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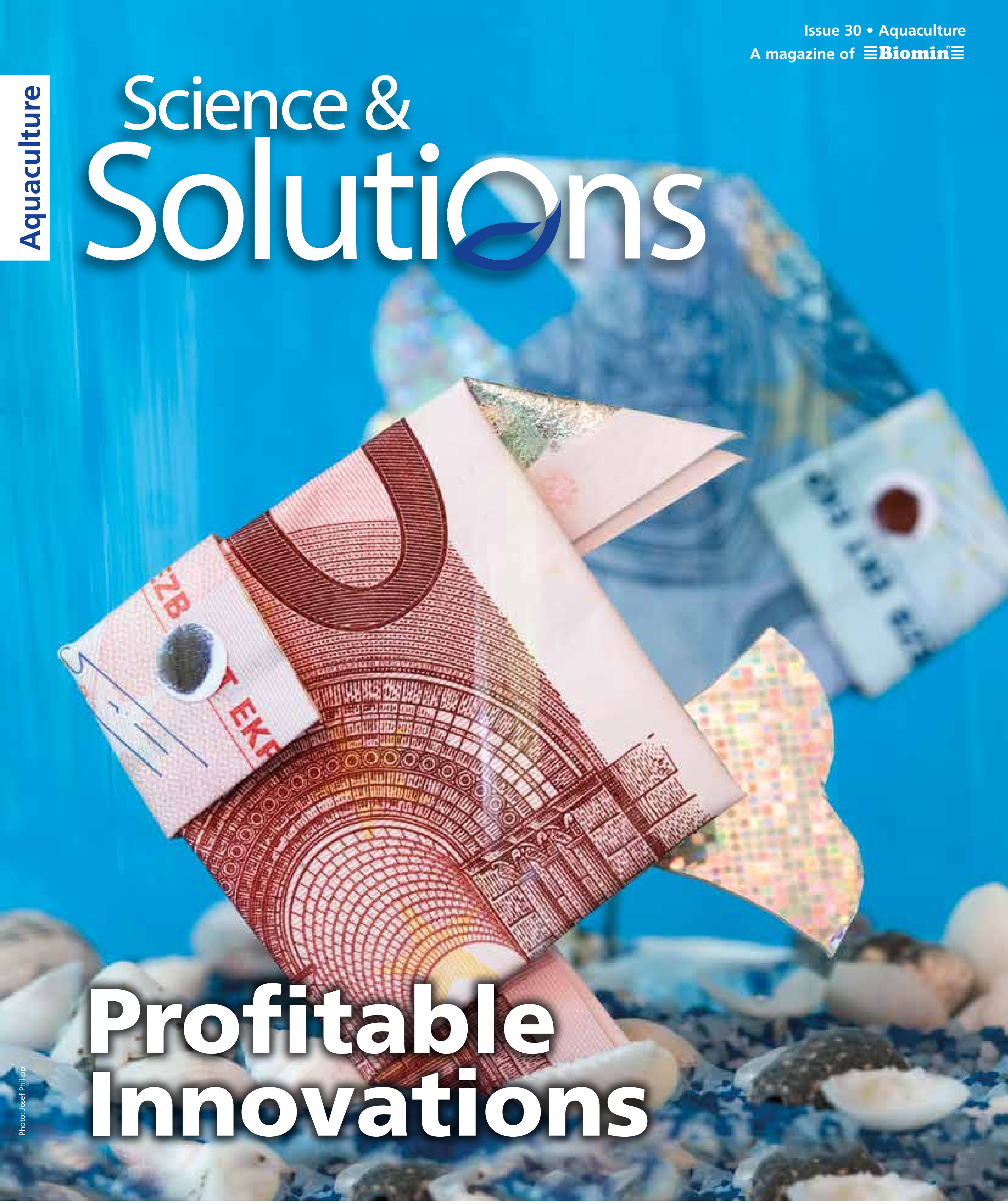
Science & Solutions



