Immunostimulants: Boon for Disease Management in Aquaculture

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Abstract

Aquaculture served as protein-rich food for rising world's population and a source of income for the population relying on fishery and fishery-related activities. However, aquaculture industry adopts intensive practices to combat the demand of rising population. And fish disease outbreak is a threat associated with the intensive practices in aquaculture industry, thereby causing production loss and economic inefficiency. Fishes and shellfish primarily depend on innate immune system to maintain its health condition. Immunostimulants are one of the prophylactic measures which are gaining wide interest for its effectiveness in fish disease management. Immunostimulants evolved as superior prophylactic measures as sources are many and inexpensive, primarily target innate immunity, are biodegradable and are effective against a wide range of fish pathogens. In this backdrop, a detailed understanding on types and use of immunostimulants in aquaculture is necessary, and this chapter discusses its different types, mechanisms, the efficiency of immunostimulant in enhancing fish and shellfish immune parameters, how it differs from vaccines, doses and time of application, efficacy and limitations.

Keywords

Immunostimulant · Mechanism · β-Glucan · Herbs · Vitamins · Limitations · Efficacy

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6.1 Introduction

Disease outbreak is one of the threats concerning world aquaculture. The rising demand for fish production due to the increase in world's population has pressurised the aquaculture sector to produce more. This has led the aquaculture sector to adopt high stocking density, feeding at higher rate, use of antibiotics and crowding of aquatic animal which ultimately resulted in stressful environment for culture organisms and disease outbreak. Shrimp industry across the globe is suffering economic loss due to disease outbreak. There is lack of effective remedial control against viral diseases, and specific vaccines for many diseases are ascertained. Fishes largely depend on non-specific defence mechanisms for their health, and thus, prophylactic measures like immunostimulants can be a solution. Immunostimulants are chemicals, drugs, stressors or action that enhances the non-specific or innate immune response by interacting directly with cells of the system activating them. According to Sakai (1999), immunostimulants are categorised based on their sources: algae-derived, bacterial, animal-derived, nutritional factors and hormones/ cytokines. Studies on the use of immunostimulants in aquaculture are under progress, and many are tested and confirmed for its efficiency in aquaculture industry.

6.2 Differences Between Vaccine and Immunostimulants

In the history, immunostimulants were considered as substances that can enhance innate/non-specific immunity (Tafalla et al. 2013), but they can induce both specific/ adaptive and non-specific/innate immunities (Ganguly et al. 2010). Immunostimulants basically increase innate immune response, but their administration along with vaccine enhances adaptive immune response which acts against the antigen. Immunostimulants are referred to as adjuvants if used along with vaccine. Immunostimulants such as β-glucans and TLR ligands can help to induce trained immunity in broodstock fish and their offspring; thus, they can serve as alternative to conventional vaccination (Zhang et al. 2019). Vaccines are nonpathogenic preparations of the pathogen that induces specific immune response in the host, enabling it to recognise and destroy the pathogen when it encounters it later (Thompson 2017). Vaccine acts on specific immune response and provides long-lasting protection in humans and animal. Vaccination imparts protection against one or two specific pathogens while immunostimulants can act against a wide range of pathogens. Generally, immunostimulants work on complement activation, phagocytosis efficiency and cytokine secretion. Immunostimulants like zymosan, glucans and lipopolysaccharides are considered as true immunostimulants as they act on non-specific immune system and do not require a specific immune mechanism (Barman et al. 2013). Though vaccines and immunostimulants act differently, their use in combination provides a better protection to the immune system of fish by facilitating the use of lower dose of vaccine. Immunostimulants increase the potential of vaccine (Wang et al. 2017). A brief account on difference between vaccine and immunostimulants is given in Table 6.1.

Table 6.1 Difference between vaccine and immunostimulants

Vaccine	Immunostimulant
Acts largely on specific immune response	Acts largely on non-specific immune response
One or two doses is required	Requires frequent doses
Provides long-term protection	Provides short-term protection in general
It can act specifically to one or two pathogens	It can act on a wide range of pathogens
Not suitable for smaller-size fish and shrimps	Suitable for smaller fishes and shrimps, for example, larvae
Costly and trained persons are required	Cost-effective process

6.3 Fish Immune System and Mechanism of Action of Immunostimulant in Fish Immune System

Fish immune system comprised of both innate and acquired immune responses. Both are associated\interdependent with humoral and cellular response mechanisms. Innate immune response is the first line of defence facing the pathogens entering a fish body. Fish possess physical barriers like scales, skin, gill's epithelial cell and mucus while exposed to aquatic environment. Pathogens may get prevented from entering these barriers; if crossed these barriers, the pathogen must encounter cellular and humoral component of innate immune response. Cellular component comprised of mononuclear (monocytes-macrophages and dendritic cells) and polymorphonuclear phagocytes (neutrophilic granulocytes). These cells facilitate phagocytosis of the pathogen by recognising the pathogen through PAMPs (pathogen-associated molecular patterns) with the help of PRRs (pathogen recognition receptors). PRRs in fishes are TLRs (Toll-like receptors), RIG-1-like receptors, C-type lectin receptors and NOD-like receptors. The binding of PRRs to PAMPs activates many cell signalling pathways in fish and induces production of nuclear factor-κβ, inflammatory cytokines, IFNs (interferons) and chemokines. After phagocytosis, the killed and digested pathogen is presented by macrophages and dendritic cells to lymphocytes. Non-specific cell-mediated cytotoxicity to kill xenogenic and allogeneic cells in fish is performed by non-specific cytotoxic cells (NCCs) and natural killer (NK)-like cells. Humoral responses in innate response of fish immune system are exhibited by different types of proteins and glycoproteins, namely, antibacterial peptides, lysozymes, cathepsins, chitinase, complement, chemokines, serum protease inhibitors (α2 macroglobulin, α1 antitrypsin) and acute phase proteins. Cytokines are secreted proteins, namely, chemokines, IFNs, ILs (interleukins), lymphokines and TNF, which are involved in innate and adaptive immune response of fish (Thompson 2017).

Adaptive immunity works based on specific response towards a specific pathogen. It memorised the pathogens and could eliminate them quickly upon reencountering. Adaptive immune response comprised of cellular response by

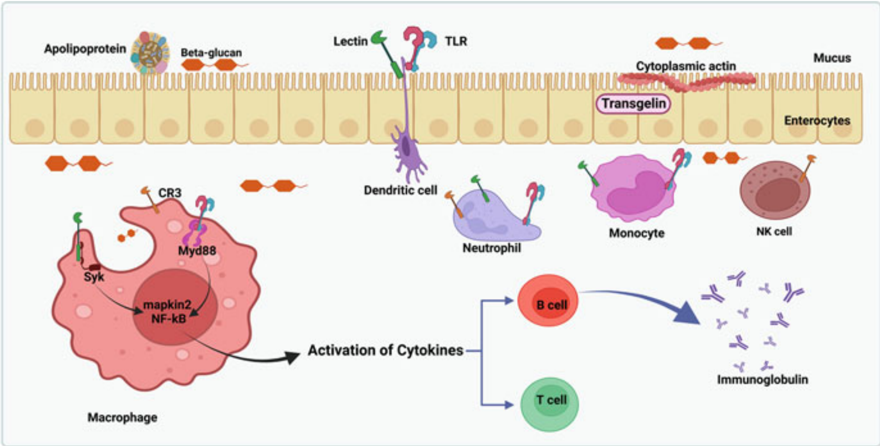

Fig. 6.1 Mechanism of action by β-glucan in fish immune system. (Adopted and modified from Machuca et al. 2022)

T- and B-lymphocytes and humoral response by immunoglobulins. The function of components in adaptive immune response is facilitated by major histocompatibility complex (MHC-I and MHC-II), B-cell receptors, T-cell receptors and recombination activator genes (*RAG1* and *RAG2*). MHC is a set of cell surface molecules to identify a foreign molecule. It facilitates the binding of appropriate T-cells on the peptide fragments of pathogen. The major function of B-lymphocytes is production of antibodies. Signals from T-helper cell induce B-cells to produce antibodies. Previously, IgM was the only reported antibody from fish; later IgD and IgT were also reported to be produced by teleosts. The function of antibody is opsonisation of pathogen and activates complement pathway and agglutination of pathogen (Thompson 2017).

