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Evaluation of Nutritional Genomes Approach for Controlling of Disease in Shrimp Farming

[1,2]Reza Nahavandi, [2]S.M. Nurul Amin, [3]Parivash Hafezamini and [4]Arash Javanmard
[1]Iranian Fisheries Research Organization (IFRO), P.O. Box: 14155-6116, Tehran, Iran
[2]Laboratory of Marine Science and Aquaculture, Institute of Bioscience, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[3]Islamic Azad University, Rasht Branch, Educational Complex Bridge, Talysh, Lake Gate, Rasht, Iran
[4]Department of Biotechnology, Animal Science Research Institute (ASRI), Karaj, Iran

Abstract: Shrimp farming is an important industry but several infectious diseases have caused high economic losses all over the world. Until now, many pathogens that include viral, bacterial and fungal species have been identified which are affecting sustainable development of this industry. Therefore, research in terms of immunity is of primary concern to control disease and to ensure long-term survival of the shrimp culture industry. There is increasing evidence indicating that nutritional genomics represents a promise to improve marine health. This goal will be reached by highlighting the mechanisms through which diet can reduce the risk of monogenic and common polygenic diseases. Indeed, nutrition is a very relevant environmental factor involved in the development and progression of metabolic disorders as well as other kind of diseases. To date, it is widely accepted that most of the effects of the nutrition on shrimp diseases and in turn, on marine health cannot be easily explained without a complete knowledge of the molecular mechanism underlying the nutrients action. In particular, the evidence shows that understanding how nutrition affects the metabolic homeostasis, influencing different cellular metabolic pathways is a crucial event. The aim of this mini review is describe of pervious nutritional genomes researches of shrimp health and improvement of immune system response.

Key words: Shrimp farming, immune system response, nutritional genomes, disease, viral, fungal species

INTRODUCTION

Aquaculture is a international industry, supplying aquatic organisms to people worldwide for eating, sport or business purposes. As the fastest growing food producing sector, aquaculture has gathered a reputation as a significant contributor to world food supplies and income generation (Tidwell and Allen, 2001) (Table 1).

A shrimp farm is an aquaculture business that cultivates marine shrimp or prawns for human consumption. Commercial shrimp farming began in the 1970s and production grew steeply particularly to service the U.S., Japan and Western Europe. Global production of farmed shrimp reached more than 1.6 million tons in 2003 representing a value of nearly $9,000,000,000 (9 billion) U.S. dollars. About 75% of farmed shrimp is produced in Asia in particular in China and Thailand. The other 25% comes mainly from Latin America where Brazil is the largest producer. Thailand is the largest exporting nation. Various diseases are still the main obstacle to global production of farmed shrimp (Jory, 2003). Significant research efforts and resources continue to be devoted to effectively address this situation and develop and improve biosecure production practices. Many diseases are linked to environmental deterioration and stress associated with farm intensification. Under poor farming conditions, it is often opportunistic diseases caused by bacteria, fungi and protozoa that are constantly present in the pond environment which cause death of the shrimp. About >15 viruses have been identified to cause diseases in shrimp during the past two decades (Bower *et al*., 1994) (Table 2).

Studies towards a better understanding of defence mechanisms in shrimp constitute one approach to overcome disease problem to be able to optimise culture conditions so that good shrimp health is retained.

Shrimp immune system: An understanding of shrimp immune defense is just beginning to emerge. Bachere provided an excellent summary of the defense systems used by shrimp in the event of infection. However, as Flegel (1997) pointed out, most of the knowledge of

Corresponding Author: Reza Nahavandi, Laboratory of Marine Science and Aquaculture, Institute of Bioscience, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Table 1: Aquaculture shrimp production by the major producer nations

