Issue 46 • Aquaculture
A magazine of Biomin

Aquaculture

Science & Solutions

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Managing the invisible symptoms of mycotoxin contamination

Editorial

Challenges are only obstacles if you let them stop you…

Global aquaculture is undergoing constant and rapid expansion, driven by the persistently increasing demand for protein derived from aquatic species. Growth has been much faster for fed species compared to non-fed species. In this scenario, a rise in productivity is crucial to fill the gap between supply and demand.

Intensive aquaculture, when compared to other sectors, is still in its infancy. Ongoing research in different fields like breeding technologies, genetics and farming technologies will likely improve profitability in the near future. However, one of the main practices currently used to increase output is the intensification of production systems. This is inevitably linked with many challenges. The demand for cheaper raw materials for feed production, the need for good quality water and the challenge of maintaining the quality of such water, and the increased risk of diseases are just some examples. Best practice gut health management and an optimized mycotoxin risk management program, combined with proper process management can help overcome many of these problems.

In this issue of **Science & Solutions**, the still unknown risks associated with mycotoxin feed contamination, related to the increased use of plant material in the diets, and the problems faced by emerging aquaculture farming in the Middle East are explored by the knowledgeable BIOMIN aqua experts.

I am sure you will enjoy reading.

Antonia TACCONI PhD
Global Product Line Manager Acids

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Science & Solutions is a publication of BIOMIN Holding GmbH, distributed free-of-charge to our customers and partners. Each issue of **Science & Solutions** presents topics on the most current scientific insights in animal nutrition and health with a focus on one species (aquaculture, poultry, swine or ruminant) per issue.
ISSN: 2309-5954

For a digital copy and details, visit: http://magazine.biomin.net
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Printed on eco-friendly paper: Austrian Ecolabel (Österreichisches Umweltzeichen)

BIOMIN is part of ERBER Group

Pioneer Shrimp Farmers in the Middle East

By **Benedict Standen**, PhD – Product Manager at BIOMIN

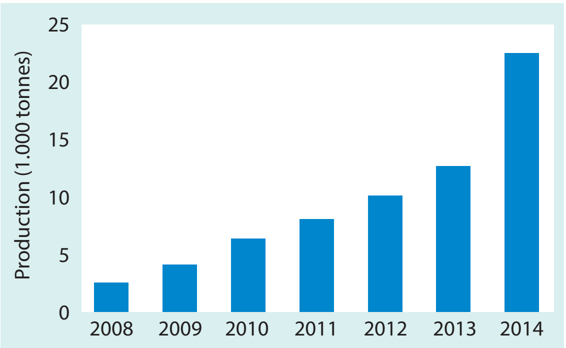
Farming aquatic species in the middle of a desert certainly brings its own challenges. But some of those challenges are more common than you think. Benedict Standen reveals the biggest problems faced by shrimp farmers in Iran.

It is a very strange experience arriving at a shrimp farm in the middle of the desert. Yet shrimp production in Iran has been growing year on year. Traditional shrimp farming has been practiced for centuries in Asia. After 1970, the industry was transformed by more intensive commercial production practices that brought a number of opportunities in terms of employment, wealth creation, and trade as well as providing local and sustainable animal protein. Now, other countries are also recognizing the potential of aquaculture, specifically shrimp farming; Iran is one of these countries.

Iranian shrimp farming is much younger than its Asian counterpart; the first farm was opened in 1994. Today shrimp farming is a common activity along the Persian Gulf and, according to FAO data, the production of Pacific white shrimp, *Litopenaeus vannamei*, in Iran was 22,500 tons in 2014 (*Figure 1*). It can certainly be argued that Iranian shrimp farmers face some unique challenges. During a recent visit speaking with producers, two issues were repeatedly raised that are surprisingly common to the industry at large: first, maintaining good water quality and second, disease control.

