

Mycotoxins In Aquaculture: An Invisible Hazard

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Introduction

Mycotoxins are compounds that occur in food, are naturally produced poisons, invisible, tasteless, chemically stable, toxic metabolic substances, produced by various secondary metabolic products of filamentous fungi (literally “fungus poisons”). Low molecular weight causes economic losses at all levels of food. Currently, 400 mycotoxins are reported. These toxins are produced by diverse groups of fungi, such as *Aspergillus*, *Penicillium*, and *Fusarium*, that contaminate crops before harvesting or during post-harvest conditions like storage.

Are Aquatic Animals Affected by Mycotoxins?

Aquaculture farmers may not see it, but a hidden hazard can wait inside every bag of pellet or grain are mycotoxins. It is increasingly recognized as a global concern in both the livestock and aquaculture industries. The rising trend of incorporating more plant-based ingredients into aquaculture feeds, driven by the high cost of fishmeal and fish oil, has heightened the risk of mycotoxin contamination. This contamination not only affects fish growth performance but also compromises the quality of the final product. The effects of t may be caused by a single toxin or the synergistic effects of multiple mycotoxins, which can be harmful even at lower contamination levels.

The five most common Mycotoxins found worldwide in aquafeed and raw material used for feed formulation are:

1. Aflatoxins (Afla)
2. Zearalenone (ZEN)
3. Trichotecenes (T-2 & HT-2)
4. Fumonisin (FUM)
5. Ochratoxin A (OTA)

Table 1. Major Mycotoxin-Producing Genera

Major Genera	Mycotoxins
<i>Aspergillus sp.</i>	Aflatoxin, ochratoxin, patulin, cyclopiazonic acid
<i>Claviceps sp.</i>	Penitrem A, clavines, lysergic acids, lysergic acid amides, ergopeptines
<i>Fusarium sp.</i>	T-2 toxin, HT-2 toxin, diacetoxyscirpenol, fumonisin fusaric acid, nivalenol, deoxynivalenol, fusarenon-X, Zearalenone
<i>Penicillium sp.</i>	Ochratoxin, citrinin, roquefortine C, PR toxin, penitrem A, cyclopiazonic acid, patulin
<i>Neotyphodium sp.</i>	Ergot alkaloids, lolines, peramine, loliterms

Source: (El-Sayed et al., 2022)

- The level of damage that can be caused by the Mycotoxins intake depends mainly on:
 - The type of toxin
 - Its concentration in the feed
 - Period of exposure
 - Animal species susceptibility
- They exhibit a wide range of effects related to carcinogenicity, neurotoxicity, and developmental toxicity (Kolpin et al., 2014).



Fig. 1: The mycotoxins and their toxic metabolites

The damage of mycotoxins to fish and shrimp

The effects of mycotoxins on fish & shrimp are gradual but pose a serious threat.

The damage of mycotoxins to shrimp

- Direct damage to the shrimp hepatopancreas
- Loss of immune system function
- Slow growth
- Poor body color
- Prone to stress
- Soft shells
- Cause damage to the digestive tract of shrimps
- Causing an empty intestine and stomach

- Red intestine, red stomach
- Hepatopancreas necrosis of shrimps will cause EMS
- Secondary infection forms enteritis, white feces, and so on

The damage of mycotoxins to fish

- Organ damage
 - Liver and kidney disease
 - Scale/shell lesions
- Reproduction
 - Altered egg production
 - Reduced embryonic survival
 - Poor sperm quality
- Immunity
 - Increased susceptibility to disease
 - Increased shell diseases
 - Reduced survivability
- Gut health
 - Feed intake reduction or refusal
 - Damage and lesions to the intestinal tract
 - Altered gut microbial functions
 - Increased gut pathogens
- Performance
 - Poor efficiency
 - Reduced growth rates
 - Smaller body weight

Key mycotoxins of concern

1. Aflatoxins (AFs)

It is produced mainly by *Aspergillus flavus* and *A. parasiticus* and other filamentous fungi of the genera *Penicillium*, *Rhizopus*, *Mucor*, and *Streptomyces* on warm-stored grains (Agag et al., 2004). It is known as the first mycotoxin to be discovered. Feedstuffs like maize, corn, wheat, cottonseed, nuts, and others are concerned with mycotoxins. The main sources of animal feed are groundnut meal, maize, and cottonseed meal. It attacks the liver and impairs growth, even at very low concentrations, causing liver damage, bleeding, and suppressed immunity (Marijani et al., 2019). Fry are more susceptible than older fish.