Region	Country	2000	2001	2002	2003	2004	2005	2006	2007
		Production in 1,000 tons year^{-1} (rounded)							
Asia	China	192	267	337	687	814	892	1'080	1'265
	Thailand	309	279	264	330	360	401	501	501
	Vietnam	90	150	181	232	276	327	349	377
	Indonesia	118	129	137	168	218	266	326	315
	India	97	103	115	113	118	131	132	108
	Bangladesh	59	55	56	56	58	63	65	64
	Philippines	41	42	37	37	37	39	40	42
	Myanmar	5	6	7	19	30	49	49	48
	Taiwan	6	8	10	13	13	13	11	11
Americas	Brazil	25	40	60	90	76	63	65	65
	Ecuador	50	45	63	77	90	119	150	150
	Mexico	33	48	46	46	62	90	112	114
	U.S.	2	3	4	5	5	4	3	2
Middle East	Saudi Arabia	2	4	5	9	9	11	12	15
	Iran	4	8	6	7	9	4	6	3
Oceania	Australia	3	3	4	3	4	3	4	3

Table 2: Estimated effects of disease on shrimp production in selected countries

Country and year of peak production	Thailand 1994	Philippines 1989	Ecuador 1992	Indonesia 1991	China 1992	Taiwan 1987	India 1994	Vietnam 1994	Bangladesh 1994	World 1994
Estimated fresh peak production (metric ton)	225	59	126	149	215	139	70	50	35	733
Estimated loss to disease (metric ton)	130	57	35	50	180	100	25	10	5	541
Percentage loss due to disease	58	45	34	50	512	400	36	20	14	74
Value of production loss (million Us $)	650	284	170	250	900	500	125	50	25	3019

Lundin, 1995

shrimp immunity is based on studies of bacterial and fungal infections. Shrimp have an open circulatory system and there is no separation between the circulatory and lymphatic systems. The fluid within this open system is called hemolymph. Haemocytes are cells present in the hemolymph. These cells play a major role in the immune response of shrimp to bacterial and fungal infections. The shrimp's immune system identifies invasion by means of specific cell wall components of the invading organisms. Peptidoglycans and lipopolysaccharides on bacterial cell walls and betaglucans on fungal cell walls are the molecules that are readily identified by specific molecules in the hemolymph. Once such a pattern recognition molecule binds to its specific molecular counterpart on the foreign body, a number of hemocyte-mediated responses follow to clear the invading organisms.

These responses include agglutination, phagocytosis and production of free radicals and antimicrobial compounds. A number of studies have shown that the shrimp immune system can be stimulated with purified peptidoglycans, lipopolysaccharides or betaglucans to achieve generalized immune protection against bacterial infections. Live or inactivated bacteria or yeast may also offer protection against bacterial infections. It is generally believed that invertebrates are incapable of adaptive immune response. This is based on the fact that inducible humoral compounds such as immunoglobulins, T cell receptors, the msajor histocompatibility complex and memory T-cells are not present in invertebrates.

Shrimp diseases: Farmed shrimp are infected by a range of disease agents including bacteria, viruses, fungi and protozoa. For comprehensive information on disease problems in shrimp farming, please refer to Lightner (1996). There are a number of viruses that infect shrimp but not all of them cause fatal diseases. Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) has been observed in most commercially farmed shrimp species. It appears to be harmless in some species such as the Asian tiger shrimp, Penaeus monodon but malicious in others causing mortality and growth retardation. There are a number of other viruses such as the Monodon Baculovirus (MBV), Hepatopancreatic Parvo-like Virus (HPV) and Baculovirus Penaei (BP) that damage the cells of the hepatopancreas and make the shrimp susceptible to other disease agents. It is believed that infection by these viruses causes a reduction in growth rates. As noted earlier, the three viruses that cause acutely fatal diseases in shrimp farming are the White Spot Syndrome Virus (WSSV), Yellow Head Virus (YHV) and Taura Syndrome Virus (TSV). All three viruses can cause extensive mortality within a few days of the first clinical signs of the disease.

