Immunoprophylactic Measures in Aquaculture

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Abstract

High intensification of aquaculture practices leads to diseases of microbial etiology of economic significance. These diseases have surfaced in rearing systems and constitute a significant threat to sustainable aquaculture which calls for proper investigation and appropriate solutions. Immunoprophylaxis focuses on tackling the effects of high intensification in aquaculture. Immunoprophylaxis is pragmatic immunology which is concerned with preventing infectious diseases through the use of vaccines, immune sera, immunostimulants, plant as well as animal products, and gamma globulins. This confers immunity in fish/shellfish during intensification of aquaculture thereby benefiting farmers and can possibly even double the output. Intensification often encourages many existing and emerging diseases and needs drugs, synthetic chemicals, and vaccination for treatments both as prophylaxis as well as curative. Immunoprophylaxis could be both active as well as passive. Vaccines (live, attenuated, inactivated,, subunit, recombinant vector, DNA-based and synthetic/peptide) are classified as active, and the microbial derivatives viz. probiotics, prebiotics as well as plant and animal extracts that boost the nonspecific immunity in both fishes and crustaceans are considered passive immunoprophylactics. Immunoprophylaxis, also known as prophylactic immunization, is one of the alternate, highly productive strategies in the primary prevention of diseases.

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18.1 Introduction

World aquaculture produces constitute around 60% of food fish supply globally which fulfills over 20% of the increasing population's animal protein requirements. Intensification of the production system through sustainable approaches is required to optimize the returns. With high stocking density, supplemental feeding and scientific pond management, following BMPs and biosecurity norms are integral to fish/shrimp farming systems' husbandry practices. Aquaculture uses various methods and techniques to cultivate aquatic organisms which involves farming of aquatic species ranging from freshwater to saltwater. As per Food and Agriculture Organization, aquaculture is a booming industry representing 46% of the total global fish production (about 172.6 million tonnes) which is worth USD 238 billion in 2017. The annual escalation in global fish consumption (3.2%) surpasses meat produced from all terrestrial farm animals (2.8%) between 1961 and 2016 (FAO 2018). Furthermore, fish accounted for about 20% of animal protein consumed by humans globally (FAO 2016). This massive global demand for fish was met by aquaculture as capture fishery which has been plateaued since the late 1980s (FAO 2018). Like freshwater aquaculture, brackish water aquaculture has a long history of the employment of traditional practices from a way of life in bheries (enclosed paddy fields) and pokkali (unique salt tolerant rice variety) fields in India, to the export-oriented aquaculture industry. In this journey of enhanced production, it is seen in India that there is a phenomenal increase of more than 25-fold from a measly 28,000 tonnes in 1988–1989 to 7,47,694 tonnes in 2019–2020; the most critical turning point was the regulated introduction and successful establishment of SPF (Specific Pathogen Free) *P. vannamei* in 2009.

Intensification in aquaculture often brings with it many existing and emerging diseases that need drugs, synthetic chemicals and vaccination for their prevention and control. However, due to the indiscriminate use of antimicrobial compounds, chemical disinfectants, anti-parasitic and other synthetic drugs, there has been a negative impact on the host and environmental health. Since there is only partial success seen in these practices, an alternative approach to stimulating the immune system of farmed aquatic animals is practised. In practicing the alternative prophylactic method in intensive sustainable aquaculture, zero-tolerance to antibiotics can be imposed while mitigating the risk of infectious diseases. Such immunoprophylactic approaches explores the beneficial role of vaccination and immunostimulant compounds, i.e., microbial components (MDP, LPS, beta-1-3-glucan, bacterin, peptidoglycan), plant products (polysaccharides, levamisole, etc.), and yeast derivatives. They are known to elicit the innate and adaptive immune systems of fish and shrimp.

18.2 Concept of Immunoprophylaxis

Immunoprophylaxis, otherwise known as prophylactic immunization, is one of the highly efficacious, alternate strategies in the primary prevention of disease (Eisenhauer et al. 2013).

General prophylactic measures followed in aquaculture operations include:

- Better management practices (BMPs) in culture operations.
- Probiotics and bioremediation process to improve host-gut microbiome and environmental health and their interaction.
- Enhancing host resistance to infectious diseases by immunostimulants.
- Nutraceutical diets for immunomodulating the host.
- Vaccination of the host against specific pathogens.
- Genetic selection/selective breeding programs for resistance to diseases.

Immunoprophylaxis is the mainstay of preventing infections via vaccines or antibody-containing preparations or immunostimulants to provide immune protection against disease. It is a branch of practical immunology which is concerned with preventing infectious diseases by injecting immunological preparations such as vaccines, immune sera, immunostimulants, and gamma globulins, to elicit immunity (Fig. 18.1). It is quite evident from the human point of view that it has greatly helped in preventing several infectious diseases, viz. poliomyelitis, yellow fever, diphtheria, tetanus, and smallpox.