Immunostimulants stimulate the activities of NK cells, T-cells, B-cells, phagocytic cells, macrophage, lysozyme, complement and inflammatory agents in fishes. They can elevate the activity of phenol oxidase, phagocyte, SOD (superoxide dismutase), respiratory burst and increase in total haemocyte count in shrimps. Some immunostimulants were found to activate Toll-like TLRs and their various functions including identification of several endogenous microorganisms and ligands and activation of ideal immune response for each antigen (Takeda et al. 2003; Heine and Lien 2003). β-Glucans can enhance the phagocytic activity of antigen-presenting cells (APCs) which is associated with the recognition of PAMP in β-glucans by PRRs in APCs. This may activate the downstream signalling cascade such as production of RNI (reactive nitrogen species), ROS (reactive oxygen species) and antibody (Hodgkinson et al. 2015). Figure 6.1 depicts how dietary β-glucan acts on fish body. Through diet, β-glucan reaches the intestine of fish. Apolipoprotein, transgelin and cytoplasmic actin 1 are some of the proteins present in enterocyte of the intestinal wall. TLR (Toll-like receptor) and lectins are present on immune cells. These proteins and receptors help β-glucan assimilation into the systemic circulation. Lectin recognises β-glucan as a foreign particle and together with spleen tyrosine kinase (Syk) activates mapkin2 (mitogen-activated protein kinase) and NF-kβ. NF-kβ is also activated by TLR and Myd88 (myeloid differentiation primary response adapter protein 88). The activation of NF-kβ led to activation of cytokines. Cytokines help in proliferation of T- and B-cells and secretion of immunoglobulin by B-cell. Thus, the fish is in a state of enhanced immune condition (Machuca et al. 2022).

Shellfish lack specific immune system, and the immune defence mechanism is rendered by non-specific cellular and humoral components. Cellular component comprised of haemocytes (hyaline, granular and semi-granular cells). Activities of haemocytes comprised of recognition, melanisation, phagocytosis, cytotoxicity and communication between cells. Humoral component comprised of lectins (agglutinins: proteins or glycoproteins), prophenoloxidase (ProPo) system, antimicrobial compounds, serine proteinase inhibitors and clotting-by-clotting proteins. The presence of β-1,3-glucans, lipopolysaccharides and peptidoglycans can activate the ProPO system in shellfish. Immunostimulation can enhance immune parameters like haemocyte count, respiratory burst, PO (phenoloxidase) activity, phagocytic activity, agglutination titre, lysozyme and SOD (superoxide dismutase) activities.

Immunostimulants are recognised as foreign body while they enter shrimp haemocoel. Following this, haemocyte has pattern recognition protein (PRP) which recognises PAMP in the immunostimulant and activates cascade of cellular signals including ProPO system, phagocytosis, respiratory burst and melanisation (Fig. 6.2). This altogether leads to a state of enhanced immune condition in shrimps. In this enhanced immune state, if a pathogen tries to enter the haemocoel, there will be recognition of PAMP (of pathogen) and PRP (of haemocyte) leading to phagocytosis, encapsulation, melanisation, nodule formation and finally death of pathogen.

6.4 Fish Mucosa as First Entry Point of Immunostimulants in Fish

Mucosa is the first line of defence in fish body towards the entry of a pathogen. It is also the first layer of fish body which interacts while subjecting to immunostimulant diet or immersion treatment. External stimuli will be recognised by the mucosal tissue; thus, initiating alterations leading to production of cytokines, peptides and hormones altogether starts an immune response. Immune response in mucosa is controlled by mucosa-associated lymphoid tissue (MALT). There are four types of MALT in teleost, that is, skin-associated lymphoid tissue (SALT), situated at the skin; gill-associated lymphoid tissue (GIALT), situated at the gill; gut-associated lymphoid tissue (GALT), situated at the gut; and nose-associated lymphoid tissue (NALT), situated at the nose. GALT possessed cells (granulocytes, macrophages, lymphocytes, plasma, T-cell, B-cell, intraperitoneal lymphocytes, goblet cells, epithelial cells and neuroendocrine cells) to regulate immune response (Vallejos-Vidal et al. 2016).

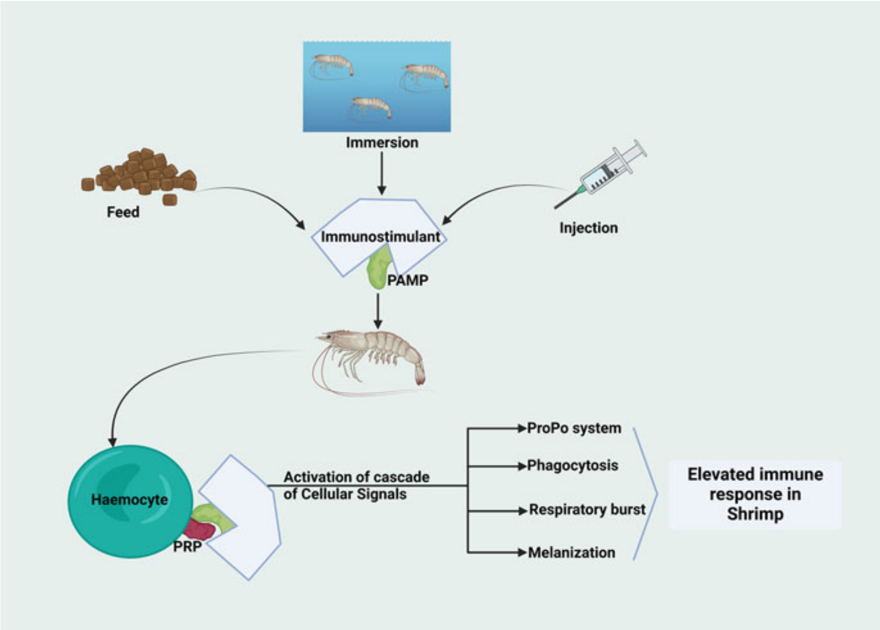

Fig. 6.2 Mechanism of action by immunostimulant in shellfish immune system. (Adopted and modified from Kumar et al. 2022)

6.5 Types of Immunostimulants Used in Aquaculture

6.5.1 Synthetic Chemicals

6.5.1.1 Levamisole

Levamisole possessed antihelminthic property. Levamisole acts to enhance phagocytic and NBT (nitroblue tetrazolium) reaction and increase antibody-producing cells. It also induces immune response in rainbow trout (Kajita et al. 1992) and *Pseudoplatystoma reticulatum* (Zanon et al. 2014). It stimulates non-specific defence mechanism in *Clarias fuscus* and provides protection against *Acinetobacter lwoffii* (Li et al. 2006). Bath treatment of rainbow trout in levamisole helps the fish in development resistant to *Yersinia ruckeri* (Ispir 2009). It enhances macrophage activity in barbel chub (*Squaliobarbus curriculus*) (Li et al. 2011). Dietary levamisole enhances immunity against infection of *Aeromonas hydrophila* and growth and survival in *Cirrhinus mrigala* (Bhatnagar and Lamba 2016).

6.5.1.2 FK-656

FK-656 is heptanoyl-y-glutamyl-(L) meso-diaminopimely-(D)-alanine. It is a peptide which is effective against bacterial infection, like *Aeromonas salmonicida*. It enhances the resistance of rainbow trout towards *A. salmonicida* and elevates humoral antibody titres and splenic-producing antibody in yellow tail (Kitao et al. 1987).

