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Prophylactics in shrimp aquaculture health management: A review

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Abstract
A shrimp farm is an aquaculture business contributes more than 50 per cent of the total shrimp exports from India for human consumption. Shrimp aquaculture is affected by the outbreak of various microbial diseases which causes severe mortality in shrimp farming industry. Viral and bacterial diseases are of major concern in shrimp farming systems. Improper use of antibiotics and chemical drugs in aquaculture is accompanied with the potential negative impacts on the environment as well as human health and is also ineffective for many pathogens, particularly virus. Although the effective control of infectious viral diseases without chemicals is important in the cultivation of aquatic organisms. Therefore, advance research is required to standardize prophylactic and control measures in shrimp aquaculture in order to reduce the risk of adverse effects of new disease introduction. Thus, all the information in this review relates to prophylactics that protect the cultivation shrimp industries.

Keywords: Aquaculture, Disease, Management, Prophylactic, Shrimp

1. Introduction

Shrimp farming is an aquaculture enterprise involving cultivation of shrimp particularly marine shrimp in seawater or brackish water for human food. Shrimp aquaculture has changed from extensive, traditional, small-scale practices to an intensive, scientific, large scale global industry in the last three decades. Technological advances in seed production, feed preparation and brood stock development have helped the change in shrimp farming. India's cultured shrimp production in the financial year 2014-15 was estimated at 426,500 MT while wild shrimp production was estimated at 450,000 MT. The major marine wild species of prawns and shrimp are White prawn (*Penaeus indicus*), Tiger prawn (*Penaeus monodon*), Pink shrimp (*Metapenaeus dobsoni*), King prawn (*Metapenaeus affinis*) and Marine shrimp (*Paraenaeopsis stylifera*). In the financial year 2014-15, the production of white leg shrimp (*Penaeus vannamei*) rose to around 353,000 MT, which was 81 percent of the total shrimp production in India [1]. FAO have reported that India has moved to the leading exporter in international shrimp trade during the first nine months of 2016, followed by Ecuador, Thailand, Indonesia and China. Compared with the same period in 2015, exports from India increased by 11.6 %, totalling 315,400 tonnes.

Major constraints of shrimp aquaculture are improper farm management, lack of technical services, lack of good quality shrimp seed, lack of infrastructure facilities, bad weather condition, conflict among the local peoples and disease risk. Contrasting terrestrial animals and plants, aquatic animals require more attention in order to monitor and manage their health. Diseases occur in aquaculture systems due to the complex interaction of three components, the farmed animal (host), the disease-causing organisms (pathogens) and the surroundings (environment) [2, 3]. The intensive shrimp aquaculture is associated with serious disease outbreaks that are responsible for the production loss either due to the reduction of growth rate or mortality caused by various pathogenic microorganisms. Pathogen control in aquatic animals is highly challenging as they are transmitted rapidly through water unlike pathogens of terrestrial animals. High stocking density is one of the major factors aiding easy transmission of pathogens. FAO reported that disease occurs in all systems, from extensive to intensive. The factors affecting host's susceptibility to diseases are stocking density, innate susceptibility and immunity [4]. Most common pathogens causing infectious diseases are bacterial pathogens, contributing over 50%, followed by viruses, parasites and fungi [5]. FAO has been reported as the estimated production of 2 million tonnes of farmed shrimp in the year 2015 was reduced due to disease problems in China, India, Ecuador, and Viet Nam [6].

Common pathogens in shrimp aquaculture

Bacteria

Bacteria cause problems ranging from growth retardation to sporadic mortalities and mass mortalities. *Vibrio* spp are the most important bacterial pathogens observed in shrimp hatcheries, grow-out ponds and sediments (eg. *Vibrio harveyi, V. spendidus, V. penaecida, V. anguillarum, V. parahaemolyticus* and *V. vulnificus*) [7, 8]. Among these bacterial species, *V. parahaemolyticus* was consistently isolated from EMS/AHPNS-infected shrimp. Filamentous bacteria such as *Leucothrix mucor, Thiothrix sp, Flexibacter sp, Flavobacterium* and *Cytophaga* sp also caused infection in penaeid shrimp larvae [9].

