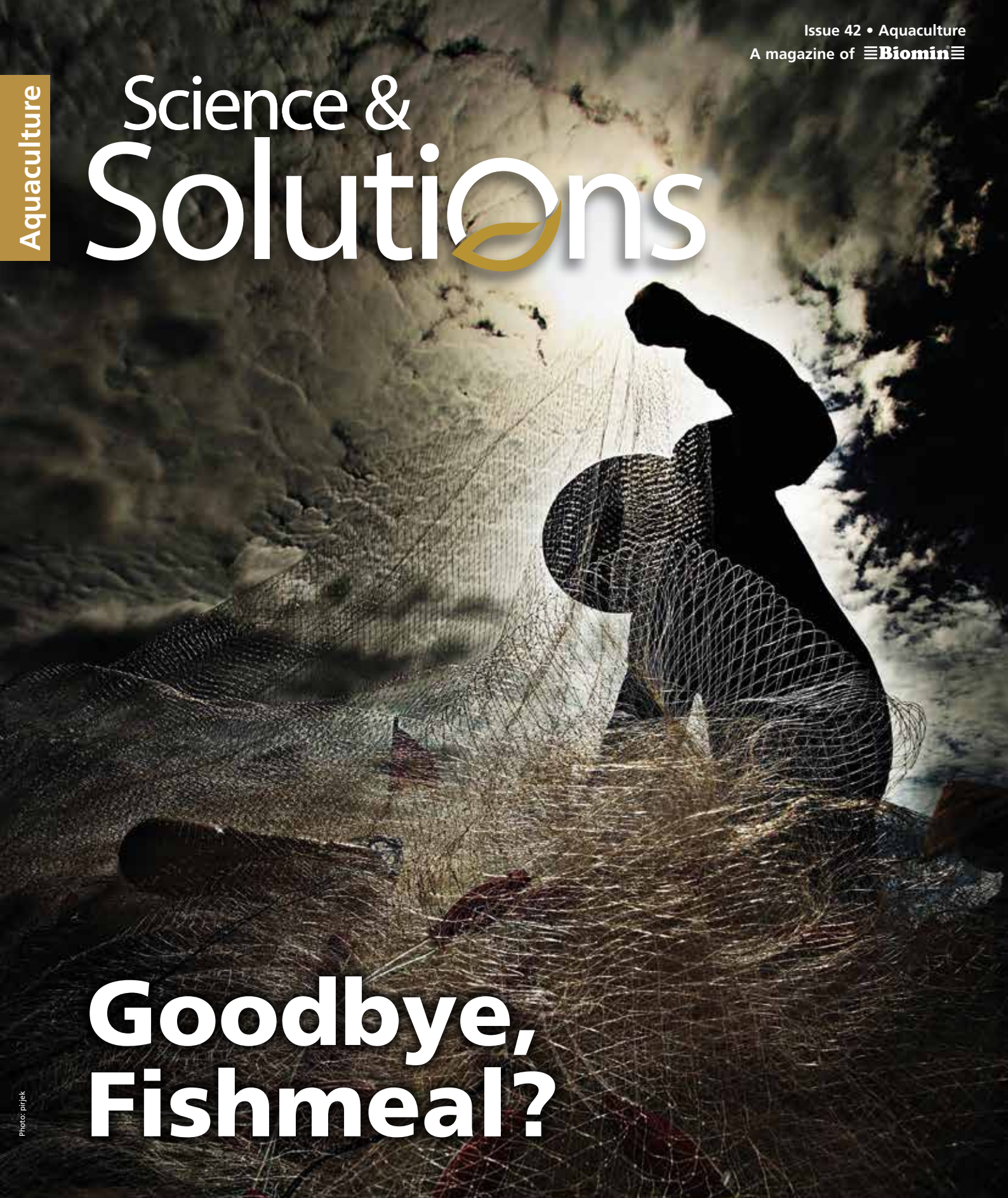


Science & Solutions



Goodbye, Fishmeal?

Photo: pitrek



Mycotoxin Levels
Put Farmed
Species at Risk

Photo: Maxphoto



Probiotics for
Better Gut
Performance

Photo: adventtr

Editorial

Hello, New Year. Goodbye, Fishmeal?

This being the first aquaculture edition of **Science & Solutions** for 2017, I would firstly like to wish all of our readers a very Happy New Year and all the best of health, happiness and business success for 2017.

