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Probiotics and prebiotics associated with aquaculture: A review

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A B S T R A C T

There is a rapidly growing literature, indicating success of probiotics and prebiotics in immunomodulation, namely the stimulation of innate, cellular and humoral immune response. Probiotics are considered to be living microorganisms administered orally and lead to health benefits. These Probiotics are microorganisms in sufficient amount to alter the microflora (by implantation or colonization) in specific host's compartment exerting beneficial health effects at this host. Nevertheless, Prebiotics are indigestible fiber which enhances beneficial commensally gut bacteria resulting in improved health of the host. The beneficial effects of prebiotics are due to by-products derived from the fermentation of intestinal commensal bacteria. Among the many health benefits attributed to probiotics and prebiotics, the modulation of the immune system is one of the most anticipated benefits and their ability to stimulate systemic and local immunity, deserves attention. They directly enhance the innate immune response, including the activation of phagocytosis, activation of neutrophils, activation of the alternative complement system, an increase in lysozyme activity, and so on. Prebiotics acting as immunosaccharides directly impact on the innate immune system of fish and shellfish. Therefore, both probiotics and prebiotics influence the immunomodulatory activity boosting up the health benefits in aquatic animals.

1. Introduction

A variety of useful feed additives, including probiotics and prebiotics having beneficial effects to the host was used in aquaculture to combat diseases such as supplements, to improve growth include increasing the size and weight gain, and in some cases, act as an alternative antimicrobial compounds [55], as well as to stimulate immunity response of the host. Probiotics live in microbial feed additives that modulate gastrointestinal microbial communities whereas Prebiotics, non-digestible forage additives stimulate the activity or abundance of beneficial gastrointestinal bacteria. Both of these have received widespread attention, showing the improved production, health and disease resistance of aquatic animals [30]. In addition increased research for the development of new strategies of food supplementation which were assessed in various health and growth promoting compounds such as; probiotics, prebiotics, synbionts, phytobiotics and other functional food supplements were also evaluated [28].

Probiotic microorganisms are served for many purposes such as increasing economic growth, promoting digestion, absorption and suppression of infectious diseases [11,76]. Apart from immunomodulation, probiotics are also believed to have diverse modes of action in living organisms. For example in terrestrial animals, they competitively exclude potential pathogens by placing repressive molecules or through direct competition for space, nutrients or oxygen in the digestive tract of an organism [39]. However, in aquatic animals, they stick with the mucosal epithelium of gastrointestinal tract, such as *Brevibacillus brevis,* lactic acid bacteria, *Vagococcus fluvialis and Vibrio harveyi* [23,65,67,73,108,111] and help to resist pathogens [70]. However, it was also reported that probiotics do not dwell in the digestive tract [90]. Another mode of action of probiotics is that, they enhance the digestibility of food [117] in the form enzymes such as alginate lyases, amylases and proteases [131]. They also boost the production of nutrients for example, fatty acids, biotin and vitamin B12 [112,113,120], which has positive effect on the health of an animal.

These are also used as a supplementary food for improving intestinal microbial balance that may offer beneficial effects to the host [40,105]. Probiotic bacteria are known to have immune stimulant effect [43,119]. Classically, certain prebiotics are non-digestible fibers that increase intestinal beneficial bacteria in a

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host [64,91]. Later proposed another type of functional saccharides; so-called immunosaccharides, which stimulate the immune system directly, rather than through the probiotic products. Prebiotics, such as fructooligosaccharide, mannanooligosaccharide, inulin or β-glucan, are known immunosaccharides. Probiotics were found to stimulate feed conversion efficiency, increase live weight gain of fish and shrimp culture [4] and by competitive exclusion to confer protection to a fixed area, to prevent pathogens [23,121], the production of organic acids, hydrogen peroxide, antibiotics, bacteriocins, siderophores and lysozyme [33,126]. They also regulate the immune response of fish [13,14].

Existing literature lack evidence that pro and prebiotics are safe for their host. Consequently, viability of an organism during its administration and retention of beneficial effects during prolonged storage or repeated sub-culturing is still questionable. A number of probiotics and prebiotics are now commercially available and in use. Although there are added costs associated with using these products, improved production efficiency and reduced disease incidence may offset such costs. Another possible way of simultaneous administration of pro and prebiotics, termed as synbiotic, is intended to improve the survival and implantation of the live microbial supplement in the GIT. However, meager information is available on the use of synbiotics in aquaculture. By understanding all the advantages that pro and prebiotics confer, it would be possible for us to apply them symbiotically only after knowing their benefits in aquaculture. So, over the last decade, the application of both probiotics and prebiotics that take advantage of their potential for pathogen control has increased in aquaculture. This document focuses on immunomodulatory action of probiotics and prebiotics, which directly stimulate the innate immune system of fish and shellfish. Furthermore, it is necessary to standardize the procedures used for the study of probiotics and prebiotics, particularly with regard to the precise nature of the congenital and or cellular immune response.