Profitable Innovations

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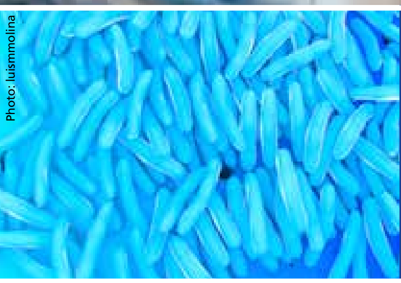

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Probiotics, Immune Fitness and Gut Health

8 Facts About Mycotoxins in Aquaculture

Editorial

Profitable innovations for Aquaculture

Our readers of **Science & Solutions** know the importance of aquaculture. Since the late 1960s, it has generated considerable economic wealth for many developing countries. It supplies a critical animal protein source to some 2 billion people, delivering health benefits associated with high omega-3 content, etc. The prominence of aquaculture in feeding humanity is set to grow in the long term.

BIOMIN has been present in the aqua business since 2005, offering practical solutions for feed mills producing aquafeed and improved management for aqua farms by developing a number of specific aquaculture products:

1. **Phytogenics** to overcome issues associated with the lower digestibility of plant proteins and to optimize the use of increasingly expensive raw materials.
2. **Probiotics** and **prebiotics** to enhance immune response, providing prophylactic protection against opportunistic pathogens.
3. **Enhanced acidifiers** to reduce antibiotic growth promoters (AGPs), preserve aquafeeds and maintain general farm health status. A comprehensive **mycotoxin risk management** product line to address the increasing issue of mycotoxins in aquafeeds.

In the articles included in this issue of **Science & Solutions**, you will find all the details to make your aqua farm and feed mill operations healthy and wealthy.

Enjoy the reading!

Paolo Doncecchi

Paolo DONCECCHI
Head of Global Gut Performance Products

Contents

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Probiotics to Boost Immune Fitness and Gut Health

By **Benedict Standen**, Product Manager - Aquaculture

This third article in our gut health series in aquaculture illustrates how AquaStar® Growout, a multi-strain probiotic can improve farmed species' natural protection, as evidenced by three recent tilapia scientific studies.

The intestine is one of the main portals of entry for invading pathogens. In order to successfully infect the host, a pathogen must navigate and survive multiple obstacles and attacks, executed by the host's immune system.

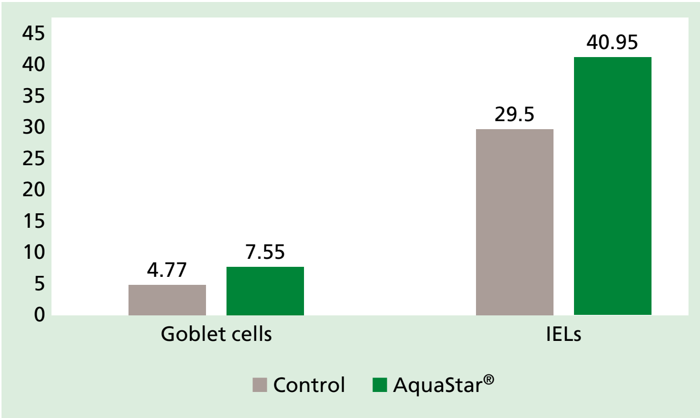
Innate immune response = first consideration

As with mammals, the fish immune system can be separated by innate (non-specific) and adaptive (specific) responses. Compared to mammals, fish are more dependent on the innate immune response for two main reasons. First, the innate immune system has developed to be non-specific and is therefore capable of mounting an immune response against a wide range of pathogens. Secondly, due to the ectothermic nature of fish, adaptive immunity can take considerable time. For example, antibody production in salmonids can take up to six weeks, compared to just hours or days for the innate immune system.

Reinforcing the first line of defense

The mucus layer produced by goblet cells provides the immediate line of defense. The mucus functions to trap and remove pathogens by providing both a physical and chemical barrier, since it contains a number of antimicrobial compounds. This mucus layer can be modified by the commensal microbiota as well as probiotic bacteria. For example, after feeding tilapia with AquaStar® Growout at 3g/kg for five weeks, it was discovered that there were approximately 60% more goblet cells in the intestine (*Figure 1*). These additional goblet cells could contribute to a greater production of mucus, thus providing a more impenetrable barrier, potentially retarding pathogens and preventing their attachment to the underlying epithelia.