The aquaculture industry continues to be an exciting business sector within the global protein economy, with the projection to account for more than two-thirds of global seafood consumption by 2030. This strong projected growth will coincide with a number of challenges, most notably in the availability of fishmeal and fish oil to support the feed demands. The focus will continue to be on effective reduction and substitution with the use of plant-based proteins and oil sources. By 2020, the overall quantity of fishmeal used in aquafeeds is projected to fall to levels last seen two decades ago.

With increasing inclusion of plant-based protein sources, additional challenges will be faced: specifically anti-nutritional factors, disturbances to the natural intestinal microbiota as well as exposure to mycotoxins coming from these new ingredient sources. In this issue of **Science & Solutions**, we review the challenges of mycotoxin contamination by addressing the aquaculture-specific results from the most recent annual BIOMIN Global Mycotoxin Survey.

The use of probiotics (beneficial bacteria) to improve the intestinal health of fish and shrimp has been well documented. In this issue, we also highlight the benefits of multi-strain probiotic applications in fish, to enhance the intestinal microbiota and improve the growth and performance in aquaculture.

I hope that you enjoy this edition of **Science & Solutions**.

Sincerely on behalf of BIOMIN



Edward Manchester

Global Head of Aqua & Regional Director



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Photo: GrigorisMoraitis

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By *Benedict Standen PhD*

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Real Mycotoxin Levels Put Farmed Species at Risk

By **Rui A. Gonçalves**, Scientist

As plant-based diets increase the risk of mycotoxin contamination in aquafeeds, a comparison of mycotoxin occurrence data and scientific literature signals that key farmed species in Europe and Asia may be at risk.



Numerous species are at risk in terms of impaired health or lower performance—undermining the industry’s profitability.

The negative effect of mycotoxins in aquatic species has been highlighted in recent publications. Mycotoxins can cause adverse effects in several aquatic species and that these effects vary greatly depending on a variety of factors including nutritional and health status prior to exposure, dose and duration of exposure, age and species. Nonetheless, the important question that remains to be answered: which species can be affected by the real mycotoxin levels detected in aquafeeds? Comparing the annual results of the BIOMIN Mycotoxin Survey with known sensitivity levels of farmed fish and shrimp reveals that numerous species are at risk in terms of

impaired health or lower performance—undermining the industry’s profitability.

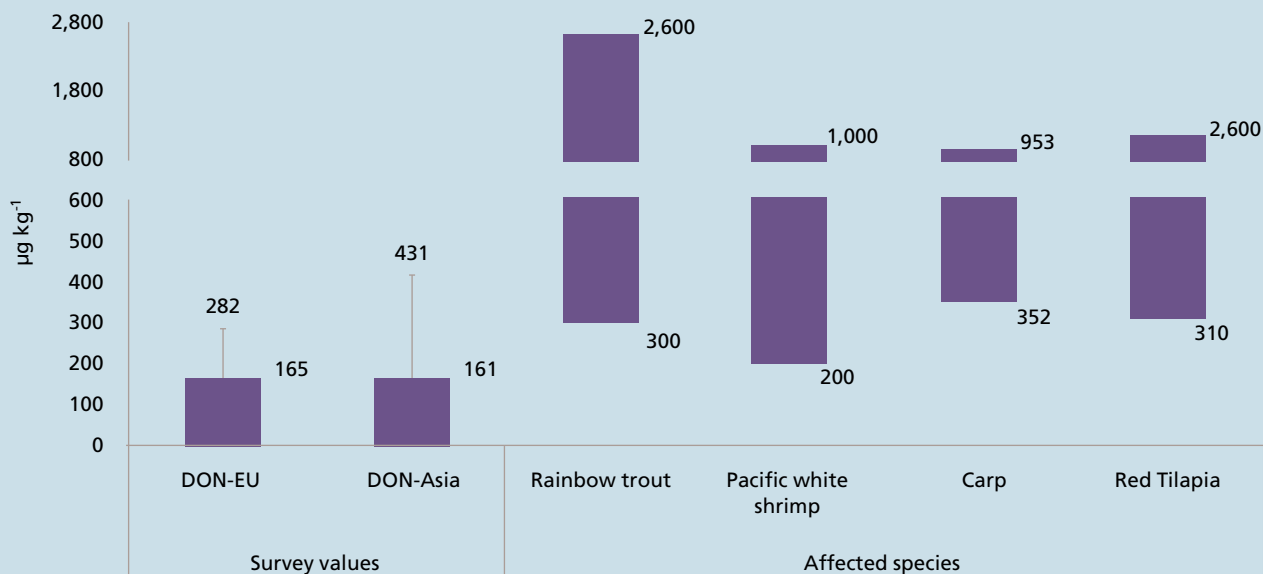
Mycotoxin occurrence

Over a period of one year, 41 samples of finished aquaculture feed, for shrimp and for fish, were analysed: 31 samples were sourced in Asia and 10 samples in Europe. In all, 154 individual analyses were conducted. The samples were tested for aflatoxins, zearalenone, deoxynivalenol, fumonisins and ochratoxin A.

