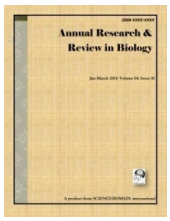

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Immunostimulants and Fish Culture: An Overview

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Authors' contributions

This work was carried out in collaboration between all authors. Author SMA designed the study and wrote the protocol, author EEM wrote the first draft of the manuscript. Author AHR managed the literature searches and author SMA managed the analyses of the study and revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aquaculture is one of the fast growing foods producing sector worldwide. Cultured marine, freshwater fish as well as shellfish species are major contributors to fish production and also increase the annual production. Fish farming constitutes unique and significant berth in industrial scale production worldwide. Due to intensive fish farming practices, infectious disease pose a major problem in aquaculture industry globally especially causing heavy loss to farmers. Fish disease is rarely a simple association between pathogen, a host fish and environmental problems, such as poor water quality or others stressors often contribute to the outbreak of disease. Various types of antibiotics, chemotherapeutic and vaccines are in use to control or prevent viral, bacterial, parasitic and fungal diseases. Unfortunately, vaccines are usually not able to confer protection on their own; especially those vaccines based on recombinant antigens or inactivated pathogens. Therefore, an alternative inexpensive and effective substitute is needed to enhance the activity of drugs. In this vista, use of adjuvants or immunostimulants is good remedy to increase the vaccine efficacy to control fish and shrimp diseases. Immunostimulants (IS) is a naturally occurring compound that modulates the pathogens via facilitate the function of phagocytic cells and also stimulate the natural killer cells (NK), complement, lysozyme and antibody responses of fish. The

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activation of the immunological functions by IS is associated with increased protection against both infectious and non-infectious diseases. In this review, we summarized the therapeutics importance of immunostimulants in fish culture and control of diseases development and progression.

Keywords: Immunostimulants; vaccine; natural killer cells (NK); complement.

1. INTRODUCTION

Fish culture is an important industry where various kinds of marine and fresh water fish are cultured with increase of annual production worldwide. Fish are usually cultured in enclosed spaces such as ponds or net cages and efforts have been made to increase productivity per unit area. Overcrowding tends to adversely affect the cultured fish health. The culture environment tends to produce poor physiological conditions for fish and increased susceptibility to infections. However, the use of chemicals and drugs in the aquaculture systems caused residual problems and poor quality in fish and its products. In the developed countries, food safety has become a major issue of concern [1,2] which should be implemented in other parts of the world.

Capture fisheries and aquaculture supplied the world with about 148 million tones of fish in 2010 (with a total value of US $217. 5 billion), where about 128 million tones was utilized as food for people and preliminary data for 2011 indicate increased production to 145 million tones, among those 131 million tones was destined as food. With sustained growth in fish production and improved distribution channels, world fish supply has grown dramatically in last five decades, with average rate of 3.2 percent per year in the period 1961-2009, out pacing the increase of 1.7 percent per year in the world's population. World per capita food fish supply increased from an average of 9.9 kg (live weight equivalent) in 1960 to 18.4 kg in 2009 and preliminary estimates for 2010 point to a further increase in fish consumption to 18.6 kg. Out of 126 million tones available for human consumption in 2009, fish consumption was lowest in Africa (9.1 million tones, with 9.1 kg per capita), while Asia accounted for two-thirds of total consumption, of 85.4 million tones (20.7 kg percapita), where 42.8 million tones were consumed outside China (15.4 per capita) [3].

The contribution of aquaculture to fish production is steadily increasing. Development of the aquaculture industry demand for fish production further provocate intensive fish culture [4]. The world aquaculture grown in last 50 years dramatically a production level of less than 1 million tones in early 1950s, with a value of US$ 78.8 billion [3]. This means that, aquaculture continues to grow rapidly than other animal food producing sectors. Aquaculture has rapidly developed from extensive system to semi-intensive, intensive and super intensive systems [2]. Fish diseases are a major constrain to aquaculture production. Fish disease is rarely a simple association between Pathogen, a host fish and environmental problems, such as poor water quality or other stressors often contribute to the outbreak of disease. Fish Disease**,** caused by pathogenic organisms present in the environment, they are mostly contagious and treatment may be necessary to control the disease outbreak. Therefore, intensive farming practices and infectious diseases induced major problems in aquaculture industry causing heavy loss to farmers. Several studies have been conducted on the modulation of fish immune system in order to prevent the outbreaks. Disease outbreaks are increasingly being recognized as a potential constraint on aquaculture production and trade and cause massive financial loss either through mortality or reduced meat quality, resulting in reduced profit margins [5]. The activation of the immunological functions is associated with increased protection against infectious diseases. Immunostimulant (IS) is a naturally occurring compound that modulates the pathogens. The different types of IS (e.g. glucan, chitin, lactoferrin, levamisole, vitamin B, C, E and growth hormone and prolactin) mainly facilitate the function of phagocytic cells. Several immunostimulants also stimulate the natural killer cells (NK), complement, lysozymend antibody responses of fish [6-8]. This review summarize the role of various types of immunostimulants in fish culture; in addition to providing the most up dated information in this regard.