The severity of a viral disease typically subsides in about 2 years after the first incidence of the given disease. This apparently indicates some type of an adaptive response to the disease agent. However, the viruses are never completely eliminated. They resurface periodically, particularly at times of stress to cause large-scale mortalities. Furthermore, growth retardation often coincides with viral infections resulting in economic losses.

Nutritional genomics: Nutritional genomics is a science studying the relationship between genome, nutrition and health. It can be divided into two disciplines: Nutrigenomics: studies the effect of nutrients on health through altering genome, proteome, metabolome and the resulting changes in physiology. Nutrigenetics studies the effect of genetic variations on the interaction between diet and health with implications to susceptible subgroups. More specifically, nutrigenomics studies how individual differences in genes influence the body's response to diet and nutrition.

Feed and feed management play a critical role in shrimp health management at a number of levels. Immune response imposes energy cost in animals. It results in changes in nutrient partitioning and directs more nutrients to the immune system (Humphrey *et al*., 2002). Therefore, nutrient requirements for optimum health status of an animal are expected to be higher than those for growth. Apart from this, certain nutrients are specifically implicated in the enhancement of immunity in many animals. These include some vitamins, trace minerals, ω-3 fatty acids, phospholipids, carotenoid pigments and nucleotides (Table 3).

Merchie *et al*. (1998) found that increasing vitamin C level from 100-3400 mg kg^{-1} diet resulted in a concomitant drop in mortality of post-larval *P. monondon* subjected to osmotic shock.

They also reported that high vitamin C or astaxanthin levels resulted in an increased resistance to salinity shock. Chien *et al*. (2003) reported that dietary supplementation with 80 mg kg^{-1} astaxanthin improved survival of *P. monondon* exposed to toxic levels of ammonia.

The positive effect of vitamin C and astaxanthin on shrimp immunity is probably related to their antioxidant properties. In addition, vitamin C also plays a role in wound healing. Lavens and Sorgeloos (2000) noted that feeding post-larvae shrimp with Artemia nauplii (brine shrimp) that have been enriched with highly unsaturated fatty acids improved their ability to survive salinity shock. Coutteau showed that phosphotidyl choline included in the diets of *P. japonicus* and *P. vannamei* at 1.5% significantly improved stress resistance in the animals. Both ω-3 and ω-6 fatty acids are precursors of eicosanoids that are potent mediators of inflammatory response in higher animals.

Phospholipids play a major role in cell membrane integrity, a significant factor in the first line of defense against antigens in all organisms. Experience with other animal species including fish shows that vitamin E, selenium and nucleotides may have a beneficial effect

Table 3: Nutrients that enhance shrimp immune system activity

Products	Role/application
Nutrients	
Vitamin C	Plays role in wounds healing; antioxidant; inclusion at high level (>2000 mg kg^{-1} improved stress tolerance in larval shrimp
Vitamin E	Antioxidant; health effect not yet established in shrimp
ω-3 fatty acid	Role in inflammatory response; health benefits proven in larval shrimp; balance between different ω-3 fatty acid as well as between ω-3 and ω-6 fatty acid perhaps important
Phospholipids	Involved in maintaining biological membrane integrity, inclusion of phosphotidyl choline, a class of phospholipids, improved stress tolerance in larval shrimp
Astaxanthin	Antioxidant; inclusion improved stress tolerance in shrimp
Nucleotides	Probably an essential nutrient at time of stress and infection
Selenium	Antioxidant
Immunostimulants	
Lipopolysaccharides	Constituent of gram-negative bacterial cell walls; activates immune system and increases resistance to bacterial infection in shrimp and may result in better growth rates, increased resistance to stress and higher survival
Glucans	Constituent of fungal cell walls may increase shrimp resistance to pathogens through immunostimulation; unreliable performance
Mannan oligosaccharides	Constituent of fungal cell walls; binds with pathogens and eliminates them from guts; immunostimulatory role may also exist
Peptidoglycan	Constituent of gram-positive bacterial cell walls; activates immune system and increases resistance to viral infections in shrimp
Fucoidan	Constituent of beown algae cell walls; activates immune system and increases resistance to viral infections in shrimp
Probiotics	
Bacteria	Compatitive exclusion of pathogenic bacteria in the guts; immunostimulation; production of antimicrobial compounds
Yeast	Strong adhesion to gut walls to limit colonization by pathogenic bacteria; immunostimulation; source nucleotides and other nutrients