2. Probiotics

The Probiotics, has been initially defined as organisms or substances that contribute to intestinal microbial balance [84]. The term probiotic was emerged from the Greek words “Pro” and “bios” meaning “for life” [47] and is often referred as a support for life, that help naturally to improve the overall health of the host organism. According to the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), probiotics are live microorganisms which, when administered in an appropriate amount provide health benefits to the host [36].

Nevertheless, aquaculture has been an expansion undertaken by FAO/WHO to include a wide range of Gram-positive and Gram-negative bacteria, bacteriophages, microalgae and yeast (Table 1) with the application through the aqueous/water route, as well as by supplying the feed. The essence of the definition of FAO/WHO is that probiotics are living organisms, which are administered orally having some tangible health benefits, and have been widely used for the control of diseases in aquaculture, especially in developing countries [11,37,56,60,76].

In practice, there is only limited evidence that the viability of probiotic cultures checked as soon as they are added to feed, and the health benefits may be inaccurately described. Of course, it may be an impact on the viability of the processing result of diet, namely pelleting/granulation and extrusion. For example, the administration of the probiotic extruded diet led to an improvement in nonspecific immunity of *Nile tilapia* compared with granulated/pelleted diet [103].

Stimulation of nonspecific host defense mechanisms, using specific biological compounds called immune stimulant, increases resistance to disease and the growth of the host [72]. The innate immune system containing physical barriers and cellular and humoral components is a self-defense weapon in fish [38]. Live bacteria as probiotics act alternatively for antibiotics and chemicals, and functions as signaling molecules to activate the immune system.

There are many beneficial effects of probiotics, one among which is the modulation of the immune system. The role of probiotics in the modulation of the immune system was extensively studied and reviewed in humans and animals [6,41]. Previous studies dealt with growth-promoting and disease protection ability of probiotics in fish. But in recent years much attention has so far been in the direction of the immunomodulatory effects of probiotics in aquaculture. Many immunological researches have been conducted in various fish, using different probiotics and their validity to stimulate fish immunity, both within invivo and invitro conditions is remarkable [13,53]. Probiotics have also been identified for use as immunomodulatory agents in shellfish like shrimp [8,51,88].

3. Strengthening immune response

The innate immune system, also known as non-specific immune system is the first line of defense which comprises the cells and mechanisms that defend the host from infection by other organisms. Probiotics interact with the immune cells such as mononuclear phagocytes (monocytes, macrophages) and polymorphonuclear leukocytes (neutrophils), natural killer (NK) cells, to enhance innate immune responses. Some probiotics can increase the number of erythrocytes, granulocytes, macrophages and lymphocytes in various fishes [55,57,62,63,66,77]. In the same

Table 1
Probiotics used in aquaculture acting on specific pathogens (updated from Ref. [79]. The pathogens controlled by the probiotics are given as Latin small letters.

Category of organism	Genus/groupings
Gram-positive bacteria	*Arthrobacter (q, s), Bacillus (b, d, q, x), Brevibacillus (m), Brochothrix (a), Clostridium (b, n), Carnobacterium (n, r, x), Enterococcus (e, q, s), Kocuria (n, r), Lactobacillus (c, g, j, l), Lactococcus (n), Leuconostoc (g), Microbacterium (n), Micrococcus (b, c), Pediococcus (h, n), Rhodococcus (n), Streptococcus (q), Streptomyces (q, u), Vagococcus (n) and Weissella*
Gram-negative bacteria	*Aeromonas (a, c, g, l, w), Agarivorans, Alteromonas (p, t, v), Bdellovibrio (b), Burkholderia, Citrobacter (b), Enterobacter (f), Neptunomonas (p, t, v), Phaeobacter (n, s), Pseudoalteromonas (n, t, v), Pseudomonas (c, h, n, q), Rhodobacter (n), Rhodopseudomonas, Roseobacter (n), Shewanella (i), Synechococcus (q), Thalassobacter,Vibrio (c, n, q, x) and Zooshikella (l)*
Non-bacterial candidates	
Bacteriophage	*Myoviridae (k) and Podoviridae (k)*
Microalgae (m, 0, u)	*Dunaliellasalina, D. tertiolecta, Isochrysisgalbana, Navicula, Phaedactylumtricornutum and Tetraselmissuecica*
Yeast	*Debaryomyceshansenii, Phaffiarhodozyma, Saccharomyces cerevisiae (b, l), S. exiguous and Yarrowialipolytica*

Pathogens:a → A. bestiarum, b → A. hydrophila, c → A. salmonicida, d → Edw. Ictaluri, e → Edw. tarda, f → F. psychrophilum, g → Lc. Garvieae, h → Photobacteriumdamselae subsp. damselae, i → Ph. damselae subsp. piscicida, j → Ps. fluorescens, k → Ps. plecoglossicida, l → Streptococcus sp./St. iniae, m → Vibrio spp., n → V. anguillarum, 0 → V. campbellii, p → V. coralliilyticus, q → V. harveyi, r → V. ordalii, s → V. parahaemolyticus, t → V. pectenicida, u → V. proteolyticus, v → V. splendidus, w → V. tubiashii, x → Y. ruckeri.

way, probiotics, both in conditions of invitro and invivo; actively induce the proliferation of B lymphocytes in fish.

