Chapter

Food Safety: The Risk of Mycotoxin Contamination in Fish

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Abstract

Mycotoxins are commonly found in animal feeds, and fish feeds are no exception to this. The need to feed fish in aquaculture with compounded feeds leads to the increasing inclusion of plant-derived feed ingredients that have a higher probability of containing mycotoxins. Since fish appear to be quite sensitive to mycotoxins, further research on mycotoxin toxicity in fish is recommended. Depending on the chemical characteristics of an individual mycotoxin and the biotransformation abilities of the different fish species, certain mycotoxins can could be found in the edible parts of a fish. Thus, the consumption of fish products increases the potential risk of mycotoxin exposure for humans. This chapter reviews the risks associated with different groups of mycotoxins and makes recommendations on how to minimize these risks in the future.

Keywords: fish, aquaculture, mycotoxin toxicity, toxin residues

1. Introduction

Estimating risk requires sufficient knowledge of the frequency with which mycotoxins occur and the levels that can be expected. However, sufficiently detailed information on the actual levels of contamination in fish feeds is often not available. In addition, there is a high degree of variability between mycotoxins due to differences in fungal distribution and climatic conditions worldwide. Nevertheless, the following sections will summarize our current knowledge of mycotoxin occurrence in feed ingredients, fish feeds, and fish tissues in order to compile sufficient evidence to prove that some mycotoxins pose a considerable risk for consumers due to their high prevalence, incidence, toxicity, and/or stability as they pass into the food chain.

2. Exposure of fish to mycotoxins

Fish production in aquaculture has increased rapidly over the previous decades. Consequently, increasing numbers of fish have to be fed in aquaculture, which requires an increasing amount of fish feed. Since the global availability of fishmeal, which is a major ingredient in fish feed, is limited, cereals are common alternatives. Based on recent estimations, it has been determined that fishmeal is still a major component in fish feed in Europe [1], despite the fact that its percentage in commercial feeds has decreased over the last decades. The disadvantage of plant-based ingredients is that there is a higher probability of them being contaminated with mycotoxins. The second most prominent feed ingredient in aquaculture feeds in

Europe is wheat flour [1], followed by soybean products. Other feed ingredients are often present in fish feeds at average percentages of less than 10%, and these ingredients may also contain considerable amounts of mycotoxins. One example of such a problematic feed ingredient may be distillers' grain with solubles (DDGS) [1, 2].

The most important mycotoxins in feed ingredients in terms of risk to fish and consumers, since they are either known to be toxic and/or occur at high concentrations, include aflatoxin B1 (AFB_1), deoxynivalenol (DON), nivalenol (NIV), zearalenone (ZEN), ochratoxin A (OTA), T-2 toxin (T2), fumonisin B_1 (FB_1), moniliformin (MON), enniatins (ENNs), and beauvericin (BEA). Nevertheless, there are a number of reasons why mycotoxin contamination levels in feed ingredients can vary widely, for example, different fungal species or strains often grow on specific feed ingredients. Especially, high OTA levels have been found in corn (up to 1850 μg/kg, [3]), followed by wheat (up to 1024 μg/kg, [4]), soybean, and sunflower products (up to 350 and 240 μg/kg, respectively, [3]). Furthermore, *Fusarium* mycotoxins can contaminate peas and soybeans [5], and FB_1 can be found in significant amounts in corn [6].

The occurrence of mycotoxins in feed ingredients is also known to vary as a result of climate effects and differences in the distribution of various fungal species and strains that have differing abilities to form toxins [7–9]. The problem with mycotoxin contamination in feed ingredients is thought to have increased as a result of climate changes and the shipping of commodities on a global scale, which has led to the worldwide distribution of many fungal species, often resulting in higher contamination in cereals [9–11]. However, the presence of mycotoxins in feed ingredients does not mean that these substances will also be present in compounded animal feeds, since a number of mycotoxins have been reported to possess different degrees of stability when thermally processed and extruded [12]. Furthermore, the processing of feed ingredients, which includes cleaning, sorting, milling, and the application of thermal processes, also influences the mycotoxin load in the final products [13–16]. Nevertheless, the extent of the reduction in mycotoxin contamination during these procedures differs widely for each mycotoxin [15, 17–20]. Generally, mycotoxins that are most stable and widely distributed and, in most cases, occur at high concentrations in certain feed ingredients are problematic for fish production. Two mycotoxins that are already problematic at relatively low concentrations in fish feeds and will be reviewed in the section on fish toxicity are AFB_1 and OTA due to their high toxicity.

