

Mycotoxins in aquaculture: Occurrence in feeds components and impact on animal performance

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Abstract

In the last years there is a trend to replace fish meal as a source of protein by less expensive sources of protein from plant origin. As a result of this trend, aquaculture feeds have a higher risk of being contaminated with one or more types of mycotoxins. Mycotoxins are secondary metabolites produced by fungi and highly toxic to animals. Despite the limited number of studies on the effect of mycotoxins on aquaculture species, there is increasing evidence that mycotoxins can cause several pathologies and growth problems in different fish and shrimp species.

A recent survey on the occurrence of mycotoxins in feed components showed that analyzed samples from different regions were contaminated with one (74%) or more (40%) mycotoxins. Despite good screening programs, selection of high quality raw materials and feed ingredients and good storage conditions it is very difficult to guarantee the absence of mycotoxins in aquaculture feeds. Therefore it is urgent to find suitable ways to face the problem through an effective management of the risks posed by mycotoxins contaminations. The current paper presents an overview of the effects of mycotoxins on fish and shrimp performance and the occurrence of these mycotoxins in feed components.

Keywords: mycotoxins, aflatoxins, ochratoxins, fumonisins, trichothecenes, fish and shrimp

Introduction

Mycotoxins are secondary metabolites produced by fungi and highly toxic to animals. The effects of mycotoxins are in general associated with reduced growth and health status of fish and other farmed animals. The most prevalent fungi responsible for the occurrence of mycotoxins are *Aspergillus*, *Penicillium* and *Fusarium* sp. At the moment more than 400 different mycotoxins have been reported that can be clustered into five major classes: aflatoxins, ochratoxins, fumonisins, zearalenone and trichothecenes (CAST 2003).

In terrestrial animals the toxic effects of mycotoxins is well known and can be of different nature such as carcinogenic (e.g. aflatoxin B1, ochratoxin A, fumonisin B1), estrogenic (zearalenone), neurotoxic (fumonisin B1), nephrotoxic (ochratoxin), dermatotoxic (trichothecenes) or immunosuppressive (aflatoxin B1, ochratoxin A and T-2 toxin).

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Aflatoxins

Aflatoxins are produced by *Aspergillus* fungi, which can infect many potential feedstuffs as corn, peanuts, rice, fish meal, shrimp and meat meals (Ellis, Clements, Tibbetts & Winfree, 2000). The number of studies addressing the effects of aflatoxins in aquatic species is very limited.

The difficulty in accurately diagnosing aflatoxicosis in fish may in part explain the lack of information regarding the incidence of aflatoxicosis in farmed aquatic species. Initial findings associated with aflatoxicosis in fish include pale gills, liver damage, poor growth rates and immune-suppression.

The susceptibility of fish towards aflatoxins seems to be age and species dependant, as fish fry are more susceptible than adults and species like Rainbow trout, *Onchorhynchus mykiss* (Walbaum) and European seabass, *Dicentrarchus labrax* (L.) seem to be more susceptible than channel catfish, *Ictalurus punctatus* (Rafinesque) (Manning, 2001, Tuan, Grizzle, Lovell, Manning & Rottinghaus 2002, El-Sayed and Khalil 2009). These species specific differences can be attributed to differences in the metabolism of Aflatoxin B1 (AFB1) in the liver and affinity of AFB1-derived metabolites to hepatic macromolecules (Ngethe, Horsberg, Mitema, & Ingebrigtsen 1993).

