# Mycotoxins in Asian aquafeeds

## Contamination in raw materials and finished aquafeeds in Asia from the Alltech Aqua Mycotoxin Survey

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The aquafeed industry is fast evolving with the development of advanced technologies, including feed processing technology, the application of novel ingredients and nutritional breakthroughs. There is a lot of interest in focusing on alternative ingredients to reduce the reliance on fishmeal and fish oil from capture fisheries in formulations to meet sustainability requirements.

The most common and economically viable option is to partially substitute marine ingredients in aquafeed, which nutritionists currently adopt, with ingredients derived from plants and their byproducts. However, the inclusion of plant-based ingredients in aquafeed presents some challenges to fish and shrimp with regards to digestibility, antinutritional factors, palatability, nutritional profiles including minerals and amino acids, and mycotoxin contamination. While information on mycotoxin contamination for grains and their byproducts is widely available, allowing feed producers to manage the mycotoxin risks in feed, information with regards to aquafeed samples is scarce.

In addition, challenges posed by mycotoxins in aquatic animals are less studied and often neglected. Similar to other vertebrates, fish are more sensitive to mycotoxins when multiple forms are present in the feed. A mycotoxin may be present at a 'safe' level in isolation, but when combined with another mycotoxin (also at a 'safe' level on its own), both can act synergistically to produce a more significant toxic effect in fish. Mycotoxins impair optimum animal performance by affecting intestinal, organ and immune systems, which, in turn, adversely impact business profitability. Although some stakeholders have begun to adopt best practices in testing mycotoxin levels in raw materials, many overlook the possible mycotoxin risks and their impacts on aquatic animal performances.

### Alltech Aqua Mycotoxin Survey 2021

To understand the landscape of the mycotoxin challenges in the Asia Pacific region, Alltech conducted a survey across the region to get an indication of the mycotoxin risks in aquaculture. During 2021, about 200 samples, including aquafeed and commonly used raw materials, were collected from six targeted countries (Bangladesh, India, China, Malaysia and Vietnam). The samples were analysed for multiple mycotoxins utilising Alltech 37+® mycotoxin analysis (an LC-MS-based system) or the Alltech RAPIREAD® system (ELISA-based system).

Six varieties of commonly used aquafeed raw materials (corn gluten meal (CGM), distiller's dried grains with solubles (DDGS), rice bran, soybean meal, wheat flour and canola meal) were analysed for six mycotoxins (aflatoxins, ochratoxin, deoxynivalenol, T-2/HT-2 toxins, fumonisins and zearalenone). These raw material samples were analysed for their mycotoxin levels and their risks in shrimp and marine and freshwater fish using RAPIREAD technology. The cumulative effect of multiple mycotoxins was expressed as a risk equivalent quantity (REQ) number. The REQ provides a single toxicity factor for the feedstuff based on the toxicity of each compound relative to aflatoxin B (the most toxic of the mycotoxins) and its concentration.

The overall REQ of all the raw materials combined was at high-risk levels across the species analysed, shrimp, and marine fish (seabass/seabream) and tilapia is shown in Table 1 and Figure 1. Note that deoxynivalenol (DON), T-2/HT-2 toxins and zearalenone were present at high-risk levels.

| Average concentration of mycotoxins above LOQ | | |
|---|---|---|
| Mycotoxins | Average (ppb) | Maximum (ppb) |
| Aflatoxins | 8 | 75 |
| Ochratoxin | 19 | 119 |
| Deoxynivalenol | 1804 | 4,400 |
| T2/HT2 toxins | <150 | 65 |
| Fumonisins | 899 | 6,149 |
| Zearalenone | 327 | 694 |
| Ergot toxins | not analysed | |

