Biomin AquaStar® to control Vibrio spp. in shrimp farming

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Early Mortality Syndrome (EMS), also known as Acute Hepato-Pancreatic Necrosis Disease (AHPND), is an emerging disease caused by Vibrio parahaemolyticus. EMS typically affects shrimp that have not reached marketable size (40 days or younger). It causes large-scale deaths among cultivated shrimp and infected shrimp ponds can be entirely wiped out.

Although several companies have already promoted solutions to EMS, the reality is that most of the products in the market have yet to prove their efficiency in the field. As such, farmers are still looking for the “silver bullet” that can effectively solve their problem.

Up to now, the most important developments for counteracting EMS/AHPND have been preventative husbandry techniques and improvements in pond management. Among those practices, screening post-larvae (PL) for quality, the introduction of nurseries, semi-biofloc systems and polyculture with tilapia seem to have led to improved results (although sometimes not on a consistent way).

Green water development is fostered by the semi-biofloc system or the early introduction of tilapia in the pond. This accelerates water maturing so that the virulent EMS Vibrios are not able to dominate the pond and therefore cannot cause EMS after stocking with shrimp. This also stabilizes the pond ecosystem and reduces the fluctuation of water parameters, in particular pH.

Another change introduced by shrimp farmers, particularly in Thailand, is the use of nursery ponds. The smaller size and volume of the nursery ponds allow better control of pond conditions during the first 30 days.

Ideally, those pond management techniques are assisted by effective feed or pond additives that can reduce the vibrio presence and their virulence.

To combat EMS, it is important to know the enemy

All vibrios are indigenous to water and adapt well to the pond environment. They have a good appetite for all nutrients that are widely available in the pond, such as chitin from shed shells,
feed waste and excreta. They are able to withstand a drastic change in their environment, such as the drying-out of the pond during a drought by entering a viable dormant state. This is why a complete eradication of vibrios in aquaculture ponds is practically impossible. Pathogenic Vibrio investigations have clearly shown that pathogenicity varies greatly and is a complex process affected by many variables, including host, Vibrio species and strain, developmental stage, physiological condition, environmental stress, dose, time and infection method.

We also know that Vibrio bacteria possess the ability to communicate. Vibrios excrete small chemical communication molecules that allow them to sense the density of the fellow Vibrios they live with. Once they reach a critical mass, virulence factors are expressed, allowing them to cause disease. Preventing Vibrios from reaching such a critical mass might therefore be a useful way to prevent EMS.

An available tool for the pond: Probiotics

The use of probiotic bacteria to improve the pond environment and control the vibrio population has been one of the most common strategies used by farmers to fight the EMS outbreaks.

Several probiotics on the market claim to counteract pathogenic Vibrio parahaemolyticus. But when many of those strains were put to the test (figure 1), it became apparent that not all organisms were able to inhibit Vibrio growth in vitro. Some probiotic species seem to be more effective than others at inhibiting the growth of the pathogenic V. parahaemolyticus.

The probiotic strains in the AquaStar® product line such as Lactobacillus sp., Pediococcus sp., Enterococcus sp. and Bacillus sp. were shown to inhibit V. parahaemolyticus. This experiment furthermore demonstrated that pathogen inhibition is a strain-specific property. Even in different strains of the same species (Bacillus subtilis), there is considerable variation (only five out of 11 B. subtilis strains were able to inhibit the growth of virulent V. parahaemolyticus by 90%). This shows the importance of selecting effective probiotics to control vibrios and that not all probiotics have similar effects.
Figure 1: The survival of pathogenic *Vibrio parahaemolyticus* was tested by adding the culture medium of many different probiotics to the *V. parahaemolyticus* growth medium (source: Biomin Research Center). The black line indicates 100% growth compared to untreated control.
How AquaStar® probiotics perform on *V. parahaemolyticus* contaminated farms

In a trial carried out in the Marine Station of Aquaculture (FURG, Rio Grande University – Brazil) we investigated the effect of the simultaneous application of AquaStar® Pond and AquaStar® Growout in shrimp cultured in an intensive biofloc technology system contaminated with *Vibrio parahaemolyticus*. In this experiment we compared two treatments: Control and AquaStar® probiotic. For AquaStar® treatment, two multi-strain products were used:

- **AquaStar® Pond**, water application of *Bacillus sp.*, *Enterococcus sp.*, *Thiobacillus sp* and *Paracoccus sp.*
- **AquaStar® Growout**, feed application of *Bacillus sp.*, *Enterococcus sp.* and *Lactobacillus sp*.

