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Science & Solutions

Aquafeeds of the Future are Fish Free

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Competition Catalyzes Development of Fish-Free Feed

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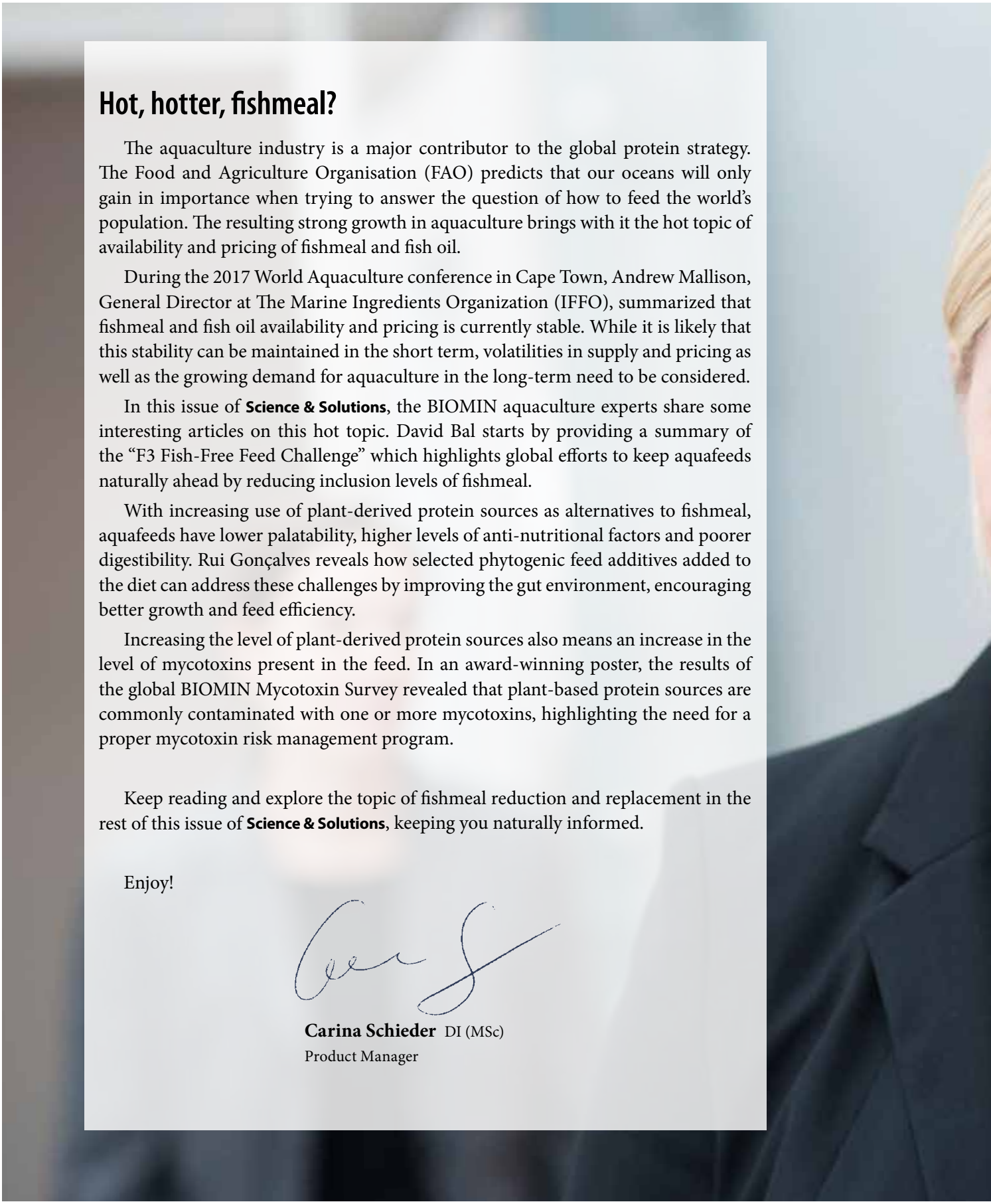
Shrimp Performance Improved by Phytogenics

Editorial



Hot, hotter, fishmeal?

The aquaculture industry is a major contributor to the global protein strategy. The Food and Agriculture Organisation (FAO) predicts that our oceans will only gain in importance when trying to answer the question of how to feed the world's population. The resulting strong growth in aquaculture brings with it the hot topic of availability and pricing of fishmeal and fish oil.

