Application and Effectiveness of Immunostimulants, Probiotics, and Prebiotics in Aquaculture: A Review

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(Received 7.11.09, Accepted 27.12.09)

Key words: immunostimulants, probiotics, prebiotics

Abstract
Immunostimulants, also called immunomodulators, adjuvants, or biological response modifiers, stimulate the immune system. They can be administered in the form of drugs or nutrients. Probiotics are organisms or substances that improve the intestinal microbial balance of a host animal. Prebiotics are indigestible components in a diet that are metabolized by specific microorganisms and prove helpful for the growth and health of the host. When provided as dietary supplements in feeds, even in small quantities, immunostimulants, probiotics, and prebiotics usually improve immunity, feed efficiency, and growth performance of crustaceans and fishes. The use of immunostimulants, probiotics, and prebiotics in aquaculture are presented in this review.

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**Introduction**

Immunomodulators are substances (e.g., drugs) that stimulate the immune system. Biological activities of immunomodulators are influenced by physicochemical parameters such as solubility, primary structure, molecular weight, branching, and polymer charge (Bohn and BeMiller, 1995). The effects of β-glucans during the development of immune reactions are well established (Vetvicka and Sima, 2004). Immunomodulators find their application in a wide variety of species, including shrimps (Duvic and Söderhäll, 1990; Das et al., 2006), fish (Anderson, 1992), rats (Feletti et al., 1992), oysters (Subhash and Lipton, 2007), rabbits (Kennedy et al., 1995), guinea pigs (Ferencik et al., 1986), pigs (Hiss et al., 2003), cattle (Buddle et al., 1988), humans (Kougias et al., 2001), and earthworms (Beschin et al., 1998).

**Immunostimulants**

Immunostimulants stimulate the immune system. The term immunostimulant can be used interchangeably with immunomodulator, adjuvant, and biological response modifier. Immunostimulators can be in the form of drugs or nutrients. They stimulate the monocyte-macrophage system and thereby modulate the immune system of the body. *Lactobacillus* spp., *Streptomyces* spp., *Aspergillus* spp., etc., can be used as immunostimulants (Ganguly et al., 2009). There are two main categories of immunostimulators: specific and non-specific.

Specific immunostimulators provide antigenic specificity in immune response and include vaccines and antigens. For specific immune response, hosts must have had prior exposure to an antigen after which recognition and subsequent activation occurs through co-ordinated action of B-lymphocytes and T-cells. B-lymphocytes play a large role in the humoral immune response, in contrast to cell-mediated immune responses which are governed by T-cells.

Non-specific immunostimulators act irrespectively of antigenic specificity to enhance the immune response of other antigens or to stimulate components of the immune system without antigenic specificity, such as glucans and the synthetic drug levamisole. Many endogenous substances are non-specific immunostimulators. For example, glucans and mannans possess non-specific immunostimulatory effect. β-glucan is a polymer of glucose consisting of a linear backbone of β-1,3 linked D-glucopyranosyl residues with varying degrees of branching from the C6 position (Bohn and BeMiller, 1995). β-glucans are major components of yeasts, mushrooms, and fungal mycelia. Mannan, a plant polysaccharide, is a polymer of the sugar mannose. Detection of mannan leads to lysis in the mannan-binding lectin pathway.

As feed additives, immunostimulants provide significant protection against pathogens and upregulate phagocytosis, bacterial killing, and oxidative burst.

**Immunostimulant Activation of Immune System**

Anti-microbial immune mechanisms in invertebrates can be induced by fungal β-glucans (Brown and Gordon, 2005). β-glucans are recognized by fish as foreign agents because of their similarity to fungal or bacterial gram-negative
polysaccharides. As a result, an inflammatory response is produced by the immune system after exposure (Robertson et al., 1994).

**Application of Immunostimulants in Aquaculture**

Glucans with a strong immunomodulating activity have been well studied in fishes (Anderson, 1992). Some investigators used *in vitro* culture of macrophages with glucan (Cook et al., 2001) but most carried out *in vivo* studies (Sahoo and Mukherjee, 2001; Ortuno et al., 2002). Fish in intensive conditions are more susceptible to microbial infection, especially in larval stages (Smith et al., 2003). During stress, immunostimulants can provide resistance to pathogens. Few immunostimulants can be used in aquaculture (Siwicki et al., 1998).