Treatments were randomly assigned to six 3.5m³ lined raceways enclosed in a greenhouse. Each tank was stocked with 10,500 *Vibrio parahaemolyticus* infected shrimp, for a final stocking density of 300 shrimps/m². Feed (38 % CP) was supplied 3 times/day using feeding trays. The experiment lasted for 70 days.

**AquaStar® probiotics for a better survival**

Results from this trial shows that the AquaStar® treatment improved survival by 37% (figure 2). In the control group only 52% of shrimp survived, compared to 83% of shrimp in the probiotic tanks. This strongly suggests that the probiotic bacteria in the BIOMIN AquaStar® probiotics were effective in preventing a *V. parahaemolyticus* associated disease outbreak. This is mediated through various modes of action:

- competitive exclusion of the pathogens through modification of the gut microbiota composition and aquatic culture environments
- direct killing / growth inhibition of vibrios by anti-vibrio substances (figure 1)
- preventing *Vibrio* spp. from switching on their virulence factors (quorum quenching)
- Improvement of the shrimp immune response, helping the animals to help themselves
Influence of AquaStar® in growth performance and the consequences for the production system

The modified microbial communities in gut and surrounding environment will have a great impact also on other factors that in last instance will improve the overall productivity of the system. The establishment of the probiotic bacteria as part of the indigenous gut microbiota will improve the nutrient digestion. It has been well described that some probiotic strains aid digestion because they synthesize extracellular enzymes (figure 3) such as proteases, amylases, and lipases as well providing growth factors such as vitamins, fatty acids, and amino acids.
Figure 3: *In vitro* tests reveal that many probiotic *Bacillus* species possess cellulase, amylase and protease activities and that they excrete these enzymes. Also for a probiotic *Pediococcus* strain cellulase activity was demonstrated. The probiotic organisms’ digestive enzymes help the host animal to improve its feed utilization.

Therefore, nutrients are absorbed more efficiently when the feed is supplemented with probiotics. In the present trial a 48% improvement of feed conversion rate (FCR) (Figure 4) became evident in the shrimps fed AquaStar® diets. Improved feed utilization is reflected also in a 3.73% improvement of the specific growth rate (Figure 5). The 48% improvement in FCR due to the better nutrient absorption will decrease ammonia emissions through decreased nitrogen losses to the environment. To better illustrate how big the impact of this 48% improvement in FCR can be on the environment, table 1 shows a simple calculation on how much we can save on excreted matter simply by using a powerful multi-strain probiotic. Based on several peer-review papers for this species, we assumed an apparent digestibility coefficient (ADC) of 80%. Considering the difference in final biomass obtained in this trial due to the 37% difference in survival, AquaStar® treatment prevented the excretion of 274 182 kg of organic matter (table 1) to the rearing system during the 70 days of this trial.

However, if we assume control treatment with a similar biomass as the AquaStar® group, the amount of organic matter being excreted from control shrimp to the rearing system for the whole trial would be 4.2 tonnes, 2 tonnes more than from the AquaStar® group.

Figure 4: The feed conversion rate during experimental period for control and AquaStar® treatment (source: FURG, Brazil).
Figure 5: The specific growth rate during experimental period for control and AquaStar® treatment (source: FURG, Brazil).