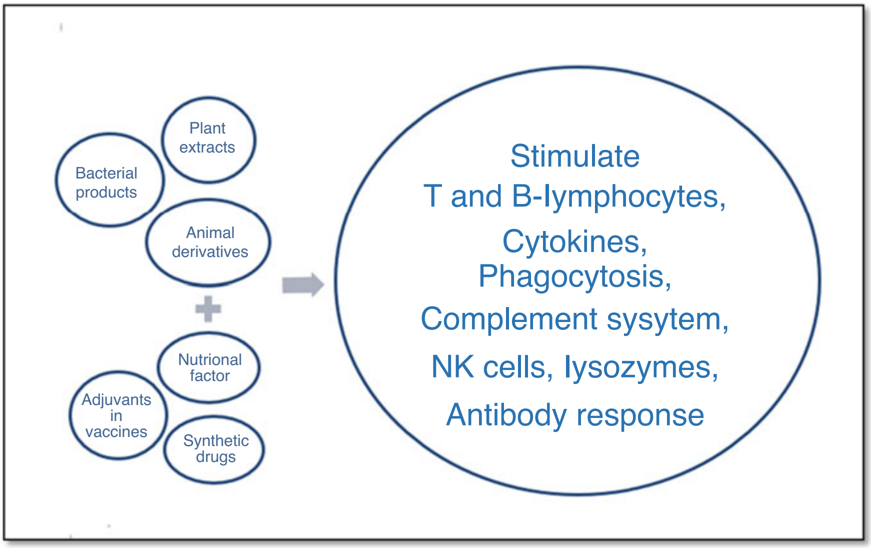

Fig. 18.1 The concept of the immune response of prophylactic treatment

Vaccination may reduce the risk of introducing and establishing any infectious agent and containing its horizontal/vertical spread to other populations/farms, thereby aiding internal biosecurity. Biosecurity is a relatively new term used to describe measures for preventing and controlling diseases (FAO 2013; OIE 2011). A commonly used definition of biosecurity mentions "a set of measures that reduce the risk of introducing and spreading disease agents." Biosecurity may be external (or bio-exclusion) or internal (or biocontainment) depending on the spread/control of pathogens to a system/population or within a population. Since aquaculture involves many individuals, boosting innate and adaptive immunity through vaccination is better as herd immunity ensures protection against diseases even in few nonvaccinated animals and poor responders.

18.3 Historical Perspective

Fish farming is entering a new era for sustainable intensification in many countries. In earlier times, antibiotics or chemotherapeutics were primarily used for disease prevention and treatment. Snieszko et al. (1938) first reported on the protective immunity in carps immunized with *Aeromonas punctata*, a manuscript in Polish. However, since this manuscript was not available elsewhere globally, the first report in English was written by Duff (1942), who demonstrated the protection in trout against *A. salmonicida* by parenteral inoculation of oral administration. Until the 20–30 year duration of World War II, very few reports on disease prevention by vaccination were published. In those days, immunoprophylaxis was disregarded because of the easy availability and use of antimicrobial compounds. Snieszko and Friddle (1949) highlighted the superiority of sulfamerazine to a vaccine for controlling furunculosis. Evelyn (1997) described the post-World War II period as "the era of chemotherapy." A Colorado company, Wildlife Vaccines, was the pioneer in vaccine production which produced bacterins. Tavolek Inc. (a subsidiary of Johnson & Johnson) was the other company licensed to produce the enteric red mouth vaccine and other bacterins. During that time, the scale of the vaccine market was too small for generating decent profits. In the eighties, Biomed Research Laboratories in Seattle entered the fray. Many professional aquaculturists and vaccinologists like Stephen Newman, Tony Novotny, James Nelson, with their expertise in disease prevention involved themselves in bacterin production in the USA. Aqua Health Inc., Charlottetown, Canada, with William D. Paterson as an expert and Aquaculture Vaccines Ltd., UK, started as a Wildlife Vaccines subsidiary with Patrick D. Smith contributed immensely during the early days of fish vaccination.

18.4 Concept of Immunoprophylaxis in Aquaculture

Intensive aquaculture is associated with severe disease outbreaks caused by various pathogenic microorganisms, resulting in loss of production due to the reduction of growth and increased mortality (Sivasankar and Kumar 2017). Different types of chemotherapeutic agents, drugs and biological products are used for preventing diseases in aquaculture. Indiscriminate use of chemical agents and natural products has potentially negative impact on the environment and human health (Sivasankar and Kumar 2017). Using antibiotics to prevent diseases could lead to Antimicrobial resistance (AMR) in microorganisms (George et al. 2006). Immunoprophylaxis is a protective management tool aiding in the prevention and treatment of diseases. The prophylactic measures for disease prevention are implemented with prebiotics, probiotics, vaccines, and immunostimulants (Panigrahi and Azad 2007; Panigrahi et al. 2011; Sivasankar and Kumar 2017).

18.5 Classification of Immunoprophylaxis

Immunity can be achieved by either active or passive immunization.

1. *Active Immunoprophylaxis (Vaccines):*
   —through vaccines (inactivated, live, attenuated, subunit, recombinant vector, DNA-based and synthetic/peptide-based)
2. *Passive Immunoprophylaxis (Immunostimulants):*
   —through immunoglobulin preparation or immunostimulants (pro-prebiotic compounds, nutritional factors, animal and plant derivatives, etc.)

18.5.1 Active Immunoprophylaxis Measure (Vaccines)

Presently, there are several commercial vaccines available that offer prevention of infectious bacterial and viral diseases (Table 18.1 and 18.2). Generally, for disease prevention, vaccines are classified as inactivated whole-cell, inactivated bacterin, live, attenuated, DNA-based, Nano-based, subunit based,, genetically modified and polyvalent.

18.5.2 Passive Immunoprophylaxis

Passive immunoprophylaxis can be achieved through immunomodulatory preparations like immunostimulants (Pro and prebiotic compounds, nutritional factors, animal and plant derivatives, etc.) or immunoglobulins (Vijayan et al. 2017).

