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Aquaculture

Science & Solutions

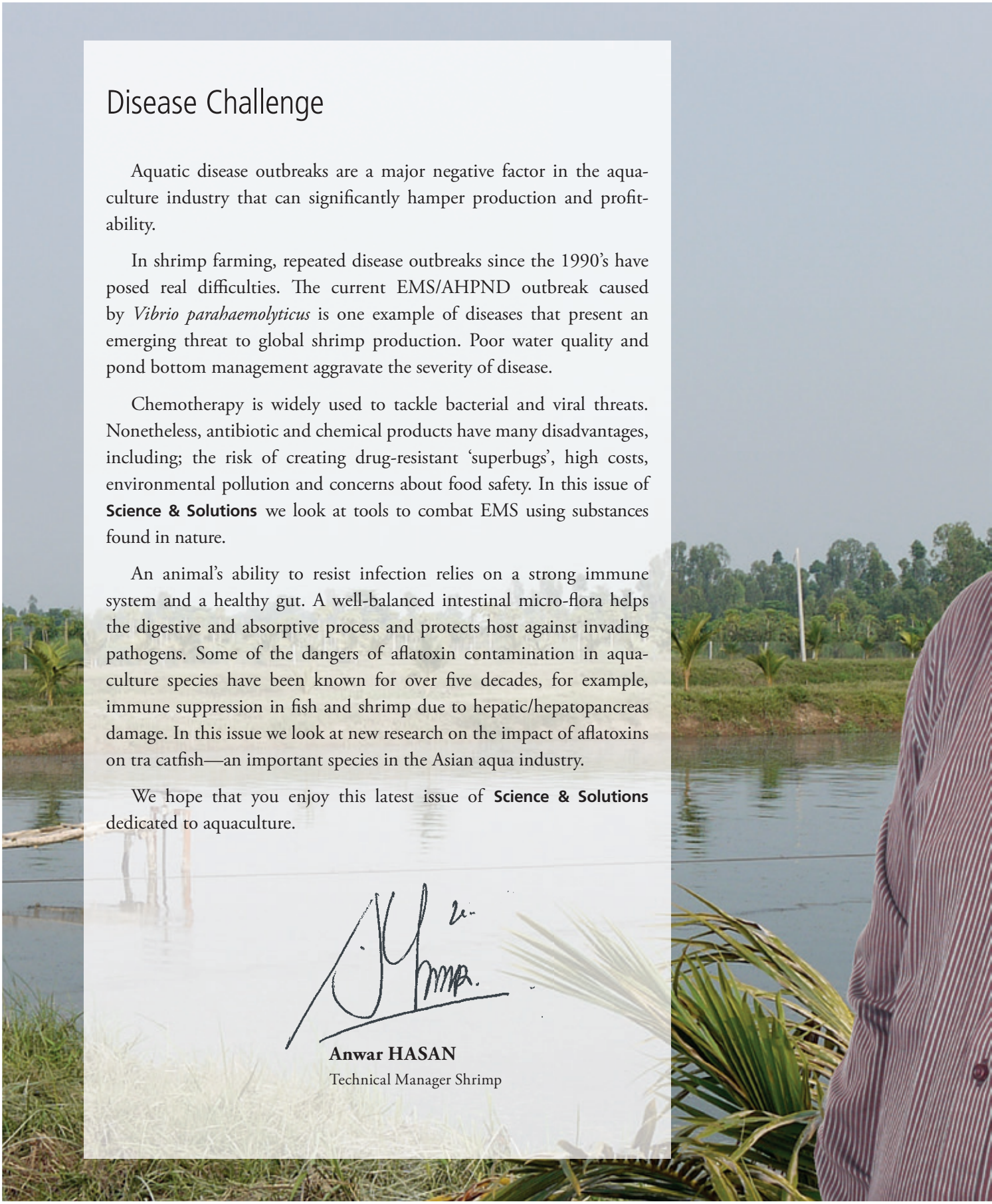
The Emerging EMS Threat

Photo: BobHemphill

Can natural strategies effectively combat EMS?