6.5.1.3 MDP

MDP (muramyl dipeptide) is a *Mycobacterium* derivative and its chemical name is *N*-acetylmuramyl-L-alanyl-D-isoglutamine, derived from *Mycobacterium*. It increases resistance in rainbow trout against *A. salmonicida*. MDP-Lys increases the activities like phagocytic, respiratory burst and migration of kidney leucocytes (Kodama et al. 1993).

6.5.2 Bacteria and Derivatives of Bacteria

6.5.2.1 Bacteria

Vibrio anguillarum bacterin is an efficient vaccine for the fishes in salmonid group. It is an inactivated whole cell vaccine which can be administered orally, bathing or injection (Sakai 1999). *V. anguillarum* bacterin can stimulate immune responses in fish and shellfish. Haemocyte activity was found to enhance in black tiger shrimp (*Penaeus monodon*) while treated with *Vibrio* bacterin (Horne et al. 1995). It stimulates disease resistance in rainbow trout against *A. salmonicida* (Norqvist et al. 1989). *Clostridium butyricum* bacterin activates leucocyte, phagocytosis and superoxide anion production in rainbow trout, thus enhancing its resistance towards vibriosis (Sakai et al. 1995). *Achromobacter stenohalis* cells are reported to use as immunostimulants in fishes. It stimulates immune responses of kidney cells and complement activity. Inactivated form of *A. stenohalis* was reported to activate immune responses while imparting resistance against *A. salmonicida*. Dietary *V. anguillarum* cells (formalin killed) can enhance immune response, growth and protection against *Vibrio harveyi* in banana shrimp (Patil et al. 2014). Inactivated cells of *V. harveyi* biofilm cells can enhance growth, survival, immune response and health of *Penaeus vannamei* (Nagaraju et al. 2019).

6.5.2.2 Lipopolysaccharides

LPS (lipopolysaccharide) is present in cell wall of Gram-negative bacteria. They are effective at very low doses, often present in bacterin preparations as contaminants and used in immunising programmes. It can induce immune responses like complement alternative pathway, phagocytic activity and superoxide anion production of macrophage in fishes. It also enhances production of macrophage-activating factor and B- and T-cell proliferation in fishes. LPS can stimulate immune responses like haemocyte activity and phagocytic activity. Immunostimulatory effects of LPS in Atlantic salmon, red sea bream (*Pagrus major*) and goldfish were discussed in the review by Barman et al. (2013). Dietary LPS (derived from cell wall of *Pantoea agglomerans*) was proven to enhance growth and non-specific immune response in fry of *Oncorhynchus mykiss* (Skalli et al. 2013). It enhances innate immune response in rainbow trout and is effective in preventing *A. hydrophila* disease (Nya and Austin 2010).

6.5.2.3 Freund's Complete Adjuvant

FCA (Freund's complete adjuvant) contains killed *Mycobacterium butyricum*. It increases activities of fish immune system. In rainbow trout, it increases activities of phagocytosis, NK cell and respiratory burst and thus enhances defence against *V. anguillarum* infection (Kajita et al. 1992). FCA was found to provide adjuvant effect on *Pasteurella piscicida* vaccine (Kawakami et al. 1998).

6.5.3 Polysaccharides

6.5.3.1 Glucans

Glucans are long chain polysaccharides extracted from yeast. They are most promising and most studied immunostimulants in aquatic species. Immunostimulatory effects of β-glucans comprised enhancement of lysozyme activity, complement activity, phagocytic and bactericidal activity of macrophages and antibody responses (Thompson 2017). Glucans used as immunostimulants in fish comprised of yeast glucan (β-1-3- and β1-6-linked glucan) (derived from *Saccharomyces cerevisiae*) and β-1,3glucan (VST) (derived from *Schizophyllum commune*) (Pais et al. 2008). β-Glucans are most commonly used immunostimulants, and sources investigated include plants, yeast, seaweed, mushrooms shiitake, maitake, reishi, fungus and bacteria (Sirimanapong et al. 2015). β-Glucan (derived from cell wall of *S. cerevisiae*) can increase resistance in Atlantic salmon against *Vibrio salmonicida*, *V. anguillarum* and *Y. ruckeri* (Robertsen et al. 1990). Dietary administration of β-glucan increases resistance to infections in fishes. Injection of 100–1000 μg glucans/fish increases resistance against *A. hydrophila* (Selvaraj et al. 2005). Glucan-induced resistance was reported to transmit maternally (Misra et al. 2004). Dietary β-1,3-glucan was found to modulate immune response (haemocyte count, superoxide anion production and phenoloxidase activity) in *P. monodon* (Chang et al. 2000). Some of the recent studies revealed the immunostimulatory impacts of β-glucans, for example, improved growth, intestinal morphology, stress resistance and immunity on fish-crowding stress were observed in β-glucan-fed *Oreochromis niloticus* (Dawood et al. 2020) and improved thermal tolerance, immune response and disease resistance against *A. salmonicida* in golden mahseer (*Tor putitora*) (Akhtar et al. 2021), etc.

6.5.3.2 Peptidoglycan

Peptidoglycan is a polymer of β-(1-4)-linked *N*-acetylglucosamine and *N*-acetylmuramic acid, derived from cell wall of bacteria. The activity of peptidoglycans increases after hydrolysis. Peptidoglycans promote growth and enhance resistance towards pathogens; thus overall immunity of aquatic animals can be improved (Wang et al. 2017). Peptidoglycan-enriched diet was able to upregulate the antimicrobial peptides (AMPs) in the skin, gills, gut and liver of rainbow trout (*O. mykiss*) (Casadei et al. 2013). In another study, it was proven that its oral administration can enhance innate immune response in rainbow trout (Casadei et al. 2015).

6.5.3.3 Chitin and Chitosan

Chitin is a polysaccharide present in exoskeletons of crustacean and insect and fungi and yeast cell wall (Sakai 1999). They are mostly administered through dietary supplementation and can be used as feed additives according to the studies conducted. Injection or dietary administration of chitin may have immunostimulatory effect on several fishes. Dietary chitin and chitosan enhance parameters like lysozyme and superoxide anion production in *Cyprinus carpio* (Gopalakannan and Arul 2006). Chitin-supplemented diet can enhance myeloperoxidase and alkaline phosphatase activity in *Labeo rohita* (Kumar et al. 2019) and ProPo and RBA of haemocytes in *Macrobrachium rosenbergii* (Kumar et al. 2015). Chitin injection has been also proven to enhance immune response, for example, enhanced RBA (respiratory burst activity), phagocytic activity and natural cytotoxic activity in *Sparus aurata* (Esteban et al. 2000) and enhanced haemocyte count, RBA, PO and phagocytic activity in *Litopenaeus vannamei* (Wang and Chen 2005). Chitosan injection or feeding can enhance immune parameters in fish and shellfish. Dietary chitosan can enhance superoxide anion production, phagocytic activity, lysozyme and alternative complement in *O. niloticus* (Abu-Elala et al. 2015).

6.5.4 Plants and Animal Extracts

6.5.4.1 Ete (Tunicate) and Hde (Abalone)

Ete is an extract from the marine tunicate, *Ecteinascidia turbinata* (Ete), while Hde is a glucoprotein fraction of water extract (Hde) from abalone, *Haliotis discus hannai*. Injection of Ete (tunicate) improves the phagocytosis in eel fish and thus increased its survival while challenged with *A. hydrophila* (Davis and Hayasaka 1984). Also, injection of Hde enhances phagocytic activity in rainbow trout and thereby increases its resistance against *V. anguillarum* infection (Sakai et al. 1991).

6.5.4.2 Firefly Squid

Extract from firefly squid, *Watasenia scintillans*, was reported to enhance immune defence mechanism in rainbow trout. It enhances production of superoxide anion, phagocytic activity of macrophages and the lymphoblastic transformation of lymphocytes in vitro.