Virus

Virus continues to be one of the most disease problems faced by the shrimp farming industry worldwide. About 20 viruses have been recognized as causative agents of diseases in shrimp [9]. These viral pathogens include white spot syndrome virus (WSSV), yellow head virus (YHV), hepatopancreatic parvovirus (HPV), Taura syndrome virus (TSV), laem-Singh Virus (LSNV), infectious myonecrosis virus (IMNV), infectious hypodermal and haematopoietic necrosis virus (IHNNV) and monodon baculovirus (MBV) [10]. WSSV is known to have a wide host range and infects most commercially important species of penaeid shrimps, leading to complete mortality within 3-7 days of onset of infection. Compared to WSSV, other viral diseases are slow killers causing moderate mortality, reduced growth rate and high feed conversion ratio of the cultured shrimps.

Fungi

About 500 fungal species have been isolated from marine and estuarine environment. *Lagenidium callinectes* and *Serolpidium* spp. are found to affect larval stages of shrimp. The protozoea and mysis stages of shrimp are affected with clinical signs of lethargy followed by mortality. Fusariosis by *Fusarium* spp. and black gill disease affect all developmental stages of penaeid shrimp. *Fusarium* spp (*F. solani* and *F. moniliformae*) are opportunistic pathogens that may lead to high mortalities (90%) [10].

Parasites

Protozoan diseases like ciliate infestation reported from the Indian penaeid prawns. Disease caused by these parasites is known as cotton or milk shrimp disease has been reported in the in the natural population of penaeid prawn in India, The metazoan parasites of penaeid prawns comprise of helminth parasites such as digenetic trematodes, cestodes and nematodes, and bopyrid isopods. A number of parasites have been found to be infecting cultured shrimp at all developmental stages. Particularly, protozoa such as *Zoothamnium, Epistylis, Vorticella, Anophrys, Acineta, Lagenophrys* and *Ephelota* are encountered as external parasites. Ciliates such as *Paranophrys* spp and *Parauronema* sp. may also cause mortalities in larvae and juveniles of shrimp. Microsporidia such as *Agmasoma sp, Microsporidium sp* and Gregarians which are endoparasitic protozoa such as *Nematopsis spp, N. litopenaeus, Paraphioidina scolecoide, Cephalobolus litopenaeus, C. petiti* and *Cephaloidophoridae stenai* are found in shrimp aquaculture [10].

Prophylactics in shrimp aquaculture

Different chemicals, drugs and biological products are used for preventing diseases in farmed aquatic animals. Indiscriminate use of the chemical and biological products is one of the major concerns, considering the potential negative impacts on the environment and human health. Chemicals or antibiotics used in preventing the disease in shrimp aquaculture could lead to the development of resistance in microorganisms [11]. Antibiotics and other therapeutic agents are widely used as common therapies in post-infection practice. Immunization, a prophylactic measure and a protective management tool is designed to aid in the prevention of disease [11]. The World Health Organization (WHO) recommended the preventative (prophylactic) approaches to disease management rather than costly post-effect treatments [12]. The prophylactic measures for disease prevention in aquaculture are the use of prebiotics, probiotics, vaccines and immunostimulants are mentioned as following.

Prebiotics in shrimp aquaculture

Prebiotics are non-digestible food ingredients that beneficially affecting the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improving host health [13]. The prebiotics is typically carbohydrates derived from plant or yeast origin. Among many food ingredients known the inulin, fructooligosaccharide (FOS), mannan oligosaccharide (MOS) and isomalto oligosaccharide (IMO) showed prebiotic characteristics [14]. Prebiotics are selectively fermented by *Bifidobacteria*, *Lactobacillus* and *Bacteroides* [14] and they increase the uptake of glucose [15] and bioavailability of trace elements [16] when included in the Pig's diet.

