# Newsletter

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**> EDITORIAL**

*In contrast to terrestrial animals, the absence of clear clinical signs that can be attributed to mycotoxicoses in aquatic species kept this topic away from the headlines. However, in recent years, the awareness of mycotoxin-related issues within the aqua industry has grown, supported by reports on the prevalence of mycotoxins in several raw materials and increasing scientific evidence of the negative impact of mycotoxins in fish and shrimp species. It is now becoming clear that such effects can have detrimental impacts not only in growth performance but also disease resistance, with obvious economic losses for producers. Moreover, this might also pose human health concerns as aflatoxin levels above the limits imposed by the European Union were recorded in the muscles of fish fed contaminated diets.*

*It is very difficult to guarantee the absence of mycotoxins in aquaculture feeds even when appropriate measures are taken, such as good screening programs, selection of high quality raw materials and feed ingredients, and good storage conditions. It is therefore imperative to find effective ways of managing the risks posed by mycotoxin contamination.*

*Enjoy reading!*

***Gonçalo Santos and Pedro Encarnação***


Mycofix® product line

**Aquaculture is the fastest growing animal food-producing sector. With growing demand for aquatic products (fish and shrimp) comes increasing concern about the reliable supply of raw materials needed to support this growth. Aqua feeds traditionally depend on fishmeal as a protein source, but the trend in recent years has moved towards replacing fish meal with less expensive sources of protein of plant origin. As a result of this trend, aquaculture feeds have a higher risk of contamination by one or more types of mycotoxins. Aquafeeds contaminated by mycotoxins occur particularly in countries with humid tropical climates owing to many factors, among which are climatic conditions conducive to mold growth and inappropriate methods of feed processing and storage. However, increasing trade globalization and the incorporation of imported raw materials in aquafeeds expose feed manufacturers and their clients to the risk of combinations of mycotoxins either from multiple mycotoxins in the same raw material or from different mycotoxins in different ingredients in the same formulation.**

**In addition, rising feedstuff prices have led feed manufacturers to look for more economical raw materials to avoid increasing feed prices. The use of more affordable raw materials of lower quality, such as DDGS, might increase the risk of mycotoxin contamination in feeds.**

# Mycotoxins in aquaculture

Among different mycotoxins, aflatoxins are the most studied group due to their prevalence in tropical climates where aquaculture is an important industry. The susceptibility of fish towards aflatoxins seems

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

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

*Fumonisins* represent a group of mycotoxins produced predominantly by the *Fusarium moniliforme* species. *Fumonisin* $B_1$ is considered the major toxic component both in corn culture and in naturally contaminated corn. Several animal diseases have been linked to this toxin, particularly those in which a disruption of the sphingolipid metabolism takes place (Wang *et al.*, 1992). In fish, the role of fumonisins as toxic agents remains unclear. On the one hand, minimal adverse effects have been reported in channel catfish fed *F. moniliforme* culture material containing 313 ppm of fumonisin $B_1$ ($FB_1$) for five weeks (Brown *et al.*, 1994). On the other hand, for the same fish species, dietary levels of $FB_1$ 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 *et al.*, 1995).

Likewise, Yildirim *et al.* (2000) found that in channel catfish, diets containing 20 ppm of moniliformin (MON) or $FB_1$ significantly reduced body weight gain after two weeks. According to the same authors, $FB_1$ is more toxic to channel catfish than MON. Another study by Tuan *et al.* (2003) with Nile tilapia also suggests a higher toxicity of $FB_1$ compared to MON as toxic symptoms appeared earlier in fingerlings exposed to $FB_1$. However, it should be noted that feeding both MON and $FB_1$ at 70 and 40 ppm respectively, negatively affected the growth performance of Nile tilapia fingerlings despite no effect on mortality or histopathological lesions. Compared to channel catfish, Nile tilapia appear 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 $FB_1$ (Li *et al.*, 1994).

Long-term exposure effects of $FB_1$ were reported in carp by Pepeljnjak *et al.* (2002). These authors showed that exposure to 0.5 and 5.0 mg per kg body weight was not lethal to young carp, but can produce adverse physiological effects with kidney and liver being the key target organs for the $FB_1$ 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.