Table 18.1 Available commercial bacterial vaccines for infectious diseases in aquaculture

S. no	Types of vaccine	Name of vaccines	Diseases prevention	Vaccinated host	References
1.	Inactivated or "killed" vaccine	*Vibrio anguillarum*-Ordalii	Vibriosis	Salmonids, rainbow trout	Sommerset et al. (2005)
2.	Inactivated or "killed" vaccine	*Vibrio salmonicida* Bacterin	Vibriosis	Salmonids Coldwater	Sommerset et al. (2005)
3.	DNA vaccine	*Vibrio anguillarum*	Vibriosis	Seabass	Kumar et al. (2007)
4.	Formalin-killed bacterin vaccine	*Aeromonas salmonicida* Bacterin	Furunculosis	Rainbow trout	Gudmundsdóttir and Björnsdóttir (2007)
5.	Live attenuated vaccine	*Aeromonas hydrophila* vaccine	Motile Aeromonas Septicemia	Common carp	Jiang et al. (2016)
6.	Live attenuated vaccine	*Edwardsiella ictalurii* vaccine	Edwardsiellosis	Catfish	Kordon et al. (2019)
7.	Recombinant GAPDH vaccine	*Edwardsiella tarda* vaccine	Edwardsiella tarda	Japanese flounder	Liu et al. (2005)
8.	Modified live vaccine	*Flavobacterium columnare* vaccine	Columnaris disease	Channel catfish	Shoemaker et al. (2011)
9.	Live attenuated vaccine	*Flavobacterium psychrophilum* vaccine	Flavobacteriosis	Salmonids	Sudheesh and Cain (2016)
10.	Live attenuated vaccine	*Renibacterium salmoninarum* vaccine	Bacterial kidney disease	Salmonids	Toranzo et al. (2009)
11.	Formalin-killed bacterin vaccine	*Yersinia ruckeri* Bacterin	Enteric redmouth disease	Salmonids	Jaafar et al. (2019)
12.	Formalin-killed bacterin vaccine	*Streptococcus agalactiae* vaccine	Streptococcosis	Tilapia	Evans et al. (2004)

Table 18.2 Available commercial viral vaccines for infectious diseases in aquaculture

S. no.	Types of vaccine	Name of vaccines	Diseases prevention	Host vaccination	References
1.	DNA vaccine	Infectious hematopoietic necrosis virus vaccine	Infectious hematopoietic necrosis	Salmonids	Garver et al. (2005)
2.	DNA vaccine	Infectious pancreatic necrosis virus vaccine	Infectious pancreatic necrosis	Salmonids	Cuesta et al. (2010)
3.	Formalin-killed vaccine	Iridoviral disease vaccine	Iridoviral disease	Red Sea bream	Nakajima and Kunita (2005)
4.	DNA vaccine	Spring viremia of carp vaccine	Spring viremia of carp	Common carp	Embregts et al. (2019)
5.	Recombinant vaccine	Nodavirus vaccine	Viral nervous necrosis	European seabass	Gonzalez-Silvera et al. (2019)

18.6 Immunostimulants

An immunostimulant is a substance that enhances the innate or nonspecific immune response by interacting directly with the cells of the immune system and activating them. Immunostimulants can be grouped under different agents based on their source, such as bacterial preparations, polysaccharides, animal or plant extracts, nutraceuticals, and cytokines (Sakai 1999). In nature, the defense mechanism in all living organisms (vertebrates and invertebrates) are common, but there still exists some dissimilarities. The vertebrate has a more complex mechanism whereas the invertebrates generally lack adaptive immunity and exclusively rely on their innate immunity. Therefore, it is necessary to inculcate products that can enhance host immunity and resistance to infectious diseases in shrimps disease prevention and control (Farzanfar 2006). Cellular and humoral immunities occur both in vertebrates and invertebrates. Immunostimulants as adjuvants (helper compounds) are effective when used with fish vaccines and as additives in aquafeeds. As immunostimulants generate passive immunity, they are used as prophylactic agents prior to the recognition of the elevated risk of disease (Raa 1996).

18.6.1 General Aspects of Immunostimulants

The success story of immunostimulants in fish/shrimp involves bacteria causing infectious diseases such as *A.salmonicida, A. hydrophila, Vibrio anguillarum, V. vulnificus, V. salmonicida, Yersinia ruckeri, Streptococcus* spp.; viruses causing infectious diseases such as hematopoietic necrosis, yellow head virus, viral hemorrhagic septicemia and the parasite like *Ichthyophthirius multifiliis.* Immunostimulants are known to elicit innate (nonspecific) defense mechanisms and improve resistance to specific pathogens (Sakai et al. 1999; Patil et al. 2014). In invertebrates, the memory factor is not fully developed and being very feeble, the memory is short-lived. There are chemical substances that activate leukocytes and lymphocytes (Lunden and Bylund 2000). Adjuvant (FCA) is one of the essential immunomodulators used in animals to enhance specific immune response and is used in conjunction with the injection of fish bacterins (Anderson 1992). The β-glucans, present in cell walls of plants, fungi, and bacteria, appear to be most promising among the immunostimulants in fish and shrimp culture ponds, although an oral application was found to be the preferred route of choice.

18.6.2 Role of Immunostimulant

Immunostimulants increase immunocompetence and disease resistance in aquatic animals (Sakai 1999). Many studies have been conducted on immunostimulants in fish and shellfish, and these include chemical agents, polysaccharides, extracts of plant and animal origins, components of bacteria, cytokines, and nutritional agents (Sakai 1999). In crustaceans, three circulating hemocytes are generally identified: hyaline cells, semigranular cells, and large granular cells. These cells form the basis for cellular immune responses, including phagocytosis and eliminating foreign bodies or microbial agents (Hose et al. 1990). Hemocytes form an integral part of the crustaceans' immune system. They are associated with enzymes prophenoloxidase (proPO), and ProPO activates systems involved in encapsulation, melanin formation and function as non-self-recognition systems (Johansson and Soderhall 1989). Phenoloxidase (PO) is activated from prophenoloxidase by a serine proteinase in the presence of a small number of microbial components such as peptidoglycan, lipopolysaccharides, or b-1.3-glucan through the recognized receptors. The PO catalyzes the stepwise oxygenation and reactions of monophenols through *O*-diphenols to *O*-quinones, which subsequently leads to melanin formation (Johansson and Soderhall 1989).

