

SCIENCE & SOLUTIONS

Keeping you naturally informed | Issue 62 | Aquaculture

Current knowledge about fumonisins in aquaculture

Why you should use a combination of beneficial bacteria in aquaculture

A summary of the research on fumonisins in aquaculture



Overcoming the challenges in aquaculture production



4

Current Knowledge about Fumonisin in Aquaculture

Rui A. Gonçalves MSc
Scientist - Aquaculture and
Michele Muccio MSc
Product Manager

Fumonisin has gained significant recognition in recent years. In 2017, the BIOMIN Mycotoxin Survey found that fumonisin was the predominant contaminant in raw material and finished feed samples across the world. Rui Gonçalves and Michele Muccio share the latest knowledge about fumonisin in aquaculture and highlight the importance of preventing synergistic interactions between different mycotoxins.

10

Why You Should Use a Combination of Beneficial Bacteria in Aquaculture

Benedict Standen PhD
Product Manager

The gut is a complex environment and needs support in a number of ways. Expecting maximum colonization from a single strain of beneficial bacteria is optimistic and often unrealistic. Benedict Standen explains why using a probiotic with a combination of species is more beneficial to aquaculture performance and production.

15

A Summary of the Research on Fumonisin in Aquaculture

All of the research on fumonisin in aquaculture is summarized in this overview table.

Performance Management



Awareness and education levels within the aquaculture industry are constantly rising, to the benefit of the industry as a whole.

Increased awareness about problems such as mycotoxins and anti-nutritional factors are of benefit to farmed species as it allows improvements in management styles and systems used based on this new knowledge. While much of the information comes from investigations and trials using terrestrial animal species, there is an increasing amount of research based on aquatic species. The available research into fumonisins is summarized on page 15 and illustrates not only the work already done, but also the gaps in knowledge that still need to be addressed.

Keeping naturally ahead in aquaculture means being open to change and innovation. Improvements in our knowledge about the complexity of the gut environment has increased our understanding of how best to support it, enabling the animal to optimize its use of high quality feed. As explained in our second article on page 10, it might be better to update the probiotic in the feed to one that contains multiple strains of beneficial bacteria rather than taking the traditional single-strain approach. When was the last time you reviewed your probiotic regiment?

In this issue of Science & Solutions, we start by reviewing current knowledge about fumonisins. This mycotoxin was found in the majority of raw material and finished feed samples tested as part of the BIOMIN Mycotoxin Survey in 2017, a trend which is likely to continue through 2018 and beyond. Are fumonisins negatively affecting your performance indicators?

I take this opportunity to wish you all a very successful and prosperous New Year on behalf of everyone at BIOMIN. We look forward to keeping you naturally ahead in 2019 and in the future.

Enjoy reading this issue of Science & Solutions, keeping you naturally informed.

Wendy Moscoso, MBA
Sales Manager

SCIENCE & SOLUTIONS

ISSN: 2309-5954

For a digital copy and details, visit:

<http://magazine.biomin.net>

For article reprints or to subscribe to

Science & Solutions, please contact us:

magazine@biomin.net

Editors: Ryan Hines, Caroline Noonan

Contributors: Wendy Moscoso MBA, Rui Gonçalves MSc, Michele Muccio MSc, Benedict Standen PhD.

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

© Copyright 2018, BIOMIN Holding GmbH.

All rights reserved. No part of this publication may be reproduced in any material form for commercial purposes without the written permission of the copyright holder except in accordance with the provisions of the Copyright, Designs and Patents Act 1998.

All photos herein are the property of BIOMIN Holding GmbH or used with license.

BIOMIN is part of ERBER Group

Current Knowledge about Fumonisin in Aquaculture

Fumonisin has gained significant recognition in recent years. In 2017, the BIOMIN Mycotoxin Survey found that fumonisins were the predominant contaminant in raw material and finished feed samples across the world.

Rui Gonçalves and Michele Muccio share the latest knowledge about fumonisins in aquaculture, and highlight the importance of preventing synergistic interactions between different mycotoxins.



Rui A. Gonçalves MSc
Scientist - Aquaculture



Michele Muccio MSc
Product Manager

What are fumonisins?

Fumonisin (FUM) is a group of mycotoxins discovered in 1988 in South Africa (Gelderblom *et al.*, 1988). The group includes FB₁, FB₂ and FB₃. They are mainly produced by a number of *Fusarium* species, notably *F. verticillioides* (formerly *F. moniliforme* = *Gibberella fujikuroi*),

F. proliferatum, and *F. nygamai*. The most abundantly produced mycotoxin of the *Fusarium* family is fumonisin B₁ (FB₁).

Fumonisin is characterized as having a long-chain hydrocarbon unit, similar to that of sphingosine and sphinganine, which plays a role in their toxicity (Wang *et al.*, 1992). Fumonisin inhibits the sphinganine (sphingosine) N-acyltransferase (ceramide synthase), a key enzyme in lipid metabolism, resulting in disruption of this pathway. This enzyme catalyzes the acylation of sphinganine in the biosynthesis of sphingolipids. Sphingolipids are important for the membrane and lipoprotein structure, and also for cell regulations and communications (Berg *et al.*, 2003).

IN BRIEF

- Fumonisin is the predominant mycotoxin contaminant in raw materials and finished feed samples worldwide.
- Aquaculture performance indicators are compromised when fumonisins are present in the feed.
- Synergies with other mycotoxins, especially aflatoxin, compound the negative impact of fumonisins in aquaculture feeds.

