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ROLE OF ENVIRONMENTAL FACTORS ON SHRIMP IMMUNITY AND DISEASE OUTBREAKS

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Diseases are the major constraints for the expansion of shrimp aquaculture worldwide. In addition to White Spot Syndrome and vibriosis, diseases like Monodon Baculo Virus (MBV),Taura syndrome virus (TSV), IHHNV, IMNV etc are causing huge economic lossesto shrimp industry. Diseases in aquaculture are result of complex interaction between host (shrimp), pathogen (bacteria/virus) and environment (pond). Severe alterations in the culture environment deviated from the optimumimpose stress on the system leading to reduced immune status of the shrimp to fight infections.

Stress and shrimp immunity

Stress is defined as a condition in which thedynamic equilibrium/ homeostasis of an organism isdisturbed as a result of intrinsic or extrinsicstimuli. In addition to extrinsic environmental factors, intrinsic factors like sex, size, moulting stages and nutritional status also influence the ability of shrimp to invading pathogen. Recent research on stress response studies has identified the levels of glucose, lactate and osmoregulatory capacity as tangible markers for measuring the stress response in crustacean species. Additionally, othermetabolic variables like hemocyanin, total proteins,total lipids, triglycerides, and cholesterol are also suggested to be of useful indicators tomonitorthe physiological condition of shrimp.

Shrimp immune system contains three types of circulating haemocytes: hyaline cells,semi-granular cells and large granular cells. In the absence of known humoral arm of immune system, these cells are responsible for cellular immune functions like phagocytosis, coagulation a encapsulation of microbes or fore particles. Bacterial clearance throu phagocytosis and respiratory burs considered as the central and m important pathway in elimination invading pathogen. Since, gills are main entry point and site accumulation for the pathogen, hea of gills significantly influence susceptibility to bacterial infecti Intact cuticular surface act as barr and prevent the entry of pathog from environment. Any damage this barrier allows pathogen entry in the system and the possible infectio In immunologically compete shrimp,haemocytes migrate damaged cuticularsite, a phagocytise the invading bacteria.

Environmental parameter stress and immunity

Shrimp under stress due to vario environmental factors show high levels of biogenic amines including noradrenaline and dopamine, which are immune suppressive in nature increasing susceptibility to pathogen infections. Pond environmental parameters like temperature, salinity, dissolved oxygen,pH, ammonia, nitrite, hydrogen sulphide and heavy metals have greater impact on the immune functions of shrimp. Extreme ranges of these parameters have proven to have adverse effect on cellular andhumoral components of shrimp immune system.

Salinity

Salinity range of 10 to 35 ppt is considered optimum for growth of tiger shrimp, *P. monodon* though it can tolerate wider range of salinity from 1 to 57 ppt. Lower salinity (below 15 ppt) and higher salinities (above 35 ppt) affects defence mechanism leading to increased susceptibility to vibriosis. At iso-osmotic salinity levels shrimp exhibit higher resistanceagainst pathogen infection due to competent immune system.

Temperature

The temperature below and above the optimum (28 to 32°C) range is known to weaken the immune status of the shrimp making it more susceptible to diseases due to vibrio. This variation in temperature is known to lower the levels of total haemocyte counts, phenoloxidase and respiratory burst in addition to reduction in the activity ofsuperoxide dismutase (SOD) responsible for scavengingsuperoxide anion. Hence it is common to expect outbreaks of diseases when environmental temperatures go beyond the optimum range of culture shrimp species.

pH

In intensive aquaculture ponds pH fluctuate between 6.6 and 10.2 due to consumption of carbon dioxide by plants for photosynthesis during day time and release of carbon dioxide from both plants and animals during the night. The influence of pH is harmful to the shrimp by indirectly increasing the unionized ammonia at pH above 7. At pH below 6.6 and above 10.2, there will be reduction in total hemocyte counts, granulocyte counts,respiratory burst, SOD activity, phagocytic activities and clearanceefficiencies leading to increase susceptibility to infections especially vibriosis. Hence the pH stress could trigger the disease outbreaks byreducing the immune defence mechanisms of the host.