Table 1: Theoretical estimation of excreted matter during experiment, assuming a ADC of 80%

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Final biomass (Kg)</th>
<th>FCR</th>
<th>Feed used (Kg)</th>
<th>ADC (%)</th>
<th>Excreted matter (Ton)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>45 973</td>
<td>2.7</td>
<td>124 127</td>
<td>80</td>
<td>2.5</td>
</tr>
<tr>
<td>AquaStar®</td>
<td>78 870</td>
<td>1.4</td>
<td>110 418</td>
<td>80</td>
<td>2.2</td>
</tr>
<tr>
<td>Control²</td>
<td>78 870</td>
<td>2.7</td>
<td>212 949</td>
<td>80</td>
<td>4.3</td>
</tr>
</tbody>
</table>

¹ADC=Apparent digestibility coefficient (%); This value was not calculated, was obtained as a mean value from several peer-review papers for the specie in study.
²Control group assuming a similar final biomass as AquaStar® (+/- same survival)

Environmental and gut probiotics: working together

The excellent results obtained in this trial are a combination of several factors that lead to an overall improvement of the production system. In this *V. parahaemolyticus* infected tank facility, the application of AquaStar® probiotics prevented a disease outbreak through several modes of action:

- Reducing nutrient excretion also means less available nutrients in the water for opportunistic pathogens to grow.
- The probiotic organisms in feed colonized the shrimp’s gut and had a chance to compete with pathogens for attachment space and nutrients.
- It is furthermore imaginable that they had direct inhibitory effects against *Vibrio* spp. as *in vitro* data shows that the probiotic AquaStar® organisms inhibit the growth of *V. parahaemolyticus*. 
A proportion of the probiotic cells present in feed (AquaStar® Growout) will pass through the digestive system and exert their effects on the environmental microbial communities together with the environmental/bioremediation bacteria in the water (AquaStar® Pond). Together they will lead to a slower accumulation of slime or organic matter in the pond bottom and compete with pathogens.

The bioremediation bacteria will optimize nitrification rates to keep ammonia concentration low; optimize denitrification rates to eliminate excess nitrogen from ponds as nitrogen gas; maximize sulfide oxidation to reduce accumulation of hydrogen sulfide; maximize carbon mineralization to carbon dioxide to minimize sludge accumulation; maximizing primary productivity that stimulates shrimp production.

Exposing shrimps to inappropriate levels of dissolved oxygen, ammonia, nitrite or hydrogen sulfide can lead to stress and diseases. Analyzing the water quality parameters like ammonia, nitrites and nitrates (Figures 6, 7 and 8), during this experiment, we can easily see the beneficial effect of the probiotic AquaStar® treatment. In the final week of the trial, where the accumulation of waste materials would become apparent as a decline in water quality, the AquaStar® treatment led to healthier environmental parameters in the AquaStar® treated tanks. This is particularly remarkable, as the AquaStar® tanks harbored approximately two times the biomass of the control treatment due to the better survival.

Figure 6: Ammonia measurements during experimental period for control and AquaStar® treatment (source: FURG, Brazil).
Figure 7: Nitrites measurements during experimental period for control and AquaStar® treatment (source: FURG, Brazil).

Figure 8: Nitrates measurements during experimental period for control and AquaStar® treatment (source: FURG, Brazil).

Probiotics - a way to achieve a sustainable industry

The increasing awareness for sustainable shrimp production has led to a run for quality and sustainability certification in the recent years. Therefore, the shrimp industry invested in the
development of sustainable shrimp farming practices in order to meet international market requirements. One of the hottest certification programs, ASC (Aquaculture Stewardship Council) is built on 7 main pillars, including:

legal compliance; land and water use; water pollution and waste management; genetics; feed management; health medicines and chemicals management and finally, social responsibility.

From those issues, aspects such as: nutrient utilization efficiency, water quality, pond environmental improvement, sludge control, reduced use of medicines and antibiotic growth promoters and survival improvement, can be achieve by using feed additives.

As the Brazilian example shows, powerful multi-strain probiotics are particularly efficient in achieving sustainable shrimp culture. As observed in this trial, AquaStar® Pond and AquaStar® Growout were able to increase the survival by 37% and to reduce the FCR by 48%. This represents 1 tonne of saved feed to produce the same amount of shrimp and a 2 tonnes reduction of excreted wastes. At the same time an overall improvement of the production system was achieved and medicine use avoided, even in *V. parahaemolyticus* contaminated shrimps.