2. IMMUNE SYSTEM OF TELEOST AND ITS RELATION TO DISEASES

Immunization has played an important role in the control of infectious disease. Both specific and

non-specific immune mechanisms are important element to protect fish against invading pathogens. In fish, the skin and mucus are the primary line of nonspecific defenses. When pathogens enter the body, cellular and humoral non-specific defense are mobilized. *Teleosts* have cellular defense system including phagocytic cells similar to macrophages, neutrophils and natural killer (NK) cells as well as T and B lymphocytes, this in addition to having various humoral defense components such as complement (classical and alternative pathways), lysozyme, natural hemolysin, transferring and C-reactive protein. Inflammatory mediators such as cytokines (Interferon, Interleukin 2, macrophage activating factors) are also discovered [6,9,10]. The innate defense includes both humoral and cellular defense mechanisms such as the complement system and the processes played by granulocytes and macrophages (Fig. 1) [7]. The innate immune system is the only defense weapon of fish where it play an instructive role in the acquired immune response and homeostasis.

The immune system is responsible to maintain the organism's homeostasis when invaded by foreign object or organisms [11]. Most pathogens and danger particles can be recognized by immune cells through expressed pathogen or danger–associated molecular patterns (PAMP or DAMPS, respectively), through non-self (e.g. allogenic or zenogenic cells) or missing major histocompatibility (MHC) class I molecules (some virus–infected target cells), presenting foreign non–self peptides of intracellular (through MHC class 1-e.g. virus–infected target cells) or extracellular (through MHC class II–e.g. from bacteria) origin. Specialized immune cells of the innate and adaptive responses are involved to eliminate invaders directly or by destroying their ability to replicate (e.g. virus–infected cells) [12].

In order to decrease the environmental stress and thus reduce the prevalence of the disease and the use of chemotherapy, fish culture management techniques are designed. Diseases are among the primary limiting factors for the growing of aquaculture, where bacterial infections are responsible for heavy mortality in both wild and cultured fish. The majority of bacterial diseases in fish are caused by short, Gram-negative rods belonging to the families *Enterobacteriaceae*, *Pseudomonadaceae* or *Vibrionacea*. They cause speticaemic and ulcerative disease conditions. The long Gram–negative Myxobacteria of the family cytophagacea that caused heavy mortality in fish stock in addition to acid–fast–Gram positive micro-organisms of less frequent encountered as dangerous for fish stocks [9]. The outbreaks of diseases are a limiting factor in fish culture. Several antibiotics, chemotherapeutic agents as well as some immunostimulants have been used at many fish farms and hatcheries to prevent viral, bacterial, parasitic and fungal diseases [6]. Despite the partially successful preventive measures including sanitary prophylaxis disinfection, antibiotics, vaccines and chemotherapy for the last 20 years, an estimated huge annual economic loss due to diseases in aquaculture is reported [13].