During the 2017 World Aquaculture conference in Cape Town, Andrew Mallison, General Director at The Marine Ingredients Organization (IFFO), summarized that fishmeal and fish oil availability and pricing is currently stable. While it is likely that this stability can be maintained in the short term, volatilities in supply and pricing as well as the growing demand for aquaculture in the long-term need to be considered.

In this issue of **Science & Solutions**, the BIOMIN aquaculture experts share some interesting articles on this hot topic. David Bal starts by providing a summary of the "F3 Fish-Free Feed Challenge" which highlights global efforts to keep aquafeeds naturally ahead by reducing inclusion levels of fishmeal.

With increasing use of plant-derived protein sources as alternatives to fishmeal, aquafeeds have lower palatability, higher levels of anti-nutritional factors and poorer digestibility. Rui Gonçalves reveals how selected phytogenic feed additives added to the diet can address these challenges by improving the gut environment, encouraging better growth and feed efficiency.

Increasing the level of plant-derived protein sources also means an increase in the level of mycotoxins present in the feed. In an award-winning poster, the results of the global BIOMIN Mycotoxin Survey revealed that plant-based protein sources are commonly contaminated with one or more mycotoxins, highlighting the need for a proper mycotoxin risk management program.

Keep reading and explore the topic of fishmeal reduction and replacement in the rest of this issue of **Science & Solutions**, keeping you naturally informed.

Enjoy!

Carina Schieder DI (MSc)
Product Manager

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Editors: Ryan Hines, Caroline Noonan
Contributors: Carina Schieder, David Bal, Rui A. Gonçalves
Marketing: Herbert Kneissl, Karin Nährer
Graphics: GraphX ERBER AG
Research: Franz Waxenecker, Ursula Hofstetter
Publisher: BIOMIN Holding GmbH
Erber Campus, 3131 Getzersdorf, Austria
Tel: +43 2782 8030
www.biomin.net

Feed Innovation Success for BIOMIN in Fish-Free Feed Competition

BIOMIN and Htoo Thit Corp. (HTC) won second prize in the Fish-Free Feed Contest that catalyzed the development and sale of competitive and viable fish-free aquafeeds around the world.

By **David Bal**, Technical Sales Manager - Aquaculture

Photo: shutterstock_Kletr

For over a year, eight companies from all around the world competed to prove the commercial viability of fishmeal-free aqua feed by selling as much of it as they could.

According to the Food and Agriculture Organization (FAO), over 18 million tons of wild-caught fish is used each year to make fishmeal and fish oil. These small schooling fish, known as forage fish, include sardines, herring, anchovies and menhaden. By 2030, it is predicted that 25% less wild-caught seafood will be available compared to today. A similar shrinkage will also occur in aquaculture unless it can overcome key constraints, such as a shortage of fishmeal for feeds.

To mitigate this issue, the F3 Fish-Free Feed Challenge was launched in November 2015 to encourage sustainable innovations such as the use of alternative ingredients for aquaculture fish feeds, and to reduce pressure on wild-caught fish to supply fish feed components. The contest was intended to help catalyze the development and sale of cost-competitive, viable aqua feeds free of fishmeal and fish oils.

For over a year, eight companies from all around the world (*Table 1*) competed to prove the commercial viability of fishmeal-free aqua feed by selling as much of it as they could. Contestants ranged from integrated multi-national with hundreds of employees, to start-up farms and ingredient companies with just a dozen employees.

The rule of F3 was simple: by September 2017, the first company to produce and sell 100,000 metric tons (MT) of aqua feeds that did not contain any marine animal meal or oil would be awarded a US$200,000 prize to support their fish-free aquafeed business.

In Myanmar particularly, the aquaculture diets targeted for the F3 contest were Rohu (*Labeo rohita*) and Tilapia (*Oreochromis* sp.) grower feeds. These two common species are often grown together in the same ponds along with other species like Pacu and Catfish. The polyculture systems in Myanmar can be time extensive with low yields (e.g. 1 - 3.5 tons/ha). They can also be combined with poultry or swine production units installed above the ponds where feces are used to boost the natural phytoplankton productivity of the water ecosystem. Hence, some systems can be semi-intensive with production yields up to 15 t/ha with fish grown in smaller ponds.