2. Fumonisin (FBs)

It is synthesized by several *Fusarium* species. *Fusarium verticillioides* is widespread in maize and generates FBs, which are often more abundant when crops are under drought stress or get significant insect damage (Mutiga et al., 2015). Fumonisin B1 is the most toxic fumonisin. In fish and shrimp, high fumonisin feed causes digestive issues and vulnerability to infections.

3. Ochratoxin (OTA)

It is generally produced by *Penicillium* spp. and *Aspergillus* spp. The most toxic form is ochratoxin A. In aquatic species, OTA exposure leads to stunted growth, lesions in the liver and kidneys, and higher death rates under disease challenge. Channel catfish fed with OTA-contaminated diets showed a substantial loss in weight increase, reduced feed conversion rate, shorter survival, and reduced haematocrit (Manning et al., 2003).

4. T-2 toxins

Produced by fungi of the genera *Fusarium*, *Myrothecium*, *Phomopsis*, *Stachybotrys*, *Trichoderma*, *Trichothecium*, and others in crops such as corn, wheat, and oats. Effect on aquatic species included decreased average weight gain, decreased production of bacterial cell wall-breaking enzymes, immunosuppression at low dosage, decreased resistance to oxidative damage, and heterogeneous growth and physiological disorders in shrimps.

5. Zearalenone

Fusarium graminearum is the main producer of the estrogenic compound zearalenone (ZEN). Zearalenone dominantly affects reproductive parameters in different aquatic species, reducing spawning frequency, accelerating sexual maturation (Schwartz et al., 2010) and leaving residues in meat.

Review about the effect of mycotoxins on aquatic species

Table 1. Impact of mycotoxins on aquatic species

Mycotoxin	Species	Dose	Duration (Week)	Effects	References
AFB1	<i>Litopenaeus vannamei</i>	100 µg/kg		Histological damages in the hepatopancreas of shrimp AFB1 level in the Pacific white shrimp diet should be <38.1 µg/kg	Fang et al (2019)
	Nile tilapia	100 ppb	12	100 ppb AFB1 negatively impacted weight gain, feed efficiency, hematological profiles, HSI, as well as liver histopathology	Mahfouz and Sherif (2005)

	Channelcatfish (<i>Ictalurus punctatus</i>)	10000µg/kg	10	Decreased leukocyte count, increased hematopoietic activity of blood-forming tissues	Jantaroaiand and Lovell (1990)
	Tilapia	2.5mg/kg	20	Abnormal Behavior	Deng et al. (2010)
Fumonisin	Gilthead seabream (<i>Sparus aurata</i>)	168 µg/kg		Significant impact on final body weight, feed conversion ratio, and protein efficiency ratio Also reduced fat and energy retention, and disrupted the respiratory system.	Goncalves et al. (2020)
	Red Tilapia			Observed growth depression	Tola et al. (2015)
OTA	Sea bass	277 µg/kg		The behavioural changes of sea bass were primarily observed as nervous and respiratory manifestations. Concluded that sea bass is a species highly sensitive to OTA	El-Sayed et al. (2022)
	Tiger shrimp	1000 µg/kg	8	A decrease in phenol oxidase (PO) activity Although no histopathological change was observed	Supamattaya et al. (2005)
	Channel catfish	4.0 or 8.0 mg/kg	8	The feed conversion ratio was significantly poorer	Manning et al. (2003b)
	Juvenile channel catfish	5mg/kg	8	Histopathological anomalies of the stomach, head, and trunk kidneys	Manning et al. (2003a)

T-2 toxin	Pacific white shrimp	2.4 and 4.8 mg/kg	8	Antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GPx), total antioxidant capacity (T AOC), and also glutathione (GSH) content increased.	Deng et al. (2010)
	Rainbow trout	1.0–1.8 mg/kg		Non-specific humoral immunity decreased significantly in both experimental groups. Influences the immunological defense mechanisms of rainbow trout	Modra et al. (2020)
ZEN	Zebrafish	1000 and 3200 ng/L	6	Exhibited a strong effect on the induction of VTG and reproduction in vivo	Schwartz et al. (2013)
	Black tiger shrimp	500 and 1000 mg/kg	10	Histological changes in hepatopancreatic tissue	Bundit et al. (2006)
	Juvenile rainbow trout	1.810 mg/kg	10	No effects on growth and may accelerate sexual maturation of female fish	Wozny et al. (2015)