6.5.4.3 Herbs and Medicinal Plants

The use of several Chinese herbs as immunostimulant has growing interest in aquaculture recently. Chinese herbs can enhance non-specific immune response such as bacteriolytic and leucocyte activities. The use of Chinese herbs has advantageous over other immunostimulants as they are natural and contain many active compounds that are tested through long screening process in humans and other animals which ensure their safety towards development of drug resistance or residue (Dugenci et al. 2003; Khanna et al. 2007). Chinese herbs (*Rheum officinale* and *Polygonum cuspidatum*) contain chrysophanic acid or chrysophanol (1,8-dihydroxy-

3-methyl-anthraquinone), and they exhibit antibacterial and anti-fungal properties. In catla, dietary chrysophanic acid provides better immunity and enhances the upregulation of immune-related genes against *A. hydrophila* (Harikrishnan et al. 2021). Chinese herbs are effective against various fish pathogens and can be supplemented through fish diet. The use of extracts from *Astragalus membranaceus* and *Lonicera japonica* is also reported to be used as immunostimulant in cultured fish species (Ardo et al. 2008). *Cynodon dactylon*, *Emblica officinalis* and *Adhatoda vasica* were known to improve the immune system in *Carassius auratus* and reduced microbial infection (Minomol 2005). Effectiveness of these herbal stimulants was studied in *Poecilia sphenops* (Ardo et al. 2008). Herbal treatment enhances phagocytosis, RBA (respiratory burst activity), plasma lysozyme activity, etc. Astragalus extract was found to enhance phagocytic activity in Nile tilapia (Yin et al. 2006) and soft-shelled turtles (*Pelodiscus sinensis*) (Yin et al. 2009). Ginger extracts, *Azadirachta indica* (neem), *R. officinale*, *A. paniculata*, *I. indigotica*, *L. japonica*, *Lonicera* extract, *A. membranaceus* (roots and stems), *Isatis tinctoria*, *Polygonum multiflorum*, *Glycyrrhiza glabra*, *Angelica membranaceus*, *A. sinensis*, green tea, cinnamon and American ginseng were some of the herbs examined in many fishes (rainbow trout, crucian carp, Nile tilapia, common carp, rainbow trout, large yellow croaker, *Catla catla*, black rock fish, *Oreochromis mossambicus*) for their immunostimulatory effects. Some of the studies reported that herbal extracts provide resistance in fishes against bacterial infection, namely, *A. hydrophila* and *Citrobacter freundii*.

The use of plant extracts is beneficial as they could lower treatment cost and be environmentally friendly, biodegradable and less likely to induce drug resistance in pathogens and typically possessed multiple mechanisms of actions. Plant extracts can induce stress reduction, appetite, growth, immunostimulation, anti-pathogen and maturation in culture species. They contain several active compounds like tannins, alkaloids, terpenoids, saponins, glycosides, flavonoids, phenolics, essential oils, etc. Herbal extracts or plant extracts used in combination of different sources or single sources were studied for their immunostimulatory effects. The extracts from plants like *Gracilaria* and *Asparagopsis* can be used in place of antibiotics to enhance immunity in shrimps. There is a growing interest over previous decades for the use of plant extracts as shrimp immunostimulants. Herbal or plant extracts should be applied in advance of the onset of diseases, and this will ensure a prior protection and prevent loss (Ghosh et al. 2021). The use of *Syzygium cumini* (leaf) powder; *Psidium guajava* leaf powder; *Forsythia suspensa* methanolic extract; *Solanum nigrum* root ethanolic extract; *Gracilaria tenuistipitata*, *Gracilaria fisheri*, *Gracilaria vermiculophylla* and *Gracilaria verrucosa* extracts; and *Sargassum hemiphyllum*, *Sargassum duplicatum*, *Sargassum fusiforme* and *Moringa oleifera* (ethanolic) extracts as immunostimulants in shrimps was studied (Ghosh et al. 2021). A combination of extracts from nine different plants (*Annona squamosa*, *Aloe vera*, *Citrus aurantifolia*, *Azadirachta indica*, *Andrographis paniculata*, *Ocimum sanctum*, *Allium cepa*, *Coriandrum sativum* and *Psidium guajava*) was found to be effective in stimulating immune response of *P. monodon* against *V. harveyi* (AftabUddin et al. 2017). Mixing of different parts of five herbs (*Hygrophila* *spinosa*, *Acalypha indica*, *Picrorhiza kurroa*, *Zingiber officinale* and *Tinospora cordifolia*) was found to enhance immune responses in *Fenneropenaeus indicus* and protects against *V. harveyi* (Rajeswari et al. 2012).

6.5.4.4 Glycyrrhizin

It is a glycosylated saponin with one molecule of glycyrrhetinic acid (animal product). It can enhance immune responses like lysozyme activity, phagocytic activity, RBA and lymphocyte count in fishes. Glycyrrhizin was tested in fishes like yellowtail and rainbow trout (Mehana et al. 2015).

6.5.5 Vitamins, Precursors of Vitamin and Trace Element

6.5.5.1 Vitamins

In fish, Vitamin C (ascorbic acid) is involved in many physiological functions including reproduction, growth and development, wound healing, response to stressors and lipid metabolism. Vitamin C at higher does was found to improve immune responses (macrophage activity, cell proliferation, NK cell activity, complement and lysozyme activity) in fish (Verlhac et al. 1996). It also imparts resistance against bacterial infections (*Edwardsiella ictaluri*, *E. tarda*, *V. anguillarum*, *A. hydrophila*, *A. salmonicida*) and parasitic infection (*Ichthyophthirius multifiliis*) as discussed in the review by Barman et al. (2013). It was observed that higher dietary inclusion of vitamin C in feed of immunocompromised *L. rohita* can neutralise immunosuppression due to aflatoxin B1-contaminated feed (Sahoo and Mukherjee 2003). Insufficient dietary level of vitamin C in juvenile shrimp leads to reduction of growth rate, reduction of feed conversion ratio and reduction of wound healing capacity and more prone to stress. It causes black dead syndrome in juvenile shrimp (Lightner et al. 1979). Vitamin C also imparts disease resistance in *P. monodon* postlarvae and juveniles against baculovirus, *V. harveyi*, and saline shock (Merchie et al. 1998). Dietary vitamin C was able to enhance non-specific immune response in Japanese eel (*Anguilla japonica*) (Shahkar et al. 2015).

Vitamin E can enhance immune response against infection in fishes, for example, Japanese flounder (*Paralichthys olivaceus*) (Villegas et al. 2006). Further, the deficiency of vitamin E was found to reduce immunity in trout against *Y. ruckeri* (Blazer and Wolke 1984). A combination of vitamin C and vitamin E was reported to have beneficial response in fishes (Wahli et al. 1998). Vitamin E supplementation in diet can enhance SOD, weight gain and total haemocyte count in *P. monodon* (Lee and Shiau 2004).

6.5.5.2 Precursors of Vitamin

Precursor of vitamin A like carotenoids was also reported to be used as immunostimulants. They play an important role in both cellular and humoral defence mechanisms in fish immune system. It can enhance phagocytosis, non-specific cytotoxicity, lysozyme activity and complement activity in fishes (Amar et al. 2004; Yanar et al. 2007). The dietary supplementation of carotenoid leads to increase phagocytic, complement and lysozyme activities in several shrimps and fishes (Wang et al. 2017), namely, β-synthetic carotenoids and astaxanthin which can enhance non-specific immune defence mechanism in rainbow trout (Amar et al. 2004); carotenoid modulates immune defence in common carp and protects against *A. hydrophila* infection (Anbazahan et al. 2014).

6.5.5.3 Trace Element

Trace elements are also reported to enhance immune responses in fish and shrimps. Trace elements such as cobalt, chromium, copper, iodine, iron, molybdenum, manganese, selenium and zinc are important components of hormones and enzymes and play an important role in biochemical processes and physiological functions (Dawood et al. 2018). Supplementation with trace elements in diet was reported to give beneficial effects, namely, dietary Zn improves growth, survival and immune parameters of *L. vannamei* (Lin et al. 2013); dietary inorganic copper resulted in enhancement of growth and immune responses of juvenile beluga (*Huso huso*) (Mohseni et al. 2014); dietary selenium enhances growth, immune response and antioxidative response in *Carassius auratus gibelio* (Zhu et al. 2017).