How aflatoxins threaten tra catfish production

Editorial



Disease Challenge

Aquatic disease outbreaks are a major negative factor in the aquaculture industry that can significantly hamper production and profitability.

In shrimp farming, repeated disease outbreaks since the 1990's have posed real difficulties. The current EMS/AHPND outbreak caused by *Vibrio parahaemolyticus* is one example of diseases that present an emerging threat to global shrimp production. Poor water quality and pond bottom management aggravate the severity of disease.

Chemotherapy is widely used to tackle bacterial and viral threats. Nonetheless, antibiotic and chemical products have many disadvantages, including; the risk of creating drug-resistant 'superbugs', high costs, environmental pollution and concerns about food safety. In this issue of **Science & Solutions** we look at tools to combat EMS using substances found in nature.

An animal's ability to resist infection relies on a strong immune system and a healthy gut. A well-balanced intestinal micro-flora helps the digestive and absorptive process and protects host against invading pathogens. Some of the dangers of aflatoxin contamination in aquaculture species have been known for over five decades, for example, immune suppression in fish and shrimp due to hepatic/hepatopancreas damage. In this issue we look at new research on the impact of aflatoxins on tra catfish—an important species in the Asian aqua industry.

We hope that you enjoy this latest issue of **Science & Solutions** dedicated to aquaculture.

Anwar HASAN
Technical Manager Shrimp

Contents

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Can natural strat effectively comb

egies at EMS?

Each year the shrimp industry suffers losses of more than US$1 billion due to early mortality syndrome or acute hepatopancreatic necrosis (EMS/AHPND), an emerging disease caused by the bacterium *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. First reported in China in 2009, the disease has spread to other countries—Vietnam (2010), Malaysia (2011), Thailand (2012) and Mexico (2013).

Pedro Encarnaçao, Director Business Development
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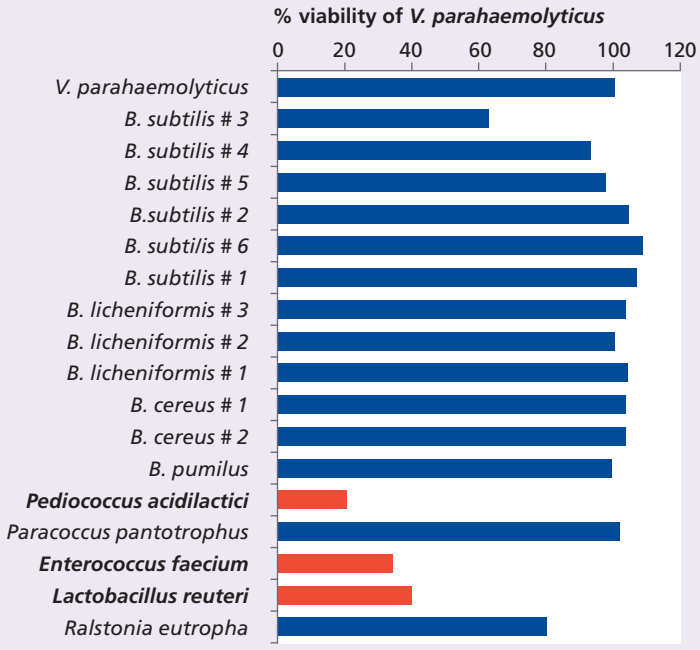
Reports of EMS

In response farmers have dedicated more attention to pond management techniques to reduce the *Vibrio* presence in their ponds. Introduction of nursery systems, disinfection, use of probiotics, polyculture with tilapia and, more alarmingly, the return to wide use of antibiotics have presented no relief to the EMS problem. This could be related to the fact that these efforts are, in many cases, generic solutions to eliminate all bacteria present in the pond and not ones designed to specifically target the precise *Vibrio* strain and its ability to survive or become virulent. In this article we focus on three main strategies for combating *Vibrio*: probiotics for pond, in-feed tools such as phytogenics and acids, and quorum quenching compounds.

Figure 1. Varying effectiveness of probiotic bacteria against pathogenic *Vibrio parahaemolyticus* after 8 hours.