Phagocytosis is an essential mechanism in crustaceans to get rid of microorganisms or foreign bodies. Reactive oxygen intermediates such as superoxides, hydroxide radicals, and peroxides are produced during the process of phagocytosis. This activity is known as respiratory burst and these products have microbicidal activities (Song and Hsieh 1994). Many of these parameters such as total hemocyte count (THC), phenoloxidase activities, superoxide dismutase (SOD), an enzyme that catalyses the two steps of rapid dismutation of the toxic superoxide anion to non-toxic molecular oxygen and hydrogen peroxide, etc. have been used to evaluate the immune status of shrimps (Maftuch et al. 2013).

Several studies have revealed the enhancement in immunological parameters in many fish species after administration of medicinal plants or extracts, including phagocytic activity, respiratory burst activity, nitrogen oxide, myeloperoxidase content, complement activity, lysozyme activity, total protein (globulin and albumin), and antiprotease activity (Dugenci et al. 2003; Talpur, 2014).

18.7 Classification of Immunostimulants

18.7.1 Synthetic Chemicals

These are the synthetic immunomodulatory compounds that are known to stimulate the immune response. Levamisole is an antihelminthic compound that combats nematodes infection in humans and animals as well. It can increase the phagocytic activity, the respiratory burst activity and increase antibody-producing cells and other immune parameters (Siwicki 1989).

18.7.2 Microbial Product as Immunoprophylaxis Measure

18.7.2.1 Bacterial Derivatives

MDP (Muramyl dipeptide): MDP (*N*-acetylmuramyl-L-alanyl-D-isoglutamine), obtained from Mycobacterium increases the phagocytic activities, respiratory burst, and migration activities of kidney leucocytes and resistance to *A. salmonicida.* Various bacterial products used for immunoprophylaxis in aquaculture is shown in Table 18.3.

Table 18.3 Bacterial products used for immunoprophylaxis in aquaculture

Lactobacillus plantarum	Increased THC, PO and SOD activities, phagocity activity, and resistance to *V. alginolyticus* infection.	Chiu et al. (2007)
Beta-1-3-glucan (*Schizophyllum commune*)	Increased PO and SOD activities, PA, superoxide anion, survival against WSSV	Chang et al. (2003)
Beta-glucan	Increased mRNA expression of lysozyme and SOD	Wang et al. (2008)
Bacterin (Vibrio)	Increased immune response	Powell et al. (2011)
Bacterial LPS	Increased immune gene expression and disease resistance, proPO activity	Rungrassamee et al. (2013)
Bacterial genomic DNA extract	Increased THC, PTP, HLAT	Amar and Faisan (2012)
Peptidoglycan	Increased survival against Yellowhead baculovirus infection	Boonyaratpalin (1995)
Mannan oligosaccharide (MOS)	Increased growth and survival, PO, SOD activities, resistance to NH_3 stress	Zhang et al. (2012)
Peptidoglycan+ MOS	Increased growth, THC, respiratory burst activity, protection against WSSV infection	Apines-Amar et al. (2014)

18.7.2.2 LPS (Lipopolysaccharide)

LPS being a cell wall component of gram-negative bacteria, is effective against *A. hydrophilla* infection in rainbow trout and stimulates the innate immune response (Nya and Austin 2010). Atlantic salmon (Salati et al. 1987), in red sea bream *Pagrus major*, in goldfish lymphocytes (Neumann et al. 1995). It is also known to elicit phagocytic, microbicidal activity, and hemocyte proliferation in shrimp (Karunasagar et al. 1996).As these substances are very potent in low doses, they can be used for fish immunizing programs.

18.7.2.3 FCA (Freund's Complete Adjuvant)

As attenuated *Mycobacterium butyricum* is found in the FCA mineral oil, it improves immune response in fish. The FCA can increase cellular and humoral immune response in rainbow trout and protect it against *V. anguillarum* infection (Kajita et al. 1992). The effect of FCA on a *P. piscicida* vaccine was observed in fish (Kawakami et al. 1998).

18.7.2.4 Vibrio Bacterin

Vibrio anguillarum bacterin (inactivated whole-cell vaccine) is the most successful vaccine for salmonid fish, administered through injection, oral, and immersion methods (Sakai et al. 1999). Immunostimulation of *V. anguillarum* bacterin was seen in fish and shellfish. Hemocyte proliferation and migration were observed in *P. monodon* treated with vibrio bacterin (Horne et al. 1995; Chou et al. 1995).

18.7.2.5 *Clostridium butyricum* Cells

C. butyricum bacterin activates leucocytes and can enhance resistance to vibriosis disease in rainbow trout and also activates phagocytosis and increased superoxide anion production when introduced orally (Sakai et al. 1995; Young et al. 1987). It also revealed that *C. butyricum* bacterin shows immunostimulatory effects like the stimulation of macrophages and NK cells and improves further protection against Candida infection.

18.7.2.6 EF203

It is a fermented product of chicken eggs and oral administration of it to rainbow trout stimulates the immune response and ensures increases protection against *Streptococcus* infection (Yoshida et al. 1993).

