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Recent trends in development and application of shrimp immunostimulants for disease management in farming systems

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1. Introduction

Crustaceans constitute a major component of the global shellfish culture and shrimp farming has been the focus of the aquaculture industry in recent years. Concomitant with the high production in farming systems has been the recognition of disease as a primary intervention limiting aquaculture production worldwide. Therefore, disease management is an integral part of all farming systems requiring rapid detection and treatment. It is generally agreed that high incidence of diseases is indicative of the deteriorating environmental conditions and the resultant stress in the cultured organisms. Crustaceans, unlike fishes, do not possess immune systems with any specificity, but have potent non-specific defense mechanisms to combat diseases as and when they occur.

A class of compounds known as immunostimulants is increasingly becoming popular and commercialised for applications in aquaculture. These compounds ranging from complex mixtures of proteins and lipids to carbohydrate based cell wall extracts are believed to increase the non-specific immunity of crustaceans so as to provide them with a variety of defensive ability to protect against the infections from pathogens. The crustacean immune system, few examples of commercially available immunostimulants, their mechanism of action and their utility in improving the crustacean defense system are discussed. There are also doubts on the consistency and effectiveness of these compounds in disease management in crustaceans.

2. Crustacean immune system

Crustaceans have both cellular and humoral defense systems and haemocytes play a major role in both these processes. Crustaceans possess one of the more 'advanced' invertebrate immune systems. Haemocytes are involved in phagocytosis of external material, confinement of pathogens by clotting, coagulation, hardening (tanning) of the cuticle and encapsulation (Noga, 2000). Haemocytes are also associated with humoral systems such as the phenoloxidase, prophenoloxidase system, bactericidins and lectins (Takahashi et al. 1995, Kopacek et al. 1993). Though specificity of haemocyte types is not well defined, they are generally classified into hyaline, semi-granular and granular cells. Generally, hyalinocytes have been associated with initiation of haemolymph coagulation (Aono and Mori, 1996), but are also phagocytic in some species (Bell and Smith, 1993). While about 10% of the haemocytes in *Penaeus japonicus* are classified as hyaline cells, no hyaline cells are reported in *Macrobrachium rosenbergii*. However,

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Yeh et al. (1998) reported high hyalinocyte count (>54%) in shrimps. Granulocytes are larger in size and are actively associated with phagocytosis in many crustaceans. They also possess lysosomal enzymes, which are important in immunodefense system of the host. Drastic reduction in circulating haemocytes may be indicative of disease, stress or starvation and therefore, haemocytes can be useful in assessing the overall health of the animal (Noga, 2000).

A variety of other haemolymph factors are also reported to be associated with immune system in crustaceans. The clotting of haemolymph is a special mechanism to protect the animal from excessive bleeding and death and also to sequester and immobilise invading microorganisms (Noga, 2000). Decreased clotting of haemolymph is often associated with diseased state. Studies conducted in crabs, lobsters, many species of shrimps, isopods and stomatopods have demonstrated the antibacterial ability of the haemolymph (Khoo et al.1999). Most of these factors reside in haemocytes and are released during clotting or lysis. These endobiotics presumably protect the animal against a variety of pathogens. In *P. monodon*, bioactive molecules such as agglutinins were shown to increase after exposure to test materials, but the effect was short lived and non-specific. Prophenoloxidase system in crustaceans constitutes an important part of the crustacean immunodefense system. Studies conducted in shrimps show that PO activity is mainly centered on granulocytes and/or serum (Sung et al. 1998; Perazzolo and Barracco, 1997). Lectins are sugar-binding proteins identified in the haemolymph of many crustaceans. While some lectins act as opsonins, others might directly protect against diseases. However, the biochemical structure of many lectins has not been well characterized (Noga, 2000).

3. Non-specific immunostimulants

Substances that allow an animal to react in a non-specific manner against a variety of pathogens are being commercialized for use in aquaculture (Newman and Deupree, 1995). These substances generally called as immunostimulants are obtained from diverse natural sources and many are chemically synthesized. The cell wall preparations that are made from bacteria, fungus and yeast are polysaccharides, lipopolysaccharides and lipopeptides. Phorbol 12-myristate 13-acetate (PMA), a surface-active agent, is used as an immunostimulant (Song and Hsieh, 1994). Dietary components such as astaxanthin, ascorbic acid-polyphosphate and HUFA are also used as immnuostimulants. Information is inadequate on the exact mode of action of these compounds.

4. Effect of immunostimulants on crustaceans

In crustaceans, immunostimulants probably act on haemocytes, possibly by causing them to rupture, producing elevated levels of highly reactive naturally occurring compounds in the haemolymph (Newman and Deupree, 1995). Oral application of some of the immunostimulants is believed to act as a growth inhibitor, which competes with *Aeromonas/Vibrio* for iron and prevents attachment of the bacteria to the gut. They may also act as an agglutinating protein or as an enzyme, which selectively destroys bacterial cell walls without damaging host cells (Kong, 1995).