Actually, these issues are interlinked. For example, *Vibrio* spp. are part of the 'normal' microbiota associated with seawater. Despite this, under normal culture conditions, healthy shrimp will grow well. However, if the shrimp are stressed due to poor water quality, they may become immunocompromised which presents an opportunity for these opportunistic pathogens. Therefore, by maintaining optimum water quality, the likelihood or severity of disease can be reduced. White spot syndrome virus (WSSV) is another major concern for Iranian producers. Luckily, early mortality syndrome (EMS) is not a problem (yet). The one thing that both these disease threats have in common (whether bacterial or viral), is that they can be mitigated, or controlled, through biosecurity and management; prevention really is better than cure. To achieve this, probiotics are a valuable management tool.

Figure 1. Production of *L. vannamei* between 2008 and 2014.



Source: FAO FISHSTAT

BIOMIN is there

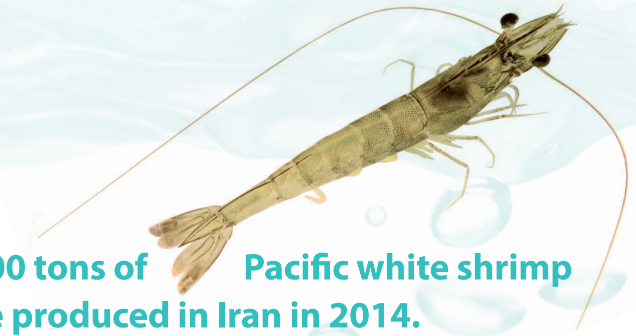
In collaboration with ETOUK FARDA, BIOMIN works with shrimp farmers across Iran to improve aquaculture pond management using innovative probiotic solutions. AquaStar® offers producers a 'one-stop-shop' for probiotic products. The formulation contains multiple beneficial bacterial species that have complimentary and synergistic benefits. For shrimp producers, it offers greater nutrient release, improved water quality and better survivability.

Greater nutrient release

The majority of commercial probiotic products are based on *Bacillus* spp., primarily because of its ability to produce large quantities of enzymes. While AquaStar® products do contain *Bacillus* sp., enzymatic digestion of organic matter is further intensified by an enzyme cocktail. This facilitates the release of highly digestible nutrients, which can be utilized by the shrimp. In addition, the enzymes break down organic matter, preventing the accumulation, and directly reducing 'black sludge' in the pond sediment. This benefit was demonstrated through a field trial. Shrimp were stocked at 50 PL/m^2 and split into two treatments; control and AquaStar®. After 57 days of culture, the ponds supple-

Discussing common challenges faced by Iranian shrimp farmers

Benedict Standen
Product Manager at BIOMIN



22500 tons of Pacific white shrimp were produced in Iran in 2014.

Table 1. Improved revenues by AquaStar® in commercial field trial.

Treatment	Production (kg)	Commercial shrimp price (USD*/kg)	Sales revenue (USD*)
Control	2,306	2.92	6,736
AquaStar®	2,687	3.54	9,503

* Exchange rate at time of print

Figure 2. Samples of pond sediment with (a and b) and without (c and d) AquaStar®. Layers of black anoxic sediments can be seen in the pond bottom when AquaStar® is not used.