Figure 1. The abundance of goblet cells and IELs in the intestine of fish fed dietary AquaStar® Growout at 3g/ kg after five weeks.

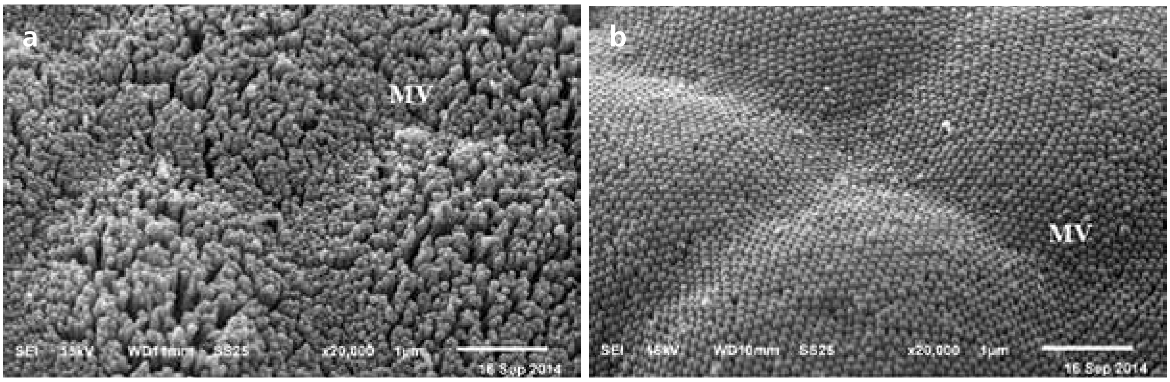


Source: BIOMIN

Strengthening the barrier: microvilli density

Beneath the mucus layer, lies the epithelia, primarily consisting of enterocytes. These cells are lined with microvilli. Using electron microscopy, it was shown that AquaStar® Growout can significantly increase microvilli density in the gut. The benefit of this increase is two-fold; firstly more numerous microvilli will increase the

Figure 2. Electron micrographs showing microvilli from fish fed a control diet (a) and AquaStar® Growout diet (b). Gaps between microvilli (MV), as seen in micrograph a, provide an entry point for opportunistic pathogens. Compared to micrograph b, the microvilli provide an impenetrable barrier, blocking pathogen entry.



Source: BIOMIN

surface area so the host can acquire more nutrients from the feed. Secondly, any gaps between microvilli present an opportunity for pathogens to penetrate the epithelia and infect the fish (*Figure 2*). Thus a higher microvilli density, caused by the probiotic contributes to a more efficient barrier between the inside of the gut and the outside, blocking pathogens.

Larger leukocyte infantry

Assuming a pathogen was able to breach the epithelia, an army of white blood cells, collectively known as intra-epithelial leucocytes (IELs), would be waiting to attack the pathogen. In all three trials, conducted at Plymouth University, the addition of AquaStar® Growout to tilapia diets resulted in significantly larger populations of IEL. This increase was between 22-38% depending on the probiotic dosage, as well as the duration of feeding (*Figure 1*).

Better immune readiness

All pathogens express pathogen associated molecular patterns (PAMPs) on their cell surface. These are recognized by their respective receptor molecules such as Toll Like Receptors (TLRs) which notify the host on the pathogen type (i.e. bacterial, viral, fungal etc.) (*Figure 3*). Gene expression analyses, conducted on tilapia intestines, show that AquaStar® Growout can up-regulate the expression of TLR2 by approximately five-fold. TLR2 is important for recognizing Gram-positive bacteria. This is particularly important because tilapia (along with many other warm water species) are susceptible to a number of Gram-positive infections, most notably *Streptococcus*.

Once activated, TLR initiate a number of molecular pathways which result in the production of pro-inflammatory cytokines. The addition of AquaStar® Growout to tilapia diets caused an increase in pro-inflammatory gene expression, IL-1β and TNFα. These data are suggestive of a fish which is more prepared to fight off potential future pathogens since the host can recognize and clear the threat much more rapidly, thus it has greater immune readiness.