Increasing the level of immunoglobulin by probiotic supplementation has been reported in a number of animals including fish [63]. It has been demonstrated that oral administration of the bacteria *Clostridium butyricum* in rainbow trout has reinforced the strength of the fish to *vibriosis*, increasing phagocytic activity of leukocytes [94]. The use of *Bacillus sp.* (Strain S11) provided protection of the disease by activating both cellular and humoral immune defense in tiger shrimp *(Penaeus monodon)* [89].

A management of mixture of bacterial strain *(Bacillus and Vibrio sp.)* positively influenced the growth and survival of young white shrimp and presents a protective effect against the pathogen *V. harveyi* and white spot syndrome virus [8]. This protection is due to the stimulation of the immune system, by increasing the phagocytosis and antibacterial activity. In addition, the administration of a lactic acid bacterium *Lactobacillus rhamnosus* (ATCC 53103) in an amount of 105 cfu g^{-1} feed; stimulates the respiratory burst in rainbow trout *(Oncorhynchus mykiss)* [81]. However [10] found only increase in immunoglobulin levels in brown trout *(Salmo trutta)* but not significant level by lactic acid bacteria (LAB) fed groups of probiotics namely *Lactococcus Lactis sp. Lactis, Lactobacillus sakei* and *leuconostoc mesenteroids* supplemented at 10 (6) CFU/g diet for a period of 2 weeks.

Therefore, probiotics are beneficial bacteria capable of inhibiting not only pathogens, but also regulate the host immune system [Fig. 1]. Immunomodulation by probiotics are considered as a community effort of the introduced microorganism, host and commensals. The host can detect whether the organism is pathogenic or not through pathogen pattern recognition receptors (PRRs). To identify these recognition receptors, the microbial associated molecular patterns (MAMPs) which are present in both pathogenic and non-pathogenic microorganism. Some MAMPs are lipopolysaccharides (LPS), peptidoglycan, flagellin, and microbial nucleic acids. The binding of MAMPs to PRRs trigger intracellular signaling cascade, urging the release of specific cytokines and transmit signals to adjacent cells, or to exert anti-viral, pro- or anti-inflammatory exercise effects. The same mechanism of recognition regulates the homeostasis of the commensal of microbiota in the mucosa. Moreover, probiotics can also manipulate the richness and diversity of commensal microbiota [76].

4. Mechanism of immune response

4.1. Phagocytic activity

Phagocytes such as neutrophils and macrophages play an important role in the anti-bacterial defense. These cells ingest and kill bacteria through the production of reactive oxygen species (ROS), including superoxide anion, hydrogen peroxide and hydroxyl radicals in the respiratory burst [34]. Therefore, probiotics can effectively cause phagocytes in host and improve phagocytosis activity by lactic acid bacteria (LAB) group of probiotics; such as *L. rhamnosus, Lactobacillus acidophilus* and *Lactobacillus Lactis* which has been observed in several animals. In tilapia *(Oreochromis niloticus)* a two weeks feeding of *L. rhamnosus* significantly stimulates phagocytosis [86]. Similarly, the oral administrations of *C. butyricum* bacteria in *O. mykiss* have also been reported to increase their phagocytosis activity [94]. In rainbow trout, increased activity was identified relative to the control after four weeks, and furthermore, the highest activity against *Vibrio anguillarum* was observed in rainbow trout two weeks after the start of probiotic feeding mode [102].

4.2. Respiratory burst activity

Respiratory burst activity is important innate defense mechanisms of fish. In fish, probiotics have been reported to improve the respiratory burst of phagocytic cells, which play a central role in the protection of non-specific cell [12,69,82]. The two weeks feeding lactic acid bacteria (LAB 4012) significantly improved the respiratory burst of peripheral blood lymphocytes (PBL) from the fed's fish