**Table 1.** Average concentration of mycotoxins in all raw materials. LOQ= Limits of quantification.

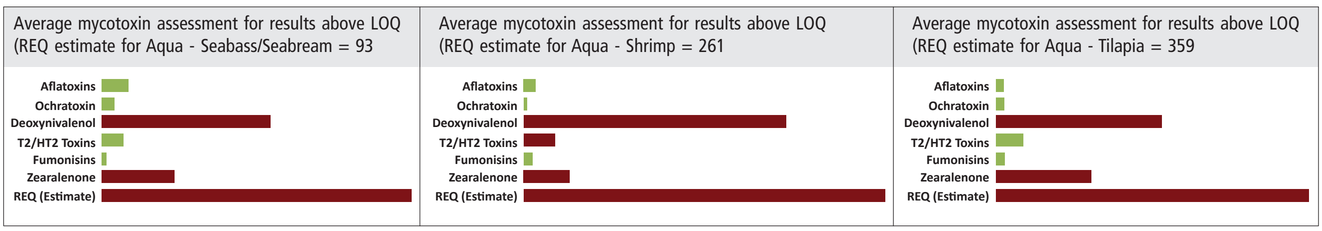



**Figure 1.** Average mycotoxin assessment and REQ estimates by species all the raw materials in marine shrimp, seabass and seabream and tilapia.

## *Plant meals (soybean meal/canola meal/rapeseed meal)*

Globally, plant proteins are typically lower in mycotoxin contamination due to their growing seasons when the mycotoxin exposure levels are lower. Although this is of less a concern, the increased inclusion of these ingredients will enhance the overall risk in aquafeed. Research shows that the synergistic effect of multiple mycotoxins can be particularly harmful.

The most prevalent form of mycotoxin is DON, with some emerging mycotoxins from *Fusarium*. In the present survey, it was observed that the plant meal samples (i.e., soybean meal, canola meal and rapeseed meal) were contaminated with significant amounts of DON and zearalenone (Table 2). Looking into the multi-mycotoxin risk, the samples contain the mycotoxin levels from low risk to high-risk in all the tested species. About 14.3% of plant meal samples pose a high risk to shrimp, and 8.6% of samples pose a high risk to freshwater and marine fish (Figure 2). Although the general inclusion rate of plant meals in aquafeeds is about 40–50%, the risk will be diluted from high level to moderate levels and it will still cause significant performance issues. So regular screening of plant meals for mycotoxins and the implementation of other mitigation plans are necessary to reduce the risk of toxicity in the first place.

| Average concentration of mycotoxins above LOQ | | |
|---|---|---|
| Mycotoxins | Average (ppb) | Maximum (ppb) |
| Aflatoxins | <3 | 5 |
| Ochratoxin | 33 | 119 |
| Deoxynivalenol | <300 | 1000 |
| T2/HT2 toxins | <50 | <LOQ |
| Fumonisins | <250 | 780 |
| Zearalenone | 144 | 276 |
| Ergot toxins | not analysed | |

**Table 2.** Average concentrations of mycotoxins in plant meals.

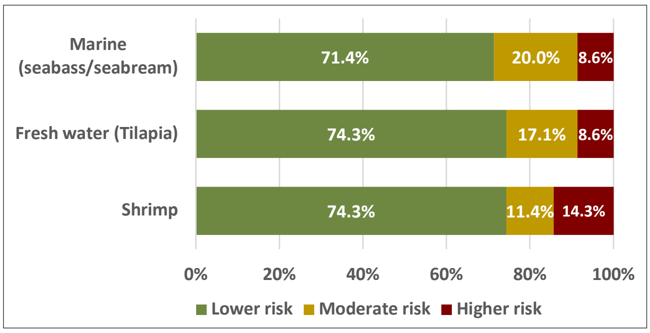



**Figure 2.** Percent of plant meal samples at lower, moderate or higher risk.

## *Bran and wheat flour*

Wheat flour and rice bran are often used in aquafeed as an energy source and gelatinising material to enhance pellet stability. RAPIREAD analysis of these samples revealed the presence of DON at significant risk levels and other mycotoxins at lower risk levels (Table 3). However, for the cumulative mycotoxin risk, REQ is lower to moderate for freshwater and marine fish, and 6.1% of samples are high-risk for shrimp (Figure 3).

| Average concentration of mycotoxins above LOQ | | |
|---|---|---|
| Mycotoxins | Average (ppb) | Maximum (ppb) |
| Aflatoxins | 6 | 9 |
| Ochratoxin | <2 | 6 |
| Deoxynivalenol | <300 | 410 |
| T2/HT2 toxins | <50 | <LOQ |
| Fumonisins | <250 | 988 |
| Zearalenone | <50 | 63 |
| Ergot toxins | not analysed | |

