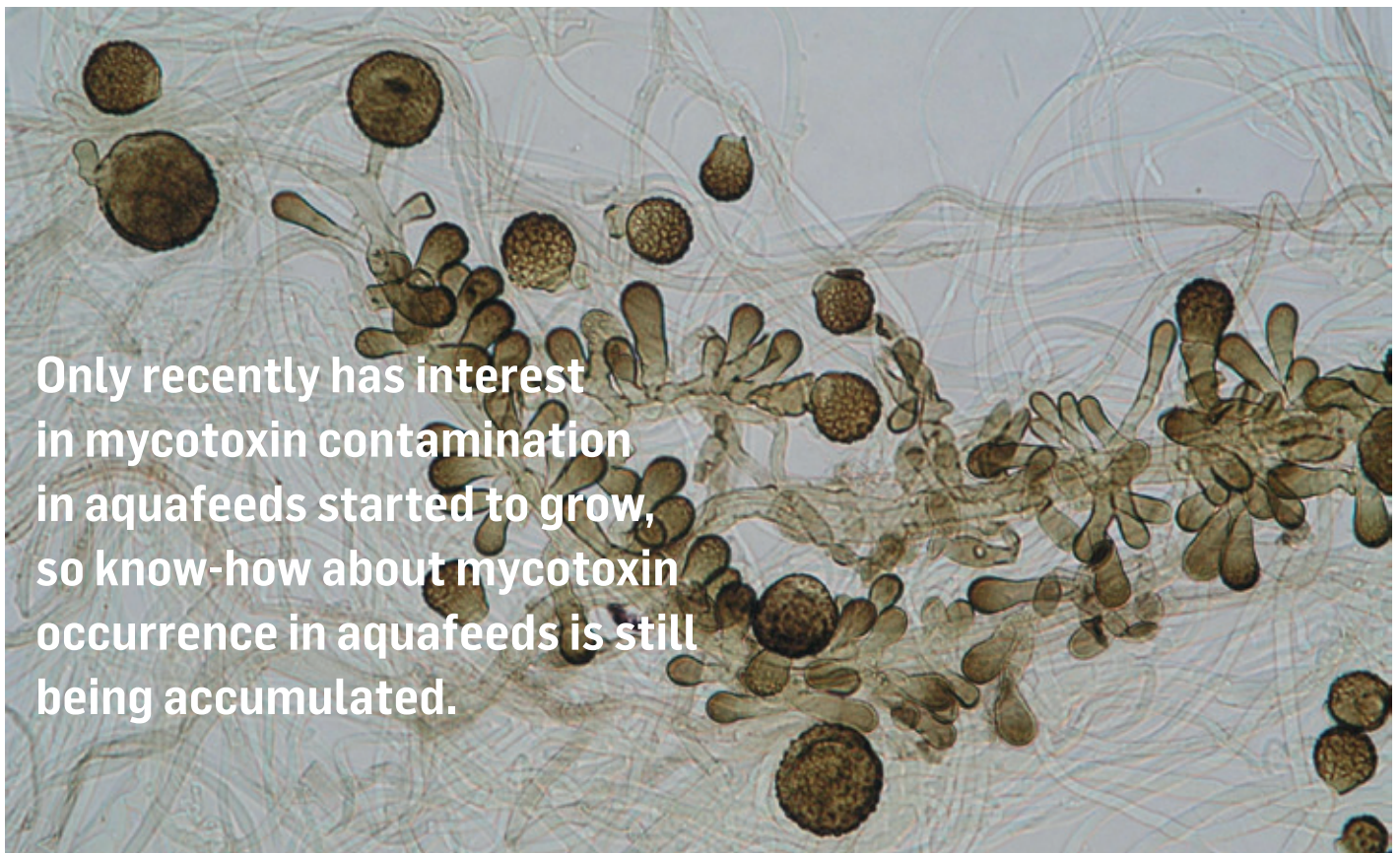


Mycotoxin Management in Livestock Production: A Model for Aquaculture?