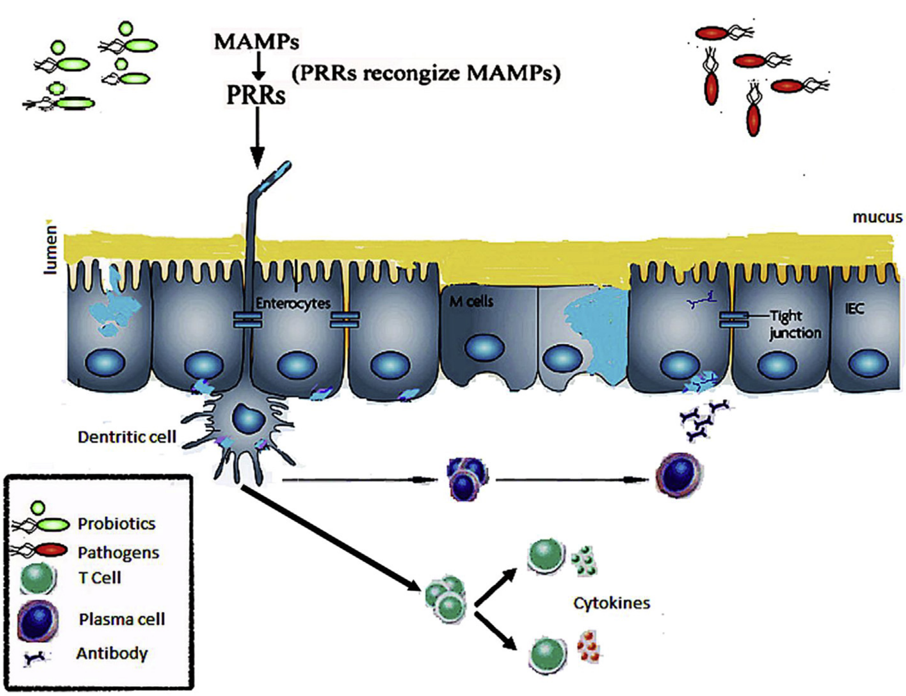

Fig. 1. Probiotics showing the activity of host immunnomodulation. Abbreviations: MAMPs → Microbe associated molecular patters, PRRs → Pathogen pattern recognition receptors, IEC → Intestinal epithelial cell.

and sparked an effective protection of Cobia *(Rachycentron canadum)* against photobacteriosis challenge (RPS = 74.3) [124]. Several invitro and invivo studies showed a significant increase in respiratory burst activity by various probiotics in many aquatic animals and fish. The activity of head kidney leucocytes of gilthead sea bream *(Sparus aurata)* has additionally been found to improve with the feeding 5×10^7 CFU/ml of heat-inactivated *Lactobacillus delbrueckii subsp. Lactis* and *B. subtilies* under invitro conditions [96]. HK, (Head kidney) respiratory burst activity of leukocytes, is important innate immune parameter, which are widely used as an indicator of immune activity, especially when caused by stimulants [101].

4.3. Lysozyme

Lysozyme is a component of the innate immune system and plays an important role in the defense mechanism because of its anti-cancer, anti-viral and opsonization properties [59]. It is one of the most important bactericidal enzymes of intrinsic immunity and is an indispensable tool for the fish to fight infectious agents [68], Probiotics are found either singly or in combination, trigger the lysozyme level in bony fish. It is noted that supplementation of *L. rhamnosus* (10^9 CFU g^{-1}) as feed additives for 30 days improved lysozyme activity in rainbow trout [82]. Stimulation of lysozyme activity in rainbow trout was observed after two weeks feeding with *Kocuria sp.* SM1 [102] and *Carnobacterium* divergens B33 [94]. Lysozyme activity significantly increased in the third and fourth week of the twelve diet group. The lysozyme activity of *Catla sp.*, serum was significantly increased (p˂0.05) after four weeks of feeding with different probiotic supplemented diets compared to that of the control [27]. In addition to serum lysozyme content probiotic can also increase the lysozyme level in the skin mucosa fish [107,116]. Taoka et al [116] reported significantly high lysozyme level in skin mucosa by supplementing commercial probiotics by water compared to oral supplementation in *O. niloticus*. These results suggest that high lysozome activity can afford to ward off bacterial diseases in probiotic-supplemented fish.

4.4. Peroxidase and anti-protease activity

The peroxidase is an important enzyme which uses oxidizing radicals to produce hypochlorous acid to kill pathogens. During the oxidative respiratory burst, peroxidase is usually released from the azurophilic granules of neutrophils. Nutritional Supplements of probiotics such as *Bacillus subtilis*, alone or in combination with *N. delbrueckii spp.* Lactis for three weeks resulted in high serum protease activity, but failed to improve the peroxidase activity of the head kidney leukocytes *S. aurata* [95]. Likewise, probiotics such as *Enteroccus faecium* also increase the serum level of peroxidase in *O. niloticus*, if by the water in every four days for forty days supplemented at 1_107 CFU/ml [123]. Anti-protease activity in serum, which contains α1-antiprotease, the α2-antiprotease and α2 macroglobulin, inhibits the activity of the proteases used by certain bacteria to invade the host. This activity is generally high and is not affected by immunization or infection [35,72]. Sharifuzzaman and Austin [102] reported significantly higher anti-protease activity in *O. mykiss* within two weeks of the completion of probiotic species *Kocuria*.