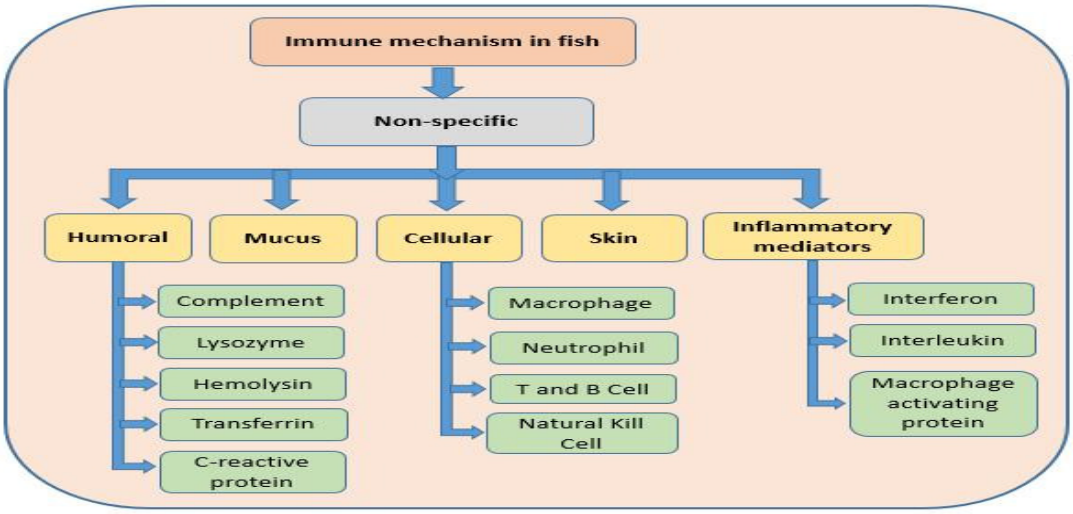

Fig. 1. Non-specific immune mechanisms in fish

The use of chemicals to control bacterial or parasitic populations brings lots of disadvantages. The application of antibiotics and other chemicals to pond culture is also environment and culture contamination as well as impairing the growth of fish [4,14]. On the other hand, the development of the fish aquaculture industry and increasing demand for fish culturing further provocate intensive fish culture, where the fishes at high risk of infectious diseases caused by bacteria, virus, parasites and fungi. Use of expensive chemotherapeutics and antibiotics for controlling disease have widely been criticized for their negative impacts like residual accumulation in the tissue, development of the drug resistance and immune-suppression, thus resulting in reduced consumer preference from food fish treated with antibiotics [4]. Moreover, as a few approved chemotherapeutic agents are available for use in food fish because of growing concern for consumer liability and for accumulation of substance in the environment together with concerns arising regarding the increase of the antibiotic-resistance strain of bacteria in the surrounding areas of aquatic farms, additional methods are needed to control these fish diseases [15]. The best way to overcome the disease problems in a system is through effective management practices, i.e. management of stock, soil, water, nutrition and environment, this in addition to the use of immunostimulants. The interest in using immunostimulants is increasing because of its safety, availability and cost efficiency together with the frequent outbreaks of bacterial, viral or parasitic diseases that are limiting factors in culture at many fish farms, hatcheries and aquaculture stations.

3. IMMUNOSTIMULANTS

Immunostimulant “IS“ is a natural occurring compound that modulates the immune system by increasing the host's resistance against diseases [7]. IS comprise a group of biological and synthetic compounds that enhance the non-specific cellular and humoral defense mechanism [4]. It may be chemical, drug or naturally occurring compound that elevates the non-specific defense mechanisms or the specific immune response of the host and may be given alone to activate non-specific defense mechanisms as well as heightening a specific immune response. Research on immunostimulants is being intensified particularly in the areas of acquired immunodeficiency syndrome (AIDS) and cancer research. There is an increasing demand to prevent and control a broad spectrum of fish diseases occurring in aquaculture systems without recourse to antibiotics and chemotherapeutics. Application of immunostimulant alone or together with vaccination has emerged as one of the more promising approaches to prevent or control fish diseases [16]. Although, oral administration is the most practical method for delivery of immunostimulants, however, the effects of long–term oral administration of IS are still unclear. The use of IS, as dietary supplements, can improve the innate defense of animals providing resistance to pathogens during period of high stress, such as grading, reproduction, sea transfer and vaccination. The application of immunostimulants is increasing currently because of the frequent outbreaks of fish diseases in culture systems and hatcheries. IS receiving major attention and claiming success in rendering enhanced protection in fish under experimental or practical conditions comprise six major types (Fig. 2), independent of their mode of action [16,17]. The immunostimulants can be divided into several groups depending on their sources and summarized in Table (1).

