

The Significance of Feed-Borne Mycotoxins in Aquaculture

Trevor K. Smith

Department of Animal and Poultry Science,

University of Guelph,

Guelph, Ontario, Canada, N1G 2W1 E-mail: tsmith@uoguelph.ca

Abstract

Mycotoxins are fungal metabolites which can reduce growth and adversely alter metabolism of aquaculture species. The pathological states arising from the consumption of feeds contaminated with mycotoxins are mycotoxicoses. Mycotoxins can be formed in the field preharvest and may continue to be formed under suboptimal storage conditions postharvest. Some fungi, such as *Aspergillus flavus*, are usually found in areas with tropical and semi-tropical climates. This mold produces the carcinogenic hepatotoxin, aflatoxin. Numerous studies have shown aquaculture species to be particularly susceptible to aflatoxicosis. *Fusarium* fungi, however, are more common in temperate climates and *Fusarium* mycotoxins are likely the most common mycotoxins on a global basis. The *Fusarium* mycotoxins are not typically carcinogenic but aquaculture species have been shown to be sensitive to the presence of feed-borne *Fusarium* mycotoxins.

There is increasing interest in mycotoxin analysis of feeds and it has recently been reported that many mycotoxins can exist in different chemical forms which are toxic but are not detected by conventional analytical techniques. The threat to aquaculture posed by the feeding of contaminated diets, therefore, is likely underestimated. The most effective tool currently available to minimize the effects of feed-borne mycotoxins is the use of an appropriate mycotoxin adsorbent.

Introduction

Mycotoxins are fungal metabolites that can reduce growth and adversely alter metabolism of aquaculture species (Spring & Burel, 2008). The pathological states arising from the consumption of feeds contaminated with mycotoxins are mycotoxicoses. Mycotoxins are found in feedstuffs of plant origin and are synthesized by fungi originating in soil. Mycotoxins can be formed in the field preharvest and may continue to be formed under suboptimal storage conditions postharvest. High moisture content often predisposes feedstuffs to fungal growth and mycotoxin formation. Another key factor influencing the degree of contamination is temperature. Some fungi, such as *Aspergillus flavus*, are typically found in regions with tropical or semi-tropical climates. This mold produces the carcinogenic hepatotoxin aflatoxin. Another carcinogenic mycotoxin is ochratoxin A, which is produced by both *Aspergillus* and *Penicillium* species, and is often considered to be a mycotoxin most often formed during feed storage. *Fusarium* fungi are common in soils of areas with temperate climates and *Fusarium* mycotoxins are likely the most common mycotoxins on a global basis (Wood, 1992).

Aflatoxin

Early studies with rainbow trout, *Oncorhynchus mykiss* (Walbaum), indicated that this species was particularly sensitive to feed-borne aflatoxin found in contaminated cottonseed meal (Halver 1969). The symptoms included a high incidence of liver tumors. Liver failure is characteristic of acute aflatoxicosis. A characteristic of chronic exposure to aflatoxin is immunosuppression. Loss of immunity and the resulting increased incidence of infectious diseases is likely the most significant economic consequence of feed borne mycotoxins. Long-term immune dysfunction has been reported in rainbow trout exposed as embryos to aflatoxin B₁ (Ottinger & Kaattari, 2000). The result was long-term alterations in mitogenesis and immunoglobulin production characterized by differential leucocyte sensitivity. Black tiger shrimp (*Penaeus monodon* Fabricius) have also been shown to be sensitive to feed-borne aflatoxin (Boonyaratpalin, Supamattaya, Verakunpiriya & Suprasert 2001). Aflatoxin B₁ concentrations in the diet (up to 2.5 mg/kg) were highly negatively correlated with average weight, weight gain and survival. There were marked histological changes in the hepatopancreas of shrimp fed 0.1 – 2.5 mg

aflatoxin B₁/kg including atrophy followed by necrosis of the tubular epithelial cells and severe degeneration of hepatopancreatic tubules.