6.5.6 Hormones

Growth hormone (GH) can stimulate immune system by activating immune competent cells like lymphocytes, macrophages and NK cells. The treatment of exogenous growth hormone (GH) in fish can enhance production of superoxide anions of leucocytes. Prolactin is a growth hormone which can enhance the immune-related activities, for example, production of superoxide anions of leucocytes in rainbow trout (Sakai et al. 1996). Lactoferrin is another growth hormone which can act as immunostimulants (Sakai 1999). It is a glycoprotein and proven to enhance non-specific immune responses of gilthead seabream (*S. aurata)* during dietary administration (Esteban et al. 2005). Cytokines can be used as immunostimulants as they act as modulators in the immune system.

6.5.7 Microalgae

Microalgae like *Arthrospira* (spirulina) *platensis*, *Haematococcus pluvialis* and *Chlorella* spp. are commonly used as nutritional supplements for fish and shellfish. They are rich in bioactive substances, proteins, vitamins, polysaccharides, etc. and emerging as good immunostimulants for fishes. Oral vaccines are developed based on microalgae like *Chlamydomonas reinhardtii* and *Dunaliella salina* and cyanobacteria. There are very few researches which evaluate microalgae as immunostimulant (Ma et al. 2020). Dietary supplementations of microalgae in fish and shellfish were studied in recent past, for example, *A. platensis* in diet leads to improve immune response and resistance against *Pseudomonas fluorescens* infection in *O. niloticus* (Mahmoud et al. 2018); *Chlorella vulgaris* can enhance ProPO activity, haemocyte count and survival of *Macrobrachium rosenbergii* postlarvae to *A. hydrophila* infection (Maliwat et al. 2017).

6.5.8 Others

6.5.8.1 Nucleotides and Metabolites

Nucleotides and metabolites are potential immunomodulators. Oral administration was shown to give beneficial impact on immune functions, vaccine efficiency or disease resistance in fishes like rainbow trout, coho salmon, Atlantic salmon, common carp, hybrid striped bass and hybrid tilapia (Barman et al. 2013). Dietary nucleotides can enhance immune response in salmonids and protect against *Piscirickettsia salmonis*, *Vibrio anguillarum*, *Piscirickettsia salmonis*, infectious salmon anaemia virus and sea lice (Burrells et al. 2001). Dietary yeast ribonucleic acid can enhance phagocyte respiratory burst, thus providing protection to juveniles of *L. rohita* against *A. hydrophila* (Choudhury et al. 2005). Growth and health performance of red sea bream (*Pagrus major*) was improved while fed with inosine and low concentration of nucleoside by-products (NBPs) (Hossain et al. 2016).

6.5.8.2 Whole Microorganism

Whole microorganisms like yeasts (*Saccharomyces cerevisiae* or *Candida utilis*) and fungus (*Mucor circinelloides*) were reported to be used as immunostimulants. Their use in the form of oral administration or injection was reported to enhance both humoral and cellular immune responses in fishes. Its use imparts protection in fishes (channel catfish, rainbow trout and gilthead sea bream) against pathogenic bacteria (Ortuno et al. 2002; Rodriguez et al. 2004).

6.5.8.3 Lentinan, Schizophyllan and Oligosaccharide

They can enhance immune response mechanisms in fish encompassing phagocyte activity, lysozyme activity and complement activity.

6.5.8.4 EF203

EF203 is product derived from fermentation of chicken eggs. It enhances activity of leucocytes, phagocytosis and chemiluminescence in rainbow trout while administered orally and protects against infection of *Streptococcus* (Yoshida et al. 1993).

6.6 Factors Affecting the Efficiency of Immunostimulants

6.6.1 Immunostimulant Administration: Timing and Dosage

Immunostimulants are administered independently or in combination with a vaccine as adjuvant. It can be administered prior to occurrence of a disease to ensure protection against a predicted seasonal disease. As an adjuvant in vaccination, immunostimulants can be administered prior to vaccination. Dosage and timing of each immunostimulant need to be determined properly. For example, alginic acid and glucan-treated sea bass showed significant increase in complement activity post 15 days of treatment, significant elevations in serum lysozyme and heat shock protein (HSP) concentration post 30 days and no difference among treated and control in terms of non-specific or specific immune parameters, growth, survival and conversion indexes post 45 days (Bagni et al. 2005; Li et al. 2010). Dosage of immunostimulants needs to be regulated properly in order to ensure its efficacy and potency. Efficacy differs according to dosage, that is, vitamin E when orally administered at different dosages showed different patterns of immunostimulation in gilthead seabream (Ortuno et al. 2000); levamisole at low-level (<500 mg kg^{-1}) supplementation in diet enhances weight gain in fishes after three weeks while higher-level (1000 mg kg^{-1}) supplementation leads to chronic toxicity like reduction in growth and feed intake (Alvarez-Pellitero et al. 2006).

6.6.2 Mode of Action

Immunostimulants can be administered orally, injection or immersion (bathing) in fishes. For example, oral administration of immunostimulants like glucans, EF203, lactoferrin, levamisole and chitosan was described. Oral administration is suitable for mass administration irrespective of fish sizes and is non-stressful. Injection of immunostimulants can be costly, time consuming, labour intensive and applicable only for large size of fish (>10–15 g in body weight). Intraperitoneal injection of chitin in fish was found to be effective to enhance humoral and cellular response while intravenous injection is ineffective (Maqsood et al. 2011). Immersion produces less innate immune response comparatively. It is cost effective in comparison to injection method and effective for intensive system, acclimatisation of juveniles; however, it can give handling stress to fish. Bathing fish in levamisole solution was reported, namely, carp immersed in levamisole solution showed activated phagocytosis and chemotaxis and enhanced protection against *A. hydrophila* (Baba et al. 1993). Immersion treatment of rainbow trout in glucan or chitosan solutions can impart protection against *A. salmonicida* (Anderson et al. 1995).

6.6.3 Particle Size

Immunostimulatory effects of chitin and chitosan were reported to be affected by particle size, that is, in vitro study showed that leucocytes of *S. aurata* can phagocytose chitin particles of size less than 10 μm but unable to do so with particle size more than 10 μm (Cuesta et al. 2003).

6.6.4 Species Being Tested

The efficacy varies according to the species tested, for example, chitin-supplemented diet at 25, 50 or 100 mg/kg body weight was able to enhance immune response in *S. aurata* while chitin supplementation at 25 and 50 mg/kg was unable to bring any alteration in *L. rohita* (Choudhury et al. 2005).

6.7 Efficacy of Immunostimulants

- Immunostimulants are widely used for fish health management.
- It provides protection to fish and shellfish prior to exposure of a pathogen and improves the survival post infection of a particular pathogen.
- Immunostimulant activity is broad-spectrum, that is, it protects fishes from several infectious diseases and provides protection against infectious bacteria (*V. anguillarum*, *V. salmonicida*, *A. salmonicida*, etc.), virus (IHN, infectious haematopoietic necrosis; YHV, yellow head virus) and parasite (white spot disease and sea lice).
- It stimulates activities of phagocytic cell, NK cells, complement system, lysozyme activity and antibody response in fish and shellfish and thus imparts protection against infectious diseases.
- It can stimulate both specific and non-specific immune system in fishes.
- Some immunostimulants are often used to increase efficacy of vaccine.

6.8 Limitations of Immunostimulants

- The use of immunostimulants is costly in few cases.
- They are not effective against all diseases, namely, some bacteria like *Renibacterium salmoninarum*, *Pseudomonas piscicida* or *Edwardsiella ictaluri* can resist phagocytosis by host cells.
- Overdose of immunostimulants can induce immunosuppression.
- Some immunostimulants failed to render protection against diseases.
- Immunostimulants are effective for short duration on non-specific immune mechanism; therefore repeated administrations are required.
- An immunostimulant may not be effective for all life stages of an aquaculture species; rather it requires different immunostimulants for different life stages.