18.7.3 Yeast Derivatives

Glucan: Glucan is a yeast-derived bioactive compound (long-chain polysaccharide extract) that stimulates the nonspecific defense mechanism in fishes and shellfishes to combat bacterial pathogens. Yeast glucan (β-1,3- and β-1,6-linked glucan) and β-1,3 glucan (VST) is derived from cell walls of baker's yeast like *Saccharomyces cerevisiae* and *Schizophyllum commune*, respectively.

18.7.4 Polysaccharides

18.7.4.1 Chitin and Chitosan

These polysaccharides are naturally abundant in the exoskeleton of crustaceans, insects, and cell walls of few fungi. They are known to elicit cellular and humoral immune responses like macrophage activity and protect the host from individual pathogenic organisms (Kawakami et al. 1998; Anderson and Siwicki 1994).

18.7.4.2 Lentinan, Schizophyllan, and Oligosaccharide

These compounds are reported to elicit cellular and noncellular defense mechanisms in fish and shrimp.

18.7.5 Medicinal Plant as Immunostimulant

Nowadays, antibiotics and chemotherapeutic agents have negative impact such as immunosuppression, development of antimicrobial resistance and bioaccumulation in the tissues and environment health. An alternative way of prophylactic measure is using a medicinal plant derivative (Table 18.4). It has rich bioactive compounds like polyphenolics compounds, flavonoids, saponins, tannins, terpenoids, alkaloids, curcumin, quinone, polypeptides, colchicine, capsaicin, and flavopiridol. These bioactive compounds trigger or modulate the immune system (Nair et al. 2019). That enhances the phagocytosis and macrophage activity, produce complementary compounds, cytokines, T lymphocytes, lysozyme activity which act on innate (nonspecific), and adaptive defense mechanisms (Farooqi and Qureshi 2018).

18.7.6 Animal Derivatives as Immunostimulant

Marine tunicate, *Ecteinascidia turbinata* (Ete) and abalone, Haliotis, *Discus hannai* extracts, and fractions (Hde) are known to have immunomodulatory roles and inhibits tumor growth in vivo. *Ete* (Tunicate) can enhance the phagocytosis and increases the rate of survival of Eel when injected against *A. hydrophila*. Rainbow trout injected with Hde against *V. anguillarum* infection showed increased survival along with enhanced phagocytic activities (Sakai et al. 1991).

18.7.6.1 Firefly Squid

Firefly squid, *Watasenia scintillans*, can stimulate the immunity of fish as observed in superoxide anion, macrophages proliferation, and the lymphoblastic transformation of lymphocytes in vitro.

Table 18.4 Report on the importance of medicinal plant extracts in enhancing growth and immune response in fish

Plants	Used parts	Route of administration	Culture species	Immune response	References
Viscum album	Leaf	Oral	*Oncorhynchus mykiss* (rainbow trout)	Enhances the phagocytic activity and respiratory burst, total protein	Dugenci et al. (2003)
Capparis spinosa	Leaf	Oral	*Oncorhynchus mykiss* (rainbow trout)	Cytokine genes (IL-1b, IL-8, TNF-a, TGF-b, -IL-10) against *A. hydrophila*	Bilen et al. (2016)
Zingiber officinale	Root	Oral	*Oncorhynchus mykiss* (rainbow trout)	Enhances phagocytic, respiratory burst, total protein	Dugenci et al. (2003)
Astragalus radix	Root	Oral	*Oreochromis niloticus* (Nile tilapia)	Increases the lysozyme activity, phagocytic, respiratory burst	Yin et al. (2006)
Solanum trilobatum	Leaf	Injection	*Oreochromis mossambicus*	Rising respiratory burst, lysozyme, serum bactericidal, serum protein against *A. hydrophila*	Divyagnaneswari et al. (2007)
Zingiber officinale	Root	Oral	*Oreochromis mossambicus*	Increases lysozyme production, Phagocytic, total protein, albumin, globulin against *V. vulnificus*	Immanuel et al. (2009)
Ocimum sanctum	Leaf	Oral	*Epinephelus tauvina*	Phagocytic, bactericidal against *V. harveyi*	Sivaram et al. (2004)
Euphorbia hirta	Leaf	Oral	*Cyprinus carpio* (common carp)	Phagocytic, respiratory burst, lysozyme, peroxidase against *A. hydrophila*	Pratheepa and Sukumaran (2014)
Lawsonia inermis	Leaf	Injection	*Cyprinus carpio* (common carp)	Lysozyme and bactericidal activity, phagocytic and respiratory burst activity against *A. hydrophila*	Soltanian and Fereidouni (2016)
Azadirachta indica	Leaf	Injection	*Carassius auratus*	Phagocytic, respiratory burst against *A. hydrophila*	Harikrishnan et al. (2009)
Cynodon dactylon	Leaf	Oral	*Catla catla* (Indian carp)	Lysozyme, antiprotease, complement, respiratory burst, nitrogen species, myeloperoxidase against *A. hydrophila*	Kaleeswaran et al. (2011)

18.7.7 Nutritional Factors

18.7.7.1 Nutrient Based Immunoprophylactic Measures

Nutritional immunostimulating compound plays a vital role in the survival, growth, physiology functions and immune response of fish/shellfish. It plays an essential role in maintaining epithelial barriers, nonspecific cellular factors such as phagocytosis by macrophages and neutrophils, nonspecific humoral factors such as lysozyme, complement, and transferrin and specific humoral and cellular immunity.