The most prominent member of the fumonisins in naturally contaminated animal feeds is FB_1 [21], which often occurs at high concentrations in feed ingredients (e.g., [22, 23]). However, since fumonisins are relatively unstable and easily affected by feed production processes, they are assumed to be less problematic than other mycotoxins. Nonetheless, feed processing may yield mycotoxin metabolites, in some cases resulting in increased toxicity [24].

ZEN is a mycotoxin that commonly occurs after crops have been infected have been infected with *Fusarium* species in the field, but this toxin can also develop during the storage of the cereals [25, 26]. ZEN contamination appears to be common in commercial fish feeds [27, 28], which raises concerns about the effects of chronic exposure to this mycotoxin, since besides exhibiting toxic characteristics, it is also a potent natural estrogen [29].

The trichothecenes include some very important mycotoxins, such as T-2 toxin, DON, and NIV. Recent research has focused on DON since it is known for its high prevalence and incidence in feed ingredients and animal feeds in Europe [30]. However, *Fusarium* fungi are also known to produce some less commonly described mycotoxins, known as emerging mycotoxins, which include BEA, ENNs, and MON [31, 32]. Although ENNs and BEA have been reported to be extremely prevalent in cereals [33], there has not been enough detailed research into their presence in feed components, compounded animal feeds, or farmed animals that have been exposed

to these mycotoxins. The other important *Fusarium*-related mycotoxin is MON. Up to 1.2 mg/kg MON has been detected in feeds for higher vertebrates [34], whereas the levels present in commercial fish feeds remain unknown.

As mentioned above, mycotoxin contamination often occurs on crop fields, but improper storage of feed ingredients and feeds also contributes to the final toxin levels in fish diets. Toxin production depends on the fungi's ability to produce certain chemical compounds as well as environmental factors, such as physical, chemical, and biological factors [35]. Accordingly, similar to the aflatoxins, the occurrence of OTA seems to be connected to temperature and humidity in the environment during growth and harvesting of crops, and the storage of feed ingredients and feeds. However, for most investigated fish feeds, low OTA levels have been observed [28]. In contrast, recent research has shown that inappropriate storage over a period of 6 weeks of a commercial feed for salmonids can lead to the development of considerable amounts of OTA (up to 400 μg/kg feed, unpublished results, C. Pietsch).

Although dietary contamination is the main route of exposure for fish in aquaculture, mycotoxins may also be introduced to aquatic environments directly. For example, levels of 90 μg/L OTA have been reported in waste water originating from wine production. Furthermore, ZEN can be found in surface waters and in waste-water treatment plants at ng/L levels, which may be environmentally relevant due to the estrogenic effects of this mycotoxin [36–38]. Thus, the stability of mycotoxins in water may also have an effect on relevant exposure concentrations in aquatic environments [39].

When data on contamination levels and incidence in common feed ingredients are compiled, there may be significant uncertainties due to the fact that these studies use different methodologies for mycotoxin detection and quantification. Another problem when compiling data from scientific studies is that several studies have not reported accuracy and reliability parameters for their methods, meaning the measured toxin values probably contain uncertainties, since the sample preparation and detection procedures differed. Furthermore, actual mycotoxin concentrations in feed components, animal feeds, and animal tissues are often underestimated, since matrix effects and the problems of detecting masked mycotoxins, which can often not be detected by routine measurement techniques. Since research is continuously improving detection methods for mycotoxins, an increased number of comparative studies addressing the advantages and disadvantages of detection methods for more commonly and emerging mycotoxins, such as can be found in the study by Pascale [40], should be conducted.