Deoxynivalenol

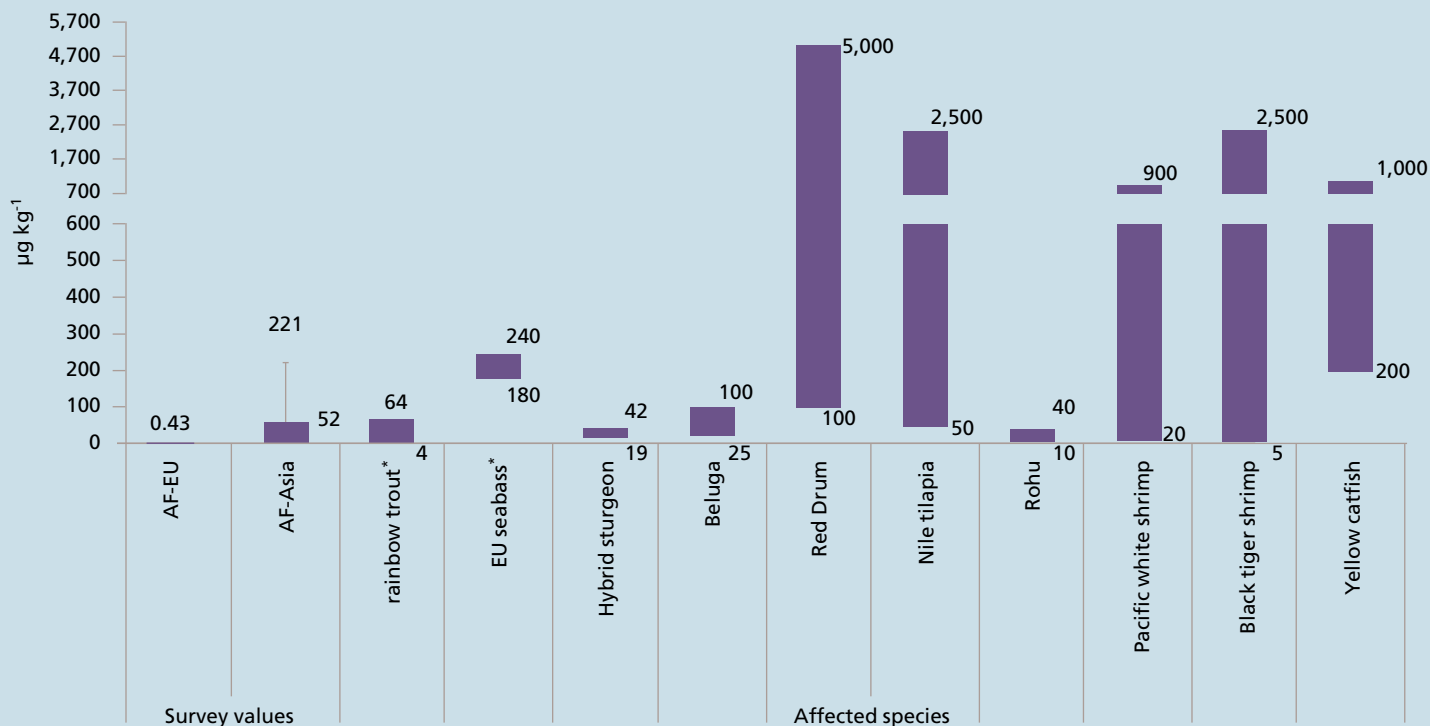
Directly comparing literature values with contamination levels found in the survey (*Figure 1*), we can observe that several species can be affected by deoxyniva-

Figure 1. Surveyed mycotoxins values for DON and affected species.



Source: Gonçalves et al., 2016

Figure 2. Surveyed mycotoxins values for AF and affected species.



Source: Gonçalves et al., 2016

lenol (DON) in real aquaculture production scenarios. In European samples, an average value of 165 ppb (parts per billion) and maximum of 282 ppb of DON was detected and in Asian samples an average value of 161 ppb and maximum of 431 ppb of DON were found. These values are within the sensitivity level of rainbow trout, pacific white shrimp, carp and red tilapia.