Detection methods

Analysis of food in search of mycotoxins is part of the mechanisms of control and prevention of poisoning. The detection method involves a series of steps:

1. Sampling

Sampling is important in determining mycotoxin levels since mycotoxigenic fungi do not grow on the substrate, making it difficult to get a representative bulk sample. Furthermore, the current mycotoxin contamination in natural samples is not homogeneous. To standardize the sampling processes for mycotoxin analysis, Commission Regulation (EC) No. 401/2006

(EC, 2006) established the sampling and analytical methodologies for official control of mycotoxin levels in foodstuffs (EU, 2014).

2. Preparation

To speed up the extraction process and identify mycotoxins, grind the sample to a particle size of around 500 µm and homogenize it to a consistency similar to whole powder (Nakhjavan et al., 2020).

3. Extraction and Purification

This process begins with an extraction in acetonitrile water, followed by liquid-liquid partitioning caused by the addition of inorganic salts. Different extraction methods like liquid-liquid extraction, liquid-solid extraction, pressurized liquid extraction, etc.

4. Detection and analysis

Thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC) are used in conjunction with a variety of detectors, including diode array, fluorescence, and UV. LC-MS/MS and GC-MS/MS have also been frequently used. When quick mycotoxin analysis is necessary, immunoassay techniques such as enzyme-linked immunosorbent assay (ELISA) and lateral flow immunoassay (LFIA) are used. The biosensor system mainly consists of a biorecognition (biosensing) element. Biorecognition elements such as antibodies, antigens, nucleic acids, and enzymes are used to identify and sense target analytes. Biosensors can be classified into electrochemical, optical, mass-sensitive, and thermal sensors (Janik et al., 2021).

Remediation Strategies for Mycotoxin Control

Mycotoxins are heat-stable compounds and difficult to eliminate once they have been synthesized. Once these molecules are present, detoxification or decontamination of food through physical, chemical, and biological methods can be reduced or controlled. Some good hygiene practices have been established and implemented regarding the activities for aquaculture food production. Currently, to guarantee the safety of aquaculture products, animal welfare, and health, various certification standards have been developed based on good hygiene practices and the implementation of HACCP and plans such as global GAP, accredited fish farm scheme, bio-Suisse, Safe Quality Food (SQF), and others (Maama, 2014).

	Methods	Commonly used and measures reagents	Decontamination efficiency	Reference
	Sorting and separation	Sieving, gravity separation, photoelectric separation, etc.	Removed at least 51%, 63%, and 93% of AFs, trichothecenes, and	Matumba et al. (2015)

Physical method			fumonisin from the shelled white maize	
	Washing and solvent extraction	solvent extraction (methanol, ethanol, hexane, acetonitrile, isopropanol, and aqueous acetone)	Removed aflatoxins, trichothecenes, ZEN, and fumonisins by 51-72%, 64-69%, 52-61%, and 73-74% from the grains	Karlovsy et al. (2016)
	Heating	High temperature, high voltage	Decomposed 78-88% of AFB1 in rice by cooking, reduced 80% of FB1 while cooking rice at 100 °C for 10 min.	Becker et al. (2013)
	Irradiation	X-rays, electron beam, ultraviolet rays, infrared, and microwave	Reduced 22.0-90.7% of AFB1 by irradiation, Decontaminated 25.0-86.0% and 60.0-100% of ZEN by ultraviolet rays	Pankaj et al. (2018)
Chemical methods	Alkaline treatment	Ammonia, sodium hydroxide, potassium hydroxide and sodium carbonate, etc.	Removed 95% of AFB1 in various cereals by ammoniation and hydroxide treatments	Bretz et al., (2006)
	Ozone treatment	Ozone, hydrogen peroxide, chlorine, sodium, and calcium hypochlorite, etc.	Reduced 92-95%, 91%, and 78% of AFBs in corn, cottonseed, and peanut meal, respectively, by ozone	Sun et al., (2016)