Another problem with estimating actual contamination levels in feeds and animal tissues is that metabolites of even commonly occurring mycotoxins are often not analyzed together with their parent compound, although metabolites may occur in significant amounts as has been shown for DON [41]. Furthermore, toxin levels in the control diets used in experimental fish studies have often been reported to contain no mycotoxins, despite the fact that the necessary toxin analyses were rarely performed to provide proof for this assumption. This may lead to an underestimation of the actual toxin levels in both control diets and experimental diets if only a restricted number of mycotoxins are measured. As a result, actual mycotoxin exposure data for fish contain various uncertainties. Therefore, more complete feed contamination databases are required so that risk assessments can be improved.

3. Presence of mycotoxins and their toxicity in fish

If the risk to humans by consuming fish products is to be calculated, the first step would be to estimate the uptake and retention of mycotoxins in different fish

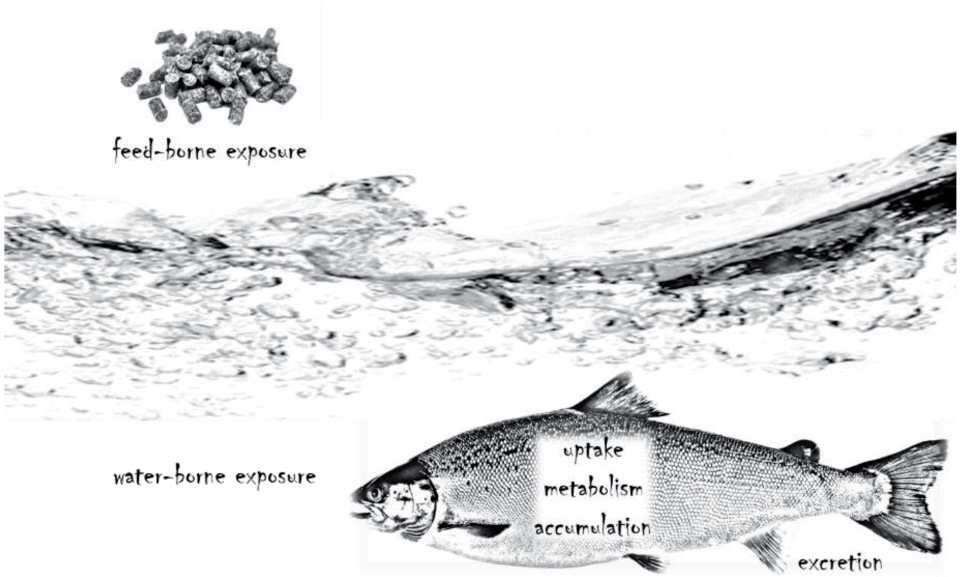

Figure 1.
Exposure routes and factors influencing mycotoxin retention in fish.

species and in different parts of the fish (**Figure 1**). Therefore, the following sections will summarize what is known about chemical characteristics in fish bodies and the toxicity in the animals resulting from the most important mycotoxins.

DON has a mean lowest-observable effect level (LOEL) in fish of 3541 ± 776 μg/kg (±SEM; **Figure 2**), whereas the contamination levels in commercial fish feeds range from 0 to 825 μg/kg [27, 28, 41]. Similar to findings in chickens, DON appears to be excreted rapidly by carp (*Cyprinus carpio*), leaving no relevant residues in the edible parts [42, 43]. FB_1 metabolization also occurs quickly in chicken and the remaining values in tissues stay low. However, exact information on the kinetics or biotransformation of fumonisins in fish is not available [44, 45]. Due to this and the large differences in the toxicity of fumonisins in fish (**Figure 2**), no exact risk can be calculated for farmed fish [1]. Typical disorders in higher vertebrates resulting from FB_1 exposure have often been linked to the disruption of the sphingolipid metabolism [46], and similar effects have also been observed in fish [47]. Nevertheless, a low potential risk has been assumed for most vertebrates, with the exception of pigs [45]. Despite the fact that the guidance values for fumonisins in complete fish feeds have been set by the European Commission and the US to 10 mg/kg based, some countries have chosen to set different guidance levels [48, 49]. Although FB_1 can affect fish at low concentrations, for example in carp (exposed to 500 μg/kg [50, 51]), the concentration range of the lowest-observable effects in fish is relatively broad, with a mean range of 26,480 ± 7124 μg/kg (±SEM; **Figure 2**), a level that is not achieved for either actual or estimated natural contamination of fish feeds [1, 52].