DO levels

In intensive aquaculture practices, dissolved oxygen (DO) is a major limiting factor especially in the bottom layers of shrimp culture ponds. The DO availability is affected by parameters like temperature, salinity, TAN and free CO_2 concentrations. At higher levels of these parameters the DO saturation reduces. In *L. vannamei,* lethal DO levels decreased with increase in body weight which might be due to reduced metabolic rate as shrimp grows. Lethal DO levels decrease as temperatureincreases from 14.5 to 24.8 °C, and then increasedafterwards. For tiger shrimp at the optimum salinity of 15-25 ppt have better resistance to low DO toxicity than the in the higher or lower salinity ranges. Hence, factors like, body weight, temperature, salinity,pH, and feeding condition have significant effect on ability of shrimp to resist different lethal DO levels.

In general, optimum conditions for minimum toxic effect of low DO levels are temperature at 22 °C, salinity at 16.6‰, pH in 7.56.Decomposition of accumulated feed and the animal faeces lead to hypoxic and sometime anoxic conditions particularly at nighttime. In intensive shrimp ponds, DO levels were above 4 mg/L during day time with aeration, whereas the levels may go less than2 to 3 mg/L during night time/early morning. DO less than 2.8 mg/l is considered hypoxic condition and it is known to influence growth, survival, feeding, moulting, behaviour,osmoregulatory capacity and immune response of penaeidshrimps. Though lethal DO levels vary from species to species, generally DO of 0.2 to 1.27 mg/l for *P.monodon* while 1 mg/l for *L. vannamei* is considered lethal after one hour of exposure.

Ammonia

Ammonia which accounts for more than half the non-faecalnitrogenous waste is formed by direct oxidationof amino acids,deamination of amides, degradation of purines/ pyrimidinesandhydrolysis of urea. Ammonia is utilizedas an energy source in the process ofnitrification by autotrophic aerobic bacteria (*Nitrosomonas*and*Nitrobacter*) and oxidized to nitriteandnitrate. Level of ammonia excretion by shrimp is altered by environmental factors like temperature, salinity and dissolved oxygen.

The concentration of total ammonia nitrogen (TAN)in intensive grow-out ponds increases as culture progress and levels of more than 1.0 ppm are toxic. In addition to immune response elevated concentration of TAN affects the growth, moulting, oxygen consumption and ammonia excretion.Continuous exposure of shrimp to ammonia leads to reduced phenoloxidase activity without affecting the number of circulating hemocytes.Increased concentration of TAN decreases the activity of superoxide dismutase responsible for the scavenging of reactive oxygen species (ROS) leading to increase in superoxide anion. Reduced phagocytic activity and clearance efficiency leads to increased susceptibility to vibrio bacterial infections.

Nitrite

Nitrite is an intermediate product both in bacterial nitrification of ammonia and in bacterial denitrification of nitrate. Imbalance in levels of denitrifying and nitrifying bacteria leads to accumulation of nitrite. Generally, the concentration of nitrite increases as culture progress and reach as high as 0.3 ppmin grow-out ponds of white shrimp *L. vannamei*. Nitrite is more toxic under low saline conditions, hence it is essential to take urgent remedial measures to prevent losses.

Among the metabolic toxicants nitrite is considered most dangerous as it can accumulate in haemolymph up to 10 fold higher than in water via active chloride uptake mechanismand passive entry.Increased concentration of nitrite in haemolymph leads to reduced levels of oxyhaemocyaninandincreased deoxyhemocyanin (indicating reduction of oxygen affinity). In addition to extracellular fluids, nitrite accumulates in gill, liver, brain and muscle tissue. Shrimps when exposed to higher concentration of nitrite,increase oxygenconsumption and ammonia excretion indicating increaseofenergy and protein catabolism and ultimately has adverse impact on growth and moulting.

These higher concentrations of the nitrite are known to decrease the levels of total haemocyte counts tothe reduced Prophenoloxidase and phagocytositic activities. Further, there is a reduction in superoxide dismutase activity consequently increasing the levels of cytotoxic superoxide anions.

Sulphide

Accumulation of excess feed in the pond bottom leads to decomposition of the organic matter under anaerobic conditions. Sulphide is considered highly toxic to shrimp, 50% of the shrimp die when exposed to 0.34 mg/l of sulhide for 96 hours. Following exposure tolower doses shrimp loose equilibrium while die immediately when exposed to 4.0 mg/l.Presence of sulpide affects the immune parameters like total haemocyte count, hyaline cells, phenoloxidase activity, phagocytic activity and clearance efficiency thereby making the shrimp more susceptible to pathogenic infections like, vibriosis.