Deoxynivalenol

Deoxynivalenol (DON, vomitoxin) is the most common of the trichothecene mycotoxins, a large family of structurally related *Fusarium* mycotoxins including T-2 toxin, HT-2 toxin, DAS, nivalenol and about 180 others (Riley & Pestka 2005). The trichothecenes are pharmacologically active and can alter behavior including reduced feed intake. These compounds also inhibit tissue protein synthesis which can result in lesions of the intestinal tract. Trichothecenes have also been shown to be immunosuppressive. Early studies of the feeding of deoxynivalenol-contaminated corn to rainbow trout indicated that juvenile trout were very sensitive to deoxynivalenol as indicated by reduced feed intake and growth rates (Woodward, Young & Lun 1983). The sensitivity was compared to that of piglets, which exhibit severe feed refusal when offered deoxynivalenol-contaminated feeds. Pacific white shrimp (*Litopenaeus vannamei*) have also been shown to be sensitive to feed-borne deoxynivalenol (Trigo-Stockli, Obaldo, Dominy & Behnke 2000). The feeding of up to 1.0 mg DON/kg feed in the form of contaminated hard red winter wheat resulted in reduced body weights and growth rates. Feed conversion ratios and survival was not affected. No changes in organ histology were seen and no deoxynivalenol residues were seen in shrimp tissues. It was concluded that feed ingredients should be monitored for deoxynivalenol contamination. This is in contrast to a more recent study in which purified deoxynivalenol and ochratoxin A were separately fed to black tiger shrimp (Supamattaya, Sukrakanchana, Boonyaratpalin, Schatzmayr & Chittiwan 2005). In this study it was concluded that shrimp feeds occasionally contaminated with deoxynivalenol or ochratoxin A have no negative impact on the shrimp culture industry. It has been shown in many other species that the feeding of materials naturally contaminated with mycotoxins are more toxic than the feeding of equivalent amounts of purified mycotoxin. This may be due to the presence of unknown mycotoxins, the presence of mycotoxins not analyzed for or due to toxicological synergy that may exist when multiple mycotoxins are present.

Fumonisin

Another family of *Fusarium* mycotoxins thought to have implications in aquaculture feeds is the fumonisins. These compounds have been shown to inhibit the synthesis of sphingolipids which can result in a variety of lesions in mammals. Channel catfish (*Ictalurus punctatus*) were fed corn-based *Fusarium* culture material containing up to 720 mg fumonisin B₁ / kg diet for up to 14 weeks. The feeding of 80 mg fumonisin B₁ / kg diet had no effect on mortality but fish fed 320 mg fumonisin B₁ / kg feed suffered 70% mortality. Histological changes in liver were seen at lower mycotoxin doses. It was concluded that diets containing 20 mg fumonisin B₁ / kg feed or more are toxic to this species. Similar conclusions could be drawn from the work of Yildirim, Manning, Lovell, Grizzle & Rottinghaus (2000).

Challenges Relating To Mycotoxin Analysis In Feeds

There are many challenges in accurately determining the mycotoxin content of feeds. Inadequate sampling of feeds is likely the greatest cause of error in mycotoxin analysis (Whitaker, Slate & Johansson 2005). Increased attention is now also being focused on the significance of conjugated mycotoxins. These are mycotoxins that are synthesized by fungi but have been subsequently modified by infested plants. An example is a glucose conjugate of deoxynivalenol that was found in samples of corn and wheat originating in Slovakia (Berthiller, Dall'Asta, Schuhmacher, Lemmens, Adam & Krska 2005). Similar glucose conjugates have previously been reported for the *Fusarium* mycotoxin zearalenone (Schnewwis, Meyer, Engelhardt & Bauer 2002). When samples of barley collected in North Dakota, U.S.A. underwent hydrolysis with trifluoroacetic acid prior to analysis for deoxynivalenol, the quantity of deoxynivalenol detected increased by up to 88% (Zhou, Li, Gillespie, He, Horsley & Schwarz 2007). Such a procedure would liberate all conjugates bound to deoxynivalenol. It is likely that such bound deoxynivalenol would revert to free form under the action of digestive enzymes. It is also likely that the conjugated forms of deoxynivalenol would not be detected by conventional analytical techniques. This means that the conjugated mycotoxins are likely toxic, but non-detectable. It is probable that current analytical techniques seriously underestimate that toxicity of contaminated feedstuffs and this greatly complicates quality control measures designed to identify potentially harmful raw materials.

New extraction protocols need to be developed to permit more accurate mycotoxin analysis of aquaculture feeds.

Towards A Solution To Mycotoxin Problems In Aquaculture

If the feeding of mycotoxin-contaminated materials cannot be avoided or goes undetected, a sometime useful strategy is the feeding of a suitable mycotoxin adsorbent. The adsorbents are high molecular weight, highly branched polymers that are non-nutritive and non-digestible. Such materials can pass down the digestive tract and adsorb small molecules such as, but not exclusively, mycotoxins to prevent them from entering the blood stream and being transported to target tissues (Ramos, Fink-Gremmels & Hernandez 1996). The categories of adsorbents include inorganic silicon-based polymers such as bentonite, zeolites and hydrated calcium aluminosilicates. The organic adsorbents are carbon-based polymers and include activated charcoal and glucomannan polymers extracted from the cell wall of yeast. In order to be effective, the level of dietary inclusion of the adsorbent must be appropriate for the level of mycotoxin challenge. Often the level of mycotoxin challenge is not well known because of incomplete mycotoxin analysis and the presence of masked mycotoxins which leads to an underestimate of mycotoxin contamination.