Use of prebiotics and their health promoting effects have been proven by many studies in human and terrestrial animals [17, 18]. The benefits are presumably accrued due to the dietary supplementation of citrus by-products (CB) formed by its fermentation by gut bacteria, which help the growth of beneficial bacteria while suppressing the growth of those harmful (Lee *et al*., 2013) [19]. Compounds like short chain FOS (scFOS) and MOS would also act as immune-stimulating saccharides by directly activating innate immune responses and thus promoting health of the shrimp. Certain compounds like β-glucan, which are currently used as nonspecific immunostimulants, are not categorized as prebiotics in shrimp because crustaceans can digest glucan and use it as an energy source [20].

Inulin is found in a variety of edible grains, fruits and vegetables such as wheat, onions, leeks, garlic, asparagus, artichokes and bananas belongs to a class of dietary fibres known as fructans [21]. Inulin promotes the growth of the gut microbiota in the colon of endothermic animals [22, 23]. In *Penaeus vannamei,* dietary supplementation of inulin decreased the prevalence of WSSV and increased the phenoloxidase activity [20].

Fructooligosaccharide (FOS) is a medium chain of β -D-fructans in which fructosyl units are bound by β - (2-1) glycosidic linkages and attached to a terminal glucose unit. FOS was found to be fermented by certain bacteria such as lactobacilli and bifidobacteria [24, 25]. Dietary supplementation of FOS has been shown to enhance the growth rate of some aquatic animals such as Atlantic salmon, hybrid tilapia, turbot larvae and soft-shell turtle [14]. Evaluation of dietary effects of short-chain FOS in two sets of *L. vannamei* juveniles (0.17 g versus 7.5 g) showed that scFOS improved specific growth rates and feed conversions in younger shrimp juveniles, and significantly affected the counts of *V. parahemolyticus, Aeromonas hydrophila*, *Lactobacillus* sp. and *Streptococcus faecals* in shrimp's gut [26] which was also supported by Li *et al*., [27].

Isomaltooligosaccharides (IMO) is a mixture of isomaltose, isomaltotriose, panose, isomaltotetraose, etc. [28]. Experiments on the effects of oral administration of probiotics (*Bacillus licheniformis* and *B. subtilis*) in combination with prebiotic isomaltooligosaccharide (IMO) on the intestinal microflora and immunological parameters of *Penaeus japonicus* and its resistence against *V. alginolyticus* [29] showed that shrimp fed the diet with both *Bacillus* probiotics and IMO produced significantly higher immune parameters such as phenoloxidase activity, lysozyme activity and nitric oxide synthase activity and superoxide dismutase activity. The study also indicated significant lower cumulative mortality after challenge with *V. alginolyticus* [29]. Dietary probiotic *Bacillus* OJ in combination with isomaltooligosaccharides have produced significantly positive synergistic effects on shrimp immune responses and disease resistance WSSV [30].

Mannan oligosaccharides (MOS) are glucomannoprotein complexes derived from the cell wall of yeast (*Saccharomyces cerevisiae*) [31]. In the past few decades, several studies have been carried out to investigate the application of MOS and their dietary beneficial effects in shrimp aquaculture. All those studies were demonstrated as improving shrimp growth, feed efficiency, protection from the pathogen, intestinal microbiota modulation and functionality, etc. [20]. MOS could affect the bacterial attachment in the intestinal tract, and a C-type lectin possessing mannose receptor was found in shrimp [32]. MOS was found to enhance the growth performance, feed conversion and survival rate of shrimp (*Penaeus semisulcatus*) at post-larval stage when fed on a diet containing 3 g MOS kg^{-1} [33].

Probiotics in shrimp aquaculture

Probiotics are live microorganisms that have a beneficial effect on the host by modifying the microbial community associated with the host, ensuring improved use of the feed or enhancing its nutritional value, enhancing the host response towards disease, or improving the quality of its ambient environment [34, 35]. The gastrointestinal microbiota of shrimp is always dependent on the external environment, due to water flow passing through the digestive tract [36]. Due to this, probiotics development mainly depends on isolating and selecting strains from the culture environment of aquatic animals. The microbes that were effectively used include vibrios, pseudomonads, lactic acid bacteria, *Bacillus* spp. and yeasts. Probiotic bacteria can also directly uptake or decompose the organic matter or toxic material in the water and sediment thereby improving the quality of water [37]. The water contains the excreta of shrimps, uneaten food materials, planktons and other organic materials can be converted to CO^2, nitrate and phosphate by the addition of beneficial bacteria into it [38].