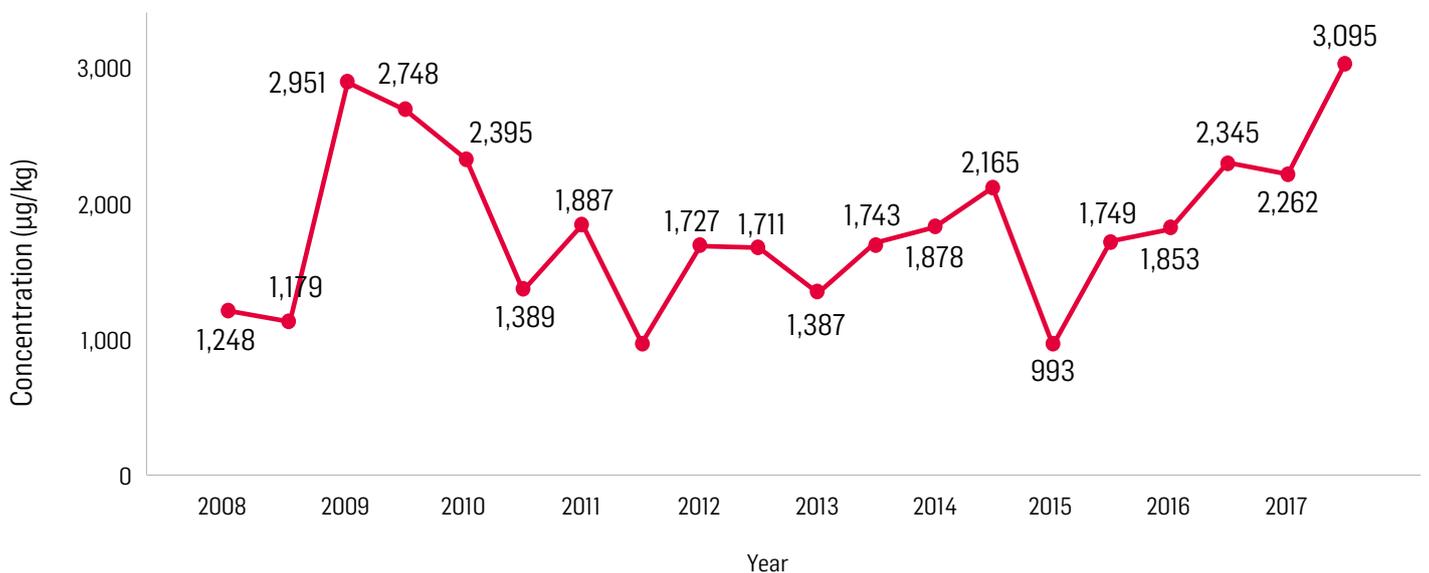
Fumonisin occurrence

The occurrence, level of contamination and implications of mycotoxins entering the feed chain through cereal grains have gained global attention in recent years. The aquaculture industry is no exception to this rise in awareness. *Figure 1* shows the occurrence of fumonisins in corn, one of the major commodities affected by this group of toxin. Since 2015, there has been a global trend towards increasing fumonisin levels



FUM was the most predominant mycotoxin, present in 81% of the collected samples.

Figure 1.
Fumonisin occurrence in corn



Source: BIOMIN

in corn. This trend is also reflected in other commodities commonly used for aquafeed production.

Table 1 shows the mycotoxin contamination in important commodities and finished feeds sampled worldwide between January and December 2017. Corn, corn gluten meal (CGM), dried distillers with solubles (DDGS) and rice bran samples presented the highest occurrence of FUM. With the exception of rice bran, which had a relatively low level of contamination (161 ppb), corn, CGM and DDGS were contaminated with FUM levels of over 2,900 ppb. As FUM is relatively stable against high temperature and processing conditions, it is expected that FUM will be found in finished feeds, as confirmed by analyses of finished feed samples taken in the same reporting period (Table 1.5). In 2017, FUM was the most predominant mycotoxin, present in 81% of the collected samples with an average contamination level of 1,352 ppb.

Mycotoxin co-occurrence

An important factor that also negatively affects aquaculture species is the co-occurrence of mycotoxins; the simultaneous presence of more than one mycotoxin in the same sample. 80% of finished feed samples collected in 2017 were contaminated by more than one mycotoxin (Figure 2).

Fumonisin occurrence in Asia: a glimpse at 2018 samples

Table 2 shows the mycotoxin contamination in plant commodities and finished feeds sampled in Asia between January and March 2018. Samples from China and India are shown in Tables 3 and 4, respectively. The trend observed in the first quarter of 2018 continues the 2017 trend. Samples from China showed the highest contamination level for FUM in both plant commodities (2,767 ppb) and finished feeds (1,765 ppb).

Table 1.

Mycotoxin contamination in important commodities and finished feeds sampled worldwide from January to December 2017

1.1 - Corn samples	Afla	ZEN	DON	FUM	OTA
Number of samples tested	5,367	5,064	4,032	4,480	1,492
Contaminated samples	23%	47%	79%	86%	5%
Average of positive (ppb)	24	154	749	3,189	25
Median of positive (ppb)	4	58	470	1,380	2
Maximum (ppb)	762	6,082	51,374	218,883	889

1.2 - DDGS samples	Afla	ZEN	DON	FUM	OTA
Number of samples tested	243	250	256	255	207
Contaminated samples	21%	84%	95%	91%	16%
Average of positive (ppb)	10	299	2,725	2,972	3
Median of positive (ppb)	3	252	2,190	1,319	2
Maximum (ppb)	277	1,329	14,252	28,605	44

1.3 - Corn gluten samples	Afla	ZEN	DON	FUM	OTA
Number of samples tested	70	79	81	69	44
Contaminated samples	40%	81%	89%	90%	32%
Average of positive (ppb)	46	1,031	1,251	3,547	9
Median of positive (ppb)	4	319	559	1,838	4
Maximum (ppb)	503	5,416	8,871	16,976	37

Can fumonisin negatively affect aquaculture species?

In aquaculture, FUM has been generally associated with reduced growth rate, lower feed consumption, poor feed efficiency ratios, and impaired sphingolipid metabolism (Goel *et al.*, 1994; Li *et al.*, 1994; Lumlertdacha and Lovell, 1995; Tuan *et al.*, 2003). However, information on the effects of FUM in the most important aquaculture species is scarce, with most of the available research focusing on freshwater species.

Channel catfish (*Ictalurus punctatus*) is the most studied species (Goel *et al.*, 1994; Li *et al.*, 1994; Lumlertdacha *et al.*, 1995; Lumlertdacha and Lovell, 1995). According to the cited authors, channel catfish can tolerate relatively high levels of FUM, with a sensitivity level of around 10 ppb.