Overall immune fitness: an extension of gut health

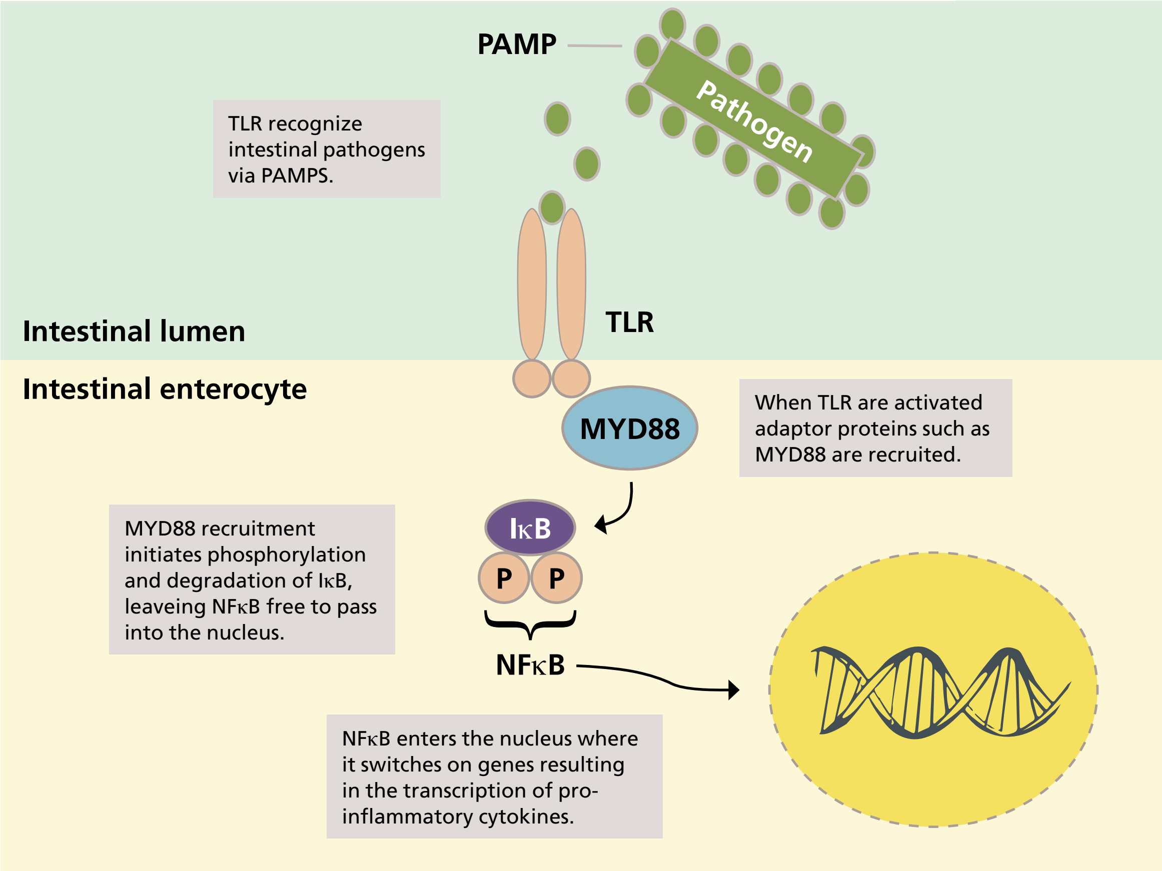
If a pathogen is successful in overcoming the localized immune system (i.e. within the gut), it is then at the mercy of the systemic immune system. In fish, this is controlled by the head kidney.

Therefore, head kidney tissues were also analyzed for immune related gene expression. Similar to the gut, RT-PCR analyses demonstrated that the gene expression of immunity genes (TLR2, pro- and anti-inflammatory) were all elevated in probiotic fed fish.

This reveals that probiotics can have a wide reaching benefit on host immunity, not just in localized tissues where the initial exposure occurred, but also at the whole organism level.