Probiotics influence the immune system of the fish, shrimp, and other aquatic species [35]. Shrimp has a poorly developed immune system compared to vertebrates and the probiotics were known to play an important role in the enhancement of immune response in shrimp. The probiotic bacteria *Lactobacillus plantarum* incorporated in the diet has reported to enhance the immune responses and gene expression in white shrimp, *L. vannamei* [39]. The bacteria enhanced the phenoloxidase (PO) activity, prophenoloxidase (ProPO) activity, respiratory bursts, superoxide dismutase (SOD) activity and clearance efficiency of *V. alginolyticus*, peroxinectin mRNA transcription and survival rate after challenge with *V. alginolyticus* [39].

Characteristics of good probiotics

Michael *et al*., [35] have described the features of good probiotic bacteria are to be the strain which is capable of exerting a beneficial effect on the host animal e.g. increased growth or resistance to disease. The probiotic bacteria should not be caused any disease or toxic effects on shrimp. They also should have capable of surviving and metabolizing in the gut environment in term of resistance to low pH and organic acid. In addition, it should be stable and capable of remaining viable for periods under storage and field conditions.

Successful and commercial probiotics

Probiotic strains were used to completely eliminate the luminous vibrio from the water column and from the sediment of ponds [40, 41]. The majority of commercial probiotic products contain one or multiple strains of lactic acid bacterial genera such as *Lactobacillus*, *Bifidobacterium*, *Lactococcus*, *Pediococcus*, *Enterococcus* or *Streptococcus* [42]. In addition, other bacterial taxa such as *Propionibacterium* spp., *Bacillus* spp. and *Escherichia coli* and the yeast *Saccharomyces boulardii* have also been used in probiotic products. Some *Bacillus* sp. (*B. megaterium*, *B. Polymyxa*, *B. subtilis*, *B. licheniformis*), lactic acid bacteria (*Lactobacillus* sp., *Carnobacterium* sp. and *Streptococcus* sp.), *Pseudomonas* sp. (*P. fluorescens*) and *Vibrio* sp. (*V. alginolyticus*, *V. salmonicida*- like) have been proposed and tested as probiotics in aquaculture [42].

Four species like *B. pumilus*, *Micrococcus luteus*, *P. fluorescens* and *P. putida* are currently included in bacterial mixtures that are marketed as probiotics for aquaculture (Prowins Biotech Private Ltd., India). Additionally, *Bacillus* sp. has been successfully used as probiotics in the aquaculture of black tiger shrimp (*Penaeus monodon*) in Thailand. When challenged with the luminous disease bacterium *V. harveyi*, the shrimp treated with probiotics showed a higher survival (13%) when compared to the control group (4%) [43].

Vaccines in shrimp aquaculture

Shrimp immune system lack immune memory characteristics as that of vertebrates. In shrimps, the immune system relies on innate or non-specific response. The immune defence is largely brought about by the activities of specialized haemocytes. These haemocytes carry out phagocytosis, encapsulation and produce antimicrobial substances to remove or neutralize foreign particles and infectious agents [44].

The ideal vaccines

An ideal vaccine should have following characteristics includes safe for shrimp and person vaccinating the animal, protect the host against a strain or pathogen type and give 100% protection. The vaccine must provide a long-lasting protection, at least as long as the production cycle. The vaccines should be easily applied, effective in a number of shrimp species, cost effective and readily licensed and registered.

Types of vaccines

A vaccine can be made in different forms namely, inactivated vaccines, live, attenuated vaccines, subunit vaccines, recombinant vector vaccines, DNA vaccines and synthetic/peptide vaccines. Efficacy of these vaccines can be improved using adjuvants, immunostimulants or vaccine carriers [45].

Vaccination against shrimp diseases

Administration of vaccines to the animal in aquaculture is generally done by oral administration through feed, by dipping or bathing the fish in a vaccine solution, or spraying the vaccine on to the animal or by injection of a small volume of antigen directly into the muscle (intramuscular (IM) injection) or into the body cavity (intracoelomic [ICe= intraperitoneal or IP] injection) [45, 46, 47]. Generally, injection method is better when compared to immersion and oral administration.

