AQUAINTECH Inc.

Managing EMS/AHPNS

There is still much to be learned about EMS/AHPNS and the nature of the disease process. The vibrio that is responsible is part of a very large group of vibrios that are not harmful for the most part. Several though are responsible for food poisoning and some very nasty diseases in aquaculture production environments. What we have learned so far is that the bacteria appears to be naturally occurring in marine ecosystems and that it's mechanism of disease production is tied in with its ability to bind to chitin and the production of a toxin. The data to date suggests that the bacteria forms a biofilm on the stomach lining and the gastric mill, both of which are sloughed off with each molt as they are chitinous in nature. This biofilm facilitates toxin production. Biofilms are notoriously difficult to get rid of since they are highly evolved mechanisms for toxin production and to protect the bacteria from the onslaught of a variety of chemicals.

One of the challenges is the delivery of anti-microbial compounds to the area where this bacterium is in the animal. Elimination of the vectors in ponds is simply not feasible as the costs of constantly eliminating rotifers and any other chitinous surfaced, bacterial consuming zooplankton would be prohibitive even if it were possible. Cysts can be moved through air and water and are often buried deep in sediments. While prudent practices can lessen the vibrio load in the environment (using products such as our PRO4000x) and lower organic loads that vibrios feed on, there is data that there are wide variations in virulence of the strains that cause this disease. This wide disparity in virulence means that efforts to lessen the impact of vibrio loads in the environment may not be useful in areas where highly virulent strains are found. On the other hand, in environments where the strains are not as virulent, it may very well be possible to lessen the severity of the impact.

Using standard feeding techniques is not a good idea if you want to deal with EMS. The bacteria are likely transmitted via vectors, which theoretically would include any chitin-containing organism such as rotifers as well as algae, etc. Any compound that is delivered via the feed must be in frequent contact with the biofilm in order to be effective. Typically antibiotics don't work because biofilms are refractory to them and the bacterium does not appear to cause disease by an internal invasion of the shrimp.

The process entails a number of steps:

Strategies for control

The first step is to address the possibility that pond reared broodstock may be carrying the bacteria as a part of their normal intestinal flora. While acute mortality in broodstock is possible, it is not common. The bacteria typically attach to the stomach although they may also be attached to external surfaces and they have been reported to occur in feces of broodstock. Broodstock should be surface disinfected as your methodology allows. Formalin, iodophors, Chlorion, and other surface disinfectants all have proven anti-vibrio activity. Spawning in water that contains the normal spawning fluids and material from adults (feces) will ensure that eggs may be contaminated. Eggs should be washed with

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clean water and surface disinfected with any of a number of compounds such as iodophors (or others previously mentioned). Nauplii should be treated the same way post collection. Washed eggs should not be returned to the spawning tanks.

In this manner the cycle can be broken if there is a false vertical transmission component to it. It is not likely that there is a real vertical transmission component given the nature of the pathogen, although our ideas about this could change as we gain more information.

Production of PLs in traditional rearing systems has not been shown to be a source of high levels of the pathogen. There are reports of low levels occurring in these systems but no active disease outbreaks. Using our tableted bacterial product, PRO4000x, one tablet per 5 to 20 MT of water daily in production tanks has been shown to be an effective tool to lower overall vibrio loads.

Once shrimp are stocked this is where the true problem of control comes. If the current picture, based on observations in all areas where the disease occurs, is true, than there is a very high probability that the bacteria is a component of what would be the "normal" flora of the pond. Strains of this bacterium have been found attached to algae, a variety of zooplankton, as well as internally in filter feeders ranging from rotifers to mussels. It may very well not be possible to prevent some ingestion of the bacteria in your shrimp regardless of what tools you use. The question is can we inhibit the bacteria to a sufficient extent to allow the animals to grow and feed normally. This is the challenge.

Phase 1

Ponds should be prepared by turning the bottom soils to at least 10-15 cms allowing the air to dry it out. Using chlorine or broadly active antimicrobials may make the problem worse although something to lessen the WSSV vector loads is prudent. For the farm, I would use a product such our PRO4000x very heavily in the beginning. One kg per ha (approximately 63 tablets) prior to filling the ponds completely. Use carbon supplementation but only after 18-20 hours post the addition of the tablets. DO NOT USE IT EARLIER THAN THIS as it has been shown that the bacterium that causes EMS may thrive on these lower molecular weight sugars. If you feed the vibrios instead of the bacillus they will outgrow the bacillus.