4. ADVANTAGES OF IMMUNOSTIMULANTS

1 - Immunostimulants are more widely and successfully applied to improve fish welfare, health and production [18].
2- It facilitate function of phagocytic cells, increase their bactericidal activities and stimulate natural killer cells, complement system, lysozyme activity and antibody response in fish and shellfish which confer enhanced protection from infectious diseases [13].
3 - It consider as enhancer to the non-specific immune responses along with the improvement of aquatic environmental quality [19].
4 - Immunostimulants are valuable for the control of fish diseases by enhancing both specific and non-specific defense mechanisms [20].
5 - Immunostimulants increase the immunocompetency and disease resistance of fish.
6 - Many IS have been developed to improve immunity of domestic animals.
7 - Some immunostimulant (levamisol) enhanced phagocytic activity and myeloperoxidase activity in neutrophils,

increased leucocytic number and serum lusozyme level [21].

8 - Immunostimulants is often necessary to increase vaccine efficacy [22].

9 - Immunostimulants are highly dependent on the receptors of the target cells recognizing them a potential high risk molecules and triggering defense pathways [23].

10 -Immunostimulants signaling pathways of the toll–like receptors (TLRs), a common adapter My88 (Myeloid differentiation factor 88) that was first characterized as an essential component for the activation of innate immunity by all TLRs (TLR1-9) [7].

11 - Immunostimulats stimulate pro-inflammatory cytokines TNF-∞, IL-1 and IL-6 up regulate the acute phase response with the production of complement components, C-reactive protein, ceruloplasmin, metallothionine etc. These cytokines also increase the epithelial response to pathogens and induce expression of adhesion molecules on vascular endothelium enhancing the diapedesis of leucocytes [23].

12- Immunostimulants have the potential to elevated the innate defense mechanisms of fish prior to exposure to a pathogen and improve survival following exposure to a specific pathogens.

- ❖ ß-glucans, FK-565 (improved bacterial resistance).
- ❖ Levamisole, CPG, ssRNA, MDP, ß-glucan and LPS improved resistance against viral infections.
- ❖ ß-glucans, MDP and Levamisole improved resistance against parasitic infections.

13 - The major responses in both vivo and vitro in fish treated with an immunostimulants are recorded (Fig. 3) [24].

14 -The use of IS for prevention of disease in fish is considered as an attractive and promising area in the field of aquaculture [25,26].

15 - IS represent an alternative and supplementary treatment to vaccination. Also have additional effects such as growth enhancement and increase in the survival rates of the fish under stress [27].

16 -IS as food additives caused an enhanced extracellular respiratory burst activity, phagocytosis and extracellular burst of blood leukocytes [6].