Benedict Standen
Product Manager - Aquaculture

Figure 3. Modulation of intestinal immunity through TLR signaling. Pathogens (and indeed probiotics) bind to TLR. Upon activation, adaptor proteins such as myeloid differentiation primary response protein 88 (MYD88) are recruited. When this happens, IκB (inhibitor of nuclear factor kappa B (NFκB)) is phosphorylated (P) and degraded by the cell. This allows NFκB to pass from the cytoplasm into the nucleus of the cell where initiates the transcription of pro-inflammatory cytokines.



Adapted from Cerf-Bensussan & Gaboriau-Routhiau 2010

Getting the right balance

The gut is home to a large number of commensal microorganisms. It is important that these are protected by the host as they provide important functions in intestinal development, nutrition and immunity. Anti-inflammatory cytokines are part of a tolerance mechanism which acts to de-sensitize the host, thus it does not initiate an immune response to attack 'good' bacteria. Furthermore, they act to balance out the pro-inflammatory cytokines, thus maintaining an equilibrium within the mucosal immune system.

In vivo trials using tilapia, demonstrates that the gene expression of two anti-inflammatory genes, IL-10 and TGFβ, can also be increased by the addition of AquaStar® Growout. This result tells us two things; firstly, that the host does not see the probiotics as a threat and secondly that the probiotics can help to promote and maintain mucosal tolerance.

Conclusion

As demonstrated by this research, AquaStar® Growout can improve the intestinal barrier function, promote a state of superior immune readiness and enhance tolerance mechanisms, both within the intestine and other immuno-important tissues.

This opens the door to healthier animals, fewer instances of disease and less chemotherapeutical intervention in aquaculture production.

Mycotoxins in Aquaculture: 8 Things You Need to Know

By **Rui Gonçalves**, Scientist Aquaculture

Mycotoxins, secondary metabolites produced by molds, can harm many varieties of aquaculture species. Here are 8 key facts about mycotoxins that reveal the real dangers for production.

1 Plant-based feed ingredients expose aquaculture to mycotoxins

Mycotoxins are found in many agricultural commodities and are produced at various stages e.g. before or after harvest, during transportation or during storage. A recent trend to replace animal-derived proteins, such as fish meal, by plant proteins sources or inclusion of other commercially available crop by-products (e.g. dried distillers grains and soluble, DDGS) increases the probability of mycotoxin contamination in aquaculture feeds.

Mycotoxins are produced by a variety of fungi

Photo: Andrew Linscott

Aflatoxins in cottonseed meal revealed the dangers of mycotoxins in aquaculture

2 Mycotoxins have been a recognized threat since 1960

In 1960 in California, aflatoxin-contaminated cottonseed meal caused an outbreak of aflatoxicosis in hatchery-reared rainbow trout (*Onchorhynchus mykiss*). This occurred around the same time as the more well-known turkey „X" disease that killed large numbers of turkeys in England (the culprit again being aflatoxins). Since then, researchers have spent considerable time working to better understand the effects of mycotoxins.

3 Mycotoxins include more than just aflatoxins

In aquaculture, aflatoxins remain the most frequently studied mycotoxin in scientific research. Scientific articles on the toxicity of aflatoxins in fish and crustacean species cover:

- Rainbow trout (*Oncorhynchus mykiss*)
- Channel catfish (*Ictalurus punctatus*)
- Nile tilapia (*Oreochromis niloticus*)
- Rohu (*Labeo rohita*)
- European seabass (*Dicentrarchus labrax*)
- Gibel carp (*Carassius auratus gibelio*)
- Shrimps

Yet, aflatoxins comprise just a small piece in the whole puzzle. Researchers have identified over 400 mycotoxins worldwide. Most consideration focuses on the main occurring metabolites, i.e., aflatoxins (AFLA), ochratoxin A (OTA), fumonisins (FUM), deoxynivalenol (DON) and zearalenone (ZEN). Though less research was performed with these mycotoxins in aquaculture species compared to aflatoxins, studies suggest that those mycotoxins can have negative effects on aquaculture species (*Table 1*).