It is known that liver tissue of rainbow trout (*Oncorhynchus mykiss*) is sensitive to FUM, inducing changes in sphingolipid metabolism at levels lower than 100 µg/kg (Meredith *et al.*, 1998) and inducing cancer in one-month-old trout (Riley *et al.*, 2001). It was observed that animals fed 1,000, 5,000, 10,000 or 20,000 µg/kg FB₁ for ten weeks appeared unaffected in terms of growth, feed intake and liver damage (García, 2013). However, the study also reported observations that all fish (including those in the control group) had very poor feed intake and growth, presenting specific growth rate values two to six times

1.4 - Rice bran samples	Afla	ZEN	DON	FUM	OTA
Number of samples tested	67	69	69	66	63
Contaminated samples	63%	68%	29%	85%	40%
Average of positive (ppb)	17	107	293	161	2
Median of positive (ppb)	3	43	47	89	1
Maximum (ppb)	306	954	1,836	878	9

1.5 - Finished feed samples	Afla	ZEN	DON	FUM	OTA
Number of samples tested	4,545	4,888	4,926	4,594	2,579
Contaminated samples	20%	46%	79%	81%	29%
Average of positive (ppb)	43	67	502	1,352	5
Median of positive (ppb)	4	34	401	740	2
Maximum (ppb)	10,918	7,080	9,805	290,517	270

Afla = Aflatoxin, ZEN = Zearalenone, DON = Deoxynivalenol, FUM = Fumonisin, OTA = Ochratoxin A, DDGS = dried distillers grains with solubles

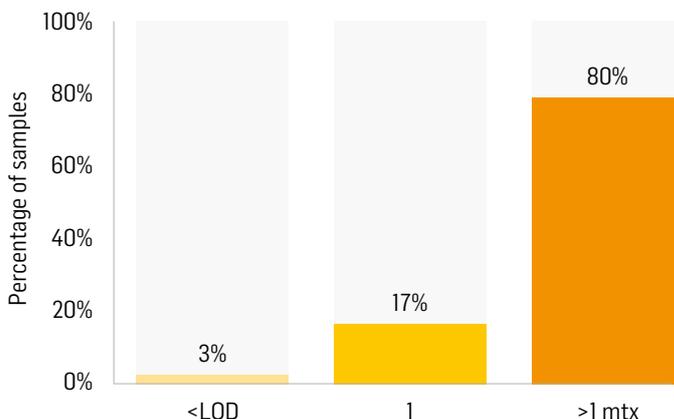
Source: BIOMIN

lower than the average reported in other studies (Farmer *et al.*, 1983; McCormick *et al.*, 1998).

The adverse effects of FUM-contaminated diets have also been reported in carp (*Cyprinus carpio L.*). One-year-old carp showed signs of toxicity at 10,000 µg FB₁/kg feed (Petrinec *et al.*, 2004). The experiments reported the presence of scattered lesions in the exocrine and endocrine pancreas and inter-renal tissue, probably due to ischemia and/or increased endothelial permeability. In another study, one-year-old carp

Figure 2.

Number of mycotoxins per sample of finished feed from January 2017 to December 2017



Source: BIOMIN



Mycotoxins entering the aquaculture feed chain through cereal grains has gained global attention.

Table 2.

Mycotoxin contamination in plant commodities and finished feeds sampled in Asia from January to March 2018

Plant meal samples	Afla	ZEN	DON	FUM	OTA	Finished feeds	Afla	ZEN	DON	FUM	OTA
Number of samples tested	338	346	346	323	292	Number of samples tested	129	137	137	129	120
Contaminated samples	48%	45%	63%	78%	33%	Contaminated samples	76%	51%	74%	91%	62%
Average of positive (ppb)	36	130	503	1,244	11	Average of positive (ppb)	29	86	315	1,103	11
Median of positive (ppb)	9	36	250	569	5	Median of positive (ppb)	10	51	139	695	5
Maximum (ppb)	917	2,612	7,715	27,352	124	Maximum (ppb)	697	2,089	5,391	27,352	124

Source: BIOMIN

Table 3.

Mycotoxin contamination in important commodities and finished feeds sampled in China from January to March 2018

Overall	Afla	ZEN	DON	FUM	OTA	Finished feeds	Afla	ZEN	DON	FUM	OTA
Number of samples tested	66	74	74	51	20	Number of samples tested	9	17	17	9	0
Contaminated samples	58%	88%	100%	98%	0%	Contaminated samples	89%	100%	100%	100%	-
Average of positive (ppb)	44	160	651	2,767	-	Average of positive (ppb)	23	85	486	1,765	-
Median of positive (ppb)	4	36	452	1,724	-	Median of positive (ppb)	12	62	461	1,750	-
Maximum (ppb)	411	2,612	2,945	21,428	-	Maximum (ppb)	54	339	1,025	2,783	-

Source: BIOMIN

Table 4.

Mycotoxin contamination in important commodities and finished feeds sampled in India from January to March 2018

Overall	Afla	ZEN	DON	FUM	OTA	Finished feeds	Afla	ZEN	DON	FUM	OTA
Number of samples tested	74	74	74	74	74	Number of samples tested	55	55	55	55	55
Contaminated samples	86%	18%	38%	89%	80%	Contaminated samples	98%	24%	51%	89%	96%
Average of positive (ppb)	32	30	72	690	15	Average of positive (ppb)	36	30	72	555	14
Median of positive (ppb)	11	22	52	488	9	Median of positive (ppb)	12	22	52	484	7
Maximum (ppb)	697	58	241	4,267	124	Maximum (ppb)	697	58	241	1,562	124

Source: BIOMIN

consumed pellets contaminated with 500, 5,000 or 150,000 µg FB₁/kg of body weight, resulting in a loss of body weight and alterations of hematological and biochemical parameters in target organs (Pepeljnjak *et al.*, 2003).

For tropical species, Tuan *et al.* (2003) demonstrated that feeding FB₁ at 10, 40, 70 or 150 mg/kg feed for eight weeks affected the growth performance of Nile tilapia (*Oreochromis niloticus*) fingerlings. In the same experiment, fish fed diets containing FB₁ at levels of 40,000 µg/kg or higher showed decreased average weight gains. Hematocrit was only decreased in tilapia fed diets containing 150,000 µg FB₁/kg. The ratio between free sphinganine and free sphingosine (Sa:So ratio) in the liver increased when 150,000 µg FB₁/kg was present in the fish feed.

Fumonisin B₁ has not been extensively studied as a shrimp feed contaminant. However, the few studies available suggest that Pacific white leg shrimp (*Litopenaeus vannamei*) are sensitive to FB₁. García-Morales *et al.* (2013) showed that white leg shrimp fed FB₁ at 20 to 200 µg/kg had a reduced soluble muscle protein concentration and reported changes in myosin thermodynamic properties after 30 days of FUM exposure. The same authors reported marked histological changes in tissue samples of shrimp fed a diet containing 200 µg FB₁/kg and meat quality changes after 12 days of ice storage when fish were fed diets containing more than 600 µg FUM per kg of feed.

Are marine species sensitive?

All the FUM-sensitive aquaculture species tested so far, are all omnivorous or herbivorous, and all are freshwater species.