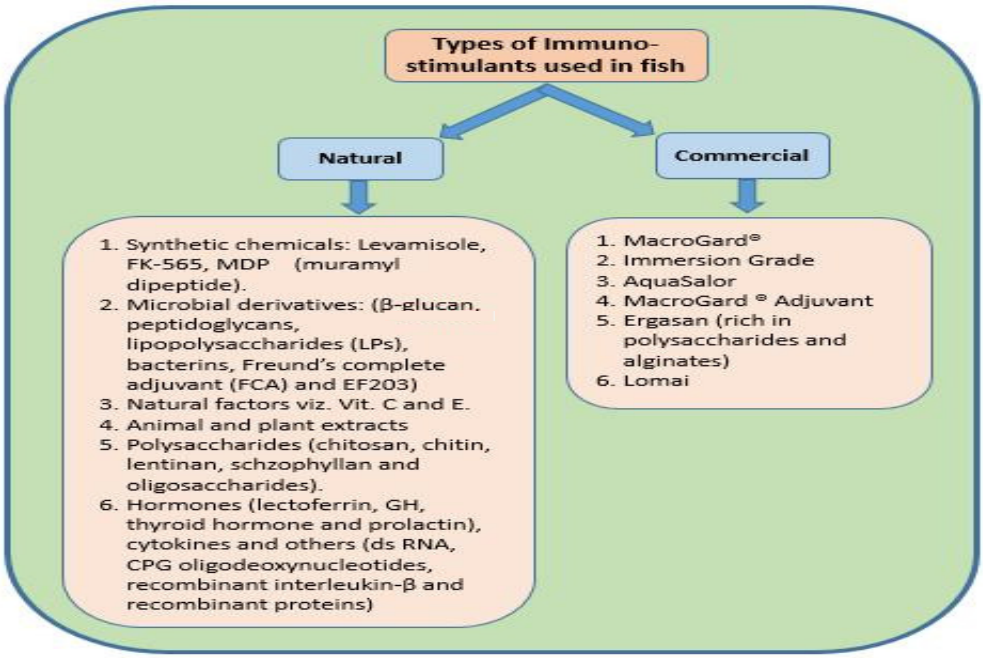

Fig. 2. Type of immunostimulatns used in fish

Table 1. Types of Immunostimulants in fish

Source	Types
I-Synthetic Chemicals:-	1-Levamisole 2-FK-565 3-MDP (Muramyldipeptide).
II-Biological Substances:- II-1-Bacterial derivatives:-	1- ß-glucan 2- Peptidoglucan 3- FCA 4- EF203 5-LPS (Lipopolysaccharides) 6- Clostridium butyricum 7- Chromobactersterohalis Vibrio anguillarum cells
II-2-Polysaccharides:-	1-Chitin 2-Chitosan 3-Lentinan 4-Schizophyllan 5-Oligosaccharide
II-3-Animal plant extracts:-	1- Ete(Tunicate) 2- Hde(Abalone) 3- Fireflysquid 4-Quillajasaponin (Scaptree) 5- Glycyrrhizin(licorice)
II-4-Nutritional factors:-	1-VitaminC 2-VitaminE.
II-5-Hormones, Cytokines and Others:-	1-Lactoferrin 2-Interferon 3-Growthhormone 4-Prolactin

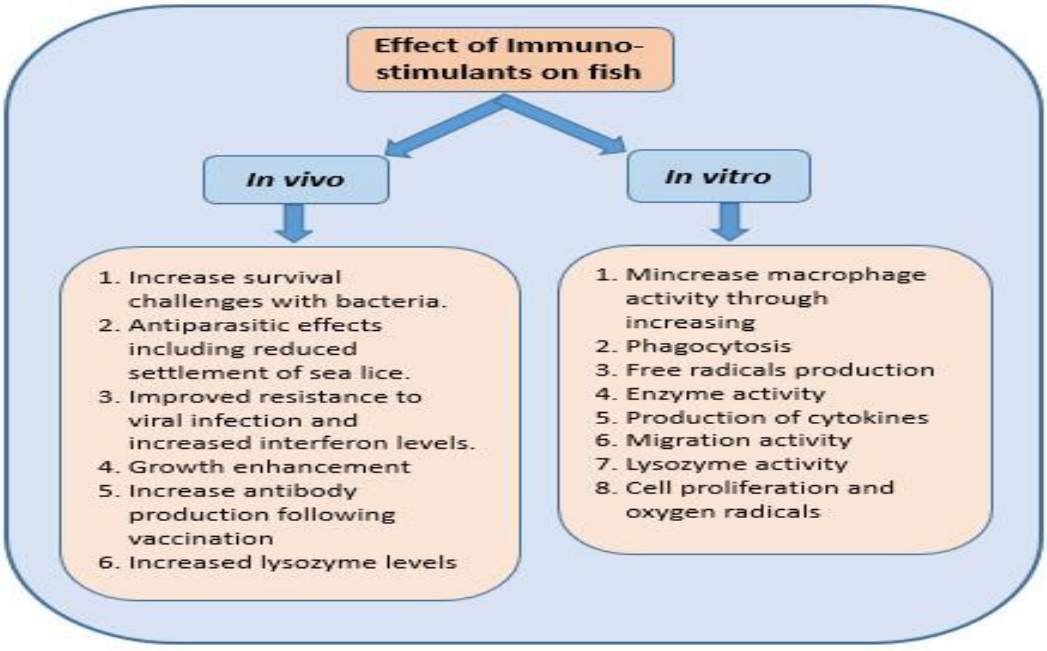

Fig. 3. Major responses in fish treated with an immunostimulants

5. DISADVANTAGES OF THE IMMUNOSTIMULANTS

1 - High cost.
2 - Limited efficiency upon parentally administration [6].
3 - Are not effective against all diseases.
4 - Over doses of IS induce immunosuppression [1].
5 - In few cases, IS failed to render enhanced protection or increase in immunity [16].
6 - Immunostimulants are used with success in aquaculture against many pathogens, but the ability to improve innate resistance to columnaris disease has not been studied [28].