Future growth and sustainability of the aquaculture industry depend on the sector's ability to identify alternative sources of protein to substitute fishmeal in aquafeeds. Consequently, many new alternatives are available, e.g. insect meal, macroalgae meal or single-cell protein. However, high costs and limited availability are still challenges to overcome. Plant-based meals seem to be one of the most promising and viable solutions, but a common problem is the presence of mycotoxins.



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Only recently has interest in mycotoxin contamination in aquafeeds started to grow, so know-how about mycotoxin occurrence in aquafeeds is still being accumulated.

Mycotoxin occurrence

The first big difference between livestock and aquaculture production is the level of knowledge about mycotoxin occurrence and co-occurrence in the plant feedstuffs used to make the diets. Only recently has interest in mycotoxin contamination in aquafeeds started to grow, so know-how about mycotoxin occurrence in aquafeeds is still being accumulated. In the past, small amounts of plant meals were included in the diets of carnivores and herbivores, which has increased the disregard for mycotoxin threats in aquafeeds. Due to the novelty of the topic, and contrary to the livestock industry, the contamination of aquaculture feedstuffs with mycotoxins is, in general, often neglected. There is a growing awareness of mycotoxin contamination in aquafeeds. However, we are still far from having solid knowledge of the mycotoxin contamination patterns in aquafeeds, and how the type of plant meal used influences it.

Tip #1: Survey your plant meals for mycotoxins to avoid any possible risk.

The wrong information may lead to employing the wrong strategies

One of the main misconceptions deeply entrenched across the aquaculture industry is that the majority of mycotoxin issues result from poor storage conditions leading to aflatoxin contamination. It is true that poor storage conditions can lead to the growth of *Aspergillus* spp. and *Penicillium* spp., which can ultimately lead to the production of aflatoxins and ochratoxin A. However, BIOMIN has observed that most of the mycotoxins found in aquaculture finished feeds are from *Fusarium* spp., i.e., resulting from field contamination of the raw materials used to produce aquafeeds. In this case, this mainly concerns deoxynivalenol (DON) and fumonisins. In some cases, aflatoxins continue to represent a challenge, especially in tropical countries and/or when storage conditions are inadequate.

Tip #2: Correctly identify the mycotoxin(s) in your diet or raw material in order to implement the correct management plan.

How do I know if my fish/shrimp are being exposed to mycotoxins?

Mycotoxins are structurally very diverse. This characteristic generates a wide range of symptoms in mycotoxin-affected animals, ranging from decreases in production efficiency to increases in mortality. In aquaculture, symptoms are generally unspecific, which makes accurate diagnosis difficult. The diagnosis of mycotoxicoses in farm animals is further complicated for two reasons. First, the synergistic

IN BRIEF

- New alternative sources of protein for aquaculture diets are now available, many of them derived from plants.
- Plant-based protein sources are often contaminated with mycotoxins, a relatively unknown and often overlooked anti-nutritional factor in the aquaculture sector.
- Symptoms of mycotoxicosis are less evident in fish and shrimp species compared to terrestrial livestock species.
- Regular testing of feed for mycotoxins will help identify threats and enable the correct mitigation strategy to be employed to keep contamination below sensitivity limits.

effects of multiple mycotoxins in feeds create a different pattern of symptoms. Second, mycotoxins are responsible for suppressing the immune system, which allows opportunistic pathogens to colonize, prompting the display of secondary symptoms in the host. Sensitivity to mycotoxins varies greatly between species and is dependent on several factors that can modify the expression of toxicity including age, gender, nutritional and health status prior to exposure, and environmental conditions.

The situation is already very complex, but in addition to this we must consider the 138 different fish species and 38 shrimp species (FAO, 2011), with different feeding behaviors (herbivorous, omnivorous and carnivorous) and inhabiting different environments (freshwater, brackish water, marine). This high number of variables tends to dilute scientific output from all aquaculture research, not only in the field of mycotoxins. The low number of experts working with mycotoxins in their research compounds the problem, making it more difficult to have comprehensive diagnoses on the effects of mycotoxins in the main species.