In contrast to freshwater species, the liver in marine fish plays an essential role in lipid metabolism. It is a sensitive organ reflecting any lipid metabolism changes, which might influence the essential pathways of n-3 long-chain polyunsaturated fatty acids (EPA and DHA) biosynthesis and metabolism (Li *et al.*, 2018). The known mode of action of FUM is inhibition of ceramide synthase, a key enzyme in lipid metabolism. It is therefore expected that FUM has a negative impact on the lipid metabolism of marine species.

Based on this theoretical rationale, BIOMIN performed some ground-breaking studies in marine species. As expected, these studies showed that marine species were highly sensitive to FUM, affecting growth performance and immune status at relatively low FUM levels (< 5,000 µg/kg).

However, according to the European Commission (EC), the guidance values for FUM (fumonisins B₁ + B₂) in complementary and complete feeding stuffs for fish is 10 ppm (European Commission, 2006). This is a reason for concern, as new BIOMIN data suggests that the guidance values might be too high, at least for marine species.

Synergism: the most important concept

FUM is the most predominant mycotoxin in plant meals and consequently in finished feeds. However, 80% of all finished feed samples tested during 2017 were contaminated with

Table 5.

Studies that observed synergism between Aflatoxin and FUM

Species	AFB ₁ level (ppb)	FB ₁ level (ppb)	Source
Rainbow trout (<i>Oncorhynchus mykiss</i>)	100	3,200	Carlson <i>et al.</i> , 2001
Pacific white leg shrimp (<i>Litopenaeus vannamei</i>)	300	1,400	Pérez-Acosta <i>et al.</i> , 2016
African catfish (<i>Clarias gariepinus</i>)	7.3	15,000	Adeyemo <i>et al.</i> , 2018

more than one mycotoxin (Figure 2). It is important to know the effects of FUM in addition to its interaction with other mycotoxins present in the feed. Synergism, i.e., the interaction of two or more mycotoxins to produce a combined effect greater than the sum of their separate effects, is not very well described in aquaculture. However, studies have shown that Aflatoxin B₁ (AFB₁) and FUM have a synergistic effect in fish (Carlson *et al.*, 2001; McKean *et al.*, 2006; Adeyemo *et al.*, 2018) and shrimp (Pérez-Acosta *et al.*, 2016).

The study conducted by McKean *et al.* (2006) in mosquitofish (*Gambusia affinis*) perfectly describes the synergistic effect between AFB₁ and FUM. The authors observed that mortality started to increase only above 2,000 ppb of FUM reaching 17%. A similar mortality rate was obtained for AFB₁ levels of 215 ppb. However, when combining both mycotoxins, the authors observed that mortality increased to 75% at concentrations of 1,740 ppb of FUM combined with 255.4 ppb of AFB₁. The same synergistic effect was also observed in other species as detailed in Table 5.

Direct mortality, especially in short-term feeding trials (up to four weeks), is an extreme consequence of the combined levels of AFB₁ and FB₁. Under commercial aquaculture conditions, lower levels of AFB₁ and FB₁ are expected to generate a decrease in growth performance and an increase in disease vulnerability.

Conclusion

Fumonisin B₁ was present in the majority of raw materials sampled in the BIOMIN Mycotoxin Survey in 2017, a trend that is likely to continue based on the analysis of samples gathered in the first quarter of 2018. Although research into fumonisins is scarce, fumonisin contamination has been directly linked to decreased performance levels in aquaculture. The majority of research is focused on freshwater species, but new BIOMIN data that is soon to be published conducted in marine species suggests that sensitivity levels may be lower than previously thought.

The majority of finished feed samples are contaminated with more than one mycotoxin, highlighting both the importance of understanding mycotoxin synergisms when diagnosing performance problems and the importance of constructing mycotoxin mitigation strategies.



Fumonisin contamination has been directly linked to decreased performance levels in aquaculture.