Some of these compounds that has been found effective in shrimps are listed in Table 1. In shrimps, immunostimulants have been shown to enhance the phagocytic activity of haemocytes (Itami et al., 1993). Enhancement of phagocytic activity was also confirmed by increasing production of reactive oxygen intermediated from haemocytes drawn from stimulated shrimp (Sung et al.1996). Immunostimulants could also probably influence clotting reaction by causing the release of TGase activity (Song and Huang, 2000). Heat or formalin killed microbial products injected in test shrimps has been shown to induce bactericidal or agglutinin activity in haemolymph and protection against a variety of pathogens (Lewis et al. 1982; Karunasagar and Karunasagar, 1999). Administration of glucans induces immunostimulation in *P. monodon,* but there are reports that the effect is short lived (Sung et al., 1994). However, recent observations show that repeated administration or by immersion of shrimp postlarvae at the PL_9-PL_{15} stage in the non-specific immunostimulant *'Penstim'* could protect the shrimp for at least 50 days from infection (Newman and Deupree, 1995). Vaccination with a formalin killed *Vibrio* sp. has proved to be effective against vibriosis for 50 days (Itami et al. 1989). In grow out system, shrimps fed regularly (once in 5 days) with a mix of vibrio bacterin and b-1,3 glucan treated feed have been shown to improve survival up to 85% (Karunasagar and Karunasagar, 1999). Boonyaratpalin et al. (1995) obtained higher survival and disease resistance in *P. monodon* fed with peptidoglycan-supplemented feed and has suggested 0.01% as the optimum level. Kong (1995) observed a number of changes on feeding shrimp with 'ENCAP' such as reduced bacterial numbers in the hepatopancreas, alteration in type of pathogens, agglutination of bacteria and increased phagocytic activity. Higher percent survival of post larvae produced from glucan-injected spawners was reported (Huang and Song, 1999). However, overfeeding with immunostimulants also can result in adverse reactions in shrimp.

Table 1. Immunostimulants and their response in shrimps

Species	Immunostimulant	Response
Penaeus monodon	Glucan	Protection against *V. vulnificus* challenge
P. monodon	Peptidoglycan	Increased phagocytic index, improved protection against WSSV
P. monodon	'ENCAP' immune enhancer	Higher survival, growth and increased production
P. monodon	'Penstim'	Increased survival and growth
P. monodon	Vibrio bacterin, yeast β-1, 3 glucan	Improved resistance to *Vibrio* spp., white spot baculovirus
P. monodon	*Vibrio alginolyticus* bacterin	Bactericidal activity
P. monodon	*Saccharomyces cerevisiae*, β1, 3-1, 6- glucan	Protection against vibriosis, growth and increased survival
P. monodon	*Brevibacterium lactofermentum, peptidoglycan*	Increased phagocytic activity, protection against vibrio, growth and survival
P. monodon	Astaxanthin and ascorbic acid polyphosphate	Tolerance to stress

Certain immunostimulants have been shown to induce non-immune-related effects. Sung et al. (1991, 1994) showed better growth in shrimps exposed to *V.vulnificus* bacterin and glucan suspension. Tiger shrimps fed with 0.01% and not 0.1% peptidoglycan (PG) supplementation have been observed to grow faster with higher feed conversion efficiency, indicating importance of application of optimum dose. PG-fed tiger shrimp would also exhibit higher tolerance to salinity (5 ppt), compared to those fed with control diet (Boonyaratpalin et al., 1995). Song et al. (1997) observed enhanced tolerance to stress including catching, transport and ammonia in shrimps treated with 15 ppm glucan for 24 hours.

There is also increased variability on the consistency and effectiveness of immunostimulants on disease resistance in crustaceans. The effect may depend upon the severity and frequency of the infection, the mode of application and the presence of other stressors. Disease being a complex process, overdependence on immunostimulants to prevent disease shall be avoided as their effectiveness will depend upon the water quality and the presence of various other stressors, which are likely to increase the susceptibility of the animal to infectious disease. Duration of 'protection' in crustaceans is likely to be shorter and repeated application timed prior to infection will be required to optimize the effectiveness of these products (Newman and Deupree, 1995). Immunostimulants should be applied to shrimps via injection to spawners, immersion to nauplii and postlarvae (PL_1, PL_{10} or PL_{20}) and feeding to PL_{30} onwards, respectively (Song and Huang, 2000).

There are several advantages of using immunostimulants for improving the immunodefense system in crustaceans, as the compounds are naturally occurring and are not antibiotics. There are indications that if used in correct doses and treated before exposing to extreme disease situations, immunostimulants may provide non-specific protection to crustaceans against a variety of pathogens.

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