Conclusions

The long term objective of the feed industry will be to eliminate the feeding of mycotoxin-contaminated feedstuffs. This may eventually be obtained with improved plant genetics to improve resistance to fungal infestation coupled with improved analytical methodologies for detection of mycotoxins in feedstuffs. In the short term, however, the most practical approach for reducing the effects of feed-borne mycotoxins is the use of a suitable mycotoxin adsorbent.

Literature Cited

- Berthiller F., Call'Asta C., Schuhmacher R., Lemmens M., Adam G. & Krska R. (2005) Masked mycotoxins: Determination of a deoxynivalenol glucoside in artificially and naturally contaminated wheat by liquid chromatography – tandem mass spectrometry. *Journal of Agricultural and Food Chemistry* **53**, 3421-3425.
- Boonyaratpalin, M., Supamattaya, K., Verakunpiriya, V. & Supraser, D. (2001) Effects of aflatoxin B₁ on growth performance, blood components, immune function and histopathological changes in black tiger shrimp (*Penaeus monodon* Fabricius). *Aquaculture Research* **32** (supplement 1), 388-398.
- Halver J.E. (1969) Aftatoxicosis and trout hepatoma. In: *Aflatoxin: Scientific Background, Control, and Implications* (ed. by L.A. Goldblatt), pp. 265-306. Academic Press, New York.
- Ottinger C.A. & Kaattari, S.L. (2000) Long-term immune dysfunction in rainbow trout (*Oncorhynchus mykiss*) exposed as embryos to aflatoxin B₁. *Fish and Shellfish Immunology* **10**, 101-106.
- Ramos A.J., Fink-Gremmels J. & Hernandez E. (1996) Prevention of toxic effects of mycotoxins by means of non-nutritive adsorbent compounds. *Journal of Food Protection* **59**: 631-641.
- Riley R.T. & Pestka, J. (2005) Mycotoxins: metabolism, mechanisms and biochemical markers. In: *The Mycotoxin Blue Book* (ed. by D. Diaz), pp. 279-294. Nottingham University Press, Nottingham.
- Schneweis I., Meyer K., Engelhardt G. & Bauer J. (2002) Occurrence of zearalenone-4-beta-D-glucopyranoside in wheat. *Journal of Agricultural and Food Chemistry* **50**: 1736-1738.
- Spring P. & Burel C. (2008) Effect of mycotoxins in aquaculture. In: *Mycotoxins in Farm Animals* (ed. by I.P. Oswald and I. Taranu), pp. 71-90. Transworld Research Network, Kerala.
- Supamattaya K., Sukrakanchana N., Boonyaratpalin M., Schatzmayr D. & Chittiwat V. (2005) Effects of ochratoxin A and deoxynivalenol on growth performance and immuno-physiological parameters in black tiger shrimp (*Penaeus monodon*). *Songklanakarin Journal of Science and Technology* **27**, 91-99.
- Trigo-Stockli D.M., Obaldo L.G., Dominy W.G. & Behnke, K.C. (2000) Utilization of deoxynivalenol-contaminated hard red winter wheat for shrimp feeds. *Journal of the World Aquaculture Society* **31**, 247-254.
- Whitaker T.B., Slate A.B. & Johansson A.S. (2005) Sampling feeds for mycotoxin analysis. In: *The Mycotoxin Blue Book* (ed. by D. Diaz), pp. 1-24. Nottingham University Press, Nottingham.
- Wood G.E. (1992) Mycotoxins in foods and feeds in the United States. *Journal of Animal Science* **70**, 3941-3949.
- Woodward B., Young L.G. & Lun A.K. (1983) Vomitoxin in diets for rainbow trout (*Salmo gairdneri*). *Aquaculture* **35**, 93-101.
- Yildirim M., Manning B.B., Lovell R.T., Grizzle J.M. & Rottinghaus, G.E. (2000) Toxicity of moniliformin and fumonisin B₁ fed singly and in combination in diets for young channel catfish *Ictalurus punctatus*. *Journal of the World Aquaculture Society* **31**, 599-608.

Zhou B., Li Y., Gillespie J., He G.-Q., Horsley R & Schwarz P (2007) Doehlert matrix design for optimization of the determination of bound deoxynivalenol in barley grain with trifluoroacetic acid (TFA). *Journal of Agricultural and Food Chemistry* **55**, 10141-10149.