Some reports describe clinical signs for the most common mycotoxins (Anater *et al.*, 2016), however, most of them are generalist parameters and can be attributed to any diverse pathologies or challenges e.g. anti-nutrition factors or lectins in the diet, or environmental changes (bacteria, environmental toxins). Some of the parameters referred to above include reduction in growth performance, alteration of blood parameters (erythrocyte/leucocyte count), changes in blood enzyme levels (Alanine Aminotransferase (ALT), Aspartate Transaminase (AST) or Alkaline Phosphatase (ALP)), alterations to the liver or the suppression of immune parameters.

Two notable exceptions are aflatoxicosis (yellowing of the body surface, (Deng *et al.*, 2010)) and ingestion of fumonisins (alteration of the sphinganine to sphingosine ratio (sa/so)

Figure 1.

Photographs illustrating classic clinical signs of mycotoxin ingestion in livestock and aquaculture production. Photographs i-iv show easily identifiable mycotoxicoses in poultry and swine. Photographs v and vi show animals fed DON at considerably high doses with no macroscopic signs of disease except anorexia (which could be attributed to other causes in a field situation).



i. Leg weakness in poultry caused by DON ingestion.



ii. Splay legs in piglets caused by zearalenone ingestion.



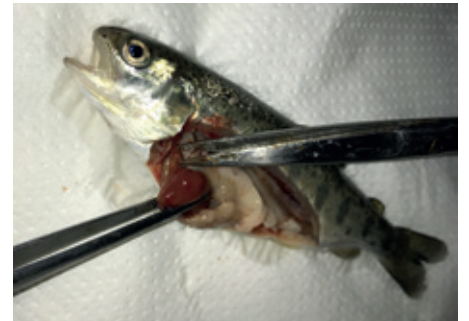
iii. Oral and dermal lesions in poultry caused by ingestion of T-2 toxin.



iv. Dermal lesions/necrosis in piglets tails caused by DON ingestion.



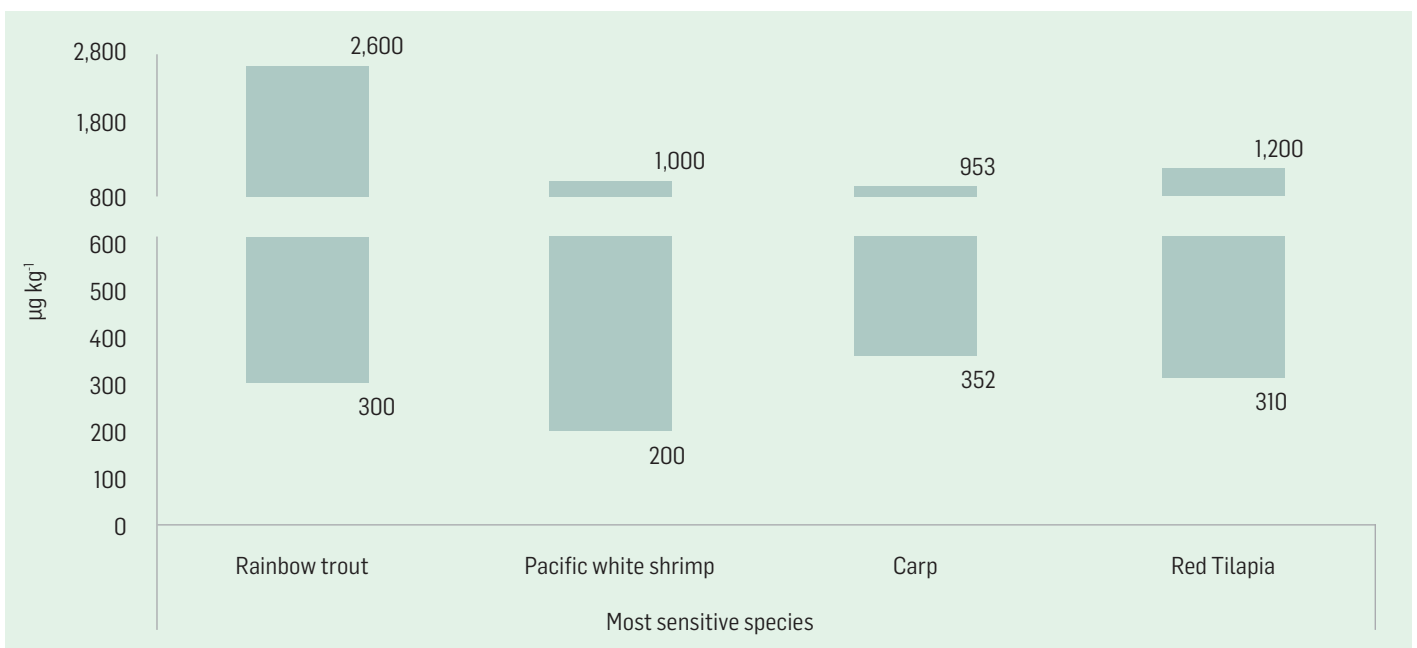
v. Rainbow trout fed non-contaminated (left), 4 ppm DON (middle) and 11 ppm DON (right). None of the treatments, even 11 ppm DON, considered a very high dose for rainbow trout, showed any observable clinical signs.