	Mycotoxins	Microorganisms	Biotransformation efficiency	Reference
Biological methods	AFB1	<i>Aspergillus niger</i> <i>Escherichia coli</i> <i>Bacillus</i> spp. <i>Pseudomonas putida</i>	98.65% 93.70% 67.20% 90.00%	Qiu et al. (2021) Wang et al. (2019) Xia et al. (2017) Samuel et al. (2014)
	ZEN	<i>Bacillus</i> spp.	87.00-100%	Ju et al. (2019); Wang et al. (2017); Xu et al. (2016)
	FB1	<i>Saccharomyces</i> sp. <i>Bacillus</i> spp.	22%-50% 43%-83%	Styriak et al. (2001) Camilo et al., (2000)

Mycotoxins Binders

Due to their importance and wide range of applications in recent years, Nano-clay particles have been significantly incorporated into various fields, including aquaculture, to

improve water quality parameters (Nathanail et al., 2016). Adsorbing agents are ultrafine nanoparticles, smaller than 100 nm, and have the potential to provide a good surface for mycotoxin binding (Fang et al., 2009). The mycotoxin removal efficiency rate of most nanoparticles or adsorbing agents is estimated to be between 20% and 80% (Nathanail et al., 2016). Still, the major drawback of mycotoxin binders is their ability also to adsorb other micronutrients in the animals' bodies, which may result in mineral deficiency (Kolosova and Stroka, 2011).

Aluminosilicates

Bentonites: Bentonites, or montmorillonites, are a group of silicate clay particles that are efficient in mycotoxin adsorption (Kolosova and Stroka, 2011). Usually incorporated into most commercially available aquafeeds to improve water quality indices (Hussain, 2018). These are mainly used for aflatoxin detoxification.

Zeolites: Zeolites are microporous tectosilicate crystalline aluminosilicates used extensively in aquaculture. In aquaculture, zeolites are used for multiple purposes, such as maintaining water quality and removing ammonia, improving feed utilization (Sava et al., 2019), and adsorbing mycotoxins (Dakovic et al., 2010).

Activated charcoal: These agents are highly porous and are one of the most efficient mycotoxin adsorbents (Avantaggiato et al., 2005). The capacity of activated charcoal to bind or sequester mycotoxins is affected by its size, shape, porosity, and structure

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Various microbes have a unique cell wall structure that can bind with different mycotoxins through different interactions such as hydrogen, ionic, and hydrophobic bonding (Haskard et al., 2001). The most commonly used microbes for this purpose are *Saccharomyces cerevisiae* and lactic acid bacteria. Microorganisms may also, at some point in their life cycle, secrete chemicals that can either: alter the structure of mycotoxins and produce fewer toxic compounds, or destroy the mycotoxins. During this degradation process, different microbes that produce various enzymes are utilized, including aflatoxin oxidase produced by *Armillariella tabescens* (Cao et al., 2011) and peroxidase produced by *Pseudomonas* species (Zaid, 2017).

Plant-based Feed Additives

Plants display a variety of essential oils, spices, herbs, and other extracted products that can play a noble role in mitigating the health effects caused by mycotoxins (Gowda et al., 2013). They are considered eco-friendly, available, and affordable products to tackle not only mycotoxins but also many other problems in the feed and food industries (Iram et al., 2016).

Bile Acids

The liver is the detoxification centre and immune control centre of aquatic animals. The

most important organ that is damaged by the damage of mycotoxins is also the liver. Bile acids are an endogenous active substance secreted by the animal. Therefore, improving the detoxification function of the hepatopancreas of the shrimp to fight against mycotoxins is the most important measure to reduce the risk of mycotoxins. Toxins can stimulate the liver to secrete a large amount of bile, leading to malfunctioning of the liver.

Conclusion

Mycotoxins represent a significant but often overlooked threat to aquaculture, posing risks to the health and productivity of aquatic organisms. With a potential for adverse effects ranging from growth inhibition to organ damage and mortality. Addressing mycotoxin contamination requires proactive monitoring, implementation of quality control measures, and the use of mitigation strategies in feed production and aquaculture management. By raising awareness, enhancing testing protocols, and adopting best practices, aquaculture stakeholders can better safeguard their operations against the silent threat of mycotoxins, ensuring the sustainability and success of the industry.

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