on the shrimp immune system. Vitamin E and selenium are highly effective antioxidants known to affect immune defense systems of vertebrates including fish. Vitamin E scavenges free radicals generated during the early stages of lipid peroxidation in cell membranes while selenium is a component of glutathione peroxidase which reduces the level of lipid peroxidation inside the cells. Given the facts that almost all highly unsaturated fatty acids are required for optimum growth of shrimp and that these fatty acids are extremely prone to oxidation, dietary provision of vitamin E and selenium becomes all the more important.

IMMUNOSTIMULANTS

Immunostimulants are compounds that stimulate the non-specific defense mechanisms in organisms. In shrimp, non-self recognition is the key to immune system stimulation and this is mediated by pattern recognition proteins that identify and bind with specific molecules on the invading organisms.

Specific cell wall carbohydrates of bacteria and fungi have been identified as molecules that are identified by the pattern recognition proteins. These molecules are widely used as immunostimulants. Newman and Bullis (2001) provided an excellent review of studies that have evaluated immunostimulants in shrimp. The three most common immunostimulants that have been tested in shrimp are Lipopolysaccharide (LPS), Glucans and Peptidoglycan (PG). LPS and PG are bacterial cell wall carbohydrates while glucans are a set of polymeric glucose molecules found on fungal cell walls.

Lipopolysaccharide is a cell wall component of Gram-negative bacteria and consists of lipids and carbohydrates. Dead vibrio cell suspensions are the most common form of LPS applications in experiments. About 20 studies have tested LPS in various species of shrimp. The results show that LPS exposure generally increases resistance to bacterial infections. Some tests show improved antiviral activity, better growth rates increased resistance to stress and higher survival. The preparations are heat stable and can be administered via feed or as immersion baths.

Peptidoglycan is a cell wall component of Grampositive bacteria. A couple of studies have found it to be effective in increasing shrimp resistance to viral infections. The most promising and conclusive study was that of Itami *et al.* (1998). The researchers found that peptidoglycan derived from Bifidobacterium thermophilum fed at the rate of 0.2 mg kg^{-1} bodyweight/day offered protection against WSSV in *P. japonicus*. Glucans are found on the cell walls of fungi. Glucans have been tested in shrimp in >10 studies. Glucans are also the most widely used immunostimulant in the shrimp farming industry. Nevertheless, the results are equivocal. While some studies show that they are effective in increasing shrimp resistance to pathogens, others show no efficacy. Some studies also show that there are adverse effects of glucan use on growth, survival and disease resistance. Scholz *et al.* (1999) reported that *P. vannamei* fed β-glucan showed poorer survival and resistance to infection by a virulent strain of Vibrio harveyi.

Newman and Bullis (2001) pointed out that glucans derived from the mycelia of a mushroom, Schizophyllum commune were much more effective in antibacterial activity than glucans derived from a baker's yeast, Saccharomyces cerevisiae. Raa (2000) suggested that β-1,3/1,6 glucan is the molecule that is recognized by the immune system and that its isolation from yeast must be done carefully in order to maintain its sidebranches intact and thereby preserve its biological activity. Glucans are heat-labile and break down at temperatures above 130°C. Crustaceans are suspected to have the ability to digest glucans which may further explain the ineffectiveness of glucans as immunostimulants.