6. THE USES OF DIFFERENT TYPES OF IMMUNOSTIMULANTS IN FISH CULTURE

1. Levamisole, an anthelmintic used for treatment of nematodes in man and animals (Synthetic Chemicals): Levamisole is enhancing metabolic and phagocytic activation of neutrophils and increase the number of phagocytes and leucocytes and the level of Lysozyme [29]. In Coho salmon, it increase resistance against *Aeromonas salmonisida* infection, In carp, it enhance phagocytic activity, myeloperoxidase activity in neutrophils, increase leukocytes number and serum lysozyme levels [30-32].
2. FK-656 (Hepatonoyl-y-glutamyl-(L) mrso-diaminopimely–(D)–alanine is a peptide Related to lactoyl tetra peptide (Synthetic Chemicals). FK-656 has been shown activity against microbial infection. It increases the resistance of Rainbow trout against *A. salmonicida*. In Yellow tail, it elevate humoral antibody titers and splenic producing Antibody [33].
3. MDP (Muramyl dipeptide) N-acetyl-muramyl-L-alanyl-D-Isoglutamine), derived from Mycobacterium (Bacterial derivatives). It activate macrophages, B lymphocytes and alternative pathway of complement [30]. MDP increase the phagocytic activities, respiratory burst and migration activities of kidney leucocytes as well as resistance of the fish to *A. salmonicida* challenge [34].
4. LPS (lipopolysaccharide) is a cell wall component of Gram-negative bacteria (Bacterial derivatives). LPS stimulate B cell proliferation and enhance macrophage phagocytic activity [35].
5. FCA Freund,s Complete adjuvant, a mineral oil adjuvent containing killed *Mycobacterium* butyricum (Bacterial derivatives). FCA enhances immune responses and increase the efficacy of vaccination in fish [36]. It increase protection against *A. salmonicida* challenge (Coho salmo), protect also against *A. hydrophila* and *Vibrio oradalii*. It activate macrophages in fish and increase respiratory burst, phagocytic and NK cell activity of leucocytes and protection against *V. anguillarium* infection [37].
6. Vibrio Bacterin (Bacterial derivatives). It increase protection in Rainbow trout against *V. anguillarum* [38]. It has immunostimulant effects in Kuruma prawns and black tiger [39].
7. Glucan (peptide-glucan-ß-1,3, glucan). It increase protection against *V. anguillarum* and *V. salmonicida*. It induce significantly protection against Furunculosis [40]. Glucan also enhances the lysozyme activity in Atlantic salmon, Rainbow trout and Turbot [41] and increase production of super oxidation by macrophages and reduced mortality against *E. ictaluri* [42].
8. Animal Extracts from marine tunicate, glycoprotein fraction of water extract from abalone, Hiliotis discus hannai (Biological Substance). It enhance killing of tumor cell and inhibited tumor growth *In vivo* [43] and stimulate phagocytosis and increased survival of eel against *A. hydrophila* [44]. It increase production of superoxide anion, potential killing activities by macrophages and lymphoblastic transformation of lymphocyte *in vitro* [45].
9. Glycyrrhizin is a glycosylated saponin, containing one molecule of glycyrretinic acid (Animal product). Glycyrrhizin has anti-inflammatory band, anti-inflammatory activities, mediated by immunomodulatory activities [46]. It increase protection against *E. seriola* infection, though lysozyme activity of blood and phagocytic activates of macrophages. It enhance respiratory burst activity of macrophages and the proliferative responses of lymphocytes from Rainbow trout [47].
10. Dietary Vitamin C (Nutritional Factor), is essential for growth and for several physiological functions in most fishes [48]. High levels of vitamin C increase resistance of channel catfish against

E. tarda and *E. ictaluri* [49], *V. anguillarum*, IHN and *Ichthyophthrius multifilis* in rainbow trout [50]. Also it was increased complement activates in catfish or Atlantic salmon [49].

11. Vitamin E (Nutritional Factor), enhances both humoral and cellular defenses in mammals [51]. Specific and cell mediated immunity and macrophage phagocytosis in rainbow trout. It increases phagocytic indices and superoxide anion production by leucocytes.
12. Other Dietary components (Such as lipids, other vitamins, trace elements etc.). They increase the immune response in fish [52].
13. Hormones regulate the neuroendocrine functions, directly effect immunocompetent cells (macrophages, lymphocytes, NK cells) [53]. Exogenous growth hormone (GH) has mitotic activity on lymphocytes and activates NK cells [54]. GH increase protection against *V. anguillarum* in Rainbow trout [54].
14. Cytokines are polypeptides or glycoproteins. Cytokines act as modulators in the immune system. Cytokines such as Interleukin-2 reported as immunostimulants [55].
15. Lactoferrin consists of a single peptide chain with a molecular weight about 87000 and possessing two-Fe-binding sites per molecule. Lactoferrin regulates free radicals production by macrophages, granulocytes and neutrophil leucocytes [56]. Enhance phagocytic activity and increase production of superoxide anion by macrophages and demonstrated high resistance to *V. anguillarum* infection. Elevated secretion of body mucus and resistance to Cryptocaryon irritants infections [54].