Currently, Htoo Thit Co (HTC) is using various plant protein sources such as imported soybean meal, whole wheat meal, groundnut cake and other local protein sources in their fish feeds. As the main aqua species in Myanmar are herbivorous, they can still perform on 100% plant diets making it possible to reduce the fishmeal content to zero as long as the necessary digestible amino acid profile and available energy level are included in the formulation.

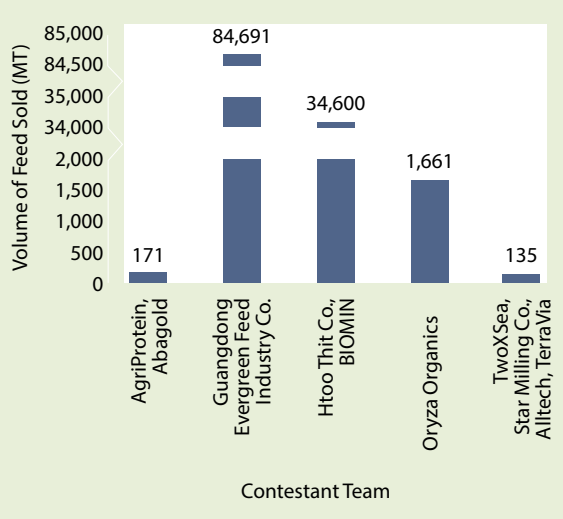
Since 2014, BIOMIN and HTC have worked closely together to improve local feed formulations and test alternative plant-based raw materials. Two remaining bottlenecks are the lack of differentiated feed formula-

Table 1. List of contestants and locations with the diets they worked on.

Team	Diets Submitted
AgriProtein (Gibraltar), Abagold (South Africa)	Rainbow Trout
Guangdong Evergreen Feed Industry Co. (China)	Tilapia, Carp, Dace
Htoo Thit Co. (Myanmar), BIOMIN (Austria)	Tilapia/Carp
JAPFA Feeds (Singapore/Indonesia)	Tilapia
Oryza Organics (Pakistan)	Tilapia (x2)
Ridley (Australia), Sureerath Prawns (Thailand)	Shrimp
TomAlgea (Belgium)	Shrimp
TwoXSea (U.S. California), Star Milling Co. (U.S. California), Alltech (U.S. Kentucky), TerraVia (U.S. California)	Trout

David Bal
Technical Sales Manager - Aquaculture

Figure 1. Fishmeal free feed sales by contestant team over the course of the competition.



Professor Kevin Fitzsimmons from the University of Arizona, former president of the World Aquaculture Society and lead spokesperson for the F3 Challenge said:

"***The contest has accelerated information sharing and partnerships between companies in all parts of the feed supply chain. The great thing about the contest has been the incredible level of publicity generated for innovative companies producing new ingredients, and to alert all aquafeed companies of the potential available.***

A second really encouraging sign was the significant shift amongst environmental NGOs when they realized that aquaculture could in fact become a much more sustainable industry. Many environmental groups have now put their efforts to actively supporting more innovative aquaculture, rather than just opposing anything related to aquaculture."

tions by species and by stages, and also the high levels of mycotoxins in raw materials.

BIOMIN is now monitoring mycotoxin contamination levels in Myanmar by analyzing several batches of raw material and finished feeds. For example, levels of deoxynivalenol (DON), fumonisin (FUM) and aflatoxin (AFLA) were all found to be far above the recommended limits. This trend is amplified with the use of 100% plant-based diets with DDGS. It is crucial for feed mills like HTC to reduce the impact of mycotoxins by introducing a mycotoxin mitigation plan in their feed manufacturing process; an area where huge improvements can be made in Myanmar.

Continuing the development of fishmeal-free fish diets, BIOMIN and HTC decided to enter the F3 competition as a team with the objective of completely eliminating the use of local fishmeal in Rohu and Tilapia diets. The F3 contest was an opportunity to accelerate the transition toward more sustainable feed. It started in May 2016 with freshwater fish species but in the future, the F3 feed concept could also be applied to other species such as Shrimp (*Vannamei*) and tropical Seabass (*Lates calcarifer*).

In September 2017, the BIOMIN / HTC team finished in second place with almost 34,000T of F3 aqua feeds sold in Myanmar (*Figure 1*). First prize went to Guangdong Evergreen Feed Industry Co. from China who sold more than 84,000T of F3 feed. Their prize was presented during a special ceremony on 4th October 2017 at the Global Aquaculture Alliance GOAL's conference in Dublin, Ireland.

