Fumonisins - mycotoxins of increasing importance in fish!

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Fumonisins are a group of recently discovered mycotoxins which belong to the family of Fusarium toxins. The contamination of feedstuffs with mycotoxins poses a serious threat to the health and productivity of animals and cause great economic losses. In the USA, the annual losses caused by mycotoxins in grain production are estimated at 900 million dollars[1]. Dependent on type of animal, sex, age as well as the nutritional and health condition of the animal, fumonisins cause different clinical symptoms. Additionally, the occurrence of several mycotoxins in feed is very likely and can amplify the toxic effects of the individual toxin (synergistic effect).

Fumonisins are mainly produced by *Fusarium verticillioides* (syn. moniliforme) as well as by *Fusarium proliferatum* (see Figure 1) and they occur predominantely in maize and maize-based feeds[2]. In 1988 they were first identified and isolated and so far there are 28 fumonisin analogues known[3,4]. Fumonisins are divided into four groups: Serial A, B, C and G. With regard to their toxicity the B-type fumonisins represent the most important ones[5]. In naturally contaminated food and feed fumonisin B1 represents about 70% - 80% of the total fumonisin content[6].

Fumonisins are very polar and water soluble compounds. Unlike other mycotoxins they have a long chain structure. Chemically they are polyhydroxyl alkylamines esterified with two carbon acids, i.e. tricarballylic acid (TCA). The four common members of the type B fumonisins differ by presence and position of the free hydroxyl groups respectively[7]. The one-sided or bilateral elimination of TCA results in partial hydrolyzed fumonisin or hydrolyzed fumonisin (HFB_1).

Fungal colonisation and growth and/or mycotoxin production are influenced by a variety of factors. Optimum conditions for fumonisin production are temperatures between 10°C and 30°C with a water activity (amount of free available water) of 0.93 aw[8].

A recently published survey about the occurrence of mycotoxins in Asia initiated by BIOMIN GmbH together with Romer laboratories in Singapore reported that 58% out of 960 feed

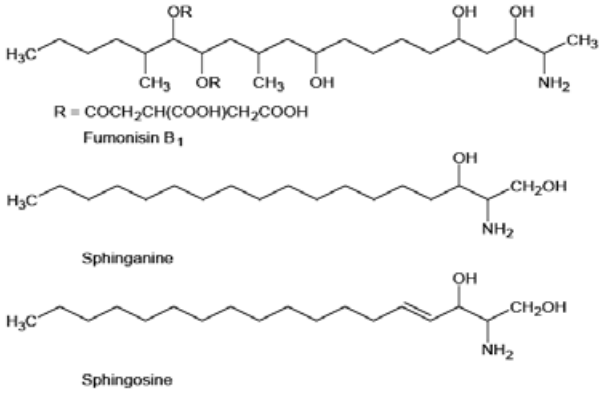

Figure 2: Structures of sphinganine, sphingosine and fumonisin B1.

Figure 1: Fusarium proliferatum contaminated maize (Source: Chamber of Agriculture, Styria, Austria).

raw material samples were contaminated with fumonisins. The highest level of fumonisins detected was 14.7 mg/kg in a corn sample from China[9]. In Europe, the maximum level of fumonisin was 3.1 mg/kg in a sample of soybean meal from Southern Europe[10]. Table 1 gives examples on high concentrations of fumonisins in maize.

Toxicity

Fumonisin toxicity is based on the structural similarity to the sphingoid bases; sphingosine and sphinganine (see Figure 2). They are inhibitors of sphinganine (sphingosine) N-acyltransferase (ceramide synthase), a key enzyme in the lipid metabolism, resulting in a disruption of this pathway. This enzyme catalyzes the acylation of sphinganine in the biosynthesis of sphingolipids and also the deacylation of dietary sphingosine and the sphingosine that is released by the degradation of complex sphingolipids (ceramid, sphingomyelin and glycosphingolipide)[11]. Sphingolipids are basically important for the membrane and lipoprotein structure and also for cell regulations and communications (second messenger for growth factors)[12].

As a consequence of this disruption many bioactive intermediates are elevated, others reduced. The main points are:

- Rapid increase of sphinganine (sometimes sphingosine).
- Increase of sphinganine degradation products like sphinganine 1-phosphate.
- Decrease of complex sphingolipids.

Free sphingoid bases are toxic to most cells by affecting cell proliferation and inducing apoptosis or necrotic cell death[13,14]. The accumulation of sphinganine is associated with hepato- and nephrotoxic effects[15]. Complex sphingolipids are important for cell growth regulation and also cell-cell

interactions. The accumulation of free sphingoid bases in the serum and urine are a useful biomarker for the exposure of fumonisins[16].

The importance of fumonisins as toxic agents in fish is still poorly understood as there have been only a few studies published. In several experiments, fumonisins are documented to be toxic for fish.

In an experiment, one-year and two-years old channel catfish were fed diets containing *Fusarium moniliforme* from maize to contain FB_1 at 20, 80, 320, and 720 mg/kg during 10 weeks and 14 weeks, respectively[17]. It was reported that dietary levels of FB_1 of 20 ppm or above are toxic to one-year and two-year channel catfish fish fed with 20 mg/kg. FB_1 did not show differences in mortalities but weight gain was significantly decreased by 15% compared to the control group. Additionally, liver lesions were noted. In another study with catfish consuming *Fusarium moniliforme* maize containing fumonisins, an increase of the Sa:So ratios in serum, liver, kidney and muscle were found at ≥10 mg FB_1/kg after 12 weeks[18]. Catfish has also been fed with FB_1 from *Fusarium* cultured maize[19]. Eight groups of 20 catfishes were fed 0, 0.7, 2.5, 5.0, 10.0, 20.0, 40.0 or 240.0 mg FB_1/kg feed, respectively, for 12 weeks. At concentrations of 40 mg FB_1/kg feed, weight gain and feed consumption were decreased and also histological changes were detected.