4.5. Complement activity

The complement system is the part of the immune system that helps or supplements the ability of phagocytes to combat pathogens by antibody opsonization. It is part of the innate immune system which is not adjustable and does not change over the lifetime of an individual [58]. However, it can be adjusted by the adaptive immune system and brought into action. Complement, is the main component of the innate humoral immune response, plays an essential role in the modification of the host immune system of the presence of potential pathogens and their clearance. Complement is initiated by one or a combination of the three ways, namely, the classical, alternative and lectin. All three paths merge into a common amplification with C3 and navigate through the terminal path that leads to the formation of the membrane attack complex, which can directly lyses pathogenic cells [17]. According to [115]; serum C3 levels of probiotic treatments were significantly higher than that of the control after 30 days of feeding on *Epinephelus coioides* grouper. Furthermore, C4, a key component of the classical and lectin pathways, compared with the control after thirty days of feeding increased slightly in the probiotic treatments. The probiotic *Pediococcus acidilactici* as a potent food additive improves the level of the alternative complement activity in the serum of the green terror during a 56-day period [78].

4.6. Cytokines

Cytokines are a broad and loose matter of small proteins that are important in cellular signaling. They are released by cells and affect the behavior of other cells, and sometimes producing cell itself. Cytokines include chemokines, interferons, interleukins, lymphokines, tumor necrosis factor [25,26]. A number of probiotics can effectively modulate the production of pro-inflammatory cytokines, such as interleukin-1 (IL-1), IL-6, IL-12, tumor necrosis factor α (TNF-α) and interferon-gamma (IFN-γ) and anti-inflammatory cytokines, such as IL-10 and transforming growth factor β (TGF β) in many organisms [24,80,122]. Probiotics such as *L rhamnosus, E faecium* and *B. subtilis* are found to up regulate the pro-inflammatory cytokines such as IL-1β1 and TGF β in the spleen and head kidney of the *O. mykiss* [83]. The cytokine gene-reaction was performed in an invitro study with head kidney cells of the Japanese pufferfish (*Takifugu rubripes*) that were exposed significantly to improve heat-killed *Lactobacillus* derived from Mongolian dairy products. Thus, the *Lactobacilli* triggered reactions of the antiviral (I-IFN-1 and IFN-γ), the cell-mediated immune regulators (IL-12p35, IL-12p40 and IL-18), pro-inflammatory (IL - 1βα, IL - 6, IL - 17A/F - 3, TNF - α and TNF - N) and regulatory (IL - 2, IL - 7, IL - 15, IL - 21, IL - 10 and TGF - β 1) cytokines [16].

Interestingly, the data pointed *Lb. subsp. paracasei* having greater potential for immunomodulation by *Lb. plantarum* [16]. With real-time polymerase chain reaction (PCR), it was determined that IL-1b, IL-10 and TNF gene expression was significantly up regulated in the head kidney after feeding with *Lb. plantarum* [85]. In a separate study, pro-inflammatory cytokine TNF-a in the Nile tilapia was up regulated after feeding with *Pediococcus acidilactici* [109]. In addition, IL-12 and IFN- c were induced in olive flounder (*Paralichthys olivaceus*) after feeding with *Lactococcus lactis* [61]. Thus, a large number of microorganisms have been considered for use as probiotics in aquaculture with a mechanism of action involving more immunomodulation, wherein some of sub cellular components act as immunostimulating agents in their own right.

5. Prebiotics

The use of immunostimulants may enhance activities in a non-specific defense mechanism, the increase resistance to infectious disease by enhancing innate humoral and cellular defense mechanisms and indirectly cause increased growth of aquatic animals [7]. Prebiotics are indigestible food ingredients that selectively and beneficially affect the host by stimulating the growth and/or activity of one or a limited number of bacteria in the colon.

Recruitment prebiotics can significantly alter the colonic microflora increasing the number of special bacteria and thus changing the composition of microflora [46]. Prebiotics are carbohydrates which can be classified according to molecular size or degree of polymerization (number of monosaccharide units) such as monosaccharides, polysaccharides or oligosaccharides. According to the International Union of Pure and Applied Chemistry nomenclature, oligosaccharides are defined as saccharides containing between three and ten meioties sugar [75]. Examples of prebiotics included fructo-oligosaccharide, mannanoligosaccharide, inulin or β-glucan. Despite potential benefits for health and performance as noted in various terrestrial animals, the use of prebiotics in the cultivation of fish and shellfish has been less investigated.

The pattern recognition receptors (PRRs), such as beta (β) glucan receptors dentin-1 receptors expressed in macrophages have direct interactions mediated with the immunmodulatory activity of prebiotics [19]. This type of receptor interaction activates signal transduction molecules, such as NF-κB, which stimulate the immune cells [125], saccharides may also interact with PRRs format of microbial associated molecular patterns (MAMPs) as teichoic acid, peptidoglycan, glycosylated protein or the capsular polysaccharide of the bacteria, causing an immune response [18].