Trichothecenes are produced by fungi of the genus *Fusarium* and can be of type A (e.g. T-2 toxin) and type B (e.g. deoxynivalenol). T-2 toxin reduced feed consumption and growth, and lowered the hematocrit and blood hemoglobin in rainbow trout at levels higher than 2.5 ppm (Poston, 1983). Also, channel catfish fed diets with levels of T-2 toxin ranging from 0.625 – 5.0 ppm had growth rates significantly reduced and mortality increasing above 2.5 ppm (Manning *et al.*, 2003). Additionally, channel catfish challenged with *Edwardsiella ictaluri* showed reduced disease resistance and survival when fed with contaminated feed (Manning *et al.*, 2005).

Deoxynivalenol (DON) is an important contaminant of wheat and corn. DON 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 *et al.*, 2000). In fish, Woodward *et al.* (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 their diets, resulting in reduced growth and feed efficiency.

Hooft (2010) studied the effect of DON contamination in trout (*Oncorhynchus mykiss*) feeds and reported that low, graded levels of DON ranging from 0.3 to 2.6 ppm from naturally contaminated corn resulted in highly significant decreases in growth, feed intake, feed efficiency, protein and energy utilization of rainbow trout. Furthermore, significant differences in growth, feed efficiency and in protein and energy utilization between fish receiving a diet containing 2.6 ppm and fish pair-fed the control diet indicated that decreases in the performance of rainbow trout associated with the consumption of DON-contaminated feed is related to direct or indirect deleterious effects on the nutrient metabolism of fish and not strictly the result of reductions in feed intake (Hooft, 2010). This finding was further supported by evidence of histopathological changes, particularly in the liver of some fish fed diets containing 1.4 and 2.6 ppm DON (Hooft, 2010). Together, these results suggest that rainbow trout are extremely sensitive to low levels of DON from naturally contaminated plant ingredients.

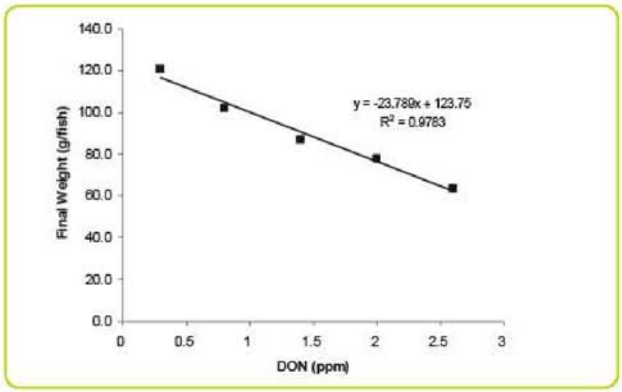


***Figure 4*** *– Final body weight (g/fish) of rainbow trout fed experimental diets containing low levels of DON from naturally contaminated corn (Hooft, 2010).*

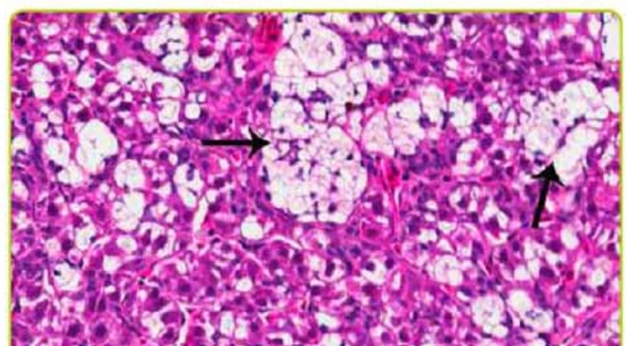

***Figure 5*** *– Liver of a rainbow trout fed diet contaminated with 2.6 ppm DON showing multifocal areas of fatty infiltration (arrows) (H&E stain; bar = 50.71 µm) (Hooft, 2010).*

For Atlantic salmon (*Salmo salar*), a study was conducted to evaluate the effects of DON, ZON and OTA (Doll *et al.*, 2010). Results from the study showed that the presence of 3700 µg/kg DON in the feed caused a 31 % decrease in specific growth rate and 18 % increase in FCR (Doll *et al.*, 2010). In addition, DON-dose dependent alteration of plasma enzymes suggested adverse effects in the to be age and species dependent, as fish fry are more susceptible than adults. Species like rainbow trout and European seabass appear more susceptible than channel catfish for example (Manning, 2001; Tuan *et al.*, 2002; El-Sayed and Khalil 2009).