Results reported by Tuan *et al*.[20] demonstrated that feeding FB_1 at levels of 10, 40, 70 and 150 mg/kg feed for 8 weeks affected growth performance of Nile tilapia fingerlings. In this experiment the mortality was low and histopathological lesions were not observed. Fish fed diets containing FB_1 at levels of 40 mg/kg or higher had decreased average weight gains. Haematocrit was decreased only in tilapia fed diets containing 150 mg FB_1/kg. The ration between free sphinganine and free sphingosine (Sa:So ratio) in liver increased at a 150 mg FB_1/kg in the fish feed.

Although research studies revealed that FB_1 is toxic to tilapia and channel catfish by suppressing growth and/or causing histopathological lesions, this fish survived mycotoxins levels up to 150 ppm. Reduction on the percentage of survival of channel catfish was observed for diets containing 240 ppm FB_1[21].

Adverse effects of fumonisin contaminated diets have also been reported in carps. One year carps indicated that signs of toxicity can be observed with 10 mg FB_1/kg feed[22]. In these experiments scattered lesions in the exocrine and endocrine pancreas, and inter-renal tissue, probably due to ischemia and/or increased endothelial permeability were reported. In one-year old carp, consumption of pellets contaminated with 0.5 and 5.0 mg FB_1 per kg body weight resulted in a loss of body weight and alterations of haematological and biochemical parameters in target organs[23].

Counteracting

Prevention measurements describe all the steps to counteract mycotoxins during the growth of the grain as well as during harvesting or storage. On the field all management practices which maximise plant performance and reduce plant stress can substantially decrease mycotoxin contamination. This includes pre-harvest practices like fertilisation, proper crop rotation, avoidance of pests, optimal crop density and high quality seeds. In addition, appropriate harvest time as well as optimal storage conditions like temperature or humidity control are important[24]. All these prevention measures can only reduce but not eliminate the risk of mycotoxin contamination. Therefore successful detoxification procedures after harvest are essential. They can be classified into three categories: physical, chemical and biological methods.

The efficacy of physical treatments depends on the level of contamination and the distribution of the mycotoxins in the grain. Additionally, the results obtained are often uncertain and associated with high losses. Various chemicals (bases, oxidizing agents, different gases etc) have been tested for their ability to detoxify mycotoxins but only a limited number of them are shown to be effective against them without reducing nutritive value, palatability of the feed or producing toxic by-products. For achieving adequate decontamination results several parameters like reaction time, temperature and moisture have to be monitored. Due to their uncertain and uneconomic results, the practical application of physical and chemical treatments is very limited.

Adsorption

Adsorbent agents are added to the feed and bind mycotoxins during digestion in the gastrointestinal tract resulting in a reduction of toxin bioavailability. Adsorption of mycotoxins requires molecule polarity and also a suitable position of the functional groups. Due to this fact only a few mycotoxins can be adsorbed efficiently without affecting essential feed ingredients. This method is especially used to counteract aflatoxins, however in the case of fumonisins it's only of limited success. In a study, different adsorbents were tested for their potential to bind FB_1. An effective adsorption of FB_1 was described with activated charcoal and cholestyramin in vitro. However, activated charcoal is a very unspecific adsorbent and binds valuable nutrients as well; therefore these results could only be confirmed for cholestyramin in vivo[25]. Avantaggiato *et al*.[26] from the Institute of Science of Food Production (ISPA) and the National Research Council (CNR), Bari, Italy, found out that among the commercially available feed additives Mycofix® Plus from BIOMIN GmbH showed good results with adsorption rates of 100% and 77% of 2 µg/ml and 20 µg/ml FB_1, respectively.

Biotransformation

Fumonisins are natural toxins, and therefore they are biodegradable by natural metabolic pathways. Compared to adsorption of mycotoxins by clay, microbial biodegradation has the advantages of being highly specific and irreversible. Several microbial strains which are capable of fumonisin biodegradation were previously isolated, and the genes encoding fumonisin detoxification enzymes were identified[27,28,29]. BIOMIN GmbH has a long standing expertise in the application of specific microbes for mycotoxin biodegradation in the gastrointestinal tract of animals[30]. Recently, BIOMIN GmbH scientists isolated and characterised new fumonisin-metabolising bacterial strains[31]. Some of these isolates were found to be active in the gastrointestinal tract of animals. One of the strains with the highest technological potential belongs to the family of the Sphingomonadaceae and was called MTA144. It degrades fumonisins by first cleaving off tricarballylic acid side chains, and subsequently catabolising the rest of the molecule into non-toxic products. Development of a novel feed additive for fumonisin detoxifica-

tion based on this strain - whose efficacy was proven in vitro - is in progress. Nevertheless in vivo trials are necessary to prove its efficacy in the animals.

Conclusion

A number of experiments reported that fumonisins are toxic for fish and the main target organs are liver and kidney. Although careful selection of raw materials, maintaining good storage conditions for feeds and raw materials, there is still a potential risk of mycotoxin contamination. As a result of their different structures mycotoxins can cause various toxic effects in animals. Therefore, there cannot be only one effective strategy against mycotoxins. Whilst adsorption is very efficient for aflatoxins, this method is quite limited for other mycotoxins and only a feed additive which combines adsorption and biotransformation will lead to success.

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