References

- Adeyemo, B.T., Tihamiyu, L.O., Ayuba, V.O., Musa, S. and Odo, J. (2018). Effects of dietary mixed aflatoxin B₁ and fumonisin B₁ on growth performance and haematology of juvenile *Clarias gariepinus* catfish. *Aquaculture* 491: 190-196.
- Berg JM, Tymoczko JL, Stryer L. *Biochemistry*. 5th edition. New York: W H Freeman; 2002. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK21154/>.
- Carlson, D.B., Williams, D.E., Spitsbergen, J.M., Ross, P.F., Bacon, C.W., Meredith, F.I. and Riley, R.T. (2001). Fumonisin B₁ Promotes Aflatoxin B₁ and N-Methyl-N'-nitro-nitrosoguanidine-Initiated Liver Tumors in Rainbow Trout. *Toxicology and Applied Pharmacology* 172(1): 29-36.
- European Commission. (2006). Commission Recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding. [On-line]. Available at: <https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:229:0007:0009:EN:PDF>. Accessed 11.09.18.
- Farmer, G.J., Ashfield, D. and Goff, T.R. (1983). A feeding guide for juvenile Atlantic salmon. *Can. MS. Rep. Fish. Aquat. Sci* 1718: 1-13.
- García-Morales, M-H., Pérez-Velázquez, M., González-Felix, M.L., Burgos-Hernández, A., Cortez-Rocha, M-O., Bringas-Alvarado, L. and Ezquerro-Brauer, J-M. (2013). Effects of Fumonisin B₁-Containing Feed on the Muscle Proteins and Ice-Storage Life of White Shrimp (*Litopenaeus vannamei*). *Journal of Aquatic Food Product Technology* 24(4): 340-353.
- García, E.C. (2013). Effects of fumonisin B₁ on performance of juvenile Baltic salmon (*Salmo salar*). *Department of Biological and Environmental Science*, University of Jyväskylä, Faculty of Science MSc.
- Gelderblom, W.C., Jaskiewicz, K., Marasas, W.F., Thiel, P.G., Horak, R.M., Vlegaar, R. and Kriek, N.P. (1988). Fumonisin - novel mycotoxins with cancer-promoting activity produced by *Fusarium moniliforme*. *Appl. Environ. Microbiol.* 54(7): 1806-1811.
- Goel, S., Lenz, S.D., Lumlertdacha, S., Lovell, R.T., Shelby, R.A., Li, M., Riley, R.T. and Kemppainen, B.W. (1994). Sphingolipid levels in catfish consuming *Fusarium moniliforme* corn culture material containing fumonisins. *Aquat. Toxicol* 30(4): 285- 294.
- Li, M.H., Raverty, S.A. and Robinson, E.H. (1994). Effects of dietary mycotoxins produced by the mold *Fusarium moniliforme* on channel catfish *Ictalurus punctatus*. *J. World Aquac. Soc.* 25(4): 512-516.
- Li, Y., Jia, Z., Liang, X., Matulic, D., Hussein, M. and Gao, J. (2018). Growth performance, fatty-acid composition, lipid deposition and hepatic-lipid metabolism-related gene expression in juvenile pond loach *Misgurnus anguillicaudatus* fed diets with different dietary soybean oil levels. *Journal of Fish Biology* 92(1): 17-33.
- Lumlertdacha, S. and Lovell, R. (1995). Fumonisin-contaminated dietary corn reduced survival and antibody production by channel catfish challenged with *Edwardsiella ictaluri*. *J Aquatic Anim Health* 7(1): 1 - 8.
- Lumlertdacha, S., Lovell, R.T., Shelby, R.A., Lenz, S.D. and Kemppainen, B.W. (1995). Growth, hematology, and histopathology of channel catfish, *Ictalurus punctatus*, fed toxins from *Fusarium moniliforme*. *Aquaculture* 130(2): 201- 218.
- McCormick, S.D., Shrimpton, J.M., Carey, J.B., O'Dea, M.F., Sloan, K.E., Moriyama, S. and Björnsson, B.Th. (1998). Repeated acute stress reduces growth rate of Atlantic salmon parr and alters plasma levels of growth hormone, insulin-like growth I and cortisol. *Aquaculture* 168: 221-235.
- McKean, C., Tang, L., Tang, M., Billam, M., Wang, Z., Theodorakis, C.W., Kendall, R.J. and Wang, J-S. (2006). Comparative acute and combinative toxicity of aflatoxin B₁ and fumonisin B₁ in animals and human cells. *Food and Chemical Toxicology* 44(6): 868-876.
- Meredith, F.I., Riley, R.T., Bacon, C.W., Williams, D.E. and Carlson, D.B. (1998). Extraction, quantification, and biological availability of fumonisin B₁ incorporated into the Oregon test diet and fed to rainbow trout. *J Food Prot* 61(8): 1034-1038.
- Pepeljnjak, S., Petrinc, Z., Kovacic, S. and Klarić, M. (2003). "Screening toxicity study in young carp (*Cyprinus carpio* L.) on feed amended with fumonisin B₁." *Mycopathologia* 156: 139-145.
- Pérez-Acosta, J.A., Burgos-Hernandez, A., Velázquez-Contreras, C.A., Márquez-Ríos, E., Torres-Arreola, W., Arvizu-Flores, A.A. and Ezquerro-Brauer, J.M. (2016). An *in vitro* study of alkaline phosphatase sensitivity to mixture of aflatoxin B₁ and fumonisin B₁ in the hepatopancreas of coastal lagoon wild and farmed shrimp *Litopenaeus vannamei*. *Mycotoxin Research* 32(3): 117-25.
- Petrinc, Z., Pepeljnjak, S., Kovacic, S. and Krznaric, A. (2004). Fumonisin B₁ causes multiple lesions in common carp (*Cyprinus carpio*). *Dtsch. Tierarztl. Wochenschr*: 358-363.
- Riley, R.T., Enongene, E., Voss, K.A., Norred, W.P., Meredith, F.I., Sharma, R.P., Spitsbergen, J., Williams, D.E., Carlson, D.B. and Merrill, A.H.Jr. (2001). Sphingolipid perturbations as mechanisms for fumonisin carcinogenesis. *Environmental Health Perspectives* 109 (Suppl 2): 301-308.
- Tuan, N.A., Manning, B.B., Lovell, R.T. and Rottinghaus, G.E. (2003). Responses of Nile tilapia (*Oreochromis niloticus*) fed diets containing different concentrations of moniliformin or fumonisin B₁. *Aquaculture* 217(1): 515-528.
- Wang, E., Norred, W.P., Bacon, C.W., Riley, R.T. and Merrill, A.H.Jr. (1991). Inhibition of sphingolipid biosynthesis by fumonisins. Implications for diseases associated with *Fusarium moniliforme*. *The Journal of Biological Chemistry* 266(22): 14486-14490.
- Wang, E., Ross, F.P., Wilson, T.M., Riley, R.T. and Merrill, A.H.Jr. (1992). Increases in serum sphingosine and sphinganine and decreases in complex sphingolipids in ponies given feed containing fumonisins, mycotoxins produced by *Fusarium moniliforme*. *J. Nutr.* 122(8): 1706-1716.

Why You Should Use a Combination of Beneficial Bacteria in Aquaculture



Benedict Standen PhD
Product Manager Microbials

The gut is a complex environment and needs support in a number of ways. Expecting maximum colonization from a single strain of beneficial bacteria is optimistic and often unrealistic. Benedict Standen explains why using a probiotic with a combination of species is more beneficial to aquaculture performance and production.

In any group of people, some have skills and talents that others do not possess. Each person has his or her own strengths, weaknesses, skills and talents that allow them to perform and successfully accomplish a task.

The same is true for beneficial bacteria, or probiotics. Different species have different characteristics that affect their efficacy, and ultimately their probiotic usefulness. This seems like common sense, but then why are the majority of commercial probiotic formulations focused on a single genera, *Bacillus*?

There are a few reasons. Firstly, most common *Bacillus* spp. are generally not too difficult to grow at moderate cost.

Secondly, they can form bacterial spores, which allows for a longer shelf life and gives greater (but not limitless) heat stability. Thirdly, they have an extensive safety record with only a couple of species known to be pathogenic or toxic to animals (*Bacillus anthracis* and *Bacillus cereus*). This safety record makes it relatively easy to register *Bacillus* spp. products, since they appear on many approved lists (e.g. GRAS - Generally Recognized as Safe in USA, and QPS – Qualified Presumption of Safety in EU). However, these benefits are more useful for probiotic producers than they are for the animal.

Although there is scientific evidence to support the use of *Bacillus*-based probiotics in aquaculture, it is unlikely that a single genera of bacteria can do everything. The intestine is an extremely complex ecosystem, and therefore specific microbial drivers are needed, in the right concentration, for specific tasks within the gut. The probiotic additive should be chosen based on the desired outcome, e.g. growth performance, immunity, disease resistance, survival rate.