6. Immunomodulatory effects

Prebiotics act as a growth factor to specific commensal bacteria, that inhibit the adhesion and invasion of pathogenic microorganisms in the colon epithelium by competing for the same glycoconjugates found on the surface of epithelial cells, altering the pH of the colon, preference the barrier function, improvement the production of mucus, production of short chain fatty acids and induce cytokine production. The effect of prebiotics directly enhances the innate immunity, such as;

Phagocytic effect; is the process which has the endocytic ability of intracellular phagocytic leukocytes and an active host defense mechanism in the spleen, kidney, head kidney (HK) or other lymphoid organs. It can be observed by measuring the level of endocytosed zymosan in phagocytes using microscopy or colorimetric detection. Phagocytosis occurs in a series of steps. Microbes are detected by PRRs such as toll like receptors (TLRs); the microbes are devoured by phagosomes; Phagosomes fuse with lysosomes, which contain a variety of proteases; and the microbes are killed by proteolysis [1]. Furthermore antigen processing steps followed by the presentation of antigen to T cells, leading to T cell activation, and consequently the whole immune system. The Japanese flounder (*P. olivaceus*) was fed 5.0 g FOS kg^{-1} for 56 days were analyzed for lysozyme activity, the percentage of phagocytic cells that assumed a marker (phagocytic percent) and phagocytic index [127]. Fructo-oligsaccharides (FOS) administration significantly improved lysozyme activity, but not the phagocytic percentage or phagocytic index, compared with the control diet group. However, when fed a mixture of FOS and mannanooligosaccharides (MOS), 5.0 g kg^{-1}, the flounder showed a marginal increase in phagocytic activity.

Macrophages; play an important role in linking the innate and adaptive connection of immune system to produce a maximum immune response and kill pathogenic microbes. Macrophages of IFN-γ and their direct interactions between MAMPs are activated on bacteria and PRRs on the host cells. Activated macrophages secrets variety of inflammatory cytokines, such as tumor necrosis factor (TNF), IL-1, IL-12, etc. [1]. The major macrophage activation steps are the cytokines and can be observed by either polymerase chain reaction (PCR) or ELISA. Phosphomonoesterase or acid phosphatase is an enzyme which removes the phosphate group from the phosphorylated molecules. In activated macrophages or dendritic cells, acid phosphatase triggers a drop in the internal pH of phagolysosomes macrophages, and increases its internal acidity. The higher internal acidity leads to activation of protease, whereby resulting microcidal activity [1].

A respiratory burst or oxidative burst is an indication of oxidative potential of reactive oxygen species such as hydrogen peroxide, superoxide anions and hydroxyl radicals. Reactive oxygen species are produced by activated phagocytes and they are responsible for killing or degrading devours materials, including microbes. Reactive oxygen species are widely used to protect the ability of the host against pathogens [1]. Respiratory bursts of innate immune cells, including blood neutrophils, are measured by using NBT (nitroblue tetrazolium) or MPO (myeloperoxidase) tests. Nile tilapia was given a dietary supplement of inulin 5 g kg^{-1} increased hematocrit NBT activity and lysozyme activity, suggesting that inulin has a stimulating effect on the innate immunity. In the group fed inulin for two months there was a statistically significant change in the NBT activity and lysozyme activity. Furthermore, the survival rate after challenge of *A. hydrophilla* increased in inulin fed group [54].

The complement system in serum is one of the most potent non-effector cell responses of the immune system and may be activated by antigen-specific antibodies, a microbial cell surface, or a lectin. Phenoloxidase can be used for measuring the state of the innate immune system of marine invertebrates, such as, Cray fish, sea cucumber, shrimp or lobster [104] [15,52]. The prophenoloxidase system is modified complement system especially for marine invertebrates comprising tyrosinases, catecholases and laccases. Phenoloxidase is critical for enhancing the antimicrobial activity through a respiratory burst and phagocytosis by opsonization. Red Swamp Cray fish proved significantly increased the activity of Phenoloxidase, superoxide dismutase (SOD) and the expression of immune genes (crustin 1, lysozyme, SOD and prop) when fed dietary FOS 8 and 10 g kg^{-1} [31]. Thus, the dietary supplement of fructooligosaccharides for thirty days is given, increasing the survival period against *Aeromonas hydrophila*.

Lysozyme is an enzyme degrading peptidoglycan in bacterial walls by hydrolyzing β- [11,56] glycosidic bonds to N-acetylmuramic acid and N-acetylglucosamine. Lysozyme is found in many areas, such as mucus, serum, intestines and eggs of marine animals [5]. Activated macrophages are the primary producers of lysozyme [48]. The effects of dietary fructo-oligosaccharides, including 10, 20, or 30 g kg^{-1} for Caspian roach fry investigated [106]. Immunoglobulin levels of lysozyme ACH50 were fed increasing the activity significantly in the groups 20 and 30 g FOS kg. Only lysozyme activity was improved in the group fed 10 g FOS kg^{-1}. Nutritional supplements FOS increases the resistance of the roast on salt stress challenges to complete, regardless of the level, but only the group fed 3% FOS had a significantly higher survival rate.