Source: BIOMIN Research Center

The bacterial culprit

Vibrio spp. are Gram-negative bacteria indigenous to water. They are difficult to eradicate because they adapt well to different environmental conditions and can adopt a dormant state when facing adverse conditions. The pathogenicity of the EMS/AHPND agent varies greatly. There are many strains of *V. parahaemolyticus*, some virulent for EMS/AHPND, others not. Even among those that can cause EMS/AHPND there is a wide range of virulence levels, with some mild strains that can cause mortalities when they reach concentration levels of 10^6 to 10^7 colony-forming units per milliliter (CFU/ml), while other more virulent strains can cause mortalities at lower levels 10^4 to 10^5 CFU/ml.

A bacteria's capacity to cause disease, or virulence, is a complex process affected by many variables, including host, *Vibrio* strain, developmental stages, physiological conditions, environmental stress, and infection method.

Probiotics for pond

The use of probiotic bacteria to improve pond environment and control *Vibrio* populations has been one of the most common strategies used by farmers to fight EMS outbreaks. Yet, not all probiotic bacteria are effective.

According to recent analysis from BIOMIN Research Center, certain probiotic species seems to be better than others in inhibiting the growth of the pathogenic *V. parahaemolyticus*. As *Figure 1* shows, probiotic strains such as *Lactobacillus reuteri*, *Pediococcus acidilactici*, *Enterococcus faecium* and *B. subtilis* (proprietary BIOMIN probiotics) were shown to inhibit *V. parahaemolyticus*. This shows that not every menace can be targeted with *Bacillus* bacteria. In fact, results demonstrate that pathogen inhibition is most effective with the three lactic acid bacteria, *L. reuteri*, *P. acidilactici*, and *E. faecium*. Within the *Bacillus* family, pathogen inhibition is both species-specific as well as strain-specific. Among different strains of the same species, *B. subtilis*, one out of six strains was able to inhibit the growth of virulent *V. parahaemolyticus*.

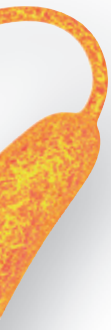
This variation highlights the importance of selecting effective probiotics in combating pathogens or pathogenic bacteria.

In-feed tools

Animal gut health is crucial to animal performance. While pond contamination by pathogenic *Vibrio* is cause for alarm, ultimately its effects are going to be exerted in the animal's digestive system, for shrimp in the hepatopancreas. As such, strategies to reduce the effects of the *V. parahaemolyticus* in the shrimp digestive system can help protect the animal.

Certain essential oil mixtures and organic acid mixtures have been shown effective for their inhibitory potential towards *V. parahaemolyticus*. These compounds can be added to the feed to have an effect in the digestive system of the animal. The acid mixture in *Figure 2* inhibited *V. parahaemolyticus* growth by 80% to 95% at a concentration of 5000 ppm. The minimal effective dose is between 1000 and 5000 ppm. Essential oils mixtures have also been demonstrated to possess an inhibitory potential, such as the one in *Figure 3* which inhibits *V. parahaemolyticus* growth by 80% to 85%. The minimal effective dose is between 100 and 500 ppm.

Pedro Encarnacao, Director Business Development
Jutta Zwielehner, Product Manager



Parliamentary pathogens

Vibrio bacteria possess the ability to communicate with each other by excreting small chemical molecules that allow them to sense the density of surrounding *Vibrios*—bacterial chatter known as quorum sensing. Once the bacteria reach a critical mass they then switch on their virulence factors, allowing them to cause disease. Preventing *Vibrios* from reaching critical mass can therefore be a useful way to prevent EMS.

Quorum quenching compounds

Whereas high dosages of phytogenic compounds restrain bacteria growth, shown in *Figures 2* and *3*, lower dosages can restrain their virulence. A wide range of phytogenic substances have been shown to inhibit quorum sensing function of bacteria—a silencing effect known as quorum quenching. Compounds found in several types of marine algae, spices, herbs and essential oils have all been found to possess quorum quenching capabilities. *Figure 4* shows how one such phytogenic substance reduces bacterial chatter *in vitro* without inhibiting growth as measured by optical density. (Higher optical density indicates higher growth: lower optical density; lower growth). Luminescence activity, or glowing, strongly correlates with quorum sensing and virulence. Suppression of luminescence at uninhibited growth therefore indicates quorum quenching activity of the phytogenic preparation. This quorum quenching can be seen in the lower luminescence of the low dosage phytogenic groups versus the control group.