Previous studies have reported lethal concentrations of OTA that lead to 50% mortality (LC_{50}) ranging from 2 to 58 mg/kg body weight in various higher vertebrate species [53, 54]. Fish species appear to be particularly sensitive to OTA, and since disposition appears to mainly take place in the kidneys of fish and not in muscles [55], this not only affects its toxicity, but is also relevant for food safety. High sensitivity to OTA in fish has been demonstrated in several studies. The LC_{50} value for OTA in adult seabass (*Dicentrarchus labrax* L.) was found to be 280 μg/kg body weight [56], 360 μg/l for zebrafish (*Danio rerio*) embryos [57], and 5.53 mg/kg

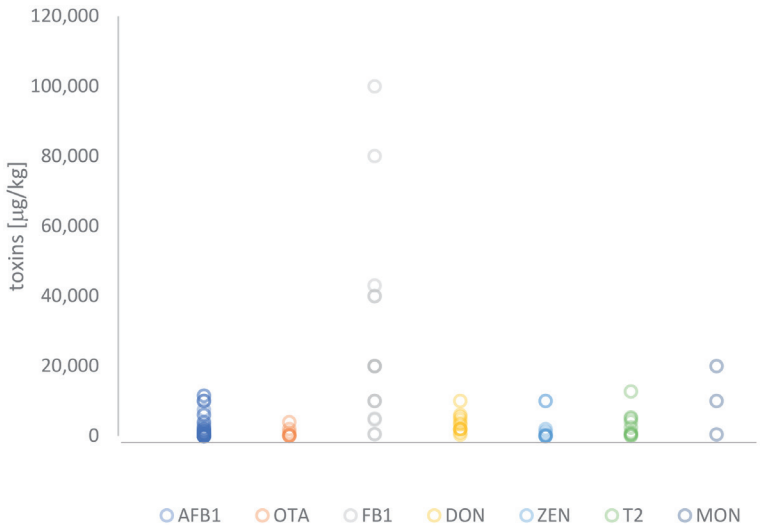

Figure 2.
Variability in mycotoxin toxicity for fish, as shown by the differences in the lowest-observable effect levels (LOEL) in different fish species. References: 92 studies for AFB_1 [63, 64, 70–149] comprising 21 different fish species, 7 studies for OTA [56–58, 94, 150–152] comprising 5 fish species, 15 studies for FB_1 [47, 50, 51, 153–165] reporting levels for 7 fish species, 12 studies for DON [42, 144, 166–175] yielding information for 5 different fish species, 10 studies for ZEN [144, 176–184] reporting LOEL for 5 different species, 10 studies [185–193] reporting effects of different levels of T-2 toxins on 4 different fish species, and 3 studies [162, 194, 195] for 3 different species exposed to MON.

body weight in rainbow trout (*Oncorhynchus mykiss*) [58]. However, the route of exposure may play a role when comparing these different studies. Furthermore, the absorption efficiency in the gut also determines the bioavailability of the mycotoxins in fish, as has been demonstrated for oral exposure to OTA in common carp [59]. If the LOEL for exposure of fish to OTA are summarized (**Figure 2**), the mean range is 1077 ± 566 µg/kg (±SEM), which indicates that the currently recommended guidance value for OTA in cereals and cereal products intended for animal feed of 250 µg/kg does not protect fish from potential damage [48]. This is in stark contrast to the guidance level of 20 µg/kg that exists in some non-EU countries [49].