Figure 1. Mycotoxigenic fungi can usually produce more than one mycotoxin.

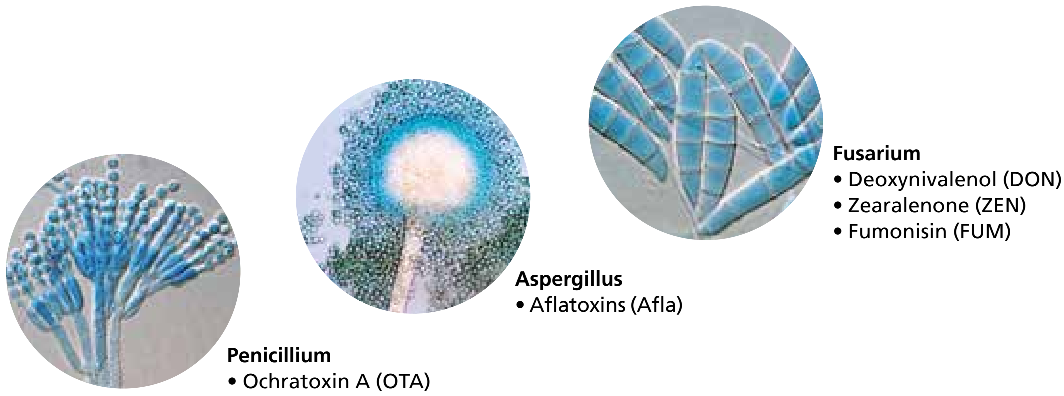


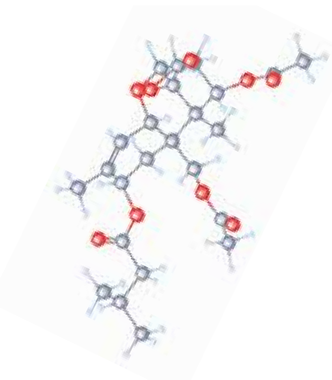
Table 1. Documented mycotoxins effects on aquaculture species.

Mycotoxin	Studied species	Tested dosage in parts per billion (ppb)	Key reference
Deoxynivalenol (DON)	**Rainbow trout** *Oncorhynchus mykiss*	300 to 2600	Hooft *et al.* 2011
	Pacific white shrimp *Litopenaeus vannamei*	200 to 1000	Trigo-Stockli *et al.* 2000
Zearalenone (ZEN)	**Black tiger shrimp** *Penaeus monodon Fabricius*	500 to 1000	Supamattaya *et al.* 2005
	Common carp *Cyprinus carpio* L.	621 to 797	Pietsch *et al.* 2015
Fumonisins (FUM)	**Rainbow trout** Oncorhynchus mykiss	100	Meredith *et al.* 1998
	Pacific white shrimp *Litopenaeus vannamei*	20 to 200	García-Morales *et al.* 2013
Ochratoxin A (OTA)	**Common carp** *Cyprinus carpio*	15	Agouz and Anwer 2011
	European seabass *Dicentrarchus labrax* L.	277	El-Sayed *et al.* 2009

Source: BIOMIN

4 Mycotoxins can harm aquaculture species

Generally, most of the mycotoxins that have the potential to reduce growth and health status of aquaculture farmed animals are produced by *Aspergillus*, *Penicillium* and *Fusarium* species (*Figure 1*). Chemically, they display a wide range of structures, differing also in biological effects, e.g. carcinogenic, teratogenic, mutagenic, estrogenic, neurotoxic, or immunotoxic.



Toxic metabolites produced by these fungi are known to be either carcinogenic (e.g. aflatoxin B_1, ochratoxin A, fumonisin B_1), estrogenic (zearalenone), neurotoxic (fumonisin B_1), nephrotoxic (ochratoxin), dermatotoxic (trichothecenes) or immunosuppressive (aflatoxin B_1, ochratoxin A and T-2 toxin).