7. UP TO DATE

Fish farming is developing from extensive to intensive high industrial scale production. Like man, fish relay on both specific and non-specific mechanisms to protect themselves against invading pathogens. When pathogens enter the body, cellular and humoral non-specific defense are mobilized. Phagocytosis is one of the main mediators of non-specific immunity to pathogens including bacteria, viruses and parasites in fish. The outbreaks of diseases are limiting factors that hindering development in the fish industry. Bacterial diseases are responsible for heavy mortality in both wild and cultured fish. Antibiotics are used to control bacterial infection but may result in development and spread of antimicrobial–resistance bacteria and resistance genes and occurrence of antimicrobial residues in fish tissues. This induces negative impact on human health, fish performance and the environment. Alternative approach employed recently to control bacterial infection in fish is the use of vaccines. However, vaccines are not available in many countries. The concept of natural. (Immunostimulants) and biological (Vaccines, Probiotics) disease control, particularly using non pathogenic agents, has received widespread attention during the last decade [57].

Vaccines have significant positive impact on the reduced usage of antibiotics. Also, the author described vaccines administrated regimes including immersion, oral and injection vaccination. Future trends for inactivated-live attenuated–and DNA vaccines will be considered [58]. Vaccines are not able to confer protection on their own; especially those vaccines based on recombinant antigens or inactivated pathogens. Therefore, the use of adjuvants or immunostimulants is often necessary to increase the vaccine efficacy.

It is well known that, the innate immune system can be triggered by many immunostimulants such as levamisole, glucan, Vitamins (C&E), lipopolysaccharides, Growth hormones, kitosan, etc. Although immunostimulants have several advantages but also have some disadvantages such as high coasts, limited effectiveness and in overdosing may cause immunosuppressive. Overall, the use of immunostimulants for prevention of diseases in fish is considered as an attractive and promising area in the field of aquaculture. Many immunostimulating substances have been developed to improve immunity of domestic animals, although their exact mode of action and effects are not clearly defined and they are now widely used in feed industry such as endotoxin, glucose, vitamin A, E and C Chromium, Selenium and Artin M as immunostimulant [59]. CPG oligodeoxynucleotides (ODNs) used as a novel adjuvant to activate the innate immune system through the interaction with Toll-like receptor (TLR9) in mammals. It suggested that, the interactions of CPGODNs with LvToll1 and LvToll3 as well as the mature of endosomes in the hemocytes of shrimp (*Litopenaeus vannamei*) were indispensable for motive and triggering the immune responses by CPGODNs

and the results provided a foundation for the application of CPGODNs as the novel immunostimulant in aquaculture [60]. Up-to-date information has been proposed that the delivery of immunostimulants as a dietary supplement to larval fish (fry and fingerlings) could be considerable benefit in enhancement the innate defense mechanisms (such as complement system and the processes played by granulocytes and macrophages). The discovery of the Toll-like receptors (TLRs) in many mammalian species has partly shed light on the mechanisms that different imuunostimulants induced. Functional analysis of mammalian TLRs has revealed that, they recognize specific patterns of microbial components that are conserved among pathogens, but are not found in mammals. In signaling pathway via TLRs, a common adapter MyD88 (myeloid differentiation factor 88) was first characterized as an essential components for activation of innate immunity by all TLRs (TLR1 -9) [9,61].

Research group concluded that black seeds, garlic and commercial Biogen as an immunostimulants improved the health conditions, the quality of the fish life, the growth and enhanced the immunity as well as enable the fish to overcome the stress induced by the artificial infection [62]. Also, concluded that, the garlic enhanced or initiate the immune response of tilapia fish besides its improvement to the quality life and shelf life of the cultured tilapia fish [8].