Several vaccines have been demonstrated towards enhancing survival, extending the duration of protection, increasing the efficacy of vaccine delivery etc. Vaccine development for shrimps was greatly hampered by absence of an established shrimp cell line, which could support the multiplication of virus. Studies have examined if changes in antibacterial activity are associated with the phenomenon of 'specific immune priming' in crustaceans [48, 49]. Powell *et al*. [49] used post-larvae of *L. vannamei* exposed to a commercial anti-vibrio vaccine (Vibromax™) and found elevated levels of antibacterial activity against *V. anguillarum* (in the vaccine) up to 14 days post-exposure; whilst Pope *et al*. [48] used the larger juveniles of *L. vannamei* and a bacterin composed of formalin inactivated *V. harveyi*. Bacterins prepared from five bacterial strains namely *Vibrio campbelli*, *V. parahaemolyticus*-like, *V. parahaemolyticus* and *V. harveyi* were used against WSSV infection in *P. monodon* and a relative percentage survival of 47% was obtained for shrimps treated with *V. harveyi* bacterin when challenged with WSSV [11].

The survivors from experimental infection study with WSSV became resistant against experimental re-challenge with WSSV at weeks 3 or 4 and the resistance continued up to 2 months [50]. Independent studies have explored different anti-WSSV strategies to protect shrimp, including inactivated vaccines [51], recombinant protein vaccines [52, 53], DNA vaccines [54, 55] and dsRNA vaccines [56, 57, 58]. Two structural protein genes such as VP19 and VP466 against WSSV have been studied in shrimp (*P. chinensis*) [59]. For the vaccination by oral feeding of rVP19 and rVP466, the cumulative mortalities were 49.2% and 89.2%, respectively. Truncated VP28 considered as one of oral vaccine candidate has been demonstrated against WSSV infection in shrimp *Penaeus monodon* [60].

A vaccination with formalin-inactivated *Vibrio* sp. has found to be effective against vibriosis in the larval culture of penaeid shrimp [60]. Shrimp that received formalin-inactivated *V. harveyi* or a commercial vaccine that contained inactivated Vibrio also exhibited enhanced phagocytosis activity [48]. Better protective effect of recombinant VP39 protein against WSSV infection in *Penaeus vannamei* has been found by intramuscular and oral administration [61]. Lin *et al*. [62] have reported that vaccination enhances early immune responses in white shrimp *L. vannamei* after secondary exposure to heat-killed *V. alginolyticus* (HVa) and formalin-inactivated *V. alginolyticus* (FVa). FVa was found as a vaccine candidate, whereas HVa functions as an immune adjuvant.

Immunostimulants in shrimp aquaculture

An immunostimulant is defined as a chemical, drug, stressor or action that enhances the innate or non-specific immune response of host by interacting directly with cells of the system [63]. Several bacterial pathogens such as *Aeromonas hydrophila, A. salmonicida, Edwardsiella tarda, E. ictaluri, Vibrio anguillarum, V. vulnificus, V. salmonicida, Yersinia ruckeri* and *Streptococcus* spp. have been successfully controlled by using immunostimulants in aquaculture [63, 64]. Immunostimulants can be classified into several categories by their origin and mode of action such as bacteria and bacterial products, complex carbohydrates, vaccines, immunity enhancing drugs, nutritional factors, animal extracts, cytokines, lectins, plant extracts etc. [65, 38].

Characteristics of immunostimulants according to Sakai [65]

Characteristics of immunostimulants are to be safe for the environment and human health, biocompatible and biodegradable. An immunostimulant triggers the immune system of shrimp to promote a health and enhance disease resistance against a broad spectrum of pathogens as well as reduce mortality due to opportunistic pathogens. It also should have potential to enhance the efficacy of vaccines, antibiotics and antimicrobial substances. In addition, they should be non-toxic, cheap and eco-friendly.