IN BRIEF

- The environment within the gut is very complex, with different groups of bacteria having different roles and bringing different benefits to the host.
- Using a single-strain probiotic is unlikely to provide a solution to the many challenges faced.
- AquaStar® from BIOMIN contains multiple species and therefore delivers multiple benefits.

Probiotic colonization

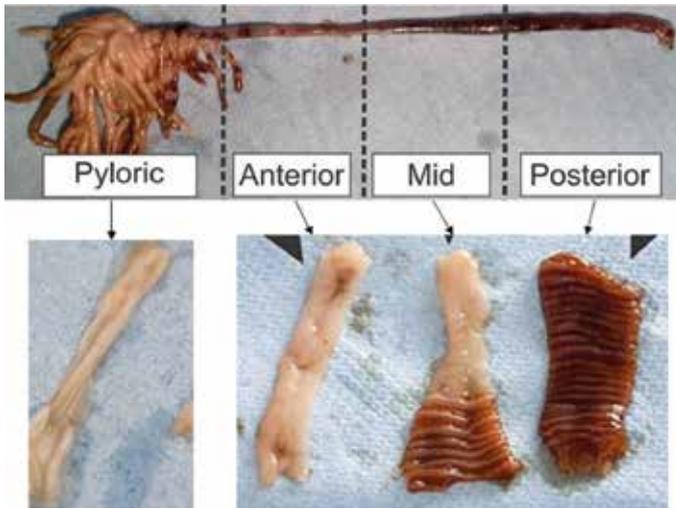
It is controversial whether 'true' probiotic colonization is possible. A number of studies have reported that once probiotic feeding has stopped, the probiotic can persist in the intestine for a certain period, therefore demonstrating temporal colonization. In aquatic species, the current literature suggests this period can be anywhere between



Photo: Getty Images, Jordan Szostak

Figure 1.

Schematic diagram of where intestinal cells were isolated from the digestive system of rainbow trout (*O. mykiss*). The intestine was split by region, opened up longitudinally and epithelial cells isolated for further *in vitro* culture



Source: Langan et al., 2018

three days to more than three weeks and is dependent on the probiotic species, host species, environmental factors, dosage and duration of probiotic supplementation.

The intestinal microbiota can be split into two distinct groups, those that are transient (allochthonous) and those associated with the epithelia (autochthonous). The best probiotic colonizers are the *autochthonous* bacteria.

By attaching to the intestinal epithelia, they compete with pathogens for adhesion sites, preventing their attachment and subsequent translocation leading to immune response, sub-acute stage of energy loss, or even acute infection. Furthermore, through a complex system of molecular receptors, autochthonous bacteria can also interact with the host immune system, improving immunity and increasing disease resistance.

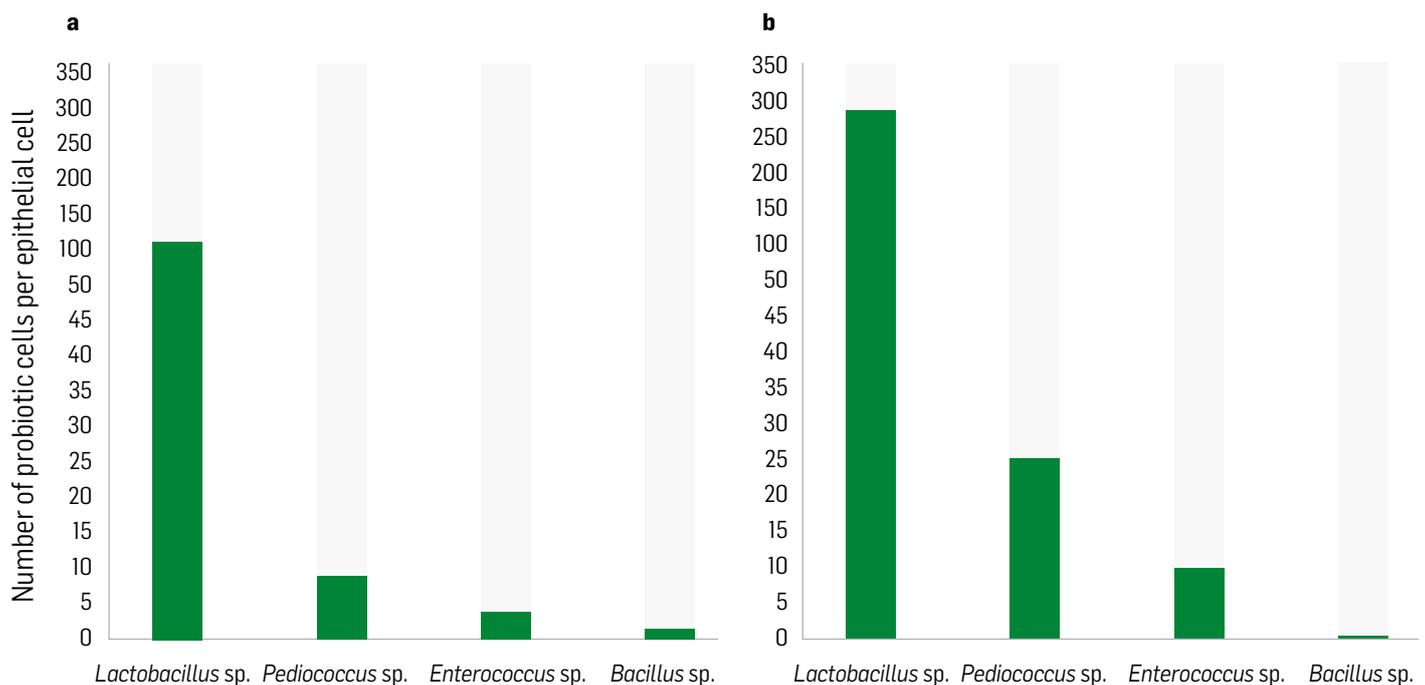
Different probiotic species possess different adhesion properties, and this can ultimately affect their ability to colonize the intestine and exert their benefits. As important mucosal surfaces, two aquatic epithelial cell lines from rainbow trout (*Oncorhynchus mykiss*) were chosen to evaluate probiotic adhesion; commercial gill cell lines (RTgill-W1, ATCC, Virginia, US) and intestinal cell lines, isolated fresh by scientists at the BIOMIN Research Centre, Austria (Figure 1). The probiotic species (*Lactobacillus* sp., *Pediococcus* sp., *Enterococcus* sp. and *Bacillus* sp.) were chosen based on their well-documented benefits in aquatic animals. Their combination makes up the commercially available probiotics from BIOMIN, AquaStar® Growout and AquaStar® Hatchery.