6.9 Conclusions

Fish disease is a major concern of crop loss in aquaculture, and in order to combat this, many therapeutic and prophylactic measures are available. Development of effective vaccine requires time and cost while the use of antibiotics possesses concern for development of antibiotic-resistant bacteria. Moreover, fishes rely more on their non-specific defence mechanisms. Under such circumstances, immunostimulants are better option as they have indefinite sources, primarily target non-specific defence system, are inexpensive in comparison to vaccines, are biodegradable and are reported to be effective against a wide range of fish pathogens. Immunostimulatory effects depend on the species tested, particle size, dose and timing of application and mode of action. Thus, optimisation of the above mentioned conditions is important. Research focus should be on safety level of immunostimulants, mechanism of action, stability in aquatic environment and synergistic effects while they are used in combination with other biological response modifiers and toxicological examination. For shrimps, efforts need to be given in compounds that do not give inflammatory reaction.

References

Abu-Elala NM, Mohamed SH, Zaki MM, Eissa AE (2015) Assessment of the immune-modulatory and antimicrobial effects of dietary chitosan on Nile tilapia (Oreochromis niloticus) with special emphasis to its bio-remediating impacts. Fish Shellfish Immunol 46(2):678–685

AftabUddin S, Siddique MAM, Romkey SS, Shelton WL (2017) Antibacterial function of herbal extracts on growth, survival and immunoprotection in the black tiger shrimp *Penaeus monodon*. Fish Shellfish Immunol 65:52–58

Akhtar MS, Tripathi PH, Pandey A, Ciji A (2021) β-glucan modulates non-specific immune gene expression, thermal tolerance and elicits disease resistance in endangered Tor putitora fry challenged with Aeromonas salmonicida. Fish Shellfish Immunol 119:154–162

Alvarez-Pellitero P, Sitjà-Bobadilla A, Bermúdez R, Quiroga MI (2006) Levamisole activates several innate immune factors in Scophthalmus maximus (L.) (Teleostei). Int J Immunopathol Pharmacol 19(4):727–738

Amar EC, Kiron V, Satoh S, Watanabe T (2004) Enhancement of innate immunity in rainbow trout (Oncorhynchus mykiss Walbaum) associated with dietary intake of carotenoids from natural products. Fish Shellfish Immunol 16(4):527–537

Anbazahan SM, Mari LSS, Yogeshwari G, Jagruthi C, Thirumurugan R, Arockiaraj J, Velanganni AAJ, Krishnamoorthy P, Balasundaram C, Harikrishnan R (2014) Immune response and disease resistance of carotenoids supplementation diet in Cyprinus carpio against Aeromonas hydrophila. Fish Shellfish Immunol 40(1):9–13

Anderson DP, Siwicki AK, Rumsey GL (1995) Injection or immersion delivery of selected immunostimulants to trout demonstrate enhancement of nonspecific defense mechanisms and protective immunity. In: Shariff M, Subasighe RP, Arthur JR (eds) Diseases in Asian aquaculture, vol 11. Fish Health Section, Asian Fisheries Society, Manila, pp 413–426

Ardo L, Yin G, Xu P, Váradi L, Szigeti G, Jeney Z, Jeney G (2008) Chinese herbs (Astragalus membranaceus and Lonicera japonica) and boron enhance the non-specific immune response of Nile tilapia (Oreochromis niloticus) and resistance against Aeromonas hydrophila. Aquaculture 275(1–4):26–33

Baba T, Watase Y, Yoshinaga Y (1993) Activation of mononuclear phagocyte function by levamisole immersion in carp. Nippon Suisan Gakkaishi 59(2):301–307

Bagni M, Romano N, Finoia MG, Abelli L, Scapigliati G, Tiscar PG, Sarti M, Marino G (2005) Short-and long-term effects of a dietary yeast β-glucan (Macrogard) and alginic acid (Ergosan) preparation on immune response in sea bass (Dicentrarchus labrax). Fish Shellfish Immunol 18(4):311–325

Barman D, Nen P, Mandal SC, Kumar V (2013) Immunostimulants for aquaculture health management. J Mar Sci Res Dev 3(3):1–11

Bhatnagar A, Lamba R (2016) Immunostimulating and growth promoting activity of dietary levamisole on Cirrhinus mrigala fingerlings. J Aquac Mar Biol 4:1–9
Blazer VS, Wolke RE (1984) The effects of α-tocopherol on the immune response and non-specific resistance factors of rainbow trout (Salmo gairdneri Richardson). Aquaculture 37(1):1–9
Burrells C, Williams PD, Forno PF (2001) Dietary nucleotides: a novel supplement in fish feeds: 1. Effects on resistance to disease in salmonids. Aquaculture 199(1–2):159–169
Casadei E, Bird S, Vecino JLG, Wadsworth S, Secombes CJ (2013) The effect of peptidoglycan enriched diets on antimicrobial peptide gene expression in rainbow trout (Oncorhynchus mykiss). Fish Shellfish Immunol 34(2):529–537
Casadei E, Bird S, Wadsworth S, Vecino JLG, Secombes CJ (2015) The longevity of the antimicrobial response in rainbow trout (Oncorhynchus mykiss) fed a peptidoglycan (PG) supplemented diet. Fish Shellfish Immunol 44(1):316–320
Chang CF, Chen HY, Su MS, Liao IC (2000) Immunomodulation by dietary β-1,3-glucan in the brooders of the black tiger shrimp Penaeus monodon. Fish Shellfish Immunol 10(6):505–514
Choudhury D, Pal AK, Sahu NP, Kumar S, Das SS, Mukherjee SC (2005) Dietary yeast RNA supplementation reduces mortality by Aeromonas hydrophila in rohu (Labeo rohita L.) juveniles. Fish Shellfish Immunol 19(3):281–291
Cuesta A, Esteban MÁ, Meseguer J (2003) In vitro effect of chitin particles on the innate cellular immune system of gilthead seabream (Sparus aurata L.). Fish Shellfish Immunol 15(1):1–11
Davis JF, Hayasaka SS (1984) The enhancement of resistance of the American eel, Anguilla rostrata Le Sueur, to a pathogenic bacterium, Aeromonas hydrophila, by an extract of the tunicate, Ecteinascidia turbinata. J Fish Dis 7(4):311–316
Dawood MA, Koshio S, Esteban MÁ (2018) Beneficial roles of feed additives as immunostimulants in aquaculture: a review. Rev Aquac 10(4):950–974
Dawood MA, Metwally AES, El-Sharawy ME, Atta AM, Elbialy ZI, Abdel-Latif HM, Paray BA (2020) The role of β-glucan in the growth, intestinal morphometry, and immune-related gene and heat shock protein expressions of Nile tilapia (Oreochromis niloticus) under different stocking densities. Aquaculture 523:735205
Dugenci SK, Arda N, Candan A (2003) Some medicinal plants as immunostimulant for fish. J Ethnopharmacol 88(1):99–106
Esteban MA, Mulero V, Cuesta A, Ortuno J, Meseguer J (2000) Effects of injecting chitin particles on the innate immune response of gilthead seabream (Sparus aurata L.). Fish Shellfish Immunol 10(6):543–554
Esteban MA, Rodríguez A, Cuesta A, Meseguer J (2005) Effects of lactoferrin on non-specific immune responses of gilthead seabream (Sparus auratus L.). Fish Shellfish Immunol 18(2): 109–124
Ganguly S, Paul I, Mukhopadhayay SK (2010) Application and effectiveness of immunostimulants, probiotics, and prebiotics in aquaculture: a review. Isr J Aquac Bamidgeh 62(3):130–138
Ghosh AK, Panda SK, Luyten W (2021) Anti-vibrio and immune-enhancing activity of medicinal plants in shrimp: a comprehensive review. Fish Shellfish Immunol 117:192–210
Gopalakannan A, Arul V (2006) Immunomodulatory effects of dietary intake of chitin, chitosan and levamisole on the immune system of Cyprinus carpio and control of Aeromonas hydrophila infection in ponds. Aquaculture 255(1–4):179–187
Harikrishnan R, Devi G, Balasundaram C, Van Doan H, Jaturasitha S, Ringø E, Faggio C (2021) Effect of chrysophanic acid on immune response and immune genes transcriptomic profile in Catla catla against Aeromonas hydrophila. Sci Rep 11(1):1–15
Heine H, Lien E (2003) Toll-like receptors and their function in innate and adaptive immunity. Int Arch Allergy Immunol 130(3):180–192
Hodgkinson JW, Grayfer L, Belosevic M (2015) Biology of bony fish macrophages. Biology 4(4): 881–906
Horne MT, Poy M, Pranthanpipat P (1995) Control of vibriosis in black tiger shrimp, *Penaeus monodon*, by vaccination. In: Shariff M, Arthur JR, Subasinghe RP (eds) Diseases in Asian aquaculture II, pp 459–467