In Brief

- In the future, there will be supply constraints on the wild-caught forage fish used to make fishmeal and fish oils
- The F3 Fish-Free Feed Challenge launched in November 2015 to catalyze the development and sale of fish-free aquafeeds
- Nearly 120,000 metric tons of fish-free aquafeed were produced and sold during the course of the competition

Phytogenics Deliver Better Performance in Low Fishmeal Shrimp Diets

In an effort to reduce costly, less sustainable fishmeal in aquafeeds, many producers are seeking more economical, alternative protein sources. Fishmeal reduction alone can compromise growth performance. Fortunately, supplementing diets with a phytogenic feed additive can recover some of this lost performance, improving bottom line results.

By **Rui A. Gonçalves**, MSc, Scientist - Aquaculture

Photo: iStockphoto: Jakub Jirsak

Table 1. Formulation and proximate composition of the experimental diets (dry matter).

	FM25	FM22	FM22+ Digestarom® P.E.P. MGE	FM19	FM19 + Digestarom® P.E.P. MGE
Ingredients (%)					
Peru Fish meal	25.00	22.00	22.00	19.00	19.00
Soybean meal (solvent extracted)	28.00	31.00	31.00	32.00	32.00
Peanut meal (groundnut meal)	10.38	11.80	11.80	14.88	14.88
Brewer's yeast	3.00	3.00	3.00	3.00	3.00
Wheat flour	22.81	22.81	22.81	22.81	22.81
Soybean oil	1.42	1.50	1.50	1.59	1.59
Fish oil	1.42	1.50	1.50	1.58	1.58
Soy lecithin	2.00	2.00	2.00	2.00	2.00
Vitamin premix	0.50	0.50	0.50	0.50	0.50
Mineral premix	1.00	1.00	1.00	1.00	1.00
$Ca(H_2PO_4)_2$	1.50	1.50	1.50	1.50	1.50
Lysine	0.00	0.03	0.03	0.06	0.06
Methionine	0.00	0.03	0.03	0.06	0.06
Digestarom® P.E.P. MGE	0.00	0.00	0.02	0.00	0.02
Cellulose	2.97	1.33	1.31	0.02	0.00
Proximate composition (%)					
Moisture	9.04	8.90	9.28	9.25	9.45
Protein	39.43	39.71	40.12	40.08	39.98
Lipid	8.52	8.55	9.00	8.65	9.09
Ash	10.07	9.70	9.82	9.34	9.60

Source: BIOMIN

Pacific white leg shrimp, *Litopenaeus vannamei*, is a popular species among aquaculture farmers due to its high economic value, rapid growth rate, and tolerance to wide ranges in salinity and temperature (Bray *et al*., 1994; Frias-Espericueta *et al*., 1999).

Concerns about fishmeal

The high dependence of Pacific white shrimp feed on fishmeal – an important protein source due to its palatability and quality – is becoming increasingly problematic due to concerns about sustainability and price. The quantity of fishmeal used by the aquaculture feed sector has increased considerably, driving up its market price over the past decade. Significant progress has been made to reduce levels of fishmeal in commercial diets for farmed aquatic animals.

Plant protein hurdle

Replacing fishmeal with alternatives such as vegetable proteins can negatively affect production performance. The use of less digestible plant raw material increases the presence of undigested nitrogenous compounds

Derived from plants, PFAs are functional feed additives used to improve animal performance.

Table 2. Growth performance parameters of juvenile Pacific white shrimp fed different experimental diets.

Treatments	Fish meal (%)	Digestarom® P.E.P. MGE	Final Weight (g)	PER	Survival (%)	FCR	SGR (%/day)	HSI (%)
FM25	25	0	15.36±1.42	2.52±0.27	98.66±2.99	1.02±0.13	6.78±0.20	5.23±0.75
FM22	22	0	12.31±2.29	2.04±0.37	98.67±1.82	1.26± 0.22	6.37±0.33	5.03±1.09
FM22+ Digestarom® P.E.P. MGE		200g/ton of feed	13.70±2.23	2.17±0.35	98.67±1.82	1.17±0.19	6.55±0.30	4.98±0.34
FM19	19	0	12.24±2.12	1.96±0.28	98.00±2.98	1.29±0.17	6.36±0.31	5.06±0.99
FM19+ Digestarom® P.E.P. MGE		200g/ton of feed	13.45±1.78	2.17±0.25	96.67±2.36	1.17±0.13	6.54±0.23	4.99±0.60

PER- Protein Efficiency Ratio; HSI – Hepatosomatic Index. Data represent mean ± S.D. of five replicates. No significant differences were found on these parameters ($P>0.05$).

