Evaluation of Vibrio control

with a multi-species probiotic in shrimp aquaculture

Competence Center Microbials

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In the economically important panaeid shrimp, members of the microorganism genus Vibrio have become a major constraint on production and trade during the past two decades. They are responsible for several diseases and mortalities of up to 100 percent, causing global losses of around US$ 3 billion. Shrimp disease prevention and control are now priority research topics. In this article BIOMIN reports on *in vivo* trials using AquaStar® as a probiotic feed additive in P. vannamei.

Penaeid shrimp culture has become an important economic activity in many countries, particularly in Asia and South America, where shrimp farming represents a substantial source of revenue.

Shrimp accounts for about 20 percent of the value of exported fishery products over the past 20 years. However, the shrimp farming industry is constantly under threat due to the outbreak of infectious diseases and environmental problems.

Bacteria are among the groups of microorganisms causing serious losses in shrimp culture throughout the world. Members of the genus Vibrio, including V. parahaemolyticus and V. harveyi, have been described as the main pathogenic species in shrimp and are responsible for most of the larval deaths. These pathogens cause serious infections, decreased production both in the hatchery and grow-out ponds, reduced feed conversion and growth rates in surviving individuals, thus having a negative impact on the overall financial efficiency of the business.

Vibriosis in shrimp aquaculture

Vibriosis is a bacterial disease caused by gram-negative, motile, facultative anaerobe bacteria of the family Vibrionaceae. It is ubiquitous throughout the world and all marine crustaceans, including shrimp, are susceptible. Vibrio species are the eminent microorganisms in the marine environment and usually constitute the majority in the normal microflora of farmed and wild panaeid shrimp. They become opportunistic pathogens when the natural defence mechanisms are suppressed (Lightner, 1993). In intensive systems, shellfish species are often exposed to stressful conditions due to the high stocking density, leading to secondary vibriosis.

Vibrio harveyi, a luminous marine bacterium, is one of the most important etiological agents of mass mortalities of black tiger shrimp (Penaeus monodon) larval rearing systems. Epizootics occur in all life stages but are more common in hatcheries.

Vibrio parahaemolyticus is a halophilic bacterium distributed in temperate and tropical coastal waters throughout the world (DePaola *et al.*, 2000). Some strains can cause acute gastroenteritis in humans, often after the consumption of contaminated seafood (Matsumoto *et al.*, 2000).

Some Vibrio species have very high growth rates under optimal conditions. Disease transmission can occur rapidly and is either via water or as a result of ingestion of infective material, although there is some evidence that wounds can also provide a means of entry. The pathogen releases exotoxins that effectively break down the wall of the gastrointestinal tract and destroy the host´s immune cells. Death can occur overnight after acute outbreaks. (Peddie and Wardle, 2005)

Signs of Vibrio disease

Vibrio infections are commonly known as black shell disease, tail rot, septic hepatopancreatic necrosis, brown gill disease, swollen hindgut syndrome and luminous bacterial disease, describing a number of clinical signs:

- Lethargy
- Loss of appetite
- Discoloured and necrotic hepatopancreas with the presence of „clumping“ (aggregation of digestive cells)
- Red discolouration of the body
- Yellowing of the gill tissue
- White patches in the abdominal muscle
- Melanisation
- Granulomatous encapsulation, necrosis and inflammation of organs (lymphoid organ, gills, heart etc.)
- Luminescence

Management strategies for shrimp disease prevention and control

Use of antibiotics to control these agents has led to problems of drug resistance and resulted in trade restrictions in export markets. Shrimp aquaculture continues to find more effective and environmentally friendly approaches of improving shrimp health and yields.

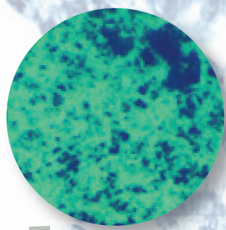
One such approach is the prevention of infection by using specific pathogen free (SPF) shrimp. Such shrimp are genetically improved stocks known to be free of one or more specified pathogens and will ensure that seed shrimp are not the conduit for introduction of pathogens (Lotz, 1997). However, SPF status is a temporary condition which isn´t passed on genetically and is lost once the SPF broodstock are transferred to a commercial facility.