Hossain MS, Koshio S, Ishikawa M, Yokoyama S, Sony NM, Dawood MA, Kader MA, Bulbul M, Fujieda T (2016) Efficacy of nucleotide related products on growth, blood chemistry, oxidative stress and growth factor gene expression of juvenile red sea bream, Pagrus major. Aquaculture 464:8–16

Ispir U (2009) Prophylactic effect of levamisole on rainbow trout (Oncorhynchus mykiss) against Yersinia ruckeri. Pesquisa Veterinária Brasileira 29:700–702

Kajita Y, Sakai M, Atsuta S (1992) Immunopotentiation activity of Freund's complete adjuvant in rainbow trout *Oncorhynchus mykiss*. Nippon Suisan Gakkaishi 58(3):433–437

Kawakami H, Shinohara N, Sakai M (1998) The non-specific immunostimulation and adjuvant effects of Vibrio anguillarum bacterin, M-glucan, chitin and Freund's complete adjuvant against Pasteurella piscicida infection in yellowtail. Fish Pathol 33(4):287–292

Khanna D, Sethi G, Ahn KS, Pandey MK, Kunnumakkara AB, Sung B, Aggarwal A, Aggarwal BB (2007) Natural products as a gold mine for arthritis treatment. Curr Opin Pharmacol 7(3): 344–351

Kitao T, Yoshida T, Anderson DP, Dixon OW, Blanch A (1987) Immunostimulation of antibody-producing cells and humoral antibody to fish bacterins by a biological response modifier. J Fish Biol 31:87–91

Kodama H, Hirota Y, Mukamoto M, Baba T, Azuma I (1993) Activation of rainbow trout (*Oncorhynchus mykiss*) phagocytes by muramyl dipeptide. Dev Comp Immunol 17(2):129–140

Kumar BN, Murthy HS, Patil P, Doddamani PL, Patil R (2015) Enhanced immune response and resistance to white tail disease in chitin-diet fed freshwater prawn, Macrobrachium rosenbergii. Aquac Rep 2:34–38

Kumar R, Kaur N, Kamilya D (2019) Chitin modulates immunity and resistance of Labeo rohita (Hamilton, 1822) against gill monogeneans. Aquaculture 498:522–527

Kumar S, Verma AK, Singh SP, Awasthi A (2022) Immunostimulants for shrimp aquaculture: paving pathway towards shrimp sustainability. Environ Sci Pollut Res 30:1–19

Lee MH, Shiau SY (2004) Vitamin E requirements of juvenile grass shrimp, Penaeus monodon, and effects on non-specific immune responses. Fish Shellfish Immunol 16(4):475–485

Li G, Guo Y, Zhao D, Qian P, Sun J, Xiao C, Liang L, Wang H (2006) Effects of levamisole on the immune response and disease resistance of *Clarias fuscus*. Aquaculture 253(1–4):212–217

Li YQ, Jian LI, Wang QY (2010) Effects of size grading on growth and non-specific immunity factors of the shrimp Litopenaeus vannamei Boone. Agric Sci China 9(3):416–422

Li GF, Liu LB, Tan YL, Liu LZ, Deng HZ, Wan H, Zhong WZ, Chen SJ (2011) In vitro effect of levamisole on the cell viability, phagocytosis and respiratory burst of Barbel chub (Squaliobarbus curriculus) macrophages. Aquac Nutr 17(2):e263–e270

Lightner DV, Hunter B, Magarelli Jr PC, Colvin LB (1979) Ascorbic acid: nutritional requirement and role in wound repair in penaeid shrimp. In: Proceedings of the World Mariculture Society, vol 10, no. 1–4. Blackwell Publishing Ltd, Oxford, pp 513–528

Lin S, Lin X, Yang Y, Li F, Luo L (2013) Comparison of chelated zinc and zinc sulfate as zinc sources for growth and immune response of shrimp (Litopenaeus vannamei). Aquaculture 406: 79–84

Ma K, Bao Q, Wu Y, Chen S, Zhao S, Wu H, Fan J (2020) Evaluation of microalgae as immunostimulants and recombinant vaccines for diseases prevention and control in aquaculture. Front Bioeng Biotechnol 8:590431

Machuca C, Méndez-Martínez Y, Reyes-Becerril M, Angulo C (2022) Yeast β-glucans as fish immunomodulators: a review. Animals 12(16):2154

Mahmoud MM, El-Lamie MM, Kilany OE, Dessouki AA (2018) Spirulina (Arthrospira platensis) supplementation improves growth performance, feed utilization, immune response, and relieves oxidative stress in Nile tilapia (Oreochromis niloticus) challenged with Pseudomonas fluorescens. Fish Shellfish Immunol 72:291–300

Maliwat GC, Velasquez S, Robil JL, Chan M, Traifalgar RF, Tayamen M, Ragaza JA (2017) Growth and immune response of giant freshwater prawn Macrobrachium rosenbergii (De Man) postlarvae fed diets containing Chlorella vulgaris (Beijerinck). Aquac Res 48(4):1666–1676