**Table 3.** Average concentrations of mycotoxins in bran and wheat flour. LOQ= Limits of quantification.

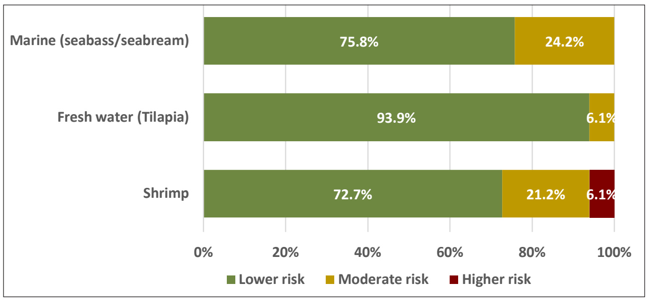



**Figure 3.** Percent of bran and wheat flour samples at lower, moderate or higher risk.

## *Corn by-products (DDGS/CGM)*

Globally, corn poses a significant mycotoxin risk. Most of the time, the greatest concern is aflatoxins, but *Fusariums* present an equal and even greater risk. The present survey revealed the presence of DON and zearalenone at high-risk levels (Table 4). The cumulative toxin effect was more alarming: about 88–94% of samples are at high REQ levels across the tested species (Figure 4). Although aquafeed millers seldom use complete grains in fish and shrimp feeds, corn by-products are often included in significant quantities. When we mill these different by-products in the manufacturing process, we decrease mass, but the mycotoxins are concentrated in a lower mass, causing risks to the animals.

| Average concentration of mycotoxins above LOQ | | |
|---|---|---|
| Mycotoxins | Average (ppb) | Maximum (ppb) |
| Aflatoxins | 14 | 75 |
| Ochratoxin | 5 | 8 |
| Deoxynivalenol | 2,403 | 4,400 |
| T2/HT2 toxins | <50 | 65 |
| Fumonisins | 1,347 | 6,149 |
| Zearalenone | 485 | 694 |
| Ergot toxins | not analysed | |

**Table 4.** Average concentrations of mycotoxins in corn

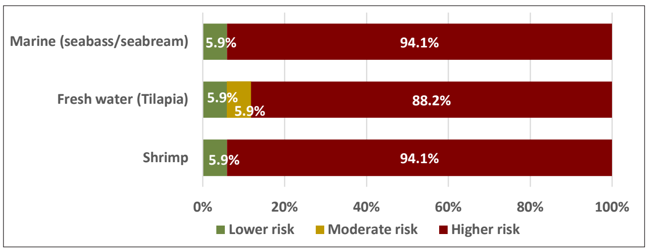
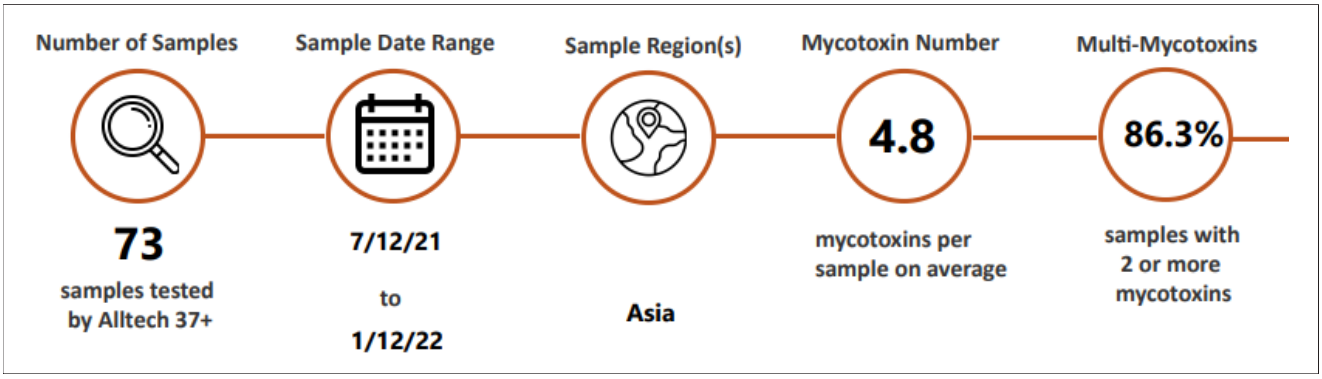



**Figure 4.** Percent corn gluten meal/DDGS samples at lower, moderate or higher risk.




## *Finished feed*

About 73 aquafeed samples were analysed for 54 mycotoxins using Alltech 37+ technology. On average, each sample contained 4.8 mycotoxins, and about 86.3% of feed samples contained more than two mycotoxins (Figure 5).