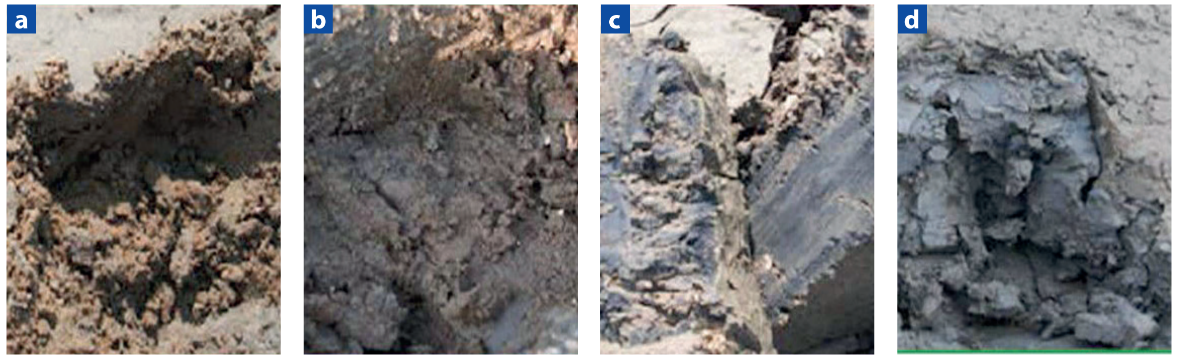


Figure 3. The effect of *Paracoccus* on the removal of nitrogen waste under aerobic (with oxygen) and anaerobic (without oxygen) conditions after 24 and 48 hours.

Dark green indicates high ammonia after 0 hours. After 48 hours the lighter green indicates this ammonia has been removed by the probiotic.

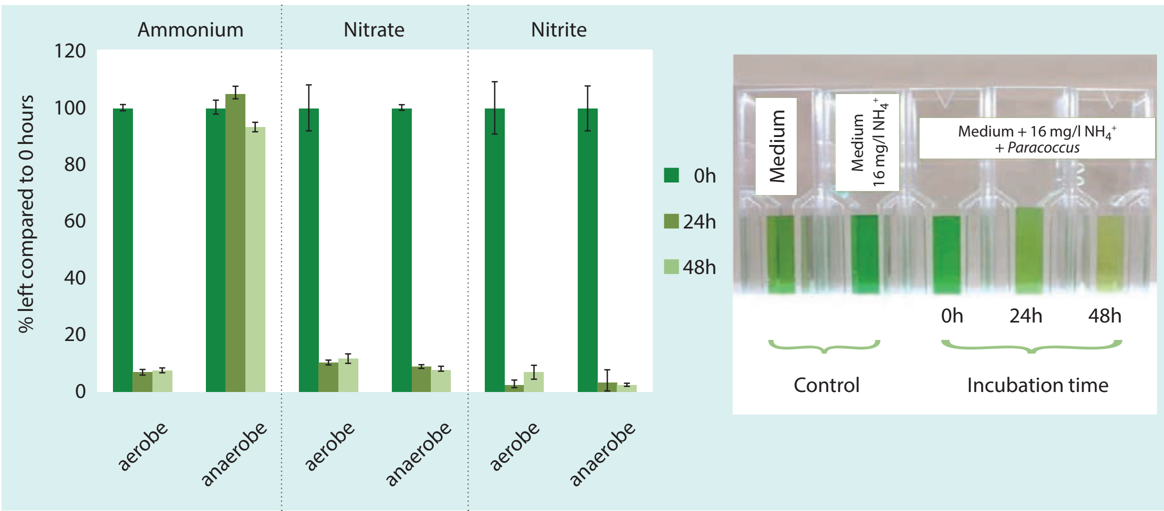
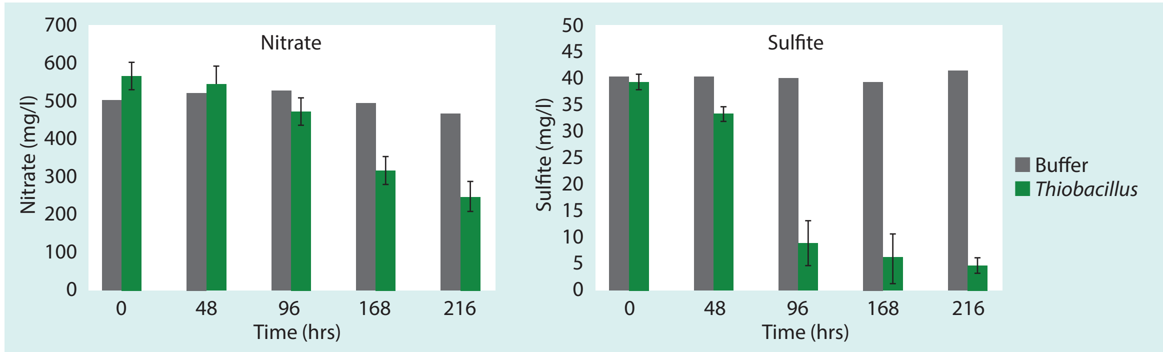