Source: BIOMIN

in the intestine, which encourages the formation of ammonia and biogenic amines by intestinal microbiota. These compounds are toxic and consequently can lead to imbalance in the intestine, resulting in inflammatory processes and accelerated turnover of the intestinal tissue, leading to poorer performance (Cabral *et al.*, 2013).

Improving performance with PFAs

Plant raw materials are less digestible and can cause negative effects directly on the gastrointestinal tract. Due to their ability to improve feed efficiency at comparatively low cost, phytogenic feed additives (PFAs) are an important addition to aquafeeds. Derived from plants, PFAs are functional feed additives used to improve animal performance. Plant essential oils have been shown to exert multiple positive effects, such as appetite stimulation, a direct reduction of gut bacteria, stimulation of gastric juices, enhancement of the immune system, as well as anti-inflammatory and anti-oxidant properties (Lambert *et al.*, 2001; Nerio *et al.*, 2010; Peterson *et al.*, 2015; Saravanan *et al.*, 2012; Yeh *et al.*, 2009).

A trial was conducted to evaluate the nutrient sparing effect of a commercially available PFA (Digestarom® P.E.P. MGE) in Pacific white shrimp feed. The trial evaluated the effect of the PFA on growth performance, feed utilization and gut intestine ultrastructure. The relationship between intestinal microorganisms and intestinal structure is complex, but it is known to have an impact on nutrient absorption (Apajalahti *et al.*, 2004).

Five diets were formulated for the experiment (*Table 1*). Three levels of fishmeal were used; the control diet contained the highest level at 25.0% (FM25). The fishmeal level was then reduced to 22.0% (FM22) and 19.0% (FM19) by substitution with soybean meal and peanut meal. Digestarom® P.E.P. MGE was supplemented to the diets with reduced fishmeal content at a dosage of 200 g/ton of feed.

Growth performance and feed utilization

The results of the trial indicate important improvements in measured parameters when using Digestarom® P.E.P. MGE. Weight gain, specific growth rate, feed conversion rate and protein efficiency ratio were all improved ($P<0.05$) for the shrimp fed low fishmeal diets supplemented with the PFA when compared to those that did not receive the PFA.

Rui A. Gonçalves
MSc, Scientist - Aquaculture

The replacement of fishmeal by plant proteins had a negative influence on feed conversion ratio (FCR), which worsened as the level of fishmeal in the diets got lower. However, when the low fishmeal diets were supplemented with Digestarom® P.E.P. MGE there was an improvement in FCR values compared with non-supplemented diets.

The growth performance and feed utilization of shrimp fed the experimental diets with lower fishmeal levels was numerically worse than other treatments (*Table 2*). Of the diets with reduced fishmeal, those supplemented with Digestarom® P.E.P. MGE numerically improved shrimp specific growth rate and FCR (*Table 2*).

Structure of the gastrointestinal tract

It is well known that the digestive tract is divided into three distinct regions, according to their relative importance in all crustaceans. While the foregut and hindgut have a chitinous lining and do not play an important role in digestive processes (Brunet *et al.*, 1994), the improvement of microvilli in the mid-gut is important for nutrient absorption. Analysis by transmission electron microscope indicated that shrimp fed the FM25 diet had a better mid-gut structure and a higher number of microvilli than those fed the other diets.

However, in shrimp fed low fishmeal diets, those fed the diet supplemented with Digestarom® P.E.P. MGE had a better gut structure and a higher number of microvilli in the mid-gut compared to those fed the non-supplemented diets.

The author would like to acknowledge Xiao-ling Huang, Ming-hong Xia and Qi-cun Zhou from the Laboratory of Fish Nutrition in Ningbo, and BIOMIN colleagues Yan Zhang, Gonçalo A. Santos, and Pedro Encarnaçao for their input to this research. Full details of the trial can be found on the BIOMIN knowledge center on www.biomin.net.