Conclusion

Early mortality syndrome/acute hepatopancreatic necrosis (EMS/AHPND) caused by *Vibrio parahaemolyticus* presents a recent and serious risk to shrimp production in many countries. Afflicting younger shrimp, it has the potential to wipe out entire pond populations. While tools to counter EMS are available, to date no single solution has proven to be 100% effective. A number of tools offer hope. Some probiotic strains significantly inhibit growth of virulent *V. parahaemolyticus*. Certain essential oil mixtures and organic acid mixtures have been shown effective. Cutting the bacteria's phone lines using quorum quenching compounds can also help in restraining virulence. A more holistic approach to fight the menace both in the pond and within the animal's digestive system is needed. Effective solutions must integrate many variables and test their effectiveness both in controlled laboratory conditions and also under the challenging field conditions.

Figure 2. *In vitro* inhibition potential of an organic acid mixture against virulent *V. parahaemolyticus*.

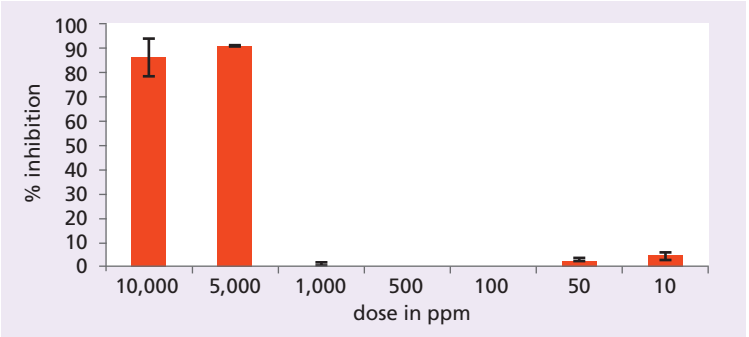


Figure 3: Growth inhibition of virulent *V. paraemolyticus* after exposure to an essential oil mixture.

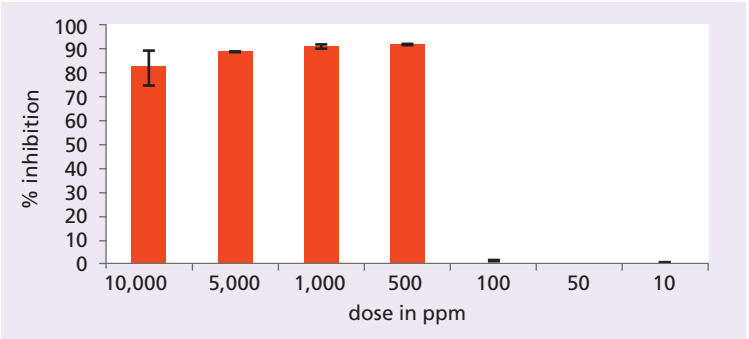
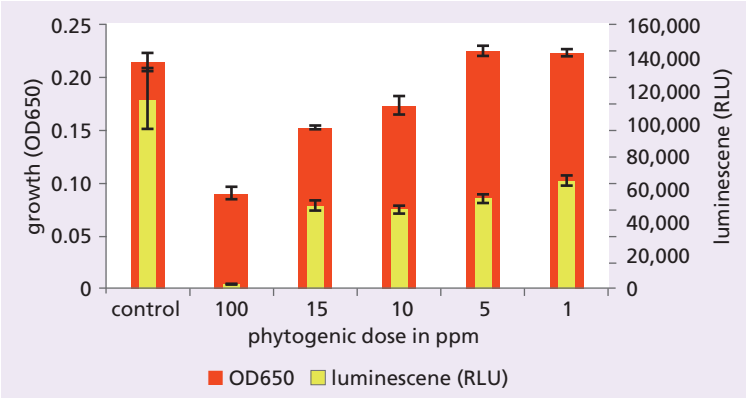


Figure 4. A phytogenic substance's quorum quenching in *V. parahaemolyticus* means less virulence of the pathogen.



of the aqua feed samples were contaminated with aflatoxins at a high average concentration of 49 parts per billion.