Vaccination or immunostimulation of shrimp is another widely accepted technology that promotes the immune response. Since shrimp possess a non-specific immune system without antibodies, they are not enabled to specifically "remember" exposure to pathogens, which is the basis of vaccination. Consequently, the efficiency of response on subsequent encounters may be limited.

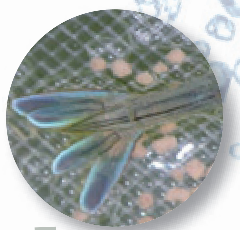
Probiotics are another means of disease control which have found use in aquaculture. The mode of action of the probiotics is rarely investigated, but possibilities include competitive exclusion, that is, the probiotics actively inhibit the colonisation of potential pathogens in the digestive tract by production of bactericidal substances, competition for nutrients and space, and modulation of the immune-system. The stimulation of host immunity and exclusion of pathogens may provide greater non-specific disease protection as a result of both immunity enhancement and competitive exclusion (Rengpipat et al., 2000).

There is accumulating evidence that the prophylactic use of beneficial bacteria is effective at inhibiting a wide range of fish pathogens.

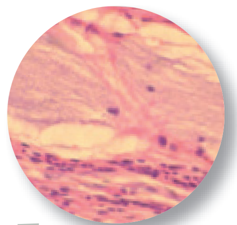
Recent data from *in vivo* experiments suggest that AquaStar® may be beneficial in the control of the Vibrio load in shrimp aquaculture. AquaStar® is a well-defined, multi-strain



Vibriosis in Shrimp



L. vannamei with greenish fluorescence on the tail (Courtesy Dariano Krummenauer)



Necrosis on the muscular fiber caused by colonies of V. parahaemolyticus (Courtesy Dariano Krummenauer)

Table 1: Enumeration of total Vibrio spp. and Enterococcus in shrimp digestive tract after feeding with test diet for 6 weeks (by FISH technique)

	CFU/g			
	Hepatopancreas		Intestine	
	Total Vibrio (x 10^4)	Enterococcus (x 10^6)	Total Vibrio (x 10^6)	Enterococcus (x 10^8)
Control	68.8 +/-19.5[a]	–	94.1 +/-68.2[ns]	–
AquaStar®	1.7 +/-0.9[b]	56.5 +/-23.2[b]	29.5 +/-19.4[b]	7.8 +/-5.7[b]

[a, b] Means within a column with different superscripts differ significantly ($p < 0.05$)

probiotic product for fish and shrimp and promotes a beneficial gut microflora as well as an improved environmental condition in shrimp and fish ponds.

Reducing the Vibrio load in the intestine of shrimp

A study by Dr Kidchakan Supamattaya (2006) at the Prince of Songkla University, Thailand, has shown that the use of AquaStar® Hatchery in feed is effective in reducing the total number of Vibrio bacteria found in the hepatopancreas and intestine of white shrimp (Panaeus vannamei), which can reduce the risk of infection *(see Table 1).*

Groups of 20 juvenile white shrimp (1 - 1.5 g) were stocked into 200 L glass aquaria and fed to satiation five times daily during a six-week period. A commercial type diet was used as a control. AquaStar® Hatchery was supplemented to the feed at an inclusion rate of 0.5 percent. Six replicates for treatment were used. During the trial all data have been recorded.

Enterococcus faecium – as part of AquaStar® Hatchery - was found along the shrimp digestive system in the group fed the diet including this probiotic strain. The total number of Vibrio spp. found in the hepatopancreas and intestine of shrimp fed AquaStar® Hatchery was lower than in the control group.