Ginger, Zingiber officinale, control the experimental infection of rainbow trout (*Onchorynchus mykiss*) with *Aeromonas hydrophila*. At 0.5 g ginger per 100 g of feed, reduce mortalities to 0% compared with the control (64%). Moreover, there was a significant increase in growth, feed conversion and protein efficiency. Also, there was proliferation in the number of neutrophils, macrophages and lymphocytes and enhanced phagocytic, respiratory burst, lysozyme, bactericidal and antiprotease activities compared with the control [63].

Investigators showed the effect of Echinacea (*Echinacea purpurea*) on the growth rate and disease resistance of the Nile tilapia cultured in earthen ponds [64]. Significant increase in body weight gain, specific growth rate, hemacrit values, lysozyme activities and total leukocytic counts, especially lymphocytes and eosinophils was noticed when compared with the control group. Moreover, Echinacea purpurea and garlic (*Allium sativum*) used as immunostimulants and improved the body weight, the survival rate of Nile tilapia (*Oreochromis niloticus*). Also, increased the resistance against *A. hydrophila* and other bacterial infections [65,66]. Earlier researcher and reported that, vitamin C has a potential effect as immunostimulant of less expensive costs and a promising supplemented dietary in fish aquaculture [67] and mentioned that, levamisole (150 mg/kg diet) increased lecocytic cell count, macrophages phagoctosis, antibody titer, total production of protein and globulin in catfish indicating its efficiency as immunostimulant to improving immune response of catfish (*Clarias gariepenus*) to infection [66].

Probiotics are usually live microorganisms which when administrated in adequate amount confer a health benefits on host. Nowadays, probiotics are also becoming an integral part of the aquaculture practices to obtain high production. The common probiotics used for aquaculture practices include lactobacillus, lactococcus, leuconostoc, Enterococcus, carnobacterium, shewanella, Bacillus, Aeromonas, Vibrio, Enterobacter, Pseudomonas, Clostridium and *Saccharomyces* species [68]. Some of the beneficial effects of probiotic consumption include: The improvement of intestinal tract health by means of regulation of microbiota and stimulation development of the immune system, synthesizing and enhancing the bioavailability of nutrients, reducing risk of certain disease [69].

Another experiment by Aly, [8] discovered that, using super biobuds as a probiotic improved the Nile tilapia body gain, the survival and enhanced the resistance against *Aeromonas hydrophila* and *Pseudomonas flourenscens* infection [70]. Survival rate of *Oreochromis niloticus* was highest with fish fed diets supplemented with *B. pumilus*, followed by mixture of ptobiotics (*B. subtilis* and *B. pumilus*) and then *C. freundii*. *B. pumilus* appears promising as a probiotic for controlling *A. hydrophila* infection among *O. niloticus* [71]. Aly et al. [62] stated that, *Bacillus pumilus* (106 and 1012 g-1feed diet) and commercial product called Organic green TM (1 and 2 kg-1feed diet) when given for 2 months showed potential effect as a probiotics to enhance immune and health status and improved disease resistance of *O. niloticus* against challenge infection by *A. hydrophila* [72]. Also, Aly et al. [62] used *Bacillus subtilis* and *Lactocbacillus acidophilus*, as potential probiotics and found that, they

enhanced the immune response, improved the hematocrit values, increased phagocytosis and increased resistance against infection in Tilapia aquaculture as a promising way to increase the productivity of Tilapia fish [72]. Biswas et al. [73] recorded the role of *Lactobacillus paracasei* (06Tca22) "cytokine" as updated immunostimulants in Japanese puffer fish. It gave a high protection against *V. harveyi* infection, enhance phagocytosis and increased lysozymal enzymes.

8. CONCLUSION

The outbreak of diseases is a limiting factor that hindering the development of the fish industry. Like man, fish relay on both specific and non-specific mechanisms to protect themselves against invading pathogens. In fish, the primarily lines of non-specific defense are skin and mucus. When pathogens enter the body, cellular and humoral non-specific defense are mobilized. Phagocytosis is one of the main mediators of non-specific immunity to pathogens including bacteria, viruses and parasites in fish. It is well known that, the innate immune system can be triggered by many immunostimulants such as levamisole, glucan, Vitamins (C&E), lipopolysaccharides, Growth hormones, kitosan– etc. Several immunostimulants also stimulate the natural killer cells (NK), complement, lysozyme and antibody responses of fish. The activation of the immunological functions by immunostimulants is associated with increased protection against both infectious and non-infectious diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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