A reduction in growth is one of the major negative effects reported due to aflatoxin B1 contamination. Several studies report reduced growth rates in channel catfish (10 ppm AFB1/kg; Jantrarotai & Lovell, 1990) and Nile Tilapia, *Oreochromis niloticus* (L.), (100 ppb AFB1 - Encarnacao Srikhum, Rodrigues & Hofstetter 2009; 1880 ppb AFB1 - Chavez-Sanches, Martinez & Moreno, 1994; 0.1 ppm AFB1 - El-Banna, Teb & Fakhry 1992). Additional effects of elevated aflatoxin levels include lower haematocrit count at levels higher than 0.25 ppm, severe hepatic necrosis in Nile tilapia with levels of 100 ppm AFB1 (Tuan et al. 2002) and immunosuppression in common carp, *Cyprinus carpio* (L.) (Sahoo and Mukherjee 2001). In addition, mortality rates of 17% were reported in Nile tilapia fed diets with 0.2 ppm AFB1 (El-Banna et al., 1992). El-Sayed and Khalil (2009) described also that a prolonged feeding of European seabass with low levels of AFB1 (0.0018 mg/Kg body weight) causes not only serious health problems in exposed-fish, but also represents a high risk to consumers through AFB1 residues in fish musculature. In line with these results Han, Xie, Zhu, Yang & Guo (2009) described that gibel carp, *Carassius auratus gibelio* (L.) fed with more than 10 µg AFB1 kg⁻¹diet showed accumulation of AFB1 residues in muscles and ovaries above the safety limitation of European Union (2 ppb).

In marine shrimp, several studies showed that AFB1 can cause poor growth, low apparent digestibility, physiological disorders and histological changes, mainly in the hepatopancreatic tissue (Wiseman, Price, Lightner & Williams 1982; Bintvihok, Ponpornpisit, Tangtrongpiros, Panichkriangkrai, Rattanapanee, Doi & Kumagai, 2003;

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Boonyaratpalin, Supamattaya, Verakunpiriya, Suprasert 2001; Burgos-Hernandez, Farias, Torres-Arreola & Ezquerra-Brauer, 2005). According to Burgos-Hernandez et al. (2005), the effect of AFB1 toxicity to white shrimp, *Litopenaeus vannamei* (Boone) results in the modification of digestive processes and abnormal development of the hepatopancreas, which can be related to alterations of trypsin and collagenase activities, and also negative effects of mycotoxins on other digestive enzymes - e.g. lipases and amylases (Burgos-Hernandez et al., 2005).

Despite one study suggesting that levels as low as 20 ppb (for 7 – 10 days consumption period) induced higher mortality rates than the control in shrimps (Bintvihok et al. 2003), other studies suggest that levels of contamination up to 100 ppb showed no effect on performance and only at levels of 2500 ppb mortality was increased (Boonyaratpalin et al., 2001).

Ochratoxins

Another important group of mycotoxins are the ochratoxins, which are a group of secondary metabolites produced by fungi belonging to *Aspergillus* and *Penicillium* genera. The most toxic and abundant mycotoxins of these groups is ochratoxin A (OA). Ochratoxin A is generally associated with contamination of corn, cereal grains and oilseeds and can affect animal performance through damage to kidney function (CAST, 2003).

Manning, Ulloa, Li, Robinson & Rottinghaus (2003a) reported a reduction in body weight gain of channel catfish fed diets with 2 ppm of OA for two weeks and 1 ppm for 8 weeks (Manning et al., 2003a). Reduced FCR was also observed in the same species with contamination levels of 4 and 8 ppm (Manning et al., 2003a). In rainbow trout pathological signs of ochratoxicosis included liver necrosis, pale, swollen kidneys and high mortality (Hendricks, 1994).

Fumonisin

The fumonisins represent a group of mycotoxins produced predominantly by *Fusarium moniliforme* species. Fumonisin B1 is considered the major toxic component both in corn culture and in naturally contaminated corn. Several animal diseases have been linked with this toxin, particularly those in which a disruption of the sphingolipid metabolism takes place (Wang, Ross, Wilson, Riley & Merrill, 1992). In fish, the role of fumonisins as toxic agents remains unclear. On one hand, minimal adverse effects have been reported in channel catfish fed *F. moniliforme* culture material containing 313 ppm of fumonisin B1 (FB1) for 5 weeks (Brown, McCoy & Rottinghaus 1994). On the other hand, for the same fish species, dietary levels of FB1 of 20 ppm or above have been shown to result in lower weight gain and significant decrease in hematocrit and red and white blood cells than those fed lower doses (Lumlertdacha, Lovell, Shelby, Lenz & Kemppainen 1995). Likewise, Yildirim, Manning, Lovell, Grizzle & Rottinghaus (2000) found that in channel catfish, diets containing 20 ppm of moniliformin (MON) or FB1 significantly reduced body weight gain after 2 weeks. According to the same authors, FB1 is more toxic than MON to channel catfish. Another study by Tuan, Manning, Lovell & Rottinghaus (2003) with Nile tilapia also suggests a higher toxicity of FB1 than MON as toxic symptoms appeared earlier in fingerlings exposed to FB1. However, it should be noted that feeding both MON and FB1 at 70 and 40 ppm, respectively, affected negatively growth performance of Nile tilapia fingerlings despite no effect on mortality or histopathological lesions. Compared to channel catfish, Nile tilapia appears to be more resistant to these two mycotoxins in the diet (Tuan et al., 2003).