Aflatoxins

Aflatoxins (AF) were the most common mycotoxin found in the survey for Asia. Aflatoxins have been considerably investigated in farmed fish and crustaceans' species due to the toxicity of aflatoxin B₁ (AFB₁). The contamination values of aflatoxins found in Europe were negligible (0.43 ppb); however, the values found in Asia (average = 52 ppb and maximum of 221 ppb) can impact several rearing species (Figure 2).

These aflatoxin levels could spell trouble for rainbow trout, European seabass, Nile tilapia, rohu, yellow catfish and white leg and black tiger shrimps, between others. While the aflatoxin contamination appears restricted to Asian samples, the global trade in raw materials and aquaculture feeds could potentially export the occurrence of mycotoxins to other regions.

Fumonisin

Fumonisin B₁ (FB₁) has not been extensively studied in aquaculture species. However, the few studies available indicate that shrimp and fish can be sensitive to fumonisins (FUM) in feed. Comparing sensitivity levels for aquaculture species found in the literature and the contamination values found in aquafeeds, we observe that some species can be affected by fumonisins, including white leg shrimp and rainbow trout (Figure 3).

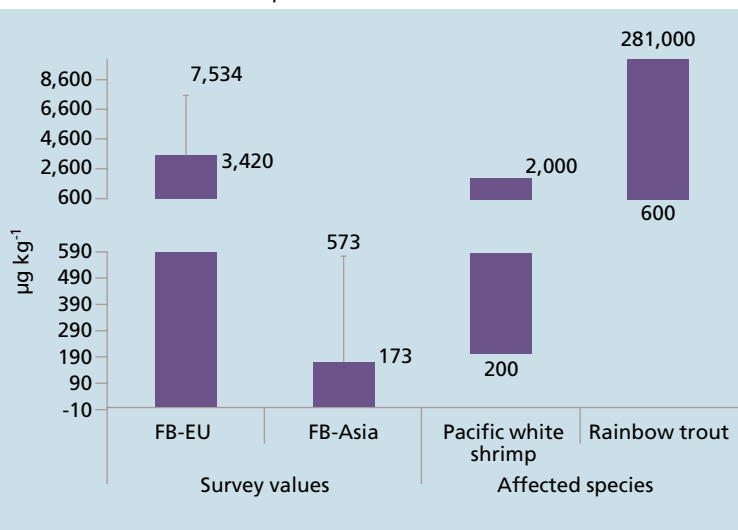
The contamination levels reported are again sufficient to harm several aquaculture species, assuming single mycotoxin contamination.

In the case of Europe, the values found are alarmingly dangerous. The prevalence of this mycotoxin in Europe was relatively low, at 30%. However, it was detected at high levels (average value of 3,420 ppb; maximum value of 7,534 ppb). At this level, fumonisins represent a serious risk for aquaculture production in Europe.

Despite the values found in Asia being much lower than in Europe, they are within the sensitivity level of one the most important Asian species: white leg shrimp. Regarding the FB₁ detected for Europe, we would

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.

Figure 3. Surveyed mycotoxins values for FUM and affected species.



Source: Gonçalves et al., 2016

assume that this extremely high value can be seen as an exception that can be explained by an unusually high raw material contamination. This highlights the need

for regular mycotoxin monitoring of raw materials and finished feeds.

Compound problems

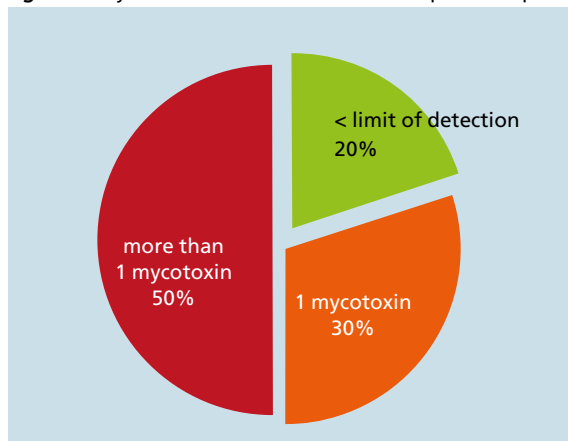
With such comparisons, we are only considering a single mycotoxin’s effects on animals. It is important to note that there are many different mycotoxins, and in many cases their simultaneous presence in feed is known to amplify the negative effects in animal—referred to as a synergistic effect.