Source: 2014 BIOMIN Mycotoxin Survey

How aflatoxins threaten Pangasius catfish production

Rui Gonçalves, Technical Manager Aquaculture

The importance of aquaculture in Asia goes beyond its relatively high contribution to the worldwide aquaculture output. Fish products feature prominently in local diets of Southeast Asia. Around 31 million people are employed in aquaculture in Asia.

In aquaculture the importance of mycotoxins –fungal metabolites toxic to animals and humans– first became apparent during the early 1960s with an outbreak of aflatoxicosis in hatchery-reared rainbow trout, *Onchorhynchus mykiss*, after being accidently fed cottonseed meal contaminated with aflatoxins.

Aflatoxins in aquaculture

Aflatoxins, a type of mycotoxin produced by Aspergillus species of fungi, can colonize many potential aquaculture feedstuffs such as corn, peanuts, rice, fish meal, shrimp and meat meals. Aflatoxin B_1 (AFB_1) is one of the most potent naturally occurring carcinogenic agents in animals. Initial findings associated with aflatoxicosis in fish include pale gills, impaired blood clotting, anemia, poor growth rates or lack of weight gain.

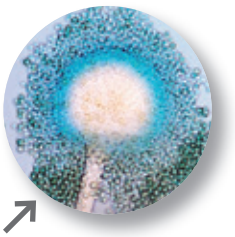
According to the annual BIOMIN Mycotoxin Survey in 2014, a look at aqua feeds for fish and shrimp showed that, out of 35 feed samples analyzed, 63% of the aqua feed samples were contaminated with aflatoxins at a high average concentration of 49 parts per billion (ppb), with some samples reaching up to 221 ppb. Furthermore, 27 of the 35 feed samples that were analyzed contained more than one mycotoxin. This poses additional risk to animals since in many cases the combined effects of two mycotoxins are higher than the individual effects of each toxin alone.

Effects on tra catfish

Due to the importance of tra catfish (*Pangasianodon hypophthalmus*) production in Asia, BIOMIN conducted complex trials with the main objective of determining the sensitivity of this species to increasing levels of AFB_1 contamination in the feed. Sensitivity to AFB_1 was evaluated in terms of growth performance, biological changes and disease resistance. An important part of the trial was to evaluate the efficiency of an aflatoxin binder, Mycofix® Secure, in order to counteract the negative effects caused by AFB_1.

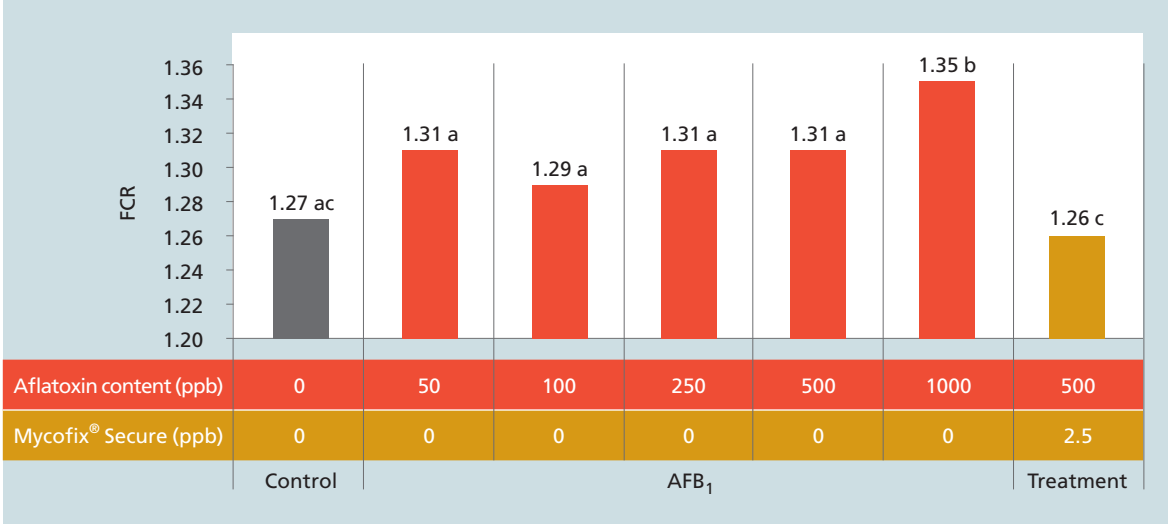
Experiment overview

The experiment was conducted at Nong Lam University in Ho Chi Minh City, Vietnam in order to test the effects of aflatoxin B_1 and the counteracting