Increased mortality was observed in channel catfish when diets contained 240 ppm FB1 (Li, Raverty & Robinson 1994).

Long-term exposure effects of FB1 were reported in carp by Pepeljnjak, Petrinec, Kovacic and Segvic (2002). These authors showed that exposure to 0.5 and 5.0 mg per kg body weight is not lethal to young carp, but can produce adverse physiological effects with

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kidney and liver being the key target organs for the FB1 action. Other changes subsequent to fumonisin exposure that have been reported for carp include scattered lesions in the exocrine and endocrine pancreas, and inter-renal tissue, probably due to ischemia and/or increased endothelial permeability (Petrinec, Pepeljnjak, Kovacic & Krznaric, 2004).

Trichothecenes

Another important group of mycotoxins are the trichothecenes, which are produced by fungi of the genus *Fusarium*. These fungi infect grains, wheat by-products and oilseed meals used in the production of animal feeds.

Trichothecenes can be of the type A (e.g. T2 toxin) and type B (e.g. deoxynivalenol, DON). T-2 toxin produced by *Fusarium tricinctum* reduced feed consumption, growth, lowered hematocrit, and lowered blood haemoglobin in rainbow trout at levels higher than 2.5 ppm (Poston, 1983). Also channel catfish fed diets with levels of T2-toxin ranging from 0.625-5.0 ppm had the growth rate significantly reduced and mortality increased above 2.5 ppm (Manning, Li, Robinson, Gaunt, Camus & Rottinghaus 2003b). Additionally disease resistance and survival of channel catfish challenged with *Edwardsiella ictaluri* was reduced when fish were fed with contaminated feed (Manning, Li, Robinson, Wise & Rottinghaus, 2005).

Deoxynivalenol (DON) is an important contaminant of wheat and corn. Deoxynivalenol levels of 0.5 and 1.0 ppm in the diet significantly reduced body weight and growth rate in white shrimp, while FCR and survival were not affected (Trigo-Stockli, Obaldo, Gominy & Behnke, 2000). In fish, Woodward, Young, and Lun (1983) showed that rainbow trout had sensitive taste acuity for DON and reduced their feed intake as the concentration of DON increased from 1 to 13 ppm of diet resulting in reduced growth and feed efficiency. Recently, Hooft, Elmor, Encarnação and Bureau (2010) observed that weight gain, feed intake and feed efficiency (FE, gain:feed) of trout decreased significantly ($p < 0.05$) with increasing levels of DON in the diets starting at levels of 0.5 ppm. Significant

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histopathological changes in liver and intestine with increasing dietary levels of DON were also reported. In Atlantic salmon diets with 3.7 mg/kg of DON resulted in 20% reduction in feed intake, 18% increase in FCR and 31% reduction in specific growth rate (Döll, Baardsen, Möller, Koppe, Stubhaug & Dänicke 2010).

Occurrence of mycotoxins worldwide

In the last years there is a trend to replace fish meal as a source of protein by less expensive sources of protein from plant origin. This trend makes it very likely that some contaminated ingredients are used in aquaculture feeds.