18.7.7.2 Vitamins and Minerals

Vitamins and minerals in negligible amount play a vital role in physiological functions and immunological responses (Table 18.5). Physiological processes such as growth, disease resistance to infections, wound healing, stress reduction and other biological processes are enhanced by vitamins. It also acts as an immunomodulator, enhances antioxidant activity, enhances calcium, phosphorus and iron absorption and regulate moult phase in shrimp and fish growth and also increases survival.

Vitamin C has a vital antioxidant and anti-stress characteristic that also induces different physiological processes, including growth, metabolism, reproduction, and protection against tissue damage, and a cofactor in cellular functions related to neuroendocrine modulation, hormone and immune systems. Ascorbic acid is found to have a significant role. Tewary and Patra (2008) revealed a positive correlation between vitamin C in diet and resistance against *A. hydrophila* in *Labeo rohita.*

Vitamin E can elicit specific and cell-mediated immunity against infection in Japanese Flounder *Paralichthys olivaceus* (Villegas et al. 2006) and macrophage phagocytosis in fish such as channel catfish Ictalurus punctatus (Wise et al. 1993) and turbot *Scophthalmus maximus* (Fernández et al. 2002). Vitamin E deficiencies in trout result in reduced protection against *Y. ruckeri* (Blazer and Wolke 1984).

18.7.8 Hormones

Growth hormones have a powerful influence on immunocompetent cells like macrophages, lymphocytes, and Natural Killer cells. Exogenous growth hormone (GH) has several activities in fish, including lymphocytes, NK cellproliferation and SOD production. Prolactin has multiple immunomodulatory roles, including the activation of immunocompetent cells and production of superoxide anions (Sakai et al. 1996).

18.7.9 Cytokines

Cytokines are polypeptides, or glycoproteins which plays a significant role as modulators in the immune system. Recombinant cytokines as immunostimulants

Table 18.5 Vitamins and minerals involved in enhancing the disease resistance and immune response in aquaculture species

Immunostimulants	Effect on host	Species	References
Vitamin C supplement with diet	Increases the growth, survival, THC, superoxide anion, PO activity, respiratory burst activity (NBT cells) resistance to *Aeromonas hydrophila* infections	Rohu (*L. rohita*)	Tewary and Patra (2008)
	Stimulates innate immune response and enhance resistance against high pH stress and protection against *Aeromonas hydrophila* infection	Megalobrama amblycephala	Liu et al. (2016)
Vitamin E	Increased growth, THC, SOD activity, and decrease TBA value	Flatfish	Pulsford et al. (1995)
	PO activity, respiratory burst activity, protect oxidative damage of tissues	Atlantic salmon	Lygren et al. (2001)
Folic acid (vitamin B9)	Improves the growth, antioxidant activity, enhance the ProPO activity and increase digestive enzymes, and better survival	*Macrobrachium rosenbergii*	Asaikkutti et al. (2016)
Copper (Cu)	Increased growth, THC, superoxide production	Tiger shrimp	Lee and Shiau (2002)
Zinc (Zn)	Increased growth, survival, THC, PO activity, phagocytic activity, alkaline phosphates, and SOD activities	White shrimp	Lin et al. (2013)
Selenium (se)	Increases superoxide anion, PO activity, respiratory burst activity, and protection against *Edwardsiella ictaluri* infection	Catfish	Paripatananont and Lovell (1997), Wang et al. (1997)
Chitin	Increased proPO activity		Sivakumar and Felix (2011)
Sodium alginate	Increased respiratory burst, PO activity, resistance to *V. alginolyticus* infection		Cheng et al. (2004)

can have a lot of utility as Immunoprophylactic agents provided they are characterized.

18.7.10 Algal Derivatives

Laminarin is a component of brown algae, e.g., Phaeophyceae, and its structure is with β(1, 6)-branched β(1, 3)-D-glucan. Laminarin extracted from *Laminaria*

Table 18.6 Other derivatives used as immunostimulants

Immunostimulants	Types	Functions	References
Levamisole	Drugs	Induction of B and T lymphocytes, monocytes, and macrophages	Patil et al. (2012), Biswajit et al. (2014)
Recombinant cytokines	Cytokines/ adjuvants	Generation of interferons and interleukins to stimulate effective immune responses	Galeotti (1998), Sirko et al. (2011)
Glucans	Carbohydrates	Stimulation of anti-tumor mechanisms and enhancement of host resistance to a variety of microbial pathogens in mammalian	Sahoo and Mukherjee (2001), Vetvicka (2011), Sajeevan et al. (2009)
Trehalose	Carbohydrates	Production of antibody, stimulation of specific immunity against different bacterial infections	Parant et al. (1978), Oswald et al. (1997)
Chitosan	Animals originated	Activating the production of cytokines such as IL-1β, TNF-α, and reactive oxygen intermediates to promote the defense system against microbial infections	Mastan (2015), Chen et al. (2009)
CpG oligonucleotides and imiquimod	Drugs/ adjuvants	Maturation and migration of DCs, and enhancement of humoral and cellular immune responses	Mizumoto et al. (2005)
Sodium alginate	Seaweed	Increased respiratory burst, PO activity, resistance to *V. alginolyticus* infection	Cheng et al. (2004)

hyperborea has immunomodulatory in aquatic animals as well. Water solubility is an issue for β-(1,3) D-glucan, making them less easy to handle than aqueous soluble laminaria.

Details of different other derivatives used as immunostimulants in aquaculture are shown in Table 18.6.