Method of administration

Administration of immunostimulant can be done in three ways in aquatic animals by injection, immersion and oral uptake. Injection of immunostimulants can produce a strong nonspecific response, but it is time-consuming and labour intensive. It has been reported that injection has wide protection against a range of pathogens [66].

Mode of action

The mode of action of immunostimulants is to enhance the immunity level of organisms against invading pathogens. The approach is very diverse in nature depending up on factors such as the type of immunostimulants, dose, route of administration, time and length of exposure [63]. Immunostimulants are generally involved phagocytosis and bacterial killing ability of macrophages, complement, lymphocytes and nonspecific cytotoxic cells, resulting in resistance and protection to various diseases and invading microorganisms [63, 64].

Common immunostimulants

Several antigens (vibrio cells, yeast glucans or their derivatives) have been experimentally tested to elucidate the innate immune mechanisms in shrimp [67]. Astaxanthin, chitosan, fucoidan, 1-3 glucan, herbal extracts, laminaria, Lipopolysaccharides, Peptidoglycans, saponins, and vitamin C have been experimentally tested as immunostimulants in shrimp [57].

Glucans: These molecules are non-specific immunostimulants in crustaceans, inducing resistance against bacterial pathogens [68]. However, crustaceans can digest glucans and use them as sources of energy. The use of LPS together with yeast glucan acts synergistically inducing a better stimulation of the crustacean immune system than when they are used separately [69]. β-glucans have found to be improving in activity of haemocyte phagocytosis, phenoloxidase and respiratory burst activity, along with enhancing the resistance of shrimp to WSSV [70].

Peptidoglycans (PGs): PGs are a mix of amino acids and carbohydrates from the cell wall of many bacteria and have been deemed as potent immunostimulants for the immune system [69]. Itami *et al*. [71] have demonstrated the potency of oral administration of peptidoglycan derived from *Bifidobacterium thermophilum* to kuruma shrimps through the diet followed by challenge with *V. penaeicida*. They obtained a higher survival rate for PG-fed group when challenged with white spot syndrome virus.

Fucoidan: These molecules are sulphated polysaccharides form microalgae cell walls which have been used as immunostimulants for shrimp. These products were moderately successful against pathogens like WSSV, *Vibrio* sp. and other bacterial species. Oral administration of crude fucoidan (CF) extracted from *Sargassum polycystum* reduced the impact of WSSV infection in *P. Monodon* [72] with the survival rates of shrimps 93% higher compared to controls.

Lipopolysaccharides (LPS): LPS affect the specific and nonspecific immune system of many animals, including crustaceans [69]. LPS are part of the cell wall of Gram -ve bacteria and are the first molecules recognized by the host's immune system, hence can be used in shrimp as a potential tool to prevent diseases which was proved in laboratory conditions [73].

Conclusion

Shrimp aquaculture has been progressing rapidly and several best management practices are being used for the successful production of each shrimp crop cycle. Howsoever, it is under continuous threat of various infectious diseases that include AHPND/EMS, WSSV, EHP, WFS, etc. which has caused devastating collapses of the shrimp industry. Prophylactic health management is one of the most essential requirements that is advocated and practised in shrimp farming. Use of prebiotics and probiotics in shrimp health management has been well documented and their application also ensures a healthy environment. Despite the lack of well developed immune system in shrimps unlike fishes, several studies on vaccine development have shown varying degrees of protection of different duration in penaeid shrimps. It goes without saying that an effective vaccine development must be preceded with the detailed study of pathogenicity mechanisms that are utilised by potential pathogens causing infection in shrimps. Apart from vaccines, application of immunostimulants has also shown to give generic protection to the shrimps albeit for short duration. Considering the fact that the crop duration extends to a maximum of four months, even short duration protection in high risk periods would help the crop to survive for successful completion of the culture cycle. Notwithstanding the shortcomings that are reported for the vaccine development, further studies for a better understanding of the immune system of the shrimps specifically to delineate the mechanistic nature of protection attained in cases of vaccine application needs to be undertaken for futuristic vaccine production endeavours. A combination of vaccines with probable addition of adjuvants, immunostimulation and probiotics to maintain the general health of the animals would go a long way in managing the shrimp culture without the problems of infectious diseases.

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