Figure 4. *Thiobacillus* can remove nitrate and hydrogen sulfide.



mented with AquaStar® showed higher productivity, improved growth performance and higher profitability (*Table 1*). Furthermore, visual differences could be seen in the quality of the pond sediment (*Figure 2*).

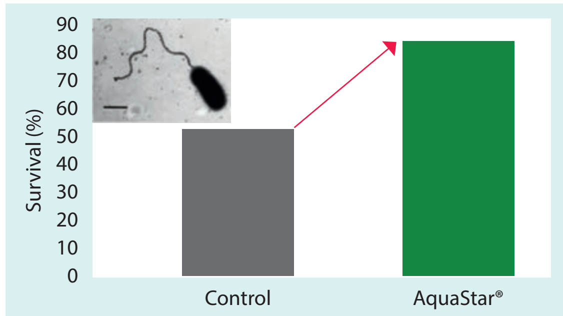
Water quality

To improve water quality, AquaStar® contains unique bioremediation strains that promote the conversion of toxic ammonia to nitrate, via nitrite (nitrification; *Figure 3*). While nitrate is not as toxic as ammonia or nitrite, its accumulation can be harmful to shrimp and acts as a fertilizer leading to dangerous phytoplankton blooms, making its removal equally as important. AquaStar® is the only commercial probiotic to use *Thiobacillus* sp., to convert nitrate to harmless nitrogen gas (denitrification). While most bacteria need a carbon source to grow, *Thiobacillus* can take its energy from an inorganic source. Thus, this species has a dual function: the removal of nitrate and hydrogen sulfide (*Figure 4*).