Maqsood S, Singh P, Samoon MH, Munir K (2011) Emerging role of immunostimulants in combating the disease outbreak in aquaculture. Int Aquat Res 3(3):147–163
Mehana EE, Rahmani AH, Aly SM (2015) Immunostimulants and fish culture: an overview. Annu Res Rev Biol 5:477–489
Merchie G, Kontara E, Lavens P, Robles R, Kurmaly K, Sorgeloos P (1998) Effect of vitamin C and astaxanthin on stress and disease resistance of postlarval tiger shrimp, Penaeus monodon (Fabricius). Aquac Res 29(8):579–585
Minomol M (2005) Culture of gold fish Carassius auratus using medicinal plants having immunostimulant characteristics (Doctoral dissertation, M. Phil Dissertation, MS University, India)
Misra CK, Das BK, Pradhan J, Pattnaik P, Sethi S, Mukherjee SC (2004) Changes in lysosomal enzyme activity and protection against Vibrio infection in Macrobrachium rosenbergii (De Man) post larvae after bath immunostimulation with β-glucan. Fish Shellfish Immunol 17(4):389–395
Mohseni M, Pourkazemi M, Bai SC (2014) Effects of dietary inorganic copper on growth performance and immune responses of juvenile beluga, Huso huso. Aquacult Nutr 20(5):547–556
Nagaraju VT, Ray AK, Avunje S, Thangaraj SK, Krishnappa H, Viswanathan B, Reddy MA, Vijayan KK, Patil PK (2019) *Vibrio harveyi* biofilm as immunostimulant candidate for high-health pacific white shrimp, Penaeus vannamei farming. Fish Shellfish Immunol 95:498–505
Norqvist A, Hagström Å, Wolf-Watz HANS (1989) Protection of rainbow trout against vibriosis and furunculosis by the use of attenuated strains of Vibrio anguillarum. Appl Environ Microbiol 55(6):1400–1405
Nya EJ, Austin B (2010) Use of bacterial lipopolysaccharide (LPS) as an immunostimulant for the control of Aeromonas hydrophila infections in rainbow trout Oncorhynchus mykiss (Walbaum). J Appl Microbiol 108(2):686–694
Ortuno J, Esteban MA, Meseguer J (2000) High dietary intake of α-tocopherol acetate enhances the non-specific immune response of gilthead seabream (Sparus aurata L.). Fish Shellfish Immunol 10(4):293–307
Ortuno J, Cuesta A, Rodríguez A, Esteban MA, Meseguer J (2002) Oral administration of yeast, Saccharomyces cerevisiae, enhances the cellular innate immune response of gilthead seabream (Sparus aurata L.). Vet Immunol Immunopathol 85(1–2):41–50
Pais R, Khushiramani R, Karunasagar I, Karunasagar I (2008) Effect of immunostimulants on the haemolymph haemagglutinins of tiger shrimp *Penaeus monodon*. Aquac Res 39(12): 1339–1345
Patil PK, Gopal C, Panigrahi A, Rajababu D, Pillai SM (2014) Oral administration of 383 formalin killed Vibrio anguillarum cells improves growth and protection against 384 challenge with Vibrio harveyi in banana shrimp. Lett Appl Microbiol 58:213–218
Rajeswari PR, Velmurugan S, Michael Babu M, Albin Dhas S, Kesavan K, Citarasu T (2012) A study on the influence of selected Indian herbal active principles on enhancing the immune system in Fenneropenaeus indicus against Vibrio harveyi infection. Aquac Int 20(5):1009–1020
Robertsen B, Rθrstad G, Engstad R, Raa J (1990) Enhancement of non-specific disease resistance in Atlantic salmon, Salmo salar L., by a glucan from Saccharomyces cerevisiae cell walls. J Fish Dis 13(5):391–400
Rodriguez A, Cuesta A, Esteban MÁ, Meseguer J (2004) The effect of dietary administration of the fungus Mucor circinelloides on non-specific immune responses of gilthead seabream. Fish Shellfish Immunol 16(2):241–249
Sahoo PK, Mukherjee SC (2003) Immunomodulation by dietary vitamin C in healthy and aflatoxin B1-induced immunocompromised rohu (Labeo rohita). Comp Immunol Microbiol Infect Dis 26(1):65–76
Sakai M (1999) Current research status of fish immunostimulants. Aquaculture 172(1–2):63–92
Sakai M, Kamiya H, Atsuta S, Kobayashi M (1991) Immunomodulatory effects on rainbow trout, Oncorhynchus mykiss, injected with the extract of abalone, Haliotis discus hannai. J Appl Ichthyol 7(1):54–59

Sakai M, Yoshida T, Atsuta S, Kobayashi M (1995) Enhancement of resistance to vibriosis in rainbow trout, Oncorhynchus mykiss (Walbaum), by oral administration of Clostridium butyricum bacterin. J Fish Dis 18(2):187–190
Sakai M, Kobayashi M, Kawauchi H (1996) In vitro activation of fish phagocytic cells by GH, prolactin and somatolactin. J Endocrinol 151(1):113–118
Selvaraj V, Sampath K, Sekar V (2005) Administration of yeast glucan enhances survival and some non-specific and specific immune parameters in carp (Cyprinus carpio) infected with Aeromonas hydrophila. Fish Shellfish Immunol 19(4):293–306
Shahkar E, Yun H, Kim DJ, Kim SK, Lee BI, Bai SC (2015) Effects of dietary vitamin C levels on tissue ascorbic acid concentration, hematology, non-specific immune response and gonad histology in broodstock Japanese eel, Anguilla japonica. Aquaculture 438:115–121
Sirimanapong W, Adams A, Ooi EL, Green DM, Nguyen DK, Browdy CL, Collet B, Thompson KD (2015) The effects of feeding immunostimulant β-glucan on the immune response of Pangasianodon hypophthalmus. Fish Shellfish Immunol 45(2):357–366
Skalli A, Castillo M, Andree KB, Tort L, Furones D, Gisbert E (2013) The LPS derived from the cell walls of the Gram-negative bacteria Pantoea agglomerans stimulates growth and immune status of rainbow trout (Oncorhynchus mykiss) juveniles. Aquaculture 416:272–279
Tafalla C, Bøgwald J, Dalmo RA (2013) Adjuvants and immunostimulants in fish vaccines: current knowledge and future perspectives. Fish Shellfish Immunol 35(6):1740–1750
Takeda K, Kaisho T, Akira S (2003) Toll-like receptors. Annu Rev Immunol 21:335–376
Thompson KD (2017) Immunology: improvement of innate and adaptive immunity. In: Fish diseases, pp 1–17
Vallejos-Vidal E, Reyes-López F, Teles M, MacKenzie S (2016) The response of fish to immunostimulant diets. Fish Shellfish Immunol 56:34–69
Verlhac V, Gabaudan J, Obach A, Schüep W, Hole R (1996) Influence of dietary glucan and vitamin C on non-specific and specific immune responses of rainbow trout (Oncorhynchus mykiss). Aquaculture 143(2):123–133
Villegas JG, Fukada H, Masumoto T, Hosokawa H (2006) Effect of dietary immunostimulants on some innate immune responses and disease resistance against Edwardsiella tarda infection in Japanese Flounder (*Paralichthys olivaceus*). Aquac Sci 54:153–162
Wahli T, Verlhac V, Gabaudan J, Schuep W, Meier W (1998) Influence of combined vitamins C and E on non-specific immunity and disease resistance of rainbow trout, Oncorhynchus mykiss (Walbaum). J Fish Dis 21:127–137
Wang SH, Chen JC (2005) The protective effect of chitin and chitosan against Vibrio alginolyticus in white shrimp Litopenaeus vannamei. Fish Shellfish Immunol 19(3):191–204
Wang W, Sun J, Liu C, Xue Z (2017) Application of immunostimulants in aquaculture: current knowledge and future perspectives. Aquac Res 48(1):1–23
Yanar Y, Büyükçapar H, Yanar M, Göcer M (2007) Effect of carotenoids from red pepper and marigold flower on pigmentation, sensory properties and fatty acid composition of rainbow trout. Food Chem 100(1):326–330
Yin G, Jeney G, Racz T, Xu P, Jun X, Jeney Z (2006) Effect of two Chinese herbs (Astragalus radix and Scutellariae radix) on non-specific immune response of tilapia, Oreochromis niloticus. Aquaculture 253(1–4):39–47
Yin G, Ardó L, Thompson KD, Adams A, Jeney Z, Jeney G (2009) Chinese herbs (Astragalus radix and Ganoderma lucidum) enhance immune response of carp, Cyprinus carpio, and protection against Aeromonas hydrophila. Fish Shellfish Immunol 26(1):140–145
Yoshida T, Sakai M, Kitao T, Khlil SM, Araki S, Saitoh R, Ineno T, Inglis V (1993) Immunomodulatory effect of the fermented products of chicken egg, EF203, on rainbow trout, Oncorhynchus mykiss. Aquaculture 109(3–4):207–214
Zanon RB, da Silva Cerozi B, Cyrino JEP, de Castro Silva TS (2014) Dietary levamisole as immunostimulant for striped surubim, Pseudoplatystoma reticulatum. J World Aquacult Soc 45(6):672–680

Zhang Z, Chi H, Dalmo RA (2019) Trained innate immunity of fish is a viable approach in larval aquaculture. Front Immunol 10(42):1–5

Zhu L, Han D, Zhu X, Yang Y, Jin J, Liu H, Xie S (2017) Dietary selenium requirement for on-growing gibel carp (Carassius auratus gibelio var. CAS III). Aquac Res 48(6):2841–2851

Vallejos-Vidal E, Reyes-López F, Teles M, MacKenzie S (2016). The response of fish to immunostimulant diets. Fish Shellfish Immunol 56:34-69.