Antibodies are produced by B lymphocytes and recognize specific microbial antigens. The antibodies neutralize pathogens by binding to their surface antigens and preventing their attachment to their target cells. Antibodies may also facilitate the phagocytosis of antibody-bound pathogens through opsonization and activate the complement system and antibody-dependent cellular cytotoxicity (ADCC). The hematocrit or total hemocyte count reflects the total number of cells in the blood, including red blood cells, white blood cells and platelets. The hematocrit count can be used as a macro-analysis of the immunological status of fish, because the number of immune cells in the immune activation increases blood. For the immune cells in the blood are neutrophils, eosinophils, basophils, monocytes and lymphocytes. Carp fry (11.12 ± 0.55 g) were given a diet supplemented with immunogen 0, 0.5, 1, 1.5 and 2.5 g kg^{-1}. Immunogen is a commercial product, which contains mannano-oligosaccharides (MOS) and β-glucan [32]. All the complement level of leukocyte counts increased significantly.

Prebiotics have a beneficial effect on the gut-associated lymphoid tissue (GALT). Inulin, Fructooligosaccahrides, mannanooligosaccahrides, galactooligosaccharides and arabinogalactans are nutritionary therapeutic preparations. They are used for optimal bowel function for preferring the proliferation of normal bacterial flora and prevent the ontogenesis of pathogens. In different species they have been investigated as prebiotics (Table 2), and are used to impede the growth of pathogenic organisms. In fact these Immunosaccharides primarily relieve the function of the phagocytic cells and increased bactericidal activities. Moreover, they also stimulate natural killer cells, complement, lysozyme and antibody response in the host.

Table 2
The following references to the table, showing related research Immunosaccharides, such as; inulin, fructooligosaccharides (FOS), mannanoligosaccharides (MOS), galactooligosaccharides (GOS) and arabinoxyline-oligosaccharides (AXOS), using as immunostimulants in aquaculture.