Mannan oligosaccharides, another component of yeast cell wall have recently gained attention as immunostimulants in shrimp culture. In other animal species, mannan oligosaccharides have been established as molecules that bind with some bacterial species that have a specific receptor to the molecules. This binding prevents the bacterial species from colonizing the intestinal cell wall of animals. It is suggested that the molecules also adsorb potentially immunosuppressive mycotoxins. In the above two respects, mannan oligosaccharides are not strictly immunostimulants. However, evidence is mounting that the molecules may have a immunostimulatory role in other animal species (Cotter *et al*., 2002).

Investigation of the effect of mannan oligosaccharides on shrimp health is required. Fucoidan, a sulfated polysaccharide has shown promise as a viral inhibitor in shrimp. Takahashi *et al.* (1998) reported that dietary administration of fucoidan derived from the brown alga, *Cladosiphon okamuranus* reduced mortality due to WSSV. Purified fucoidan is too expensive to justify its use in shrimp feeds; however the use of crude extracts of brown algae is a possibility. In Japan, shrimp farmers already use a combination of peptidoglycan and an algal extract to protect shrimp against another form of WSSV.

Although, immunostimulants are widely used in the shrimp culture industry at present, there are several unresolved issues concerning their field application. The primary concern is related to dosage: how much to apply and how long should application continue? Some believe that long-term and continuous exposure to immunostimulants exerts an energy cost on the animal and may even weaken or desensitize the immune system. Administration of immunostimulants during or after an infection may also be deleterious.

It is widely accepted that immunostimulants exert their protective effect only on a short-term basis because they elicit only non-specific defense mechanisms. So, farmers need to understand how frequently the products need to be used.

Finally, the interactive effects between two types of immunostimulants need to be understood as well. For example, beneficial effects of combining lipopolysaccharides and glucans have been noted.

PROBIOTICS

Several probiotic products are used in shrimp farming, particularly in Asia. Unlike in terrestrial animal farming, probiotic application in aquaculture extends beyond maintaining the intestinal microbial balance of host animals (Gatesoupe, 1999). Maintaining optimal microbial balance and eliminating pathogenic bacteria in the pond is one of the stated applications. Bioremediation of organic wastes produced by the animal in the pond water and thereby enabling good water quality is another major application.

In fact, a majority of probiotic products in aquaculture are intended for application in water not through feed. It is assumed that the probiotic microbes added in water eventually enter the animal's intestine. While probiotic bacteria added in the culture media has conferred disease resistance to shrimp (Uma *et al*., 1999), it is not clear whether the benefit is due to intestinal colonization. On the other hand, dietary delivery of probiotic organisms has shown positive effect on shrimp health in a number of studies (Rengpipat *et al*., 1998; Uma *et al*., 1999; Scholz *et al*., 1999; Rengipipat *et al*., 2000).

Probiotic bacteria that have been shown effective in shrimp health include the following groups: Vibrio (particularly, *V. alginolyticus*) and various *Bacillus* and *Lactobacillus* species and strains. Rengpipat *et al.* (1998) showed that even the lyophilized form of bacteria (Bacillus) can be effective. The effect that has been demonstrated in almost all studies is the control of infection by the pathogenic bacteria *V. harveyi*. In addition increase in phagocytosis, melanization activity and antibacterial activities have also been demonstrated (Rengipipat *et al*., 2000). The mechanisms suggested for the beneficial effects of probiotic bacteria in improving shrimp health are:

- Competitive exclusion of pathogenic bacteria in the gut
- Cell wall components of probiotic bacteria stimulating the innate immune system of shrimp
- Enzymes and antibacterial compounds produced by the probiotic bacteria being detrimental to the pathogenic bacteria

Scholz *et al.* (1999) showed that yeasts (*S. cerevisiae* and *Phaffia rhodozyma*) enhanced shrimp resistance to vibriosis. It is surprising that only limited attention has been paid to yeasts as probiotic organisms in shrimp. Their cell wall is a rich source of glucans and mannans that stimulate the immune system. They are rich in nucleotides, vitamins and trace minerals that are essential nutrients for optimum immune system function. Yeasts certainly deserve more attention in the context of shrimp health management. As noted above, probiotic organisms have been shown to be effective only for pathogenic bacterial control. Efforts are underway to identify bacteria with antiviral effects (Horowitz and Horowitz, 2001). Certain cyanobacteria and Pseudomonas strains with antiviral activity have been identified and are being tested.