In shrimp feeds, the risk was high due to a broad range of toxins, but type B trichothecenes stood out. Marine fish feeds have a lower overall risk, which was primarily from type B trichothecenes, zearalenone and fusaric acid. The lower risk was probably due to the lesser use of plant materials in marine fish feeds. Freshwater fish feed samples were recorded with high REQ from aflatoxins, type B trichothecenes and ergot toxins (Table 5). Little is known about the impact of ergot toxins in aquaculture, while in other species, ergot impacts distal blood flows. About 96% of shrimp feed samples contained at least one mycotoxin. Overall, type B trichothecenes in finished feed (Figure 5) and DON (a type B trichothecene) in the raw materials were of concern across the species tested (Figure 1). This clearly indicates the mycotoxin threat was from the raw materials.

**Average Assessment for Performance Impairment for Aqua – Shrimp (100% Inclusion of Finished Feeds)**

| Mycotoxin Group | Average Based on Inclusion, ppb | Lower, ppb | Moderate, ppb | Higher, ppb |
|---|---|---|---|---|
| Aflatoxin B1 | 1.2 | 5 | 10 | 20 |
| Aflatoxins, Total | 1.8 | 5 | 10 | 20 |
| Ochratoxins/Citrinin | 2.3 | 50 | 100 | 200 |
| Type B Trichothecenes | 534.4 | 50 | 100 | 200 |
| Type A Trichothecenes | 0.0 | 15 | 30 | 60 |
| Fumonisins | 209.5 | 750 | 1500 | 3000 |
| Zearalenones | 54.4 | 50 | 100 | 200 |
| Fusaric Acid | 153.4 | 500 | 1000 | 2000 |
| Emerging Mycotoxins | 5.5 | 500 | 1000 | 2000 |
| Other Penicillium Mycotoxins | 0.0 | 25 | 50 | 100 |
| Other Aspergillus Mycotoxins | 0.6 | 20 | 40 | 60 |
| Ergot Toxins | 0.0 | 50 | 100 | 200 |
| REQ | 63.3 | 5 | 10 | 20 |

**Average Assessment for Performance Impairment for Aqua – Seabass/Seabream (100% Inclusion of Finished Feeds)**

| Mycotoxin Group | Average Based on Inclusion, ppb | Lower, ppb | Moderate, ppb | Higher, ppb |
|---|---|---|---|---|
| Aflatoxin B1 | 0.0 | 5 | 10 | 20 |
| Aflatoxins, Total | 0.0 | 5 | 10 | 20 |
| Ochratoxins/Citrinin | 0.0 | 20 | 50 | 100 |
| Type B Trichothecenes | 61.5 | 250 | 500 | 750 |
| Type A Trichothecenes | 0.0 | 50 | 100 | 200 |
| Fumonisins | 19.3 | 2500 | 5000 | 10000 |
| Zearalenones | 9.4 | 75 | 150 | 300 |
| Fusaric Acid | 43.0 | 500 | 1000 | 2000 |
| Emerging Mycotoxins | 0.0 | 500 | 1000 | 2000 |
| Other Penicillium Mycotoxins | 0.0 | 40 | 70 | 100 |
| Other Aspergillus Mycotoxins | 0.0 | 20 | 40 | 60 |
| Ergot Toxins | 0.0 | 50 | 100 | 200 |
| REQ | 2.5 | 5 | 10 | 20 |