Improved survivability

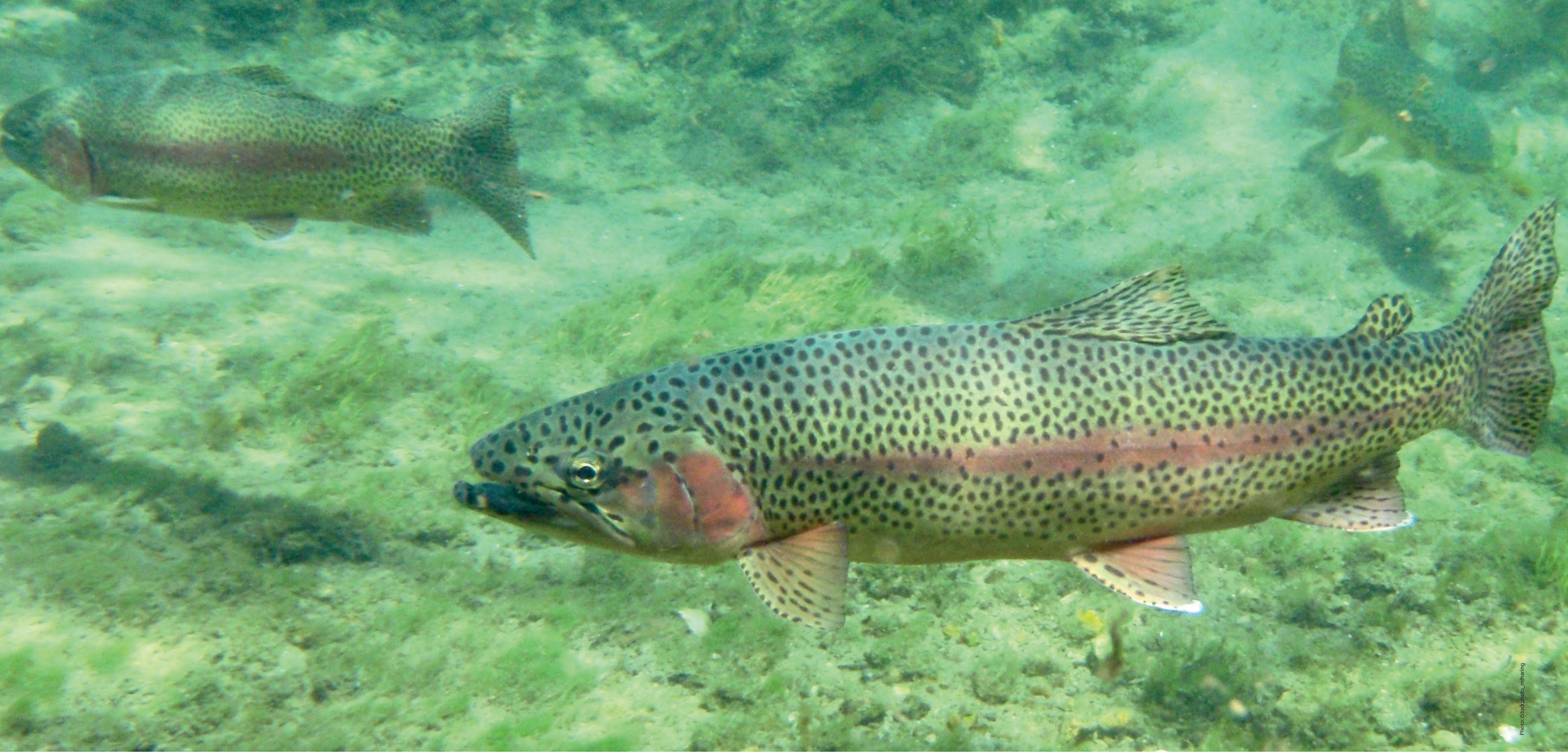
While other probiotic species are capable of reducing pathogens, lactic acid bacteria (LAB) are by far the best candidates for this role. The LAB in AquaStar® directly reduce a wide range of pathogens by producing numerous potent antibacterial substances, termed bacteriocins.

Figure 5. Survival of shrimp in control treatments and AquaStar® treatments when challenged with *V. parahaemolyticus* under field trial conditions. Inlay shows a single *V. parahaemolyticus* cell.



Furthermore, when LAB are ingested, they can colonize the intestinal epithelia where they drive immunity and enable the shrimp to fight pathogens using its own immune response, a benefit demonstrated in the field. When shrimp were challenged by *Vibrio parahaemolyticus*, survival was significantly higher in those ponds that had received AquaStar® probiotics (*Figure 5*).

To conclude, AquaStar® is a valuable tool for shrimp producers. The multi-species formula is designed to bring multiple benefits to the producer; better water quality, reduced organic matter, reduced sludge, pathogen control and increased pond productivity.



The occurrence of mycotoxins and their impact on rainbow trout (*Oncorhynchus mykiss*)

By **Rui A. Gonçalves**, MSc – Scientist – Aquaculture at BIOMIN

Mycotoxins might not always cause visible symptoms but their impact on production and profitability is significant. Rui A. Gonçalves shares two trials highlighting the importance of mycotoxin management.