A reduction in growth is one of the major negative effects reported due to aflatoxin $B_1$ ($AFB_1$) contamination. Several studies report reduced growth rates in channel catfish (10 ppm $AFB_1$/kg feed; Jantrarotai and Lovell, 1990) and Nile Tilapia, (100 ppb $AFB_1$ – Encarnação *et al.*, 2009; 1,880 ppb $AFB_1$ - Chavez-Sanches *et al.*, 1994; 0.1 ppm $AFB_1$ - El-Banna *et al.*, 1992). Additional effects of elevated aflatoxin levels in fish include lower hematocrit count at levels higher than 0.25 ppm, severe hepatic necrosis in Nile tilapia with levels of 100 ppm $AFB_1$ (Tuan *et al*,. 2002) and immunosupression in common carp (Sahoo and Mukherjee, 2001). In addition, mortality rates of 17 % were reported in Nile tilapia fed diets with 0.2 ppm $AFB_1$ (El-Banna *et al.*, 1992). In a recent study on the effects of $AFB_1$ in tilapia, Deng *et al.* (2010), focused on the toxic effects of $AFB_1$ during long-term dietary exposure and came to the conclusion that under good culture conditions, tilapia is a rather tolerant species for dietary $AFB_1$. No toxic effects of $AFB_1$ were found during the first 10 weeks, but after 20 weeks, the diet with 245 µg $AFB_1$/kg or higher concentrations reduced growth and induced hepatic disorder, resulting in decreased lipid content, hepatosomatic index, cytochrome P450 $A_1$ activity, elevated plasma alanine aminotransferase activity and abnormal hepatic morphology.

Another important issue is consumer safety and whether residues of mycotoxins can be found in fish. Han *et al.* (2009) described that gibel carp, fed with more than 10 µg $AFB_1$/kg diet showed accumulation of $AFB_1$ residues in muscles and ovaries above the safety limits (2 ppb) imposed by the European Union. These results are also in line with similar studies on marine fish. El-Sayed and Khalil (2009) also described that prolonged feeding of European seabass with low levels of $AFB_1$ (0.0018 mg/kg body weight) causes not only serious health problems in exposed fish, but also represents a high risk to humans through $AFB_1$ residues in the fish muscles.

In marine shrimp, several studies showed that $AFB_1$ can cause poor growth, low apparent digestibility, physiological disorders and histological changes, mainly in the hepatopancreatic tissue (Bintvihok *et al.*, 2003; Boonyaratpalin *et al.*, 2001). $AFB_1$ toxicity of white shrimp results in the modification of digestive processes and the 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- Hernadez *et al.*, 2005). A study by Bintvihok *et al.* (2003) suggests that levels as low as 20 ppb $AFB_1$ (for a seven-to-10- day consumption period) induced higher mortality rates in shrimp (2003).

In a study carried out in Vietnam, Tu (2010) tested the sensitivity of Tra catfish (*Pangasionadon hypophthalmus*) to $AFB_1$ and the effectiveness of Mycofix® Secure as a binder. After 8 weeks of feeding, reduction in weight gain ($P<0.05$) was observed for fish fed diets contaminated with 50 µg $AFB_1$/kg. This weight gain was further reduced with increasing levels of $AFB_1$ in the diets. Fish fed diets contaminated with 500 and 1000 µg $AFB_1$/kg showed an increased ($P>0.05$) hepatosomatic index (HIS), while an increase in adipose somatic index (ASI) was already observed in fish fed 50 µg $AFB_1$/kg when compared to the control (Tu, 2010). After 12 weeks, blood serum analysis revealed higher alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in fish fed 50, 100 and 250 µg $AFB_1$/kg, suggesting occurrence of liver damage in particular for those fish fed the 250 µg $AFB_1$/kg. (Tu, 2010).

A bacteria challenge was also carried out in the same study, in which fish were exposed to *Edwardsiela ictaluri*. Survival was also compromised by the presence of $AFB_1$ in the feed and was directly related to the contamination level. Seven days after exposure, survival rates were 50, 41.7, 31.7 and 8.3 % for fish fed control, 50, 100 and 250 µg $AFB_1$/kg, respectively. This trial showed that $AFB_1$ contamination in tra catfish diets at a level of 50 µg $AFB_1$/kg and above can affect fish performance and disease resistance. The study also evaluated the application of Mycofix® Secure to counteract the negative effects of $AFB_1$ and reported that the application of 1.5 kg/t Mycofix® Secure was effective in reducing the negative effects of $AFB_1$ in diets containing 500 µg $AFB_1$/kg (*Figure 1 and 2*).