The danger of mycotoxins to aquaculture production first became apparent in the early 1960s.

Figure 1. Feed conversion ratio (FCR) during the first 8 weeks of the trial. Values with different letters are significantly different (P < 0.05).



Source: BIOMIN

properties of the binder Mycofix® Secure. For 8 weeks a total of 100 fish with an average initial weight of 8 g were fed seven diets: one control, 5 with varying amounts of aflatoxins, and one with aflatoxins and Mycofix® Secure.

Growth performance

After the 8-week feeding period tra catfish showed sensitivity towards AFB_1. The presence of aflatoxins raised the feed conversion ratio (FCR), resulting in lower feed efficiency (*Figure 1*).

The specific growth rate (SGR) after 8 weeks was reduced in all dietary AFB_1 groups (*Figure 2*). Here the relationship between aflatoxin contamination and lower growth appeared more linear: higher concentrations of aflatoxins further slowed growth. Fortunately, treatment proved effective. The inclusion of Mycofix® Secure in a contaminated diet significantly improved the results of FCR and SGR. In both cases, similar results were obtained compared to the control group, despite the ingestion of AFB_1.

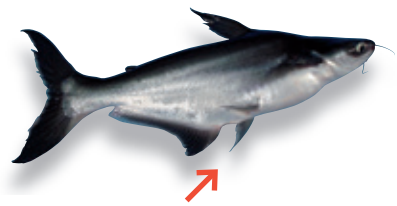
Liver damage

As in mammals, in fish some enzymes can be used as indicators of hepatotoxic effects. One such enzyme –aspartate aminotransferase, or AST– is found in high concentrations in the liver, heart, muscles and kidney. Tissue damage causes the release of AST. Alanine aminotransferase, or ALT, is present primarily in liver cells and is highly associated with hepatic necrosis, or liver damage.

In the first 8 weeks of trial, no significant difference in the activities of AST and ALT were found compared to the control. However, after 12 weeks a statistically significant increase of the enzymatic activity associated with liver damage was verified in fish fed diets containing AFB_1 (*Table 1*). Fish supplemented with Mycofix® Secure had a statistically significant decrease in the activity of these enzymes, indicating the preservation of liver tissue.