This matters because multiple kinds of mycotoxins are generally found together.

Co-occurrence

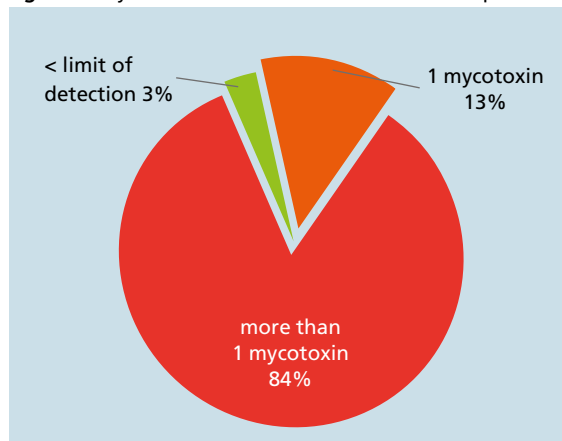
According to the BIOMIN Mycotoxin Survey, mycotoxin co-occurrence for Europe was 50% and 84% for Asia (Figures 4 and 5). This essentially means that threats to farmed species cited in this article are likely to understate the real risk to aquaculture production. Farmers would do well to regularly test feed materials for mycotoxins and use a proven mycotoxin risk management solution in order to maintain health and profitability.

Figure 4. Mycotoxins co-occurrence for European samples.



Source: Gonçalves et al., 2016

Figure 5. Mycotoxins co-occurrence for Asian samples.



Source: Gonçalves et al., 2016

A detailed scanning electron micrograph (SEM) showing a dense population of green, rod-shaped bacteria. The bacteria are oriented in various directions, some appearing in parallel rows, others more randomly. The surface of the bacteria shows fine, textured details, possibly flagella or surface proteins. The background is dark, making the green bacteria stand out prominently.

Probiotics for Better Gut Performance

By **Benedict Standen**, Product Manager

Probiotics can improve intestinal defense, stimulate digestive enzyme secretions and bring nutritional benefits in farmed aquatic species.

Probiotics have many functional benefits in aquatic species,” noted Dr. Daniel Merrifield, a world-renowned probiotic researcher, in his opening message at the recent World Nutrition Forum.

Probiotics are important modulators of intestinal microbiology. Microbial populations in the intestine are extremely complex and are highly influenced by environment and nutrition. The microbiota is very important to the animal and plays pivotal roles in development, immunity and tolerance, regulation of stress, disease resistance and nutrition.

The intestine – a ‘living’ organ

Although bacteria are typically the most abundant organisms in the gut, Archaea, viruses, protozoa and yeasts are also present. Microbial abundance in the intestine of fish varies. It is not uncommon to find more than 10¹¹ cells per gram of intestinal material (100 billion cells per gram). It is estimated that around 30% to 40% of the intestinal mass is comprised of biologically active microbial cells. It is not only the number of cells which is impressive, but also the diversity. There are hundreds of different bacterial strains within the gut, and new ones are being identified as technology improves.

Keeping the bad guys in check

Not all microbiota can be considered beneficial. Many opportunistic pathogens naturally occur in the gut, even in healthy

Therefore, at all times, the host must be aware of any potential pathogens that may pose a risk.

In this way, the host is continuously scanning the intestinal microbiota. Macrophages (a type of white blood cell) ‘taste’ the microbiota (*Figure 1*) and are in constant communication with other leucocytes in the epithelia.

These cells are primed to attack and break down pathogens before they can reach the blood system. To further block the entry of pathogens, fish have developed a number of protective mechanisms in the gut. For example, intestinal mucus, produced by goblet cells, acts as a physical (glycoproteins) and a chemical (antimicrobial proteins, defensins etc.) barrier.