ZEN has a mean toxicity value of 2389 ± 1285 µg/kg (±SEM), based on the LOEL calculations for five different fish species shown in **Figure 2**. Although the number of studies reporting effects of ZEN in fish is very limited, they may indicate that fish are more sensitive to water-borne ZEN than to dietary ZEN, which is why the mean LOEL level, including both, dietary and water-borne exposure for fish, shows quite a high standard error of the mean. ZEN concentrations above the LOEL levels in water samples have not been reported for aquatic environments [36–38]. Although the actual ZEN contamination of commercial fish feeds appears not to exceed the current guidance level for this mycotoxin in cereals and cereal products in the EU of 2000 µg/kg [27, 48], dietary exposure to this mycotoxin may still do harm to farmed fish. The guidance values in other countries that recommend maximum ZEN levels of 20–1000 µg/kg have a higher probability of protecting fish from damage [49], since the ZEN levels in fish feeds often do not exceed concentrations of 200 µg/kg [27, 60]. Nevertheless, more exact reports on ZEN toxicity in fish and the actual contamination levels in commercial fish feeds are needed to support these assumptions.

T-2 toxin has a mean toxicity of 3201 ± 1236 µg/kg (±SEM) in fish, based on the currently available LOEL for different fish species (**Figure 2**). This level is considerably higher than the actual contamination level found in salmonid fish feed

in South America [28], and much lower than the guidance levels of 250 mg/kg for T-2 toxin set by the European Commission for cereal products in compound feeds [61] and individual recommendations in other countries (max. 80–100 mg/kg) for T-2 toxin in complete feed and all grains [49]. From these data, it can be assumed that fish do not regularly suffer from T-2 toxicity, and there have been no reports of accumulation of this mycotoxin in edible parts of the fish.

The situation for AFB_1 is, however, quite different. The mean LOEL for fish has been calculated to be 1248 ± 275 μg/kg (±SEM) (**Figure 2**). However, AFB_1 appears to be readily absorbed by the intestine [62] and a LOEL of less than 1 μg/kg has been observed in Nile tilapia (*Oreochromis niloticus*) and rainbow trout [63, 64], which shows that this mycotoxin can be a problem for farmed fish. In commercial fish feeds, AFB_1 levels are commonly less than 10 μg/kg [65, 66], but may be considerably higher in some cases [67–69]. Critical levels for fish have been estimated to be a mean of 4.30 μg/kg in commercial feeds [1], which indicates that farmed fish are exposed to a risk from AFB_1 intoxication.

Less information is available on the toxicity of ENNs and BEA in fish, but from initial experiments it can be assumed that at least some ENN toxins have toxic effects on zebrafish embryos (unpublished results, C. Pietsch). However, how relevant this toxicity is in comparison to the actual ENN contamination in commercial feeds remains unclear. Similar to other emerging mycotoxins, these substances have already been detected in the plasma of pigs after exposure to ENNs [196], indicating that the uptake of these substances occurs in vertebrates. In addition, it has been shown that food processing affects the presence on ENNs and BEA in bread [197, 198], and thermal processes, in particular, also appear to influence the ENN content in fish tissue [199]. Finally, the presence of high ENN and BEA levels in feed ingredients appears to overestimate the actual risk of fish feed contamination and the potential effects on farmed fish [1]. Thus, more research is needed on the toxicology and the biotransformation of ENNs and BEA in vertebrates.

An issue that also makes mycotoxin research difficult is the fact that we do not know enough about mycotoxin mixtures and their effects. Natural contamination of feed ingredients leads to the occurrence of several mycotoxins at the same time and their interactions remain mostly unknown.

4. Fish products and food safety

Exposure assessments are often based on a deterministic approach, which obtains the estimated daily intake (EDI) levels by assuming a human body weight of 60 kg for an adult. The EDI of each mycotoxin is commonly calculated as μg/kg body weight per day for each mycotoxin. Accordingly, the Joint FAO/WHO Expert Committee and Food Additives and Scientific Committee on Food have established a tolerable weekly intake (TWI) levels for humans for OTA of 120 ng/kg body weight and tolerable daily intake (TDI) levels of 250 ng/kg body weight for ZEN, 100 ng/kg body weight for T-2 and HT-2 toxins together, and 1000 ng/kg body weight for DON [200, 201]. For aflatoxins, no tolerable intake levels have been set since these toxins are listed as human carcinogens. The tolerable intake levels should be compared to the actual contamination levels found in fish products. However, the frequency of mycotoxin occurrence in fish products has not been investigated in detail. Recent studies indicate that less than 10% of fish and meat food samples are contaminated with mycotoxins, with DON contamination occurring in 17% of the 29 fish samples [202]. In addition, the accuracy of the reports also strongly depends on the accuracy and the number of samples that were analyzed.