18.8 Combating Diseases Through Immunoprophylaxis Measures

Shellfishes lack adaptive immune systems and instead, have nonspecific immune system to combat pathogenic infections. Immunostimulants are dietary additives that enhance the innate (non-specific) defense mechanisms and increase resistance to pathogens in shrimp. Beta-glucans are increasingly used in aquaculture and are seemingly very promising in immune enhancement in aquaculture (Ajadi et al. 2016; Barman et al. 2013). Mode of action of various immunoprophylactic agents are summarized in Table 18.7.

Table 18.7 Mechanism of action of immunoprophylactic agents

Immunostimulants	Mechanism of action
Freund's complete adjuvant (FCA), glucans, FK-565 (Lactoyl tetrapeptide from *Streptomyces olivaceogriseus*)	Stimulators of T lymphocytes
Bacterial endotoxins, lipopolysaccharides, and chitin and chitosan	Stimulates of B cells and macrophage activity
Cytokines	Leukotriene, interferon
Detergents and sodium dodecyl sulfate, quarternary ammonium compounds (QAC), saponins	Cell membrane modifiers
Muramyl dipeptide (a purified form of mycobacteria)	Enhances the antibody activity, stimulation of polyclonal activation of lymphocytes, and activation of macrophages
Lentinan, Schizophyllan, and oligosaccharide	Increases the lysozyme activity, phagocyte activity, and complement activity
Levamisole	Enhancement of cell-mediated cytotoxicity, lymphokine production, and suppressor cell function and stimulation of the phagocytic activity of macrophages and neutrophils.
Yeast derivatives glucans (β1-3- and β1-6-linked glucan)	Increased lysozyme activity, phagocyte activity, complement activity, and bactericidal activity of macrophages
Plant based immnunostimulant (e.g., *Ocimum sanctum*, *Emblica officinalis*, *Cynodon dactylon*, and *Adathoda vasica*	Improved the phagocytic activity, serum bactericidal activity, albumin–Globulin (A/G) ratio
Nutritional factor—vitamin C	Collagen synthesis and cellular functions related to neuromodulation, hormone, and immune systems

18.8.1 Combating Vibriosis

Shellfish/finfish have always been affected by vibriosis, and it has a globally high mortality rate. Vibrio species' distribution is worldwide, and infections due to Vibrios are frequently encountered in hatcheries, but outbreaks are widespread in pond-reared shrimps (Lavilla-pitogo et al. 1998). Vibriosis is caused by a group of gram-negative bacteria, especially members of the family Vibrioceae.

Pores or wounds on the exoskeleton may also be a good source of entry of vibrios into the host system (Alday-Sanz et al. 2002). Bacterial penetration through the gills is typical because they are covered by a thin exoskeleton (Taylor and Janney 1992). The intestines which are not lined by exoskeleton also predispose the shrimp to Vibrio attack. The bacteria present in food, water and the environment can easily penetrate the system (Jayabalan et al. 1982). Over 280 shrimp hatcheries along the coastline of Thailand suffered a setback from seed production due to luminescent bacterial diseases caused by *V. harveyi*. Many researchers have reported vibriosis infections in penaeid shrimps and at least 14 species of *Vibrio* have been implicated as the cause of the bacteria diseases. They include *Vibrio harveyi, Vibrio* *parahaemolyticus, Vibrio alginolyticus, Vibrio splendidus, Vibrio mimicus, Vibrio vulnificus, Vibrio anguillarum, Vibrio damsela, Vibrio cambelli, Vibrio fischeri, Vibrio ordalli, Vibrio orientalis, Vibrio logei,* and *Vibrio mediterranei* (Eaves and Ketterer 1994; Lavilla-pitogo et al. 1998).

18.8.2 Combating *Aeromonas hydrophila*

The effect of *Astragalus membranaceus*, *Portulaca oleracea*, *Flavescent sophora,* and *A. paniculata* on immunomodulation in *Cyprinus carpio* was studied by Wu et al. (2000) and the effect of two Chinese medicinal herbs (*A. membranaceus* and *L. japonica*) on the immune response of Nile tilapia (*O. niloticus*) has been reported by Ardo et al. (2008).

18.9 Immunostimulation Act on Nonspecific Defense Mechanisms

Many immunomodulatory materials elicit the nonspecific immune response in crustaceans, which is regarded as the first defense line against challenging pathogens. The hemocytes present in hemolymph of crustaceans (granular and semigranular) and the prophenoloxidase (Propo) system are the primary immune mechanisms (Johansson and Soderhall 1989). Phenoloxidase being the terminal enzyme in this activation system, is activated by the cell wall components like lipopolysaccharides or peptidoglycans from bacteria and β-1, 3 glucan from fungi pattern recognition system (Smith et al. 1984).

18.10 Diet and Husbandry Practices Toward Immunoprophylaxis

The health of the animal is a reflection of its diet and farming practices. Domesticated animals resistant to specific pathogens can be reared in a proper environment; feed and health management form an integral part of aquaculture. The interaction between the pathogens, stress factor, microbiota and the immune response can be superimposed to design healthy feed and additives. Three main strategies, (a) combating pathogens by following hygiene practices, (b) providing a stress-free environment, and (c) efficient feed to optimize fish/shrimp immune system, need to be addressed by fish farmers to enhance the defense of fish and shellfish.

18.10.1 Husbandry Practices Followed for Better Prophylaxis

(a) Fighting the pathogens.
(b) Preserving the intestinal vital microbiota.

(c) Improving strict hygiene to avoid infection.
(d) Antimicrobial preparations and compounds from plant and animal origin.
(e) Improving the welfare of fish through healthy rearing module.
(f) Addressing interaction between neuroendocrine and immune functions in fish and shellfish.
(g) Improving water quality and bioremediation.
(h) Improving rearing conditions through carrying capacity approach.
(i) Fatty acid and antioxidants and their importance for health.