Moreover the contamination of feeds and raw materials by mycotoxins is increasing on a global scale. The status of raw materials used in feeds in regards to contamination with mycotoxins is therefore a topic of great interest. A survey (BIOMIN mycotoxin survey 2009, Rodrigues and Griessler, 2010) was conducted on diverse samples ranging from cereals such as corn, wheat and rice to processing by-products, namely soybean meal, corn gluten meal, dried distillers grains with solubles (DDGS) and other fodder such as straw, silage and finished feed. From January 2009 until December 2009, a total of 9 030 analyses were carried out for the most important mycotoxins in terms of agriculture and animal production – aflatoxins (Afla), zearalenone (ZON), deoxynivalenol (DON), fumonisins (FUM) and ochratoxin A (OTA). In total, 2 660 different samples were analysed. Samples derived from different countries/regions all over the world, namely the Asian-Pacific region, Europe, Middle-East and Africa and the Americas (North and South America). The majority of analyses were performed at ROMER Labs Diagnostic GmbH (Austria), ROMER Labs Singapore Pte Ltd (Singapore), ROMER Labs Inc (USA) and SAMITEC (Brazil). 75% of the samples were analyzed by HPLC, 25% by ELISA. Samples were mostly taken as part of routine analysis. Overall results showed that a high percentage of samples were contaminated (over detection limit) with at least one type of mycotoxins: 33% of samples with Afla, 30% with ZON, 49% with DON, 54% FUM and 27% with OTA. Data were additionally grouped according to occurrence in different geographical

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regions, on the occurrence in different raw materials and on the co-occurrence of different mycotoxins.

Highest contaminations (in % of total analysed) of aflatoxins were found in Africa (85% positive, 91 ppb average contamination), highest zearalenone contaminations were found in North Asia (59%, 326 ppb average) and South America (mainly Brazil 51% pos., 185 ppb average) and in North America (40% pos., 324 ppb average). In most regions more than 50% of samples were affected with deoxynivalenol, with highest levels in South Africa (88% pos., 1403 ppb). Fumonisin was most prevalent in North America (82% pos., 1800 ppb average), Brazil (87% pos, 4965 ppb average), South Europe (81%, 3411 ppb), and Africa (80%, 1488 ppb). South Asia showed highest ochratoxin A contamination (63% pos., 34 ppb average).

Comparing different materials analysed it can be stated that maximum contaminations (for each mycotoxin) for aflatoxins were found in Vietnamese corn (6105 ppb), Japanese corn for zearalenone (7422 ppb), corn gluten meal from Malaysia (11836 ppb deoxynivalenol and 32510 ppb fumonisin). Highest contamination for ochratoxin was found in a finished feed sample from Pakistan (1582 ppb).

Overall 74% of all analyzed samples were contaminated with one mycotoxin and 40% were contaminated with more than one mycotoxin.

Strategies for mycotoxin risk management

Despite good screening programs, selection of high quality raw materials and feed ingredients and good storage conditions it is very difficult to guarantee the absence of mycotoxins in aquaculture feeds. Therefore it is most important to find suitable ways to face the problem through an effective management of the risks posed by mycotoxin contaminations.

One strategy already widely used in mycotoxins risk management is the inclusion of mycotoxin binders or adsorbents into the feed. Binders or adsorbents have been used to neutralize the effects of mycotoxins by preventing their absorption from the animal's digestive tract. The most common binders are clays, bentonites, zeolites, silicas and aluminum silicates. However this strategy does not work well for all mycotoxin groups. Adsorption works perfectly for aflatoxin but less- or non-adsorbable mycotoxins (like ochratoxins, zearalenone and the whole group of trichothecenes) have to be deactivated by using a different strategy which is the use enzymatic deactivation to target functional groups of mycotoxins such as trichothecenes, ochratoxin A and zearalenone. Moreover, all mycotoxins are known to negatively affect the liver and cause immunosuppression in animals. The addition of plant and algae extracts to the diets helps to overcome these negative influences.

Conclusion

On a global scale a high percentage of feed ingredients and raw materials are contaminated with mycotoxins.

The available studies on the effects of mycotoxins in fish and shrimp show that performance and health status are negatively affected which have economical losses. The awareness of mycotoxin problems in aquaculture farms must be developed to minimize the negative impact of mycotoxins on the performance and health of exposed fish. Moreover the risk for consumers should be also be addressed as mycotoxin residues were found in fish muscle beyond acceptable levels. This should be done through increased research in this topic and the development of effective mycotoxins risk management.

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