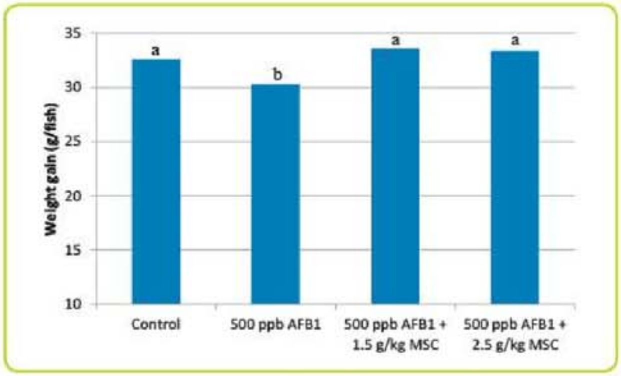


***Figure 1*** *– Weight gain (g/fish) of pangasius fed contaminated diets with $AFB_1$ at 500 ppb alone and in combination with Mycofix® Secure (MSC) at 1.5 and 2.5 g/kg diet (Tu, 2010).*

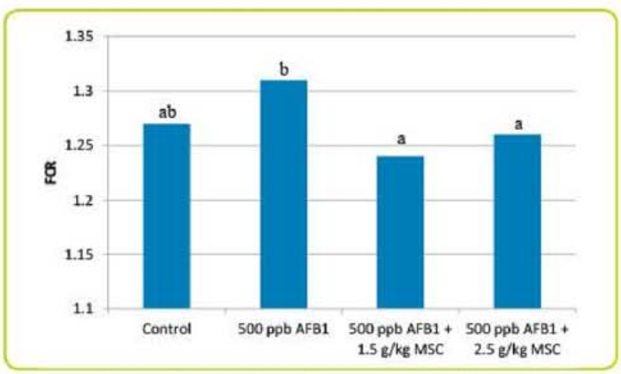


***Figure 2*** *– Feed conversion ratio of pangasius fed contaminated diets with $AFB_1$ at 500 ppb alone and in combination with Mycofix® Secure (MSC) at 1.5 and 2.5 g/kg diet (Tu, 2010).*

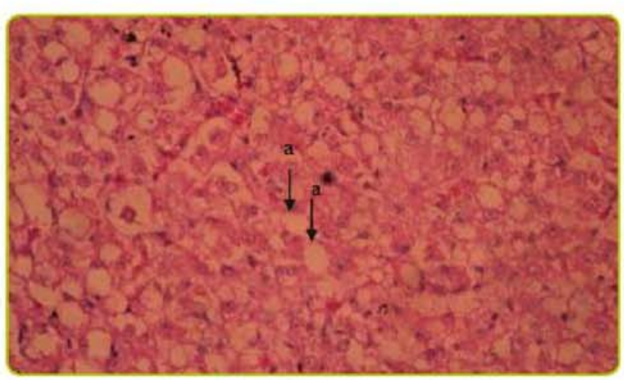


***Figure 3*** *– Accumulation of lipids in liver cells (a) affected by $AFB_1$ at 100 ppb for 12 weeks (H&E x 400, Tu, 2010).*

liver, which need further confirmation by histological evaluation (Doll *et al.*, 2010). The authors concluded that the present EU recommendations for DON levels in feed (5000 µg/kg) do not mitigate the adverse effects on the performance and health of Atlantic salmon. Another study on the effects of OTA was conducted in European sea bass (*Dicentrarchus labrax*). In this study it was concluded that this species was highly sensitive to OTA with a 96 h $LC_{50}$ of 9.23 mg/kg diet (El Sayed *et al.*, 2009).

## CONCLUSION

Despite good screening programs, the 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. Available studies on the effects of mycotoxins in fish and shrimp show that performance and health status are negatively affected, all of which can have an adverse economic impact. The broad range of contamination levels that can negatively affect fish performance and health can be related to the intrinsic differences in the species, its age, nutritional and health status. The awareness of mycotoxin problems in aquaculture must be developed to minimize the negative impact of mycotoxins on the performance and health of exposed fish and shrimp. Moreover the risk for consumers should also be addressed as mycotoxin residues were found in fish muscle beyond acceptable levels. More research should be conducted on this topic along with the development of effective mycotoxin risk management.

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