18.10.2 Probiotics and Prebiotics

The microorganisms in the cultural environment play a fundamental role in maintaining balance in the living creatures and in the environment by recycling natural resources. The utilization of microbial community in improving the health of the host and the environment has been investigated quite intensively. Microbes in probiotics have been extensively used globally in medicine, animal husbandry and agriculture.

18.10.3 Nutraceuticals

Nutraceuticals have been used in the last decade due to the increasing cost and side effects of therapeutic pharmaceutical agents. This concept of food for health and well-being was the primary basis of the Indian system of medicine for ages, gaining importance following the drawbacks of modern medicine systems. Stress is the common factor affecting humans (due to contemporary lifestyles) and plants and animals (due to farm intensification). These products containing dietary fiber, prebiotics, probiotics, poly-unsaturated fatty acids, antioxidants and other different types of herbal/ natural foods generally improve the antioxidant defense mechanism and innate immunity. Though the beneficial effects of nutraceuticals have been well established in humans and terrestrial farm animals, similar reports in aquaculture are scarce.

18.10.4 Genetic Selection

Genetic selection for disease resistance has been a milestone in agriculture/animal husbandry to meet the global hunger for food. Breeding for disease resistance is a well-established science in crop protection and has shown mixed success in the livestock sector. However, such programs for cultured aquatic species are limited. The ever-increasing cost of disease prevention and control has shifted the focus of health management from prophylactic and therapeutic intervention to genetic selection. Since the genetic selection programs are time-consuming, involving huge costs, the essential factor to be considered is the economic cost of the disease in question.

Selection for production traits can be easily achieved by measuring growth and survival. Selection for disease resistance is complicated due to the possibility of increased susceptibility to non-target pathogen and the loss of production traits as observed in dairy/beef cattle and poultry. Hence, such selection programs may ideally employ a straight forward multi-disciplinary approach with biologists, microbiologists, immunologists, epidemiologists, virologists, pathologists, environmental experts, and specialists in production systems management. At times, it may not be possible to select for resistance to certain diseases; a classic example being WSSV and TSV in shrimp. It is indeed possible to select for families having resistance to TSV whereas it is not possible to select for resistance to WSSV due to its low heritability.

18.11 CIBAstim, a Shrimp Immunostimulant: A Case Study

CIBASTIM, an immunostimulant product prepared from whole-cell *Vibrio,* has been proven to improve growth, survival and immunity in shrimps. This microbial product (MP) was obtained from heat-killed beneficial bacterial isolate developed and refined by ICAR–CIBA through ICAR- & NFDB-funded research projects. The product was extensively field-tested in different shrimp farming regions of India and the results indicated that it improved immunity in shrimps there by providing better growth, survival and production benefits. Field trial studies revealed substantial growth, survival (6–15%) and net profit returns upto Rs. 40,000/ha.

Benefits/uniqueness

- The product improves the efficacy of feed consumption, which results in higher growth and survival in shrimps and also improves immunity factors in shrimp.
- Field trial studies revealed that CIBASTIM fed shrimps exhibited uniform size, reduced tail rot incidence and other bacterial problems.
- The product maintains good water quality during culture operation.
- The product usage by shrimp farmers revealed higher production with the minimum cost of application.

The product has been commercialized and Technology transfer of CIBASTIM has generated scientific publications, revenue and employment to several entrepreneurs.

18.12 Biofloc-based Farming System

Beneficial bacteria are recognized as a useful tool to fight diseases, minimizing the rampant use of drugs and avoiding usage of antibiotics. Biofloc is a consortium of particulate matter formed predominantly by a biota of aerobic and heterotrophic bacteria, protozoa, microalgae, metazoan, exoskeletons, feces, and detritus. The diverse microbial community present in the biofloc system acts like natural probiotics and stimulates non-specific immune activity. The biofloc system acts as a natural source of probiotic bacteria (Ferreira et al. 2015; Panigrahi et al. 2019d, 2020b) which act as an immunostimulant source (Xu and Pan 2014, Anand et al. 2017; Kumar et al. 2018). This improves the immunity of fish/shellfish and reduces the prevalence of disease. The complex microbial interaction in the biofloc enhances the immune response of the cultured animals (Kim et al. 2014; Panigrahi et al. 2019a, b, c, 2020a, b). Biofloc technology can be seen more as a mechanism, which provides shrimp/fish a chance to keep the immune system active at all times as they get exposed to various microbes.

These microbes facilitate competitive strategies in complex communities, like competitive exclusion or harm the other microbes either by challenging or producing toxins (Panigrahi et al. 2018; Hibbing et al. 2010). Further, they can inhibit the proliferation of pathogens along with immunomodulation in the host.

18.13 Conclusion

Immmunoprophylatic approaches in aquaculture are to be followed in a most judicious way, exploring the beneficial role of vaccination and immunostimulant compounds. This is more important in present-day aquaculture as it is evolving toward sustainable intensification. We have standardized various eco-based, economically feasible models like Organic farming technology, Biofloc-based farming technology, Low input sustainable farming technology, Probiotic based seed, and grow-out production system, Bio-secured Zero water exchange shrimp Farming technology, Green water technology and Polyculture and Zero stocking model where a healthy system of farming is practiced often with immunoprophylactic measures. The sustainable intensification in aquaculture is undoubtedly feasible, thus increasing productivity through eco-friendly approaches to incorporate immunoprophylactic modules.

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