vi. The liver of fish fed 11 ppm DON does not show any macroscopic lesions, and the hepatosomatic index was similar to the control group (Gonçalves *et al.*, 2018).

Figure 2.

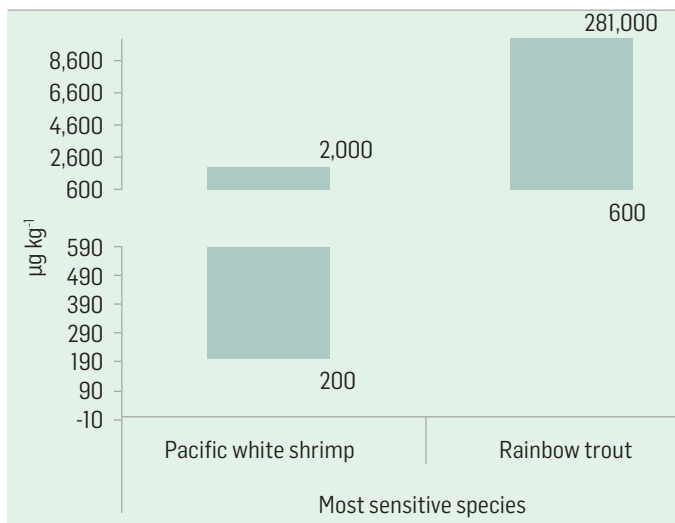
Sensitivity levels to DON of some sensitive species. DON has been studied in several important aquaculture species including rainbow trout, which is the most sensitive species, and white leg shrimp.



Sources: Hooft *et al.*, 2011 (Rainbow Trout (*Oncorhynchus mykiss*)); Tola *et al.*, 2015 (Red tilapia (*Oreochromis niloticus* x *O. Mossambicus*)); Pietsch *et al.*, 2014 (Carp (*Cyprinus carpio*, L)); Trigo-Stockli *et al.*, 2000 (White leg shrimp (*Litopenaeus vannamei*)).

Figure 3.

Sensitivity levels to fumonisins of some sensitive species. Fumonisin have not been extensively studied in aquaculture species, however, the few studies available indicated that white leg shrimps and rainbow trout can be sensitive to fumonisins in feed.



Fumonisin is the sum of FB₁ and FB₂.

Sources: Garcia-Morales *et al.*, 2013 (White leg shrimp); Meredith *et al.*, 1998 and Riley *et al.*, 2001 (Rainbow trout).

(Tuan *et al.*, 2003)). Only aflatoxicosis can be visually identified so to correctly diagnose a change in the *sa/so* ratio, blood or haemolymph samples need to be collected and analyzed.

Compared to livestock, there is a lack of any clear, clinical signs of mycotoxin ingestion in aquatic species (Figure 1i-v).