In general, lactic acid bacteria (LAB; e.g. *Lactobacillus* spp., *Pediococcus* spp. and *Enterococcus* spp.) can be colonizing bacteria in the gut. As this is one crucial criterion for an efficacious probiotic, all LAB strains selected for AquaStar® products are able to attach to the gill and gut epithelial cells, with stronger adhesion observed in the intestinal cell lines (Figure 2).

AquaStar® *Lactobacillus* sp. was extremely good at attaching to epithelial cells, with an average of >100 and

Figure 2.

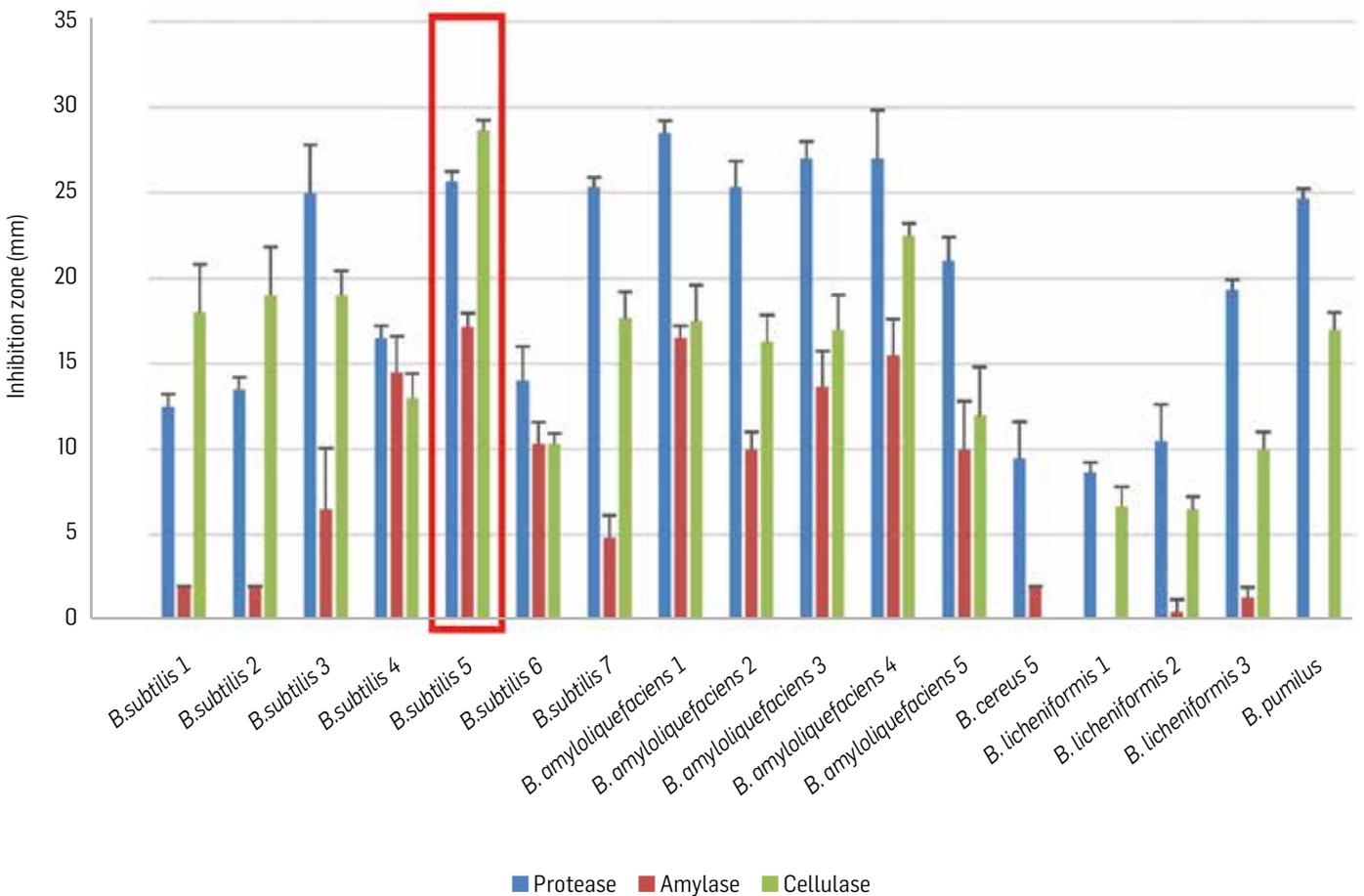
Adhesion properties of selected probiotic bacteria to gill (a) and gut (b) epithelial cell lines. The data indicate the number of probiotic cells attached to a single epithelial cell



Source: BIOMIN

Figure 3.

Ability of *Bacillus* spp. to produce extracellular proteolytic, amylolytic and cellulolytic enzymes. Higher bars indicate greater production or activity of the respective enzyme. The red box indicates the *B. subtilis* strain in AquaStar® products



Source: BIOMIN

>300 probiotic cells attaching to an individual gill and gut epithelial cell, respectively. AquaStar® *Pediococcus* sp. exhibited the second strongest adhesion properties, with more than 30 probiotic cells attaching to a single enterocyte (gut epithelial cell). *Enterococcus* sp. also displayed adhesion abilities, albeit at a lower level than *Lactobacillus* and *Pediococcus*.

Also of interest was the complete inability of *Bacillus* sp. to attach to either epithelial cell types (less than one *Bacillus* cell per epithelial cell). In the intestinal environment, this suggests that their main function is in the lumen of the gut. It is hypothesized that, due to their proteolytic nature (discussed below), it would actually be negative and potentially harmful for the animal if a *Bacillus* probiotic were to attach to epithelial cells. These data suggest that for better colonization of the intestine, an in-feed probiotic should favor LAB, instead of *Bacillus* spp.

Enzyme production by *Bacillus* spp.

Bacillus spp. are well known for their ability to produce enzymes. In the intestine, these enzymes can improve the digestibility of feeds, contributing to better feed conversion

and growth performance, whilst in the environment they can help break down organic matter in the water and sediments.

Using *in vitro* techniques, BIOMIN scientists have documented the ability of numerous *Bacillus* probiotic candidates to produce proteolytic, amylolytic and cellulolytic enzymes. It was clear from the variation that the ability to produce enzymes was not universal within the *Bacillus* genus (Figure 3) and therefore caution must be applied when choosing a commercial probiotic. Most interesting was the variability within a single species. For example, when looking at protease production, the inhibition zone from *B. subtilis* 1 was approximately half that of *B. subtilis* 5, indicating considerably lower protease production.