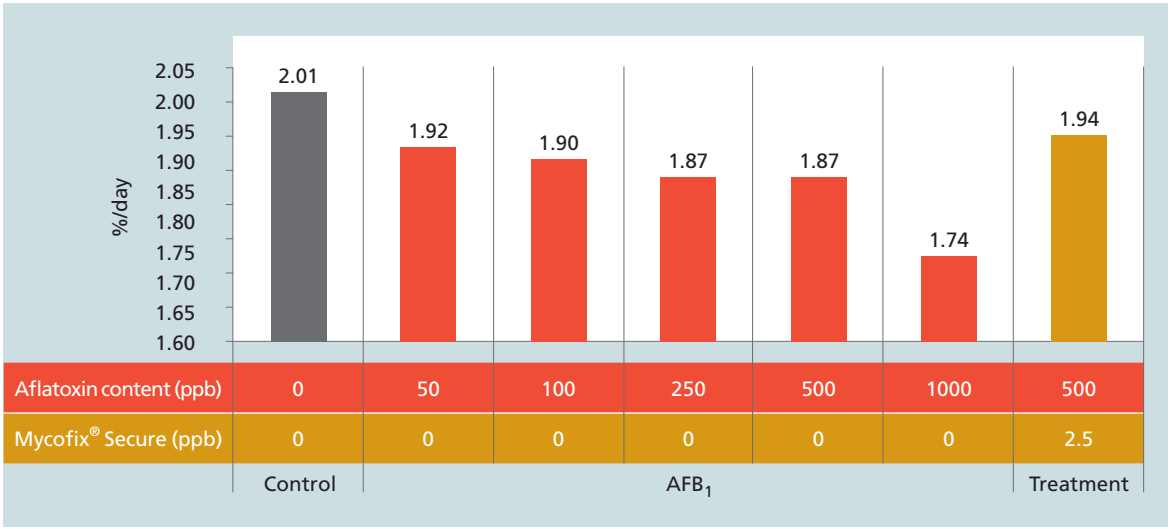
Challenge test with *E. ictaluri*

In addition to these negative health impacts, AFB_1 is also a potent immune-modulator leading to the suppression of the immune system. In other words, aflatoxin contamination makes animals more prone to infection. In the study, fish fed aflatoxin contaminated diets were evaluated for an additional 4 weeks period (total of 12 weeks) to analyze subclinical



BIOMIN conducted complex trials to determine the sensitivity of *Pangasianodon hypophthalmus* to aflatoxin contamination.

Figure 2. Specific growth rate (SGR) during the first 8 weeks of the trial.



Source: BIOMIN

effects and disease resistance under the challenge with the gram negative bacterial strain *Edwardsiella ictaluri* (4.4 x 10^6 CFU/mL). Fish fed diets containing 250 ppb of AFB_1 had a significantly lower survival rate after 1 week of challenging with *Edwarsiella ictaluri* bacteria. After 2 weeks without any treatment, the survival rates of all dietary AFB_1 were significantly lower than the control (*Figure 3*). It is clear that a long-term feeding of AFB_1 can significantly affect tra catfish resistance to diseases.

Conclusion

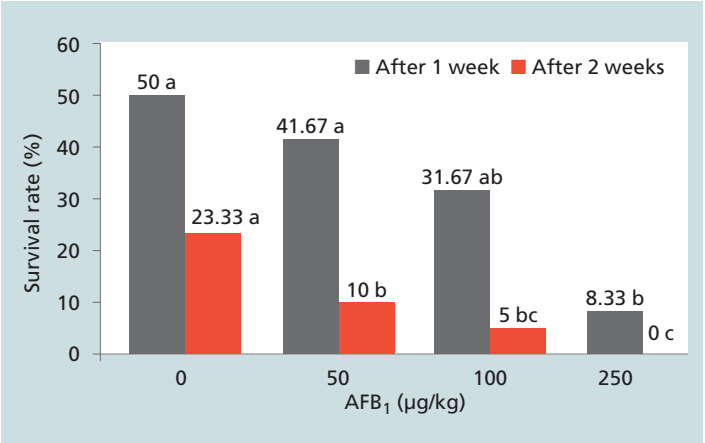
The danger posed by aflatoxins to aquaculture became apparent in the 1960s. While much has been learned since that time, further research regarding the effects of aflatoxins on health and performance of aqua species is still needed. In fish, aflatoxin exposure results in pale gills, impaired blood clotting, anemia, poor growth rates or lack of weight gain. Our research shows that in tra catfish —an important species in the Asian aquaculture industry– aflatoxin contamination results in; lower feed efficiency, lower growth, the elevated presence of enzymes associated with liver and tissue damage, and lower disease resistance. Consequently, constant monitoring, prevention and mitigation of aflatoxin contamination are therefore necessary in the aquaculture industry and in tra catfish production in particular.

Table 1. Enzymatic activity indicates the effectiveness of treatment in preserving liver tissue. Values with different letters are significantly different (P < 0.05).

	AST (IU/ml)	ALT (IU/ml)
Control	402[a]	27.7[a]
AFB_1 (500µg/kg)	498.2[b]	41.4[b]
AFB_1 (500µg/kg) + Mycofix® Secure (2.5 kg/t)	377.1[a]	26.6[a]

Source: BIOMIN

Figure 3. Survival rate (%) after a challenge test with *Edwarsiella ictaluri*. Values with different letters are significantly different (P < 0.05).



Source: BIOMIN

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