There are two types of glucans: α- and β-, the numbers of which clarify the type of O-glycosidic bond. Glucans are commercially significant as immunostimulating agents. Different types of β-glucans have been used to increase resistance of fish and crustaceans against bacterial and viral infections (Paulsen et al., 2001; Bagni et al., 2005). The health, growth, and general performance of farmed shrimp and fish may be improved by the use of β-glucans. Product source, animal species, development stage of the target organism, dose and type of glucan, route, time schedule of administration, and association with other immunostimulants affect the immunomodulatory effects of glucans (Guselle et al., 2007).

The immunostimulatory effects of glucan, chitin, lactoferrin, levamisole, vitamins B and C, growth hormones, and prolactin have been reported in fish and shrimp. These immunostimulants mainly facilitate the function of phagocytic cells and increase their bactericidal activities. Several immunostimulants also stimulate natural killer cells or complement lysozyme and antibody responses of fish. The most effective method of administration of immunostimulants to fish is by injection. The efficacy of oral and immersion methods decreases with long-term administration. In some cases, overdoses of immunostimulants induce immunosuppression in fish. Growth promoting activity has been noted in fish or shrimp treated with glucan or lactoferrin. Immunostimulants can overcome immune suppression by sex hormones.

For the effective use of immunostimulants, the timing, dosage, method of administration, and physiological condition of the fish need to be taken into consideration. Immunostimulants can reduce the losses caused by disease in aquaculture, however, they may not be effective against all diseases.

**Probiotics**

The term probiotics was coined by Parker (1974) to describe organisms and substances which contribute to intestinal microbial balance. They affect the host animal by improving its intestinal microbial balance (Fuller, 1989). Probiotics are viable cultures of bacteria and fungi which, when introduced through feed, have a positive effect on health. According to the currently adopted definition by FAO/WHO, probiotics are: "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2001). Some reside in the digestive tracts of individuals while
others derive from an external origin. They are sometimes referred to as direct fed microbials (DFM). Probiotics can be used as growth promoters and for therapeutic purposes (Ramakrishnan et al., 2008)

A variety of population of microorganisms are present in the gut and their population is affected by various factors including age, diet, environment, stress, and medication. The most commonly used organisms in probiotic preparations are lactobacilli, streptococci, and bifidobacteria. In addition, Bacillus spp., yeasts, Saccharomyces spp., and filamentous fungi (Aspergillus oryzae) are used as probiotics. Probiotic preparations are available as tablets, powders, capsules, pastes, or sprays.

**Characteristics of Probiotics**
Efficient probiotics must be (a) resistant to pH and bile acids, (b) have no pathogenicity, (c) be viable, (d) be stable in storage and in field, (e) survive and potentially colonize in the gut, (f) be cultivable on a large scale, (g) be able to adhere to the epithelial lining of the gut, and (h) affect host animals beneficially. All new strains used for probiotic development should possess all the aforementioned characteristics (De et al., 2009).

**Mode of Augmentation of Probiotics in the Immune System**
Probiotic microorganisms in the gut stimulate the immune response of host systems in two ways (De et al., 2009). They can migrate through the gut wall as viable cells, multiplying to a limited extent, or antigens released by the dead organisms are absorbed and directly stimulate the immune response. Probiotics are generally applied in aquafeed because of their positive effects on growth rate, feed conversion, and disease resistance. Probiotics have a positive effect on host immune response through increased activity of macrophages, shown by enhanced phagocytosis of organisms or carbon particles, increased production of systemic antibodies, e.g., IgM and interferon, and increased effects of local antibodies on mucosal surfaces such as the gut wall. The effect of probiotics on the host immune system can be measured by estimating the levels of macrophage enzymes.

**Application of Probiotics in Aquaculture**
Lactic acid bacteria have received priority as probiotics in fish feed (Hagi et al., 2004). Lactic acid bacteria produce acetate and lactate which inhibit the growth of several Vibrio species (Vazquez et al., 2005). Lactic acid bacteria in the diet of Atlantic cod increased their survival rate when challenged with the pathogen Vibrio anguillarum. Probiotics influence the specific and non-specific immunity in many fish species such as rainbow trout (Nikoskelainen et al., 2003; Panigrahi et al., 2005) and gilthead seabream (Salinas et al., 2005). Probiotics help reduce mortality of larval and pathogen-challenged fishes and provide enzymes needed for digestion. However, the effectiveness of probiotics is adversely affected by harsh conditions of extrusion or pellet manufacturing. There are also many regulatory issues regarding the application of probiotics in aquafeed.
Sometimes desired outcomes are not obtained after the use of probiotics in feed. This is attributed to the fact that different probiotics contain different microorganisms which may act differently in different situations, and that they each have their own metabolic pathway (De et al., 2009).