In a recent study, the Marine Station of Aquaculture at the Federal University of Rio Grande (FURG) in Brazil, investigated the effect of the simultaneous application of AquaStar® Pond and AquaStar® Growout in Litopenaeus vannamei cultured in a biofloc technology system contaminated with Vibrio parahaemolyticus (Krummenauer *et al.*, 2009).

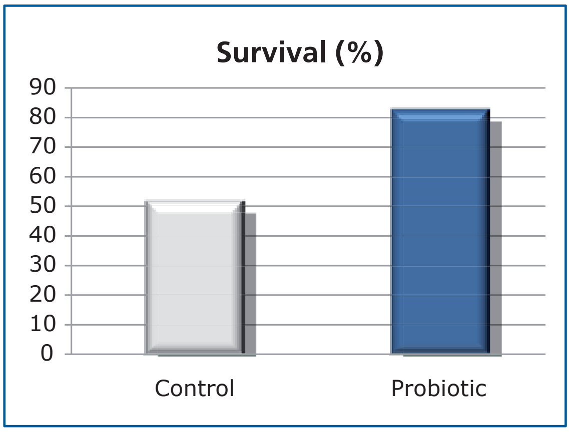
Table 2: Mean survival, growth rate, final weight, final biomass and FCR

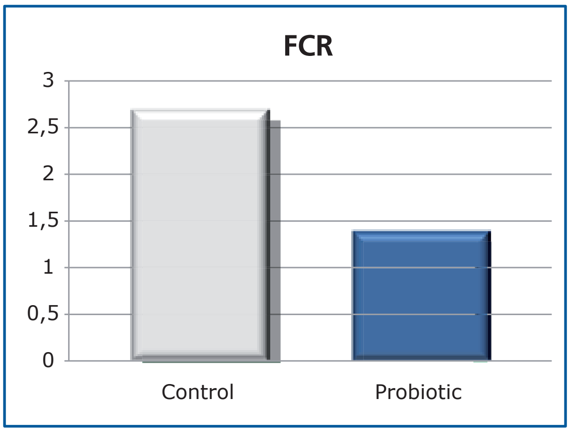
	Control	AquaStar®
Survival (%)	52.0[a]	83.0[b]
Growth rate (g/week)	0.85[a]	0.92[b]
Final weight (g)	8.42[a]	9.05[a]
Final biomass (kg/tank)	45.97[a]	78.87[b]
FCR	2.70[a]	1.40[b]
Productivity (kg/m²)	1.31[a]	2.25[b]

[a, b] Means within a row with different superscripts differ significantly ($p < 0.05$); analyzed by one-way analysis of variance (ANOVA).

The juvenile white shrimp were stocked in tanks at the high density of 300 shrimp/m². Feed (38 percent crude protein) was supplied three times/day. The experimental group additionally received three grams of AquaStar® Growout/kg feed and 0.5 ppm/week of

Figure 1 and 2: Survival and FCR of juvenile shrimp in biofloc culture system ($p < 0.05$)





AquaStar® Pond during the rearing period. Biological parameters, growth, weight gain, FCR, and survival were evaluated throughout the study for each group. The experiment lasted for 70 days. The results showed that AquaStar® was effective in controlling Vibrio parahaemolyticus in a biofloc culture system and improved the overall productivity of the system. Survival was increased by 30 percent and FCR improved significantly as well. Despite the high density, the final weight of shrimp supplemented with AquaStar® was also slightly increased (8.42 g versus 9.05 g). Thus, final biomass was significantly higher in the group receiving AquaStar® resulting in a 70 percent increase in production (79 kg versus 46 kg).

Conclusion

In order to withstand the high stocking densities in shrimp production (hatcheries and pond grow-out) and related stress situations, directly-fed probiotics are a promising additive to stimulate shrimp growth and secure a low disease response. The data of these studies suggest that the use of AquaStar® improved survival, growth rates, and the general health status of juvenile Litopenaeus vannamai while also reducing pathogenic Vibrio spp.

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