Phase II

Shrimp, like all other animals have mechanisms that allow them to differentiate self from non-self materials. While they do not produce antibodies and there are other components of their immune system that are much different than phylogenetically more evolved animals, such as fish and mammals, they nonetheless have very sophisticated mechanisms to defend themselves. Shrimp cannot be vaccinated in the classic sense of the word. There is some evidence that suggests that exposure to a variety of materials induces the production of Chaperone molecules. These molecules have been shown to have a protective effect. We marketed a product some years ago that we believe likely

acted in this manner. Animals were protected in a non-specific manner. There are a number of materials that could do this, although I am not aware of any that has of yet been tested against EMS. I would suggest feeding our product to PLs during transport or in nursery ponds immediately prior to stocking. The product. MEGA X, is sold as liquid dietary supplement.

Phase III

Compound L has potent anti-vibrio activity. Feeding shrimp three times a day will not necessarily ensure adequate contact between the compound and the bacteria. The shrimp need to be consuming feed that is filling, satisfying and minimizing the additional grazing that they do as well as minimizing the cannibalism of weakened shrimp that most certainly contributes to the spread of the less virulent forms of this microbe. There is no specific manner in which this can be approached due to the absence of data.

Feed compound L using an automatic feeder, at often as you can, the more times daily the better. The less food that they consume in the ponds the less likely they are to get a mouth full of the bacteria. Consistent contact is required between compound L and the structures in the shrimp where the bacteria colonize. If shrimp are fed using the same methods that are traditional then they will be pulse fed. They consume pelleted feeds for only a short period of time during the day and the rest of the day forage for feed, which will include vectors containing high loads of the bacterium. I would begin to feed compound L as soon as you can, starting in the hatchery and moving onto the farm. There are some pesticides that could be used to kill rotifers or even better some fish species that will not eat the shrimp but will feast on the rotifers present in the ponds. Lowering the bacteria load through multiple mechanisms is an important step in trying to reduce the impact of this bacterium.

Feed shrimp continuously at 1% of their diet with compound L. Feed as frequently as is possible. The more often the greater the chance is that you can keep the bacteria on the stomach at bay. Use automatic feeders if possible or hand feeding. Material is not soluble in water. Its melting point though is 63 C (145 F) and it should be possible to heat the material to liquefy it and then spray this material on the feed or mill it into the feed. Frequency of feeding will be an important element of how well this works (if at all).

At this time we still do not have a good understanding of where in the environment the bacteria is and what the reservoirs are. There are reports that rotifers and other zooplanktonic forms may be a major source of the bacterium. This presents several challenges. The first being how to eliminate these from the ponds. While the use of pesticides and disinfectants toxic to rotifers might be useful, typically rotifers produce cysts that survive these onslaughts and that are present in sediments. Biological control is an option if one were to stock ponds with certain fish species that aggressively feed on these forms. As long as there are significant reservoirs that contain high levels of the etiologic agent of AHPNS, control will be problematic. If the bacteria entered shrimp and caused a sepsis as part of the disease process then feeding antibiotics might be a

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useful tool. However no one has yet to suggest that this is the case and there are no reports where the use of antibiotics has helped. Certain antimicrobials such as that contained in Compound L might be useful as well although they require direct contact between the compound and the bacteria to be effective. Pulse feeding shrimp three times a day, one would expect, would not be adequate.

Compound L costs between $5 and $6 per kg FOB warehouse. Comes in 25 kg bags. For 1%, you would need 10 kgs per MT of feed. It is insoluble in water. Dissolving it in ethanol would be too costly. It does melt though above 63 C so it can be liquefied. It can be ground up and added to the feed this way although bear in mind that it has a relatively low melting point. This material is a member of the glyceride family. It is GRAS.

Note: EMS is complicated and multifactorial. We have no idea yet how to control it although many things are being tested in the lab and the field. To date none have worked. There are several challenges that must be met and many unanswered questions. The proposed approach is one step. Each of the tools has proven anti-vibrio activities.

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