Horowitz and Horowitz (2001) advocate caution in the application of probiotics. Continued exposure to probiotics may lower the sensitivity of pathogenic bacteria to the antibiotic compounds produced by the probiotic organism and may even lead to the development of resistant strains. Some of the antibiotic residues found in shrimp may actually originate from the probiotic organisms. Given the stringent regulations on antibiotic residues in food by major shrimp-consuming countries, this speculation is worth further investigation. There is also a risk that the otherwise benign probiotic organisms may become pathogenic due to genetic exchange between microbes. Alternatively, genes that provide the probiotic organism's superior survival skills may transfer to the pathogenic bacteria making the latter even more resistant to destruction.

Characterisation of some immune genes in shrimp: Under physiological conditions, arthropod proPOs require a proteolytic cleavage by a specific protease for activation; the inactive proPO in the freshwater crayfish with a molecular mass of 76 kDa is converted into an active form with a molecular mass of 62 kDa by the

Prophenoloxidase Activating enzyme (ppA). A proppA becomes activated by the presence of PRPs (Aspan *et al*., 1990, 1995; Aspan and Soderhall, 1991). ProppAs cloned from insects and crustaceans have been shown to be homologous to tachypleus clotting enzyme activated factor B and drosophila easter (Muta *et al*., 1991; Lee *et al*., 1998; Jiang *et al*., 1998).

The common feature of arthropod ppA enzymes are that they are serine proteinases and have clip-like domains (Lee *et al*., 1998; Wang *et al*., 2001). The clip-like domain seems to play several biological functions. The clip-domain of clotting enzyme and factor B in horseshoe crab is proposed to mediate the functional conversion of hemocyanin to phenoloxidase (Nagai and Kawabata, 2000). Wang *et al.* (2001) has recently shown that the recombinant peptide from the clip-like domain (defensins) of crayfish proppA has an antibacterial activity *in vitro*.

Peroxinectin, an associated factor of the proPO system several cell adhesion molecules have been discovered and characterised during the past few years in invertebrates and have shown to participate in immunological processes. These processes include cell attachment and spreading, nodule formation, encapsulation, agglutination (or aggregation) and phagocytosis (Johansson *et al*., 1999). So far, a few blood cell adhesion molecules in arthropods have been cloned.

The addition of bioactive compounds to shrimp diet and comparison of control and different level of these bioactive compounds in mRNA level and in immune gene expression view can be good horizon for prevent of disease in shrimp farming and controlling of environment by formulation of diet.

CONCLUSION

The aquaculture of shrimp has rapidly grown to a major industry which on a worldwide basis provides not only economic income and a high quality food product. Diseases have emerged as a major constraint to the sustainable growth of shrimp aquaculture. Many diseases are linked to environmental deterioration and stress associated with farm intensification. Under poor farming conditions, it is often opportunistic diseases caused by bacteria, fungi and protozoa that are constantly present in the pond environment which cause death of the shrimp.

Disease prevention is more important than treatment. Studies towards a better understanding of defence mechanisms in shrimp constitute one approach to overcome disease problem to be able to optimise culture conditions so that good shrimp health is retained. Discovery of new source of nutrients which can improve immune system response against of decease and pathogens is good horizon for shrimp farming.

For this purpose, Designing of experiments on effect of these immune stimulants on immune gene expression is per-require. Result of gene expression of study can be good indicator for formulation of diet in shrimp industry.

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