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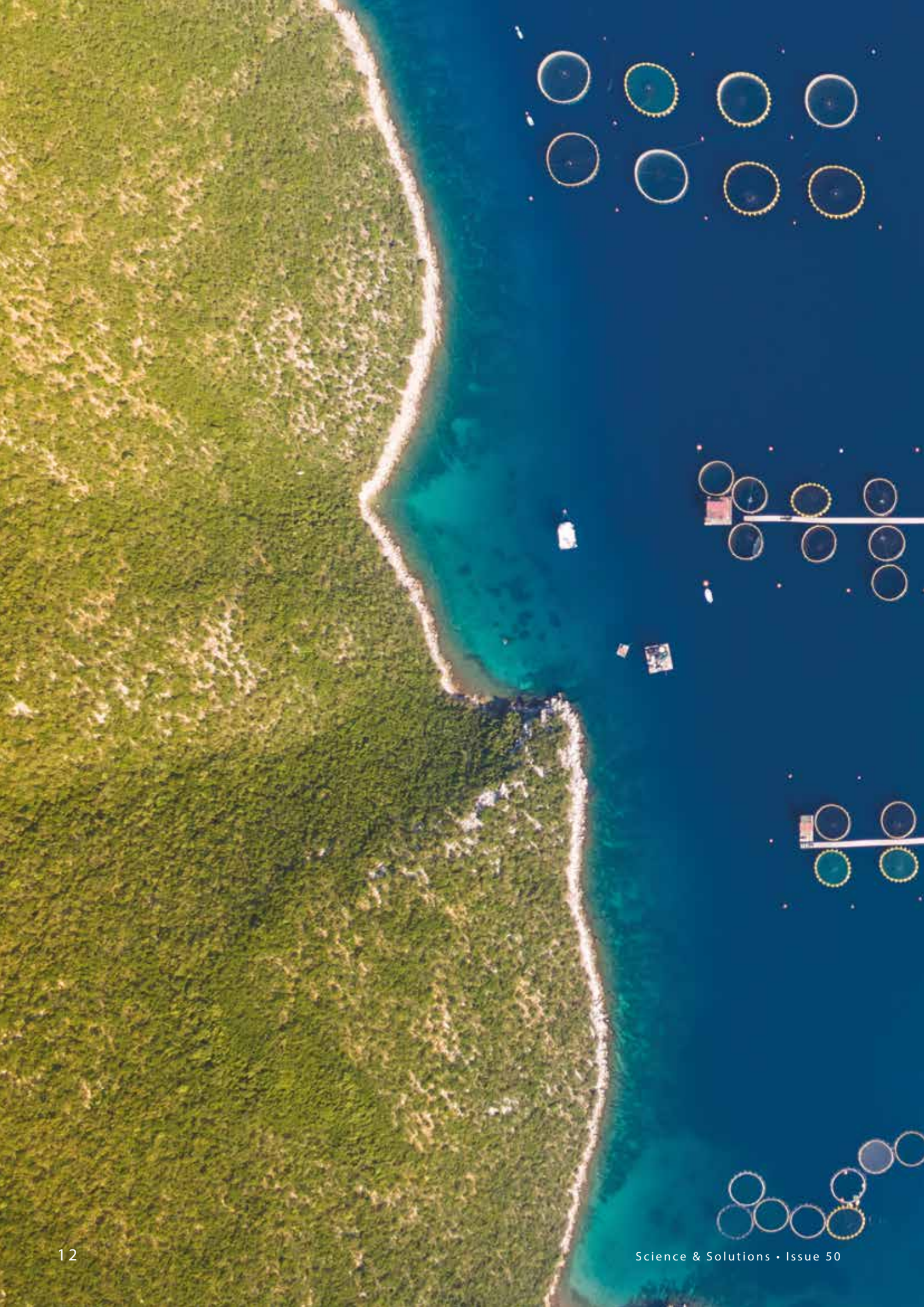

Worldwide Mycotoxin Occurrence in Plant Meals: A Real Risk to Aquaculture Development?

Awarded 'best poster by a PhD student' at Aquaculture Europe 2017, held from 17-20 October in Dubrovnik, Croatia, a team of BIOMIN experts, led by Rui Gonçalves, reveal the potential economic consequences of mycotoxins for the aquaculture industry.

Photo: iStockphoto_ paulprescott72

Fusarium mycotoxins were the most prevalent, followed by aflatoxins and deoxynivalenol

Mycotoxins are a diverse group of toxic secondary metabolites produced mainly by filamentous fungi, on agricultural products before or after harvest, during transportation or storage. Research characterizing the adverse effects of mycotoxins on the performance and health of animals has largely focused on terrestrial livestock species (D'Mello and Macdonald, 1997; Rotter *et al.*, 1996). However, in recent years, research has been carried out on the effects of mycotoxins in aquaculture species. Understanding these effects has become even more important with the rising cost of fishmeal and the need to identify and use more economical protein sources such as plant protein or other commercially available plant by-products.