Heavy metals

Generally heavy metals in the pond water are well below the toxic levels and their concentration may exceed the permissible limit due to excessive addition, one such example is copper. Excess growth of blue-green algae releasesgeosmin in low salineponds.Shrimp culturedin such waters will have unpleasant flavour. Farmers often apply excess amount of copper sulphate due to lack of information to eradicate the filamentous and blue-green algae. Dose of copper sulphate application varies from 0.1 to 0.2 ppm- and depends mainly on the total alkalinity of pond water.Environmental deteriorationdue to Cu accumulation in the pond sediments poses serious concern to shrimp health and growth.

Being heavy metal, excess copper concentration is known to decrease the haemocyte count, phenoloxidase activity, phagocytic activity and respiratory burst in cultured shrimp. Exposure of copper sulphate, as low as 5 mg/l for 24 hr leads to cytotoxic levels of superoxide anion.

This immune suppression is correlated with the increased susceptibility to *Vibrio* challenge. In addition to immune suppression, Cu-exposure causes oxidative stress leading to structural damages in the gills andhepatopancreas. Cu^{2+} disturbs the cell calcium homeostasis leading to altered mechanism of apoptosis. Mechanism of cupper toxicity has been attributed to generation of reactive oxygen species, over production of these causeoxidative damage to tissuemacromolecules including DNA, proteins and lipids.

Moulting stress

Generally, the moulting is classified as three primary moult stages: postmoult (A, B),intermoult (C) and premoult (D) which are easily identified by the degree of hardness of the exoskeleton.Moulting stages are known to influence degree of susceptibility to infection. At pre and post moult stages, the immune cells in hemolymph, granular cells and hyaline cells are reportedly lower in numbers. Since, the granular cells and hyaline cells are responsible for phenoloxidase activity and phagocytic activity respectively, any reduction in their number obviously makes the host susceptible to infection. During the intermoult stage cuticle is sclerotinized and not permeable invading pathogens but it is comparatively easy for bacteria to enter thought the new cuticle which is in making after the detachment of old cuticle during the premoult stage increasing the chances of infection during this vulnerable period.

Managing environmental parameters and stress

In view of the observed effects of environmental stress on immune system of cultured shrimp, the management strategies should include, maintaining optimum condition of pond environmental parameters. Regular monitoring of water and bottom soil in culture ponds for pH, DO, ammonia, nitrite and H_2S is the key in protecting the losses due to diseases. Since, it is not possible to go for water exchange in zero water exchange system maintaining the parameters within the permissible ranges is a challenge to farmers/farm managers. Several commercial products are available in the market for improving the quality of pond bottom soil and water.There are range of probiotics containing nitrogen and sulphur recycling bacteria which mitigate the accumulation of toxicants in water and soil respectively. Effective use of scientifically proven products helps in maintaining the optimum pond environment.

Improving immunity of shrimp

It is necessary to maintain the shrimp defence system as the ability of shrimp to withstand environmental stress and fight infection depends largely on the levels of immune function.Scientific application of commercial products having immune stimulatory effects helps the host to maintain heightened immune status. Shrimp with higher levels of immune status effectively fight environmental stress and invading pathogens. Probiotics with *Bacillus* species colonize the shrimp gut and exclude the entry of pathogens in addition to improve the immunity. Range of plant and microbial based immune stimulating products when administered help in preventing the immune suppression due to environmentalfactors. Some of our studies in different shrimp farms on utility of plant based immune stimulating agents have proven to be effective. Additionally, the microbial products like, Vibrio bactericin has also shown to improve the immune status of the shrimp under pond conditions in filed studies conducted in states like, Tamil Nadu, Andhra Pradesh and Gujarat.

Conclusion : Higher levels of metabolic ammonia and nitrite, depleting DO levels, variation in pH by above 0.5 and salinity by 5 ppt within a day in water increases the susceptibility of shrimp to pathogens. Better control of water quality within the ponds become vital when farms reporting incidence of shrimp coming upto the surface and problems of mortality. The two pronged approach of combining pond management and health monitoring is the key for sustainable shrimp production. Regular monitoring of environmental parameters and timely mitigation using appropriate biological agents is the key to protectpotential losses due stress and opportunistic bacterial infections. The understanding on ecological process occurring in shrimp culture ponds through regular monitoring will help to solve some of the disease issues faced by shrimp farms.