Even if fish are exposed to feed-borne mycotoxins, and the resulting effects are not great, possible retention of these toxins in edible parts of the fish may pose a risk for human consumption. A risk to humans is assumed when the toxin concentrations in food exceed the safety limits. For AFB_1, this level has been set at 2 μg/kg by the European Union for food designated for human consumption [49]. However, the exact risk to humans is difficult to predict, since the behavior of the chemicals in the fish strongly depends on the chemical structures of the mycotoxins. In addition, toxin concentration in the feeds and duration of exposure also play an important role, therefore different studies may lead to different results. One example is the absence of accumulation of aflatoxin in the musculature of common carp in the study by Svobodova and Piskac [136], which contradicts the findings of Akter et al. [91]. The AFB_1 content in the hepatopancreas of gibel carp (*Carassius auratus gibelio*) was found to be considerably higher than in their muscle tissues (2.4–11.8 μg/kg) after 12 weeks of oral exposure [104]. An extrahepatic deposition of AFB_1 has also been confirmed in trout [62, 203], but the detection of this toxin in kidneys is more relevant from a toxicological point of view than from a food safety point of view. The study by Selim et al. [121] showed that exposure to 200 μg/kg AFB_1 for 2 weeks was sufficient to lead to detectable toxin residues in fish musculature (>20 μg/kg AFB_1), which increased to levels of more than 90 μg/kg AFB_1 after 10 weeks of exposure. Furthermore, feeding European seabass (*Dicentrarchus labrax* L.) with 18 μg/kg body weight AFB_1 resulted in toxin concentrations of 2.5 μg/kg AFB_1 in the fish musculature after 28 days of feeding, and even higher levels of 4.25 μg/kg AFB_1 after 42 days of exposure [94]. Compared to this, oral exposure of lambari fish (*Astyanax altiparanae*) to AFB_1 increased the body residues after feeding for at least 90 days [204]. In addition, this study showed that feeding an AFB_1 concentration of 50 μg/kg feed for 120 days also resulted in aflatoxin accumulation in muscle and liver tissues that were as high as in the feed. In other fish species, residues exceeding the safety limit were detected in the liver but not in the fish musculature [89, 104]. From these studies, it can be concluded that aflatoxin contamination can be a threat to humans after fish have been fed AFB_1 contaminated diets for certain duration. These values show that consuming fish can considerably add to the toxicological burden that can already be expected from consuming cereals, for which the daily intake through consumption of cereal-based products has been reported to reach levels of up to 7.9 ng/kg body weight [205] and 3 ng/kg body weight if peanuts are consumed [206]. An interesting finding was described in a study using walleye (*Sander vitreus*) which had been exposed to considerable amounts of AFB_1 that had accumulated in their edible parts. The accumulation of AFB_1 in the musculature may be reversible by feeding mycotoxin-free diets for 2 weeks [107], which also confirms similar findings in other fish species [104].

Fish muscle did not contain OTA in a Polish study [207]. In seabass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) muscles, only low OTA levels have been detected [208]. It has already been reported that contaminated cereals and feed ingredients lead to the introduction of OTA into the food chain, posing a risk for humans [209]. Consuming fish appears to contribute to the presence of OTA in the food chain and also adds to the detectable levels of OTA in humans [2]. However, compared to the daily intake through direct consumption of cereal-based products that has been reported to be up to 22.2 ng/kg body weight for OTA [205], the amount that fish products may contribute to the toxicological burden appears to be lower. Nevertheless, this adds to the earlier assumption that naturally contaminated feeds also lead to the introduction of this mycotoxin into the food chain which may pose a risk to human consumers [210, 211]. The knowledge presented here on the presence and toxicity of this toxin in fish supports this assumption. The potential risk due to OTA exposure is probably caused by the fact that OTA is even more stable in the environment than aflatoxins [212, 213].