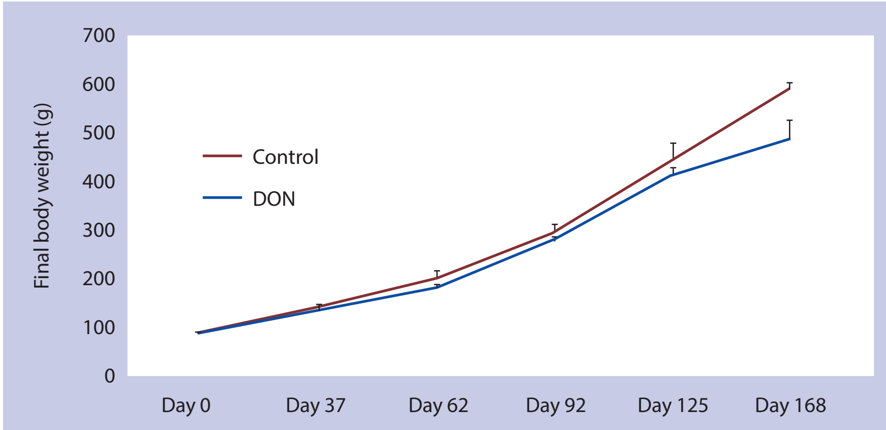
Concern about mycotoxins in aquaculture has been growing, partly due to the gradual replacement of animal-derived proteins, such as fishmeal, by plant sources. Plant-based ingredients already represent the major dietary protein source used within feeds for lower trophic level fish species and the second major source of dietary protein and lipids after fishmeal and fish oil for shrimp and high trophic level fish species. The tendency to use plant-based ingredients in aquafeeds is set to increase due to sustainability issues and the price of fishmeal. Plant ingredients used in aquaculture are of varying origin and quality and recent reports show the risk of mycotoxin contamination in aquafeeds.

Mycotoxin occurrence: more than just aflatoxins

Generally, in SE Asia, it has been shown that raw materials such as: soy bean meal, wheat, wheat bran (WB), corn, corn gluten meal (CGM), rapeseeds/canola meal and rice bran are mostly contaminated with *Fusarium* mycotoxins (zearalenone (ZEA), deoxynivalenol (DON) and fumonisins

Mycotoxins are found in the raw materials used to make aquafeed all year round so trout are exposed to low levels of contamination over a long period.

Figure 1. Final body weight at different sampling points. Values displayed as averages ± standard deviation.



(FB). Only cotton seed meal was observed to be contaminated primarily by aflatoxins (AF) and *Fusarium* toxins (ZEA and DON) at lower concentrations (data from BIOMIN mycotoxin survey 2015/16). European aqua feedstuffs are mainly contaminated by *Fusarium* mycotoxins. Moreover, the majority of mycotoxin contamination found in finished feeds are *Fusarium* mycotoxins, i.e., they come mainly from the raw materials used to produce feeds (so, from crops) and not aflatoxin contamination as is commonly believed within the aquaculture industry. An important factor is also the co-occurrence of mycotoxins which is high for all commodities, raising the probability of co-occurrence in finished feeds. An accumulation of mycotoxins on processed plant-based ingredients, (e.g., CGM and WB) has been observed when compared to the respective whole grains (C and WH, respectively). Regarding finished feeds, contamination detected in recent years poses a risk for several important aquaculture species, assuming single mycotoxin contamination, i.e. excluding any possible additive and synergetic effects between mycotoxins.

Relevance of mycotoxin occurrence to rainbow trout

To evaluate the consequences of DON contamination in European aquaculture finished feeds, two experiments were performed with rainbow trout (*Oncorhynchus mykiss*). First, the effect of short term feeding of high levels of DON (50 days; 1,166 μg/kg DON and 2,745 μg/kg DON) was tested. Moreover, the influence of mycotoxins against *Yersinia ruckeri* susceptibility was also evaluated. A second experiment studied the long term feeding of low levels of DON to rainbow trout (168 days; 367 μg/kg DON).

The experimental design tried to replicate two possible scenarios commonly observed. First, a contamination higher than 1000 ppb due to an inclusion of a highly contaminated raw material in the diets, which normally affects only a few batches of feed (short term). Second, a lower contamination, around 300-500ppb, which is commonly found all year round according to the BIOMIN aquafeeds survey, and can be ingested by animals over long periods of time.