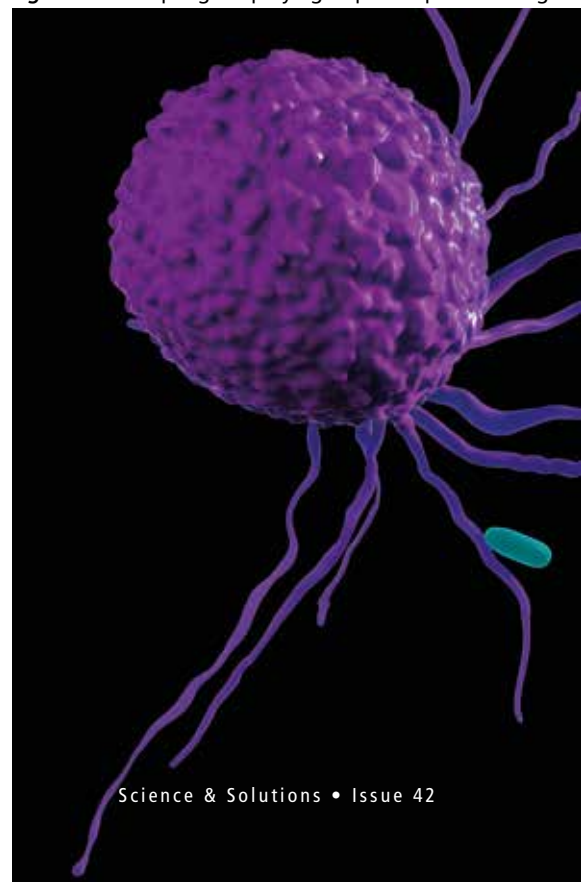
Probiotic colonization and benefits

By using probiotics, we can manipulate microbial populations to favor good bacteria. A number of probiotic candidates have been explored in aquatic animals, some successfully and some not. The concept of probiotic ‘colonization’ is an interesting one; it is clear that a probiotic

Around 30% to 40% of the intestinal mass is comprised of biologically active microbial cells.

animals. When aquatic animals are under stress, they may become immunocompromised; this provides a perfect opportunity for these bacteria to make the transition from harmless commensal organisms to disease-causing pathogens.

Figure 1. Macrophage deploying its pseudopods in the gut



does not have to be present in huge numbers to bring benefits to the host. Typically, an artificially high level of probiotics in the intestine will decrease over time, so it is important that supplementation is continuously provided. Some of the immune localized benefits of probiotic supplementation include reduced adhesion of pathogens to intestinal mucus, increased abundance of goblet cells and elevated mucus lysozyme.

The **improved intestinal defense** brought about by probiotics can be demonstrated using *ex vivo* models. When fish intestines are artificially exposed to pathogens, typically a loss of membrane integrity, necrotic and irregular enterocytes and intracellular spaces are observed, creating a 'leaky gut'. If probiotics are added to the intestine, this level of damage can be prevented and can even be reversed, suggesting that they offer greater barrier defense efficiency and reduce pathogen translocation.

Probiotics can bring **nutritional benefits** to aquatic animals too. At the cellular level of the gut, each gut cell (enterocyte) is covered with many micro-

villi constituting the 'brushborder', which functions to improve nutrient absorption by increasing the surface area. In many cases, probiotic supplementation can increase the length and number of microvilli, which can have a net benefit on

Probiotics can stimulate digestive enzyme secretions and recent working indicates that they can mediate intestinal enteritis.


nutrient uptake and a positive effect on growth performance.

Furthermore, probiotics can **stimulate digestive enzyme secretions** and recent working indicates that they can mediate intestinal enteritis.

The positive effects of probiotic supplementation can manifest at different time points. Positive changes at the macroscopic level (organism, organ, tissue) may be seen after three or more weeks of continuous feeding, depending on the species. However, at the microscopic (sub-cellular) level, these effects occur much earlier. For example, inflammatory cytokine expression, which regulate the innate immune response, can be improved after hours or days.

Commercial probiotics

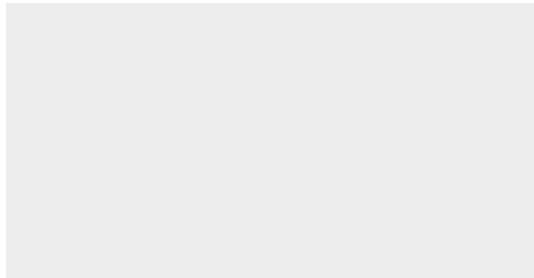
A word of warning: Dr Merrifield highlighted that not all probiotics can induce these benefits. Therefore, it is of paramount importance that probiotic products, whether commercialized or not, are well defined, fully characterized and are supported by robust scientific and field tested data.

BIOMIN addresses the need for a specific aquaculture probiotic through the AquaStar® range. AquaStar® offers producers a scientifically proved probiotic solution to support gut health (AquaStar® Hatchery and AquaStar® Growout) and improve water quality through bioremediation (AquaStar® Pond and AquaStar® PondZyme). 

as it 'tastes' the intestinal microbiota.



Photo: Eraxion



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