C significantly increased the protection against *A. hydrophila*.

Vitamin E

Vitamin E can enhance specific and cell-mediated immunity against infection in Japanese Flounder *Paralichthys olivaceus* [20] and macrophage phagocytosis in fish such as channel catfish *Ictalurus punctatus* [21] and turbot *Scophthalmus maximus* [12]. Vitamin E deficiencies in trout result in reduced protection against *Y. ruckeri* [5].

3.2 Timing of Administration

It is important to apply immunostimulants at the right time. Anderson [1] proposed that the application of immunostimulants should be implemented before the outbreak of disease to reduce disease related losses. Effective dosage and exposure time will be further more complicated based on different culture systems and their feeding regime [7]. In Atlantic salmon injection with high dose of glucans @ 100 mg/ kg led to the absence of protection for 1 week, but maximum benefits only occur after 3-4 weeks. Also, at low dose of injection @ 2-10 mg/kg, give protection only for 1 week [13]. Similarly, it has been noticed that

increase in the number of NBT positive cells in African catfish fed with glucan or oligosaccharide over 30 days, but not over 45 days. Overdoses of several immunostimulants induce immunosuppression in fish. The side effects of immunostimulants have not been well-studied.

3.3 Mode of action

The mode of action of immunostimulants is to activate the immune systems of organisms, to enhance the immunity level against invading pathogens. The approach is very diverse in nature or may be poorly understood and also depends on the type of immunostimulants, dose, and route of administration, time and period of exposure.

3.4 Mechanism of action of immunostimulants

- Levamisole, Freund's Complete Adjuvant (FCA), Glucans, Muramyl dipeptide, FK-565 (Lactoyl tetrapeptide from *Streptomyces olivaceogriseus*)- stimulators of T-lymphocytes
- Bacterial endotoxions, Lipopolysaccharides, Macrophage activator- Glucans, Chitin and Chitosan- stimulates of B-cells.
- Inflammatory agents including chaemotoxins
- Detergents and Sodium dodecyl sulphate, Quarternary ammonium compounds (QAC), saponins- Cell membrane modifiers
- Vitamin C and E, n-3 fatty acids- Nutritional factors
- Cytokines- Leukotriene, Interferon
- Animal and fish extracts- Mitogens

4. Conclusion

Immunostimulants are substances that control the fish and shellfish diseases in aquaculture. The immunostimulatory effects of chitin, glucan and levamisole for fish and shrimp have been reported. Nutritional factors such as Vitamins C and E have also been reported to be immunostimulators. These immunostimulants mainly facilitate the function of phagocytic cells and increase their bactericidal activities. Many immunostimulants also stimulate the natural killer cells, complement, lysozyme and antibody responses of fish. The effective method of administration of immunostimulants to fish is by injection. Oral and immersion methods have also been observed, but the efficacy of these methods decreases with long-term administration. Growth-promoting activity has been observed in fish or shrimp treated with glucan or lactoferrin. In conclusion, immunostimulants can reduce the losses caused by various diseases in aquaculture; but they may not be effective against all diseases. For the effective use of immunostimulants, the timing, dosages, method of administration and health status of animal need to be taken into consideration.

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