5 Mycotoxins can raise production costs

Mycotoxins' impact on fish growth performance has been estimated to increase the feed conversion ratio (FCR) by 5% on average. Given the prominence of aquafeeds in aquaculture, this loss of efficiency could easily translate into a production cost increase of 4% or more. (*See Mycotoxins and their economic impact on aquaculture in* **Science & Solutions** *Issue 10 for more*).

6 Mycotoxin occurrence in aquafeeds quite high

According to the BIOMIN Mycotoxin Survey's full year data spanning January to December 2014, 41 samples of finished aquaculture feed for both shrimp

Rui Gonçalves
Scientist Aquaculture

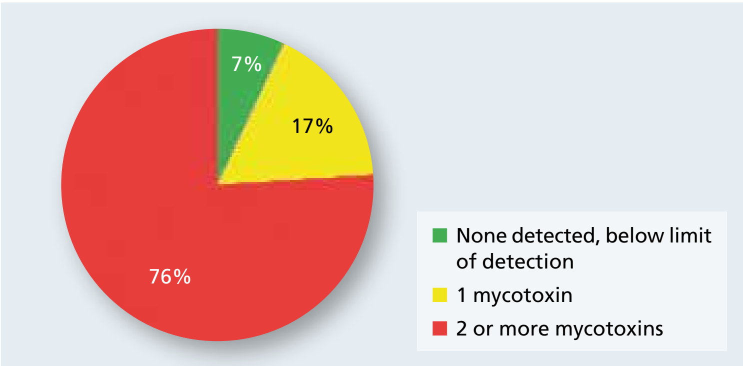
Mycotoxins can impair farmed species' health and raise production costs.

Table 2. 2014 results of BIOMIN Mycotoxin Survey for aquafeed.

Mycotoxins	AFLA	ZEN	DON	FUM	OTA
Number samples tested (n)	37	37	37	41	37
Positive samples (n)	22	22	25	21	21
% Positive	59%	59%	68%	51%	57%
Average of positive (ppb)	49	71	162	637	2
Maximum (ppb)	221	306	413	7,534	5
Average (ppb)	29	42	109	326	1

Source: BIOMIN

Figure 2. Co-occurrence of mycotoxins in aquafeeds in 2014.



Source: BIOMIN

and fish in aquaculture revealed widespread mycotoxin contamination. According to these results (*Table 2*), deoxynivalenol was the most prevalent mycotoxin globally, with 68% of the samples testing positive, followed by AFLA and ZEN (both 59% positive), OTA (57%) and FUM (51%). The fumonisin levels were rather high in terms of concentration, at an average of 637 ppb for the 21 positive samples with a maximum value of 7,534 ppb.

When comparing the existing literature about the sensitive levels of certain species to mycotoxins we can find several aquaculture species e.g., rainbow trout, white leg shrimp, black shrimp and Nile tilapia that are reported to be sensitive to the found levels.

7

Aquafeeds normally contain several different mycotoxins

While a single mycotoxin alone, in sufficient quantities, can harm farmed species, the reality is such that most animals face contamination by multiple mycotoxins at the same time.

The BIOMIN Mycotoxin Survey data in *Figure 2* reveals that three out of four samples tested contained two or more mycotoxins. Seventeen percent of samples were contaminated by one mycotoxin, and just 7% of samples did not contain detectable levels of any of the five mycotoxins.

8

Multiple mycotoxins can be even worse than a single one

Mycotoxins can have compound effects on living organisms in one of three ways. Additive effects boil down to basic addition: the impacts of two mycotoxins equal the sum of the harm posed to the animal by each one separately.

Synergistic effects mean that the adverse impacts are magnified and thus result in greater overall harm to the animal. Antagonistic effects entail a disruption or 'cancelling out' of impacts, though this is not common. Few studies address the combined effects of mycotoxins in aquaculture. In one study (Carlson, 2001), fumonisin B_1 was shown not to be carcinogenic in rainbow trout at 0, 3.2, 23, and 104 mg/kg over 34 weeks. When trout were fed a diet containing fumonisin B_1 and aflatoxin B_1 over 42 weeks, liver tumors resulted—indicating a synergistic effect.

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