**Average Assessment for Performance Impairment for Aqua – Carp (100% Inclusion of Finished Feeds)**

| Mycotoxin Group | Average Based on Inclusion, ppb | Lower, ppb | Moderate, ppb | Higher, ppb |
|---|---|---|---|---|
| Aflatoxin B1 | 8.5 | 25 | 50 | 100 |
| Aflatoxins, Total | 8.5 | 25 | 50 | 100 |
| Ochratoxins/Citrinin | 0.4 | 50 | 100 | 200 |
| Type B Trichothecenes | 666.2 | 250 | 500 | 1000 |
| Type A Trichothecenes | 0.0 | 50 | 100 | 200 |
| Fumonisins | 303.8 | 2500 | 5000 | 10000 |
| Zearalenones | 59.8 | 75 | 150 | 300 |
| Fusaric Acid | 109.0 | 500 | 1000 | 2000 |
| Emerging Mycotoxins | 6.3 | 500 | 1000 | 2000 |
| Other Penicillium Mycotoxins | 2.4 | 40 | 70 | 100 |
| Other Aspergillus Mycotoxins | 0.7 | 40 | 60 | 80 |
| Ergot Toxins | 51.2 | 50 | 100 | 200 |
| REQ | 130.8 | 25 | 50 | 100 |

**Table 5.** Mycotoxin contamination in shrimp feed, marine fish feed -seabass/seabream and freshwater fish feed -carp.

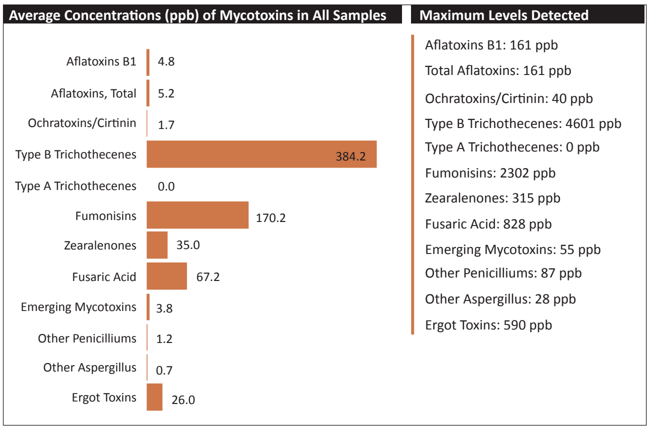



**Figure 5.** Mycotoxin contamination in all finished feed samples.

Farmers in Asia are facing serious production challenges, including those from masked mycotoxins in feeds. Many experimental studies revealed the adverse effects of mycotoxins on shrimp and fish health. In a laboratory experiment, when juvenile whiteleg shrimp (*Litopenaeus vannamei*) were fed with diets containing 250–1,000ppb of DON, weight gain decreased with the increasing dietary DON levels (Xie *et al*., 2018). Survival was lower in shrimp fed with high levels of DON-contaminated wheat flour. Intestinal mucosae and the microstructure of intestinal epithelial cells were harmed by dietary DON (Xie et al., 2018). Also, exposure of whiteleg shrimp to T-2 toxin significantly decreased specific growth rate and weight gain. T-2 toxin damaged the hepatopancreas in a dose-responsive manner (Qiu et al., 2016). Exposure of tilapia (*Oreochromis niloticus*) to increasing T-2 toxin doses significantly decreased the survival rate, weight gain and hepatosomatic index, and induced liver cell and myofibre damage (Deng et al., 2019).

Based on a review article from Koletsi et al. (2021) on mycotoxins in European aquafeed stuff, 80% of the wheat samples were contaminated with mycotoxins. The same was found in 95% of corn and 87% of soybean meal samples. *Fusarium sp.* mycotoxins are the most common and dominant among the raw materials. There was also a high level of mycotoxin co-occurrence – from 63–88% of samples. In fish feeds, the frequency of mycotoxin co-occurrence was from 36–55% for fusaric acid, DON and fumonisin $B_1$. DON is the most important mycotoxin in European aquaculture. In a meta-analysis on the effects of DON on fish species, DON reduces feed intake exponentially by 13.2% and growth by 16.5% (Koletsi *et al*., 2021). In Koletsi's wet laboratory experiments with rainbow trout, they found that the responses were more severe; feed intake dropped by 18.8% and growth by 20%. A histopathological study revealed clear liver necrosis and haemorrhage upon feeding with a DON-contaminated diet for one week.