**Prebiotics**

Prebiotics are indigestible components of a diet that are metabolized by specific microorganisms which prove to be helpful for growth and health of the host (Manning and Gibson, 2004). Nutrients such as linoleic acid, linolenic acid, and soluble carbohydrates were studied for their effects on the aerobic/facultative anaerobic intestinal microbiota of Arctic char *Salvelinus alpinus* (Ringo and Olsen, 1999). Prebiotics shift the microbial community to one dominated by beneficial bacteria, such as *Lactobacillus* spp. and *Bifidobacterium* spp. (Manning and Gibson, 2004). To date, no significant data is available related to the use of prebiotics in fish feed. When linoleic acid was supplemented to the diet of Arctic char, the total viable counts of digestive enzyme-secreting bacteria increased by 10-fold as compared with fish fed a diet without linoleic acid (Ringo, 1993). Polyunsaturated fatty acids of the n-3 and n-6 series also were shown to alter the microbial population of Arctic char, with the lactic acid bacteria *Carnobacterium* spp. being the dominant facultative anaerobe cultivated (Ringo and Gatesoupe, 1998).

The effects of a potential prebiotic were also investigated on hybrid striped bass *Morone chrysops x M. saxatilis* (Li and Gatlin, 2004, 2005) by application of GroBiotic®-A, a mixture of partially autolysed brewers yeast, dairy ingredient components, and dried fermentation products. It was encouraging that fish fed a diet containing GroBiotic®-A had significantly higher feed efficiency and significantly lower mortality when challenged with the bacterial pathogens *Streptococcus iniae* and *Mycobacterium marinum* (Li and Gatlin, 2004, 2005).

**Mechanism of Action of Prebiotics on the Immune System**

Prebiotics have the potential to enhance many host biological responses and reduce the mortality of fishes caused by invasion of pathogens. However, the anaerobic intestinal tract microbiota of commercially important fishes, such as channel catfish, hybrid striped bass, tilapia, and salmonids, need to be investigated to determine if there are particular bacterial species to be enhanced by the use of prebiotics. By increasing the production of volatile fatty acids (VFA) in the gastrointestinal (GI) tract, hosts benefit by recovering some of the lost energy from indigestible dietary constituents and by inhibiting potential pathogenic bacteria (Manning and Gibson, 2004; Vazquez et al., 2005). The produced VFA are also indicative of the microbial population present in the GI tract (Nisbet, 2002).

Herbivorous fishes such as the sea chubs *Kyphosus cornelii* and *K. sydneyanus* were the first species shown to have VFA as bacterial metabolic by-products in their intestinal tracts (Choat and Clements, 1998). Other fishes found with bacterial VFA in their intestinal tracts include the tilapia *Oreochromis mossambicus* (Titus and Ahern, 1988). Prebiotics have many
beneficial effects such as increased disease resistance and improved nutrient availability (Schley and Field, 2002). As such, prebiotics have the potential to increase the efficiency and sustainability of aquaculture production.

The most commonly used prebiotic preparations in aquaculture are fructooligosaccharide (FOS), transgalactooligosaccharide (TOS), inulin, glucoooligosaccharide, xyloooligosaccharide, isomaltooligosaccharide, soybean oligosaccharide, polydextrose, and lactosucrose (Vulevic et al., 2004; Propulla, 2008). Natural sources of prebiotics in vertebrates include chicory, onion, garlic, leek, tomato, and honey.

**Properties of Prebiotics**

Prebiotics should have the following properties: (a) be easy to incorporate in the feed or ration, (b) regulate gut viscosity, (c) be non-carcinogenic, (d) derive from dietary polysaccharides, (e) have low calorific value, (f) reduce harmful microbial loads, (g) be effective at low concentration, (h) exert anti-adhesive properties against harmful gut microbes, (i) stimulate beneficial gut microbes, and (j) produce no residual effects.

**Popular Prebiotics**

Currently, the most popular targets for prebiotics use are lactobacilli and bifidobacteria, based largely on the success of potential prebiotics. Prebiotics can be used as unique tools to create gut microflora with a controlled composition that can eventually be correlated with specific physiological conditions.

**Conclusion**

Investigated immunostimulants, probiotics, and prebiotics have numerous beneficial effects in aquaculture including improved disease resistance and nutrient availability, leading to increased sustainability and profitability of fish and crustacean production. Therefore, more research regarding the use and effects of different immunostimulants is warranted.

**References**


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