Prebiotic	Species	Dose, route and duration of administration	Weight	Response	Reference
Inulin	*Beluga [Great sturgeon]*	Dietary 1.0, 2.0, 3.0% inulin 8 weeks	(16.14 ± 0.38 g)	RBC count →, Total WBC count ↑, MCH → Dose dependent alkaline phosphatase ↓.	[2]
	Gilthead seabream	Dietary 1.0% (10 g kg^{-1}) inulin 2 and 4 weeks	(50 g)	Serum complement activity ↑, IgM level ↑, Leucocyte phagocytic activity ↑, Leucocyte respiratory burst activity ↑, Protection from P. damselae challenge ↑.	[20,21]
	Hybrid surubim	Dietary 0.5% inulin 15 days	(73.6 ± 19.5 g)	Lactic acid bacteria ↑, Vibrio spp. ↓, Pseudomonas spp. ↓, Total Ig ↑ (restricted to symbiotic treatment), Serum protein or lysozyme →.	[74]
	Nile tilapia	Dietary 0.5% (5 g kg^{-1}) inulin 1 and 2 months	(11.00 ± 0.2 g)	Hematocrit ↑, NBT (Superoxide activity) ↑, lysozyme activity ↑, Protection from A. hydrophila challenge ↑.	[54]
Fructooligosaccharides	*Atlantic salmon*	Dietary 1.0% (10 g kg^{-1}) 4 months	(200.2 ± 0.6 g)	Whole Blood Neutrophil Oxidative Radical Production →, Serum Lysozyme Activity →.	[49]
	Black amur bream	Dietary 0.3 and 0.6%, 8 weeks	(30.5 ± 0.5 g)	ACP ↑, PO ↑, ACH50 ↑, Ig M ↑.	[128]
	Caspian roach	Dietary 1, 2, 3%, 7 weeks	(0.67 ± 0.03 g)	Serum Ig ↑, Lysozyme activity ↑, ACH50 ↑Resistance to salinity challenge ↑.	[105,107]
	Japanese flounder	Dietary 0.005% (5.0 g kg^{-1}) 56 days	(21 g)	Lysozyme activity ↑, Phagocytic percentage and index →.	[127]
	Red swamp cray fish	Dietary 0.008, 0.01% (8, 10 g kg^{-1}) 30 days	(15–17 g)	Immune related genes (crustin1, lysozyme, SOD, and proPO) ↑, Phagocytic activity ↑, SOD ↑, Survival against A. hydrophila ↑	[31]
	Sea cucumber	Dietary 0.25, 0.5%, 8 weeks	(5.06 ± 0.10 g)	0.5% FOS TCC ↑, Phagocytosis ↑, PO ↑.	[129]
	Sea cucumber	Dietary 0.4, 0.8, 1.6%., 50 days	(3.72 ± 0.16 g)	No significant immunological improvement observed.	[114]
	Stellate sturgeon	Dietary 1, 2%. 11 weeks	(30.16 ± 0.14 g)	1% FOS;; serum lysozyme activity ↑.	[3]
	Yellow croaker	Dietary 0.2, 0.4%, 10 weeks	(7.82 ± 0.68 g)	No statistically significant innate immunity improvement observed.	[87]
Nanoligosaccharides	*Atlantic salmon*	Dietary 1.0% (10 g kg^{-1}) 4 months	(200.2 ± 0.6 g)	Whole Blood Neutrophil Oxidative Radical Production↓, Serum Lysozyme Activity ↓	[49]
	Cray fish; common yabby	Dietary 4%, 56 days	(35.14 ± 0.48 g)	THC ↑, GC ↑, Semi-GC ↑, Hyaline cells ↓, Bacteremia ↓	[97,99]
	European sea bass	Dietary 2, 4% 8 weeks	(116 g)	4% MOS head kidney (HK) macrophage phagocytic activity ↑, 2% MOS HK macrophage phagocytic activity ↓, Eosinophilic granulocytes ↑, levels of V. alginolyticus after exposure ↓	[118]
	Japanese flounder	Dietary 0.005% (5.0 g kg^{-1}) 56 days	(21 g)	Lysozyme activity ↑, Phagocytic percentage and index→	[127]
	Marron	Dietary 0.2, 0.4%/0.05, 0.1, 0.2, 0.4, 0.8%, 30–112 days	(4.44 ± 0.20 g, 10.44 ± 0.20 g, 94 ± 2.17 g)	Survival in V. mimicus challenge ↑, Live transport; GCs ↑, Bacteremia ↓	[97,99, 100]
	Rainbow trout	Dietary 0.2% (2 g kg^{-1}) 42 days	(30 g)	Antibody titer ↑, Lysozyme activity ↑	[110]
	Rainbow trout	Dietary 0.4% 12 weeks	(13.2 g)	Hemolytic activity ↑, Phagocytic activity ↑, Survival in V. anguillarum challenge ↑	[92]
	Rainbow trout	Dietary 0.25, 0.5% 12 weeks	(36.27 ± 0.42 g)	0.5% MOS Hematocrit ↑, Phagocytic activity ↑, Survival in A. salmonicida challenge ↑	[93]
	Sea cucumber	Dietary 0.1, 0.2% 4 weeks	(3.8 ± 0.2 g)	TCC ↑, Phagocytosis of coelomocytes ↑, Superoxide anion production ↑, SOD activity ↑, Survival in V. splendidus challenge ↑	[50]
	Sea cucumber	Dietary 0.002% (2 g kg^{-1}) 4 weeks	(6.80 ± 0.30 g)	TCC ↑, Superoxide anion production ↑ at 15 days	[9]
	Tropical spiny lobster	Dietary 0.4%, 8 weeks	(1.28 ± 0.01 g)	THC ↑, GC count ↑, Bacteremia ↓	[98]
	Common carp	Dietary (Immunogen®) 0.0005, 0.001, 0.0015, 0.0025%, (0.5, 1, 1.5, 2.5 g kg^{-1}8 weeks	(11.12 ± 0.55 g)	Leucocyte counts ↑, Dose dependent survival rate in A. hydrophila challenge ↑	[32]
Galactooligosaccharides	*Atlantic salmon*	Dietary 1.0% (10 g/kg) 4 months	(200.2 ± 0.6 g)	Whole Blood Neutrophil Oxidative Radical Production →, Serum Lysozyme Activity→	[49]
	Red drum	Dietary 1% 8 weeks	(7 g)	Lysozyme level ↑, NBT reduction level →, Microvilli heights in the pyloric caeca, proximal and mid intestine ↑	[130]
Arabinoxylan	*Siberian sturgeon*	Dietary 2% 12 weeks	(25.9 ± 0.9 g)	Phagocytic activity ↑, ACH50 ↑ (AXO-32-0.30 only), Serum peroxidase content ↑ (AXO-32-0.30 only)	[45]
	Siberian sturgeon	Dietary 2% 28 days	(48.4 ± 1.4 g)	Phagocytic activity ↑, Respiratory burst activity ↓	[44]

Abbreviations: ACP-plasma alkaline phosphatase, GC-granular cells, PO-phenoloxidase, SOD-superoxide dismutase, TCC-total coelomocyte count, THC-total hemocyte count, ACH50-alternative complement activity, NBT-nitroblue tetrazolium. ↑-increase, →-no change, ↓-decrease.

7. Conclusion

Fish has become one of the major aquaculture industries. In natural conditions, fish consume a larger variety of food compared to cultured fish. An indicator in this regard may be farmed fish to reduce the intestinal microbial community diversity [29]. Many dietary supplements such as probiotics, prebiotics, vitamins or plant crude extracts can compensate for this problem, resulting in prosperous result in aquaculture [42]. However, most of probiotics and prebiotics can exert immunomodulatory effects in aquatic animals. Therefore, in order to improve the probiotics and prebiotics for aquaculture, further work needs to be carried out in the exact nature of humoral innate or cellular immune response and to determine the protective probiotic life of bacteria. Finally, this paper proposes a long-term perspective of natural dietary supplement, such as probiotics and prebiotics that can be used as strategic control of diseases in aquaculture.

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