Exposure of juvenile turbot (*Scophthalmus maximus*) to DON at 3ppm significantly decreased weight gain, specific growth rate and feed efficiency ratio and reduced levels of IgM and complement four concentrations in serum. DON also decreased the abundance of potential helpful bacteria and increased the abundance of potential pathogens in the intestine (Wang et al., 2021). The addition of Mycosorb® (yeast cell wall extract) at 0.2% and 0.4% to the DON-contaminated diets significantly improved growth performance and immune response and enhanced the intestinal health of turbot (Wang *et al*., 2021). In another experiment by Yang et al. (2020), turbot juvenile exposure to aflatoxin B1 at 500ppb significantly hampered the immune system and health. However, supplementation with Mycosorb at 0.2% and 0.4% levels restored the immune response, relieved adverse effects in the liver, lowered the AfB1 residues in turbot tissues, promoted intestinal microbiota diversity and lowered the abundance of potential pathogens.

## Conclusion

The present survey reinforces the risk of multiple mycotoxins from plant raw materials in shrimp, marine and freshwater fish. Mycotoxins in plant material typically accumulate during crop harvest due to adverse weather conditions, such as unseasonal rains, moisture, heat and drought. Effective mycotoxin management is about seeing the whole challenge – from the farm to the feed mill – and from risk assessment to feed management.

The Alltech® Mycotoxin Management program provides several solutions to mitigate the threat. The Alltech 37+ analytical laboratory conducts an ISO-accredited procedure that analyses 54 mycotoxins in a sample using UPLC-MS/MS to accurately determine mycotoxins with the highest level of sensitivity.

Guideline limits for mycotoxins in aquatic species to reduce negative effects on health and performance was published in Aqua Culture Asia Pacific November/December 2020, pages 54-58.

**References**

Koletsi P, Schrama J.W, Graat E.A.M, Wiegertjes G.F, Lyons P, Pietsch C. (2021). The occurrence of mycotoxins in raw materials and fish feeds in Europe and the potential effects of deoxynivalenol (DON) on the health and growth of farmed fish species—a review. *Toxins*. 13(6):403.

Xie, S, Lu Zheng, Migen Wan, Jin Niu, Yongjian Liu, Lixia, Tian. (2018). Effect of deoxynivalenol on growth performance, histological morphology, anti-oxidative ability and immune response of juvenile Pacific white shrimp, *Litopenaeus vannamei*. Fish & Shellfish Immunology, 82, 442-452.

Deng, Y., Mei Qiu, Yaling Wang, Rundong Wang, Pengli Lu, Lijun Sun, Xuepeng Li, Ravi Gooneratne. Protective effect of antioxidant-enriched diets on T-2-toxin-induced damage in tilapia (*Oreochromis niloticus*). Aquaculture, 506, 341-349.

Qiu, M., Yaling Wang, Xiaobo Wang, Lijun Sun, Riying Ye, Defeng Xu, Zhe Dai, Ying Liu, Siyuan Bi, Yanpo Yao, Ravi Gooneratne. 2016. Effects of T-2 toxin on growth, immune function, and hepatopancreas microstructure of shrimp (*Litopenaeus vannamei*). Aquaculture, 462, 35-39

Jinzhu Yang, Tiantian Wang, Gang Lin, Mingzhu Li, Ronghua Zhu, Alexandros Yiannikouris, Yanjiao Zhang, and Kangsen Mai. 2020. The assessment of diet contaminated with aflatoxin $B_1$ in juvenile turbot (*Scophthalmus maximus*) and the evaluation of the efficacy of mitigation of a yeast cell wall extract. Toxins 2020, 12(9), 597.

Tiantian Wang, Jinzhu Yang, Gang Lin, Mingzhu Li, Ronghua Zhu, Alexandros Yiannikouris, Ruiguo Wang, Yanjiao Zhang, Kangsen Mai. 2021. Evaluation of the mitigation efficacy of a yeast cell wall extract toward deoxynivalenol contaminated diet fed to turbot (*Scophthalmus maximus*). Ecotoxicology and Environmental Safety, 216, 112221.

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