In contrast, the presence of fumonisins in fish appears not to be relevant for consumers, since they rarely occur in farmed fish (e.g., in a survey in Switzerland in only one fillet sample containing less than 0.06 μg/kg FB_1 + FB_2, personal communication C. Pietsch). In addition, it was not possible to identify a high risk to humans as a result of consuming fish products contaminated with other mycotoxins, such as ZEN and DON, since no relevant toxin levels could be detected in the musculature of DON- or ZEN-treated rainbow trout and common carp [42, 214, 215]. Interestingly, ZEN exposure did result in retention in the ovaries of farmed trout [184]. Furthermore, the study by Nácher-Mestre et al. [216] found no detectable mycotoxin levels in gilthead sea bream or Atlantic salmon (*Salmo salar*) after 8 months of dietary exposure to DON levels of up to 79.2 μg/kg and fumonisins at levels of up to 754 μg/kg. A study into fish as food reported mean DON levels of 1.19 μg/kg [202]; and since DON was the major mycotoxin in the fish samples analyzed in this study, it was also assumed to be the main contributor to the daily human mycotoxin exposure. ZEN retention in human breast milk has already been related to consuming meat, fish, dry fruits, and spices [217]. However, compared to the presence of *Fusarium* toxins in cereals, it can still be assumed, based on the fact that rapid metabolization takes place in fish, that the retention of DON and ZEN in fish is low. Therefore, there can be no assumption of a higher risk to humans of consuming these mycotoxins in fish compared to the risk of exceeding the toxicological reference values by consuming cereal products directly [202, 206, 218].

In the 29 fish samples in the study by Carballo et al. [202], mean ENN A concentrations of 0.89 μg/kg were observed. ENNs were also detected in 20% of the salmon flesh samples and 10% of rainbow trout samples in the study by Tolosa et al. [199], but further processing including cooking or smoking appears to mitigate the toxin content [219]. In contrast, fish from Egypt contained predominant xerophilic molds with *Aspergillus* species being the major ones (58.2%), followed by *Penicillium* species (32.7%) in salted products and also in smoke-cured bonga shad and African catfish (*Ethmalosa fimbriata* and *Clarias gariepinus*) [220, 221]. However, a study in Kenya only showed aflatoxins in dried fish, and not in fresh ones [222]. Smoked-dried fish from Nigeria may also contain potential mycotoxin producing fungi and aflatoxins [223–226]. Similar results from Egyptian smoked fish confirmed that the moisture and salt concentrations that occur during food processing influence the OTA and AFB_1 contents in the fish products, possibly exceeding the permissible limits for both mycotoxins [227].

Mycotoxins can also occur in sun-dried fish products, which are typically found in tropical and subtropical regions where high temperatures and humidity considerably influence fungal growth and toxin formation. Accordingly, samples of dried seafood contained high levels of ZEN and OTA (317.3 and 1.9 μg/kg, respectively). Furthermore, low amounts of AFB_2 (1.2 μg/kg) were also observed in the muscle of crucian carp (*Carassius carassius*), even after storage for 3 months at room temperature [228], emphasizing the high stability of aflatoxins.

5. Conclusions

Taken together, mycotoxin contamination in feed ingredients and fish feeds is an increasing problem that will have to be addressed by crop farmers, feed producers, and researchers. One step that could be taken is to prevent heavily contaminated raw materials being introduced into the feed production processes, which would lower potential mycotoxin contamination levels. Nevertheless, other mycotoxins are still formed during storage, and improved guidelines and recommendations for storage of feed ingredients and animal feeds should be published. Since mycotoxins

are present in animal feeds, in some cases at toxicological relevant levels, this may cause health problems in fish and limit production in aquaculture. More data on the presence of mycotoxins in fish would allow better risk assessments for human consumers to be carried out. Furthermore, the data sets for some mycotoxins indicate that more strict guidance levels are needed for fish feeds to protect farm animals from harm and prevent accumulation of potentially problematic mycotoxins such as AFB_1 and OTA in the food chain.

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Conflict of interest

The author declares that there are no conflicts of interest regarding the publication of this chapter.

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