Similarly, amylase production differed between the *Bacillus* spp. tested. Certain *Bacillus* candidates were not able to produce amylase at all (e.g. *B. licheniformis* 1), whereas others (e.g. *B. subtilis* 5) could produce more amylase. While carbohydrates are not fully utilized in aquatic animals, starches help with pellet binding and expansion in extruded aqua feeds, affecting pellet density. For example, sinking feeds will typically have 6-8% starch, while floating feeds will have over 20%.



Photo: Gettyimages, paardtun

Conclusion

The intestinal microbiota is infinitely complex, with different groups of bacteria having different roles and bringing different benefits to the host. Therefore, it is extremely unlikely that a single probiotic species, or even genera, can offer a “silver bullet” solution to the diverse challenges in aquaculture. Producers and feed millers can address these complexities by choosing formulations that utilize a multi-genus and multi-benefit concept, such as AquaStar®. *Bacillus* sp. can produce high volumes and activity of enzymes, contributing to improved digestibility and feed conversion, whilst AquaStar® containing LAB can colonize the intestine, reducing pathogen load, improving immunity and increasing disease resistance.

Clearly, there is a place for simple *Bacillus* probiotics, but perhaps it is time that they were substituted, in favor of alternative and maybe more promising, systematic probiotic products.

Reference

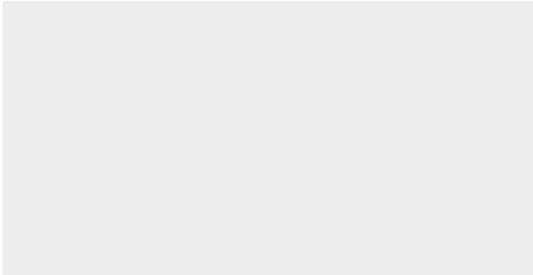
Langan, L.M., Owen, S.F., and Jha, A.N. (2018). Establishment and long-term maintenance of primary intestinal epithelial cells cultured from the rainbow trout, *Oncorhynchus mykiss*. *Biology Open* 2018 7. doi: 10.1242/bio.032870

A Summary of the Research on Fumonisin in Aquaculture

The first article in this issue of Science & Solutions explained the current knowledge on fumonisins in aquaculture. Here is a summary of the research on fumonisins in different aquaculture species. If you have any questions about fumonisins and how they might be impacting your business, please get in touch with the BIOMIN aquaculture team.

Species	Tested Range [µg/kg]	References	Tissue alterations	Performance alterations	Hematopoietic alterations	Increased mortality
Channel catfish (<i>Ictalurus punctatus</i>)	300 to 720,000	Lumlertdacha <i>et al.</i> , 1995; Goel <i>et al.</i> , 1994; Li <i>et al.</i> , 1994; Lumlertdacha and Lovell, 1995	<ul style="list-style-type: none"> • Liver • Brain 	<ul style="list-style-type: none"> • Reduced weight gain • Reduced feed intake 	<ul style="list-style-type: none"> • Reduced white and red blood cell count as well as a lower hematocrit • Increased Sa:So ratio in kidney, serum, liver, muscle and brain • Reduced IgM during a <i>Edwardsiella ictaluri</i> challenge 	Yes
Rainbow trout (<i>Oncorhynchus mykiss</i>)	600 to 281,000	Meredith <i>et al.</i> , 1998; Riley <i>et al.</i> , 2001	n/a	n/a	<ul style="list-style-type: none"> • Increased Sa:So ratio in kidney, liver, and serum 	n/a
Nile tilapia (<i>Oreochromis niloticus</i>)	10,000 to 150,000	Tuan <i>et al.</i> , 2003; Claudino-Silva <i>et al.</i> , 2018	<ul style="list-style-type: none"> • Decrease of growth hormone receptor and insulin growth factor levels 	<ul style="list-style-type: none"> • Reduced weight gain • Increased feed conversion ratio 	<ul style="list-style-type: none"> • Reduced hematocrit • Increased Sa:So ratio 	Yes
Common Carp (<i>Cyprinus carpio</i>)	500 to 100,000	Pepeljnjak <i>et al.</i> , 2003; Petrinc <i>et al.</i> , 2004; Kovacic <i>et al.</i> , 2009	<ul style="list-style-type: none"> • Dilation of sinusoids • Alterations in liver, gall bladder, head kidney, kidney and brain 	<ul style="list-style-type: none"> • Reduced weight gain 	<ul style="list-style-type: none"> • Reduced mean erythrocyte volume • Increased red blood cell count and Aspartic aminotransferase levels 	No
African catfish (<i>Clarias gariepinus</i>)	2 to 82,770	Adeyemo <i>et al.</i> , 2016	n/a	<ul style="list-style-type: none"> • Reduced weight gain, final weight and specific growth rate • Increased feed conversion ratio 	<ul style="list-style-type: none"> • Reduced leucocyte count, hemoglobin and packed-cell volume • Increased erythrocyte count 	n/a
African catfish (<i>Clarias gariepinus</i>)	5,000 to 15,000	Gbore <i>et al.</i> , 2010	n/a	<ul style="list-style-type: none"> • Reduced final weight 	<ul style="list-style-type: none"> • Reduced hemoglobin count, total protein, mean erythrocyte volume and erythrocyte count • Increased leukocyte count, albumin: globulin ratio and glucose 	n/a
Shrimp studies						
Pacific white leg shrimp (<i>Litopenaeus vannamei</i>)	250 to 2,000	García-Morales <i>et al.</i> , 2015; Burgos-Hernandez <i>et al.</i> , 2005; Mexía-Salazar <i>et al.</i> , 2008	<ul style="list-style-type: none"> • Alteration of muscle myofibrillar structure • Increased protein degradation • Decrease percentage of residual activity of trypsin-like protease and collagen • Alteration of hepatopancreas structure 	<ul style="list-style-type: none"> • Reduced final weight 	<ul style="list-style-type: none"> • Reduced Pro-PO, phenoloxidase, superoxide anion and hematocyte count 	No

n/a – parameter not assessed



Mycofix[®]

Absolute protection

Powered by science to actively defend against multiple mycotoxins*

With 3 combined strategies



ADSORPTION



BIOTRANSFORMATION



BIOPROTECTION

*Authorized by EU Regulations No 1060/2013

mycofix.biomin.net