Tip #3: Keep a detailed and up-to-date record of your farm activities

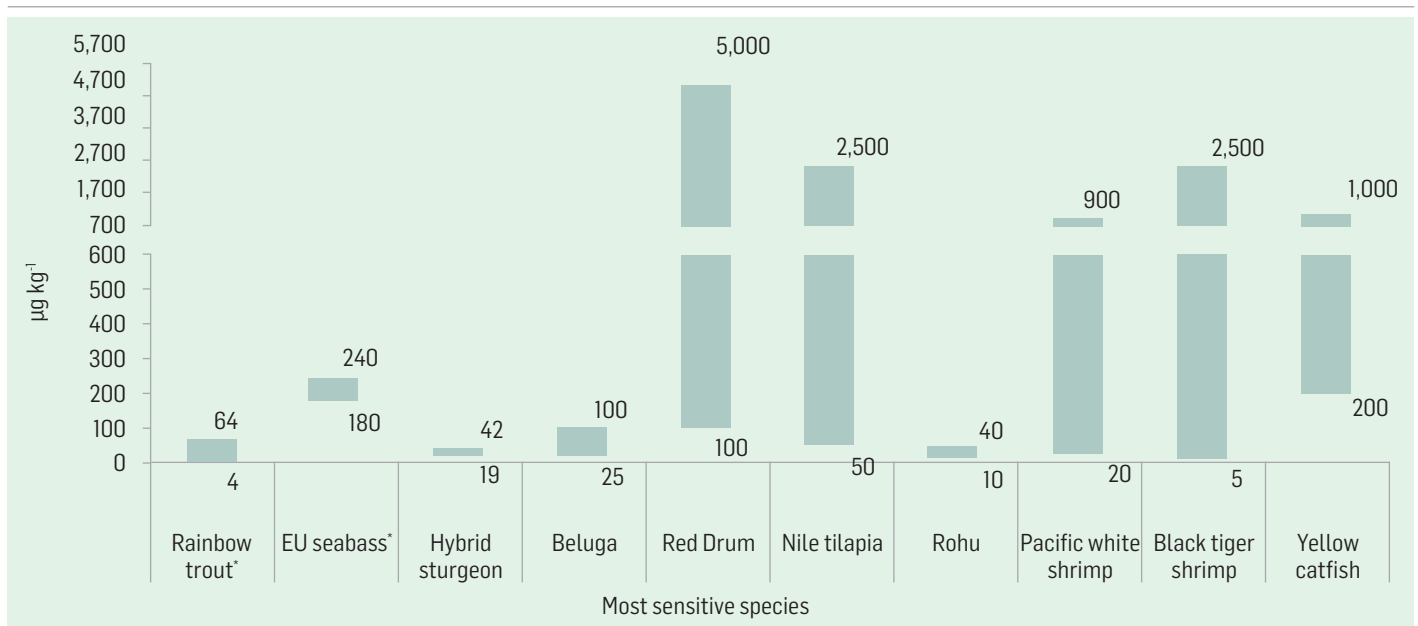
The lack of any clear clinical signs of mycotoxicoses makes it very important to have a rigid mycotoxin management plan and a good record of farm activities. For example, an up-to-date record of environmental parameters (salinity, temperature, N-compounds, oxygen) and feed management (feed intake, identification of feed batches) could be fundamental to identifying the causes of a sudden decrease in feed intake or growth performance or an increase in mortality. While analyzing environmental and feed management parameters, you may also consider mycotoxin contamination depending on the success of your mycotoxin management plan.

Impact of mycotoxins: Are my contamination levels critical?

In aquaculture, it is common practice to study the impact of anti-nutritional factors (ANFs) present in the plant meals, and try to overcome these limitations. However, mycotoxins are often overlooked as ANFs. It is not yet common practice in academia to evaluate the raw materials used to formulate test diets for the presence of mycotoxins. As a result, when comparing to livestock species, much less is known about the effects of mycotoxins in aquaculture species. The efforts of the aquaculture scientific community are even more diluted when taking into account the much higher number of aquaculture species compared to the number of livestock species. As reported previously, sensitivity to mycotoxins

Figure 4.

Sensitivity levels to aflatoxins for some sensitive species. Aflatoxins have been extensively studied in farmed fish and crustacean species due to the toxicity of AFB₁. Several species are extremely sensitive to aflatoxins. While aflatoxin contamination is more common in tropical countries, the global trade of raw materials and aquaculture feeds could potentially export the occurrence of mycotoxins to other regions.