Generally, most of the mycotoxins that have the potential to reduce growth and compromise the health status of aquaculture farmed animals are produced by *Aspergillus*, *Penicillium* and *Fusarium* species. Toxic metabolites produced by these fungi are known to be either carcinogenic (e.g. aflatoxin (AF) B_1, ochratoxin A (OTA), fumonisin (FUM) B_1), estrogenic (zearalenone (ZEN)), neurotoxic (FUM B_1), nephrotoxic (ochratoxin), dermatotoxic (trichothecenes) or immunosuppressive (AFB_1, OTA and T-2 toxin). The tendency, and the economic need, to replace expensive animal-derived proteins such as fishmeal, with less expensive plant protein sources, has increased the impact of mycotoxin contamination in aquaculture feeds (Gonçalves *et al.*, 2017).

Mycotoxin contamination levels found were high, and in 74% of samples, there were two or more mycotoxins present.

Materials & methods

From January to June 2017, 8,345 samples of plant meals were analysed 33,370 times within the scope of the BIOMIN® mycotoxin survey program (*Table 1*). The study focused on corn, corn gluten meal, corn DDGS, soybean meal, wheat, wheat bran rice and rice bran. The samples were tested for aflatoxins (sum of AFB_1, AFB_2, AFG_1 and AFG_2), ZEN, deoxynivalenol (DON), FUM (sum of FB_1 and FB_2), T-2 toxin and OTA (full toxin screen). Sample providers were instructed to follow good sampling procedures according to Richard (2000). The analyses were carried out as described by Binder *et al.*, (2007).

Table 1. Origin of samples.

Location	Number of samples
Europe	1480
North America	527
South America	4344
Asia	1663
Middle East	118
Africa	213
Total	8345

Source: BIOMIN

Results

Globally, *Fusarium* mycotoxins were the most prevalent compounds found in the samples, followed by aflatoxins. Some of the plant meals that are commonly used in aquaculture feeds, such as corn gluten meal and corn DDGS, showed high levels of mycotoxin contamination, commonly with DON and FUM. The results are presented in *Table 2*. Mycotoxin co-occurrence was generally very high; on average, 74% of the samples contained more than one mycotoxin.

Discussion

Deoxynivalenol, one of the most prevalent mycotoxins in the samples analysed, is known to cause adverse effects in several aquatic species, but especially rainbow trout (*Oncorhynchus mykiss*) (Hooft *et al.*, 2011).

Rui A. Gonçalves
MSc, Scientist - Aquaculture

Table 2. Analysis results.

	Average (µg/kg)		Maximum (µg/kg)	
	FUM	DON	FUM	DON
Corn DDGS	3,844	2,791	28,605	10,445
Corn gluten meal	2,250	1,688	11,882	8,871

Source: BIOMIN

Mycotoxins have the potential to reduce growth rates and compromise the health status of aquaculture species

DON is responsible for decreases in growth, feed intake, feed efficiency, protein and energy utilization. The levels of DON found in some commodities might represent a threat for aquaculture species, depending on the inclusion levels of the plant meals in the finished feeds. FUM, also very prevalent among the collected samples, was found in considerably high concentrations, especially in corn gluten meal and corn DDGS.

Fumonisins inhibit the sphinganine (sphingosine) N-acyltransferase (ceramide synthase), a key enzyme in lipid metabolism, resulting in the disruption of this pathway. It is known that rainbow trout liver is sensitive to FUM, inducing changes in sphingolipid metabolism even when contamination levels are lower than 100 µg/kg (Meredith *et al*., 1998) and inducing cancer in one-month old trout (Riley *et al*., 2001).

Crustaceous can be very sensitive to FUM as well, being reported that *Litopenaeus vannamei* are sensitive to FB_1 at levels below 200 µg/kg (García-Morales *et al*., 2013).

Conclusion

The contamination levels found in plant meals commonly used in aquaculture were high, and in 74% of samples there were two or more mycotoxins present, potentially leading to additive or synergistic effects. These results highlight the mycotoxin-related risks associated with growth performance and immunosuppression that can lead to significant economic impacts in the aquaculture sector.

The economical need to replace fishmeal with plant protein sources has increased the impact of mycotoxin contamination in aquaculture

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