Impact of short term exposure to DON on rainbow trout

As previously described by other authors, rainbow trout growth performance is affected by dietary contamination of DON. At the levels tested (1,166 μg/kg DON and 2,745 μg/kg DON), the thermal growth coefficient decreased 17% (p = 0.001) and the specific growth rate decreased by 13% ($p < 0.001$). Also, important numerical differences ($p > 0.05$) were found for protein efficiency rate and feed efficiency rate. Ingestion of DON did not influence the trout's *Yersinia ruckeri* susceptibility, however the ingestion of DON resulted in gut and liver tissue destruction, confirmed by blood enzyme values and histology. Experiment results confirmed that the ingestion of DON at levels higher than 1,166 μg/kg, even if during short periods (50 days) can lead to an overall decline in performance which ultimately results in economic losses.

Long term exposure to DON

The second experiment aimed to study the impact of low mycotoxin contamination (367 μg/kg DON) over a longer period. Despite no statistical differences being found for final body weight (FBW) and other performance parameters, after 92 days of DON exposure, a more accentuated difference between the control and DON fed animals was observable. Actually, at day 168, the differences between the two treatments were relatively high, with a p-value at this sampling point of 0.053 (*Figure 1*). Feed conversion rate (FCR) showed a similar pattern to FBW and after 168 days, animals fed DON presented a FCR 25% higher when compared to the control (*Figure 2*). Growth performance reductions observed, even if not statistically different in certain cases, can negatively affect the profit of farmers, especially if we think that feed costs account for 60% of total production costs for salmonids. FCR showed an important numerical increase, which results in economic but also environmental consequences for the salmonids industry, especially so because European countries have legal limits for N-compound emissions.

As well as the negative impact described above, mycotoxins caused size dispersion, probably due to the individual history (health/nutritional status) of the trout. This size dispersion is extremely negative for famers, who need to invest resources in size sorting.

Authors believe that the ingestion of low levels of mycotoxins is often a reality in the aquaculture industry, however due to the lack of clinical signs of mycotoxicoses and the lack of regular analyzes of mycotoxins in feeds, it is difficult to evaluate the possible impact of these mycotoxins in feed.

Lack of clinical signs makes diagnosis difficult

In aquatic organisms, it is difficult to prove that a disease is a mycotoxicosis. Even when mycotoxins are detected, it is not easy to show that they are the etiological agents in a given veterinary health problem. In the short-term period experiment, it was observed that animals fed the higher dosage of DON (2,745 μg/kg) presented haemorrhaging in the abdominal cavity and rectal hemorrhaging and irritation (*Figure 2* and *3*). Due to the controlled rearing conditions and the known ingested levels of DON, the authors associated the presented clinical signs to DON. However, in complex environmental conditions, such as those found on commercial sites, these clinical signs could easily be attributed to other etiological factors.

In the case of feeding DON chronically, it is even more difficult to detect any signs of mycotoxicoses. This experiment did not detect any evident clinical sign that could be associated to the ingestion of DON. However, interestingly, high levels of individual variability in sizes within the fish fed DON were observed, suggesting that the individual immune/nutritional status of each animal might influence the DON susceptibility.

Possible synergism still to evaluate

In both experiments, we were only considering the effect of a single mycotoxin (effect of DON). It is important to note that there are many different mycotoxins, and in many cases they appear simultaneously in feed. This is known to amplify the negative effects in animals, referred to as a synergistic effect. This essentially means that the sensitivity levels found with the present experiments can decrease in practice. Farmers would do well to regularly test feed materials for mycotoxins and use a proven mycotoxin risk management solution in order to maintain health and profitability.

Rui A. Gonçalves
Scientist – Aquaculture at BIOMIN

Figure 2. Trout fed 2.7 DON showing a slight hemorrhage in the abdominal cavity.

Figure 3. Trout fed 2.7 DON showing a rectal hemorrhage/irritation.

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