Sources: El-Banna *et al.*, 1992, Cagauan *et al.*, 2004, and Selim *et al.*, 2014 (Nile tilapia (*Oreochromis niloticus*)); El-Sayed and Khalil, 2009 (EU Sea Bass (*Dicentrarchus labrax* L.)); Bintvihok *et al.*, 2003, and Boonyaratpalin *et al.*, 2001 (Black tiger shrimp (*Penaeus monodon* Fabricius)); Ostrowski-Meissner *et al.*, 1995 (White leg shrimp); Wang *et al.*, 2016 (Yellow catfish (*Pelteobagrus fulvidraco*)).

The lack of any clear clinical signs of mycotoxicoses makes it very important to have a rigid mycotoxin management plan

varies greatly between species and is dependent on several factors which can modify the expression of toxicity including age, gender, nutritional and health status prior to exposure and environmental conditions. However, for some species we can already provide some advice. The figures on pages 12 and 13 show some of the sensitivity levels (minimum and maximum) of DON (Figure 2), fumonisins (Figure 3), and aflatoxins (Figure 4).

Tip #4: Ensure mycotoxin contamination stays below sensitivity levels.

Most published studies address the effects of single mycotoxin contamination. Thus, it is assumed that values reported in the literature are quite conservative when taking into account that most aquafeeds are contaminated with more than one mycotoxin (Gonçalves *et al.*, 2016, 2017, 2018). The interaction between several mycotoxins might decrease the sensitivity levels reported.

There are still several gaps that need to be addressed in order to understand how to better manage mycotoxin risks in aquaculture. In recent years, the awareness of mycotoxin-related issues within the aquaculture industry has grown significantly. This is driven by increasing scientific evidence of the negative impacts of mycotoxins in aquatic species, and by frequent reports of the prevalence of mycotoxins in many raw materials.

Aquaculture: raised in a complex environment

One of the first challenges faced in aquaculture production is the environment where the fish lives, breathes, eats and defecates: the water. In aquaculture, fish and shrimp live in close connection with the surrounding environment. Through the ingestion of water, aquatic farmed animals are constantly exposed to pathogens and environmental stress. There are approximately one million bacteria per milliliter of water in coastal areas, and in aquaculture systems, especially in intensive systems, this number will be considerably higher. Most bacteria found in aquatic environments are opportunistic, therefore the slightest unbalance in the animal's immune system will be used by these opportunistic bacteria to become pathogenic.

Due to this complex interaction between the environment and the animal, two main challenges emerge. First, the fact that the animals are in water makes the rapid perception of any macroscopic clinical signs (e.g. skin lesions, lethargy or other common visual control points) very difficult. This is particularly true for animals raised in highly turbid water (i.e. most of the aquaculture in Asia and South America). Second, as soon as an animal has a suppressed immune system or its immune defense is affected (e.g., a skin lesion), opportunistic bacteria rapidly infect. When the farmer realizes that something is wrong, there is a high probability that the animals are already contaminated with *Vibrio* spp. and, depending on the environment, many other bacteria. The question of whether the animals are sick due to the initial bacterial infection or whether they are the target of secondary opportunistic bacterial infections arises.

Tip #5: Maintain high levels of biosecurity, ensure good feed management, and frequently monitor the health status and behaviour of your animals.

The best way to investigate production problems is to examine biosecurity and feed management. Keep information on environmental parameters (e.g., salinity, temperature, N-compounds, oxygen, rain), and feed management (e.g., feed intake, identification of feed batches, feed ingredients, purchase date, date of first use, and storage temperature and humidity). Take regular samples to assess growth performance. Make sure the samples are properly stored and updated to reduce reaction times to potential problems.

Mycotoxin management in livestock production: a model for aquaculture?

Both production sectors have their own challenges. However, the aquaculture industry may learn from the mycotoxin management plans already in place for livestock. Furthermore, some plant meals used in livestock are also commonly used in aquaculture, so there are benefits in sharing information regarding occurrence and co-occurrence levels.

Regarding sensitivity levels, in aquaculture there is a great disparity of vulnerability between the already studied species. Research should continue to better understand which are the most sensitive species and to which mycotoxins. We also need to understand why some species (e.g. channel catfish) are extremely resistant to some mycotoxins (in this case DON), to help us improve the resistance of other sensitive species.

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References available on request.



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