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# Food, Fish and Mycotoxins

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**Review Article**

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**Abstract**

Due to their incidence and negative effects on public health, foodborne diseases are considered an issue of international relevance. Fish is considered a nutritious food and highly susceptible to contamination by physical, chemical, and biological agents responsible for diseases in animals and humans. Mycotoxins are biological contaminants of food with various harmful effects on human and animal health derived from their consumption, concentration, and exposure time. The presence of mycotoxins has been reported in different foods, mainly cereals, which are intended for human and animal consumption, such as feed processing. The presence of mycotoxins in fish is generally by way of feeding by supplying contaminated feed in aquaculture production activities, and the transfer can be generated in the food chain and affect human health. The objective of this bibliographic review is to provide relevant general information about mycotoxins in foods including fish, harmful effects on fish, especially tilapia, prevention, control, and regulation of these contaminants in foods intended for human and animal consumption, its negative impact on animal production and health, as well as human health through its transfer in the food chain.

*Keywords:* Aquaculture, *Tilapia,* feed, food safety

**Gıda, Balık ve Mikotoksinler**

Halk sağlığı üzerindeki olumsuz etkileri ve sık görülmeleri nedeniyle, gıda kaynaklı hastalıklar uluslararası önem taşıyan bir konu olarak kabul edilmektedir. Balık, besleyici bir gıda olarak kabul edilir ve hayvanlarda ve insanlarda hastalıklara neden olan fiziksel, kimyasal ve biyolojik ajanların bulaşmasına karşı oldukça hassastır. Mikotoksinler, tüketim, konsantrasyon ve maruz kalma sürelerine bağlı olarak insan ve hayvan sağlığı üzerinde çeşitli zararlı etkileri olan biyolojik gıda kirleticileridir. Mikotoksinlerin varlığı, insan ve hayvan tüketimine yönelik olarak üretilen tahıllar başta olmak üzere farklı gıdaların yem olarak işlenmesi aşamalarında bildirilmiştir. Balıklarda mikotoksinlerin varlığı, su ürünleri yetiştiriciliği faaliyetlerinde genellikle kontamine yem temin edilerek beslenme yoluyladır ve mikotoksinlerin transferi besin zinciri yoluyla oluşur ve bu durum insan sağlığını olumsuz etkileyebilir. Bu bibliyografik incelemenin amacı, balık dahil gıdalardaki mikotoksinler, özellikle tilapia olmak üzere balıklar üzerindeki zararlı etkileri, insan ve hayvan tüketimine yönelik gıdalardaki bu kirleticilerin önlenmesi, kontrolü ve düzenlenmesi, hayvansal üretim üzerindeki olumsuz etkisi, gıda zincirine aktarılması yoluyla insan sağlığı.üzerindeki etkileri hakkında genel bilgi sağlamaktır.

*Anahtar Kelimeler:* Su ürünleri yetiştiriciliği, *Tilapia*, beslenme, gıda güvenliği

## INTRODUCTION

For some years the concept of food quality and safety has become increasingly important worldwide (Rojas et al., 2017). Consumers have the right to access safe and quality food, placing them as the basis of a nutritious diet (FAO, 2019). Food safety is considered as the absence, or safe and acceptable levels, of food hazards that can harm the health of consumers (FAO, 2019). Annually, 600 million people get sick, and 420,000 die from Foodborne Diseases (FD); these diseases affect the general population, and being the children and low-income people the most vulnerable groups; besides, the impact of unhealthy food causes production losses for around USD 95 billion in low- and middle-income economies (FAO, 2019).

The products of animal origin (meat and by-products, eggs, fish, shellfish, milk, dairy products, among others), vegetables, water, and even ready-to-eat foods, are the main causes of foodborne illnesses since they are an available route of food and nutrition for consumers; during production, food can be subjected to scarce or zero hygiene conditions, short thermal processes and high handling, being these activities a potential risk to public health by having a high probability of acquiring various hazardous pollutants (Espinosa et al., 2014; Soto et al., 2016; Huertas et al., 2019).

It has been established that education and improvement in hygiene practices focused on food production and handling by consumers contributes to the reduction and control of foodborne diseases; highlighting that food safety is a shared responsibility among government, food industry, and consumers (FAO, 2019).

The objective of this bibliographic review is to provide general yet relevant information about mycotoxins in foods including fish, harmful effects on fish, especially tilapia, prevention, control, and regulation of these contaminants in foods intended for human and animal consumption, its negative impact on animal production and health, as well as human health through its transfer to the food chain.

**Foodborne diseases**

Foodborne diseases are considered a major public health problem as it is a major cause of morbidity, mortality and negative economic effects due to a decrease of productivity, health services, and surveillance measures in food safety, as well as a commercial detriment of food (Zuñiga and Caro, 2017; Palomino et al., 2018; Huertas et al., 2019).

These diseases are defined as those symptoms derived from the consumption of water or food contaminated with physical, chemical, or microbiological agents (Huertas et al., 2019). More than 250 foodborne diseases have been reported whose occurrence has been increasing in recent years, due to factors such as the globalization of the food market, changes in eating habits, and increased migration of infected people favoring the spread, reemergence, and appearance of pathogens in food (Zuñiga and Caro, 2017; Palomino et al., 2018; Huertas et al., 2019); it has even been established that factors such as the climate change affect the geographical occurrence and prevalence of contamination hazards in foods (FAO, 2019). Foodborne diseases can be infectious or toxic, whose casual agents are bacteria, viruses, fungi, parasites, heavy metals, or chemicals that penetrate the body through contaminated food or water (Ortega, 2014; Zuñiga and Caro, 2017; WHO, 2019).

Among the chemicals that involve health risks are those natural toxins and environmental pollutants (lead, mercury, cadmium, dioxins, and polychlorinated biphenyls) (WHO, 2019). Natural toxins include mycotoxins, marine biotoxins, cyanogenic glycosides, and toxins present in poisonous mushrooms. Basic foods such as cereals may contain high levels of mycotoxins, and prolonged exposure to these toxins can affect the immune system, normal development, or be a cause of cancer (WHO, 2019). Likewise, the consumption of foods of animal origin that were exposed to mycotoxins during their production (livestock and aquaculture) through feeding can constitute a risk to human health when consumed (Abrunhosa et al., 2012; Tolosa et al., 2013).

**Fish**

Fish is a perishable and very nutritious food because it is a source of biological value protein, vitamins, minerals, and polyunsaturated lipids (Sartori et al., 2012; Fuertes et al., 2014; Castillo et al., 2017). The nutritional quality, freshness, and safety of the fish is a function of factors such as species, age, medium in which they live, food, conditions of capture, cultivation, processing, conservation, storage, transport, and distribution (Fuertes et al., 2014; Avdalov, 2015).

Fish and products are the most commercialized food in the world mainly due to availability, access, and price in relation to the consumption of beef, pork and poultry (Anater et al., 2016; FAO, 2018). Worldwide, in 2016, the production in fishing activities was 90.9 million tons, while aquaculture production reached 80 million tons, with a total of 171 million tons, of which 88% was used for direct human consumption, reaching a *per capita* consumption of 20.3 kg; the remaining 12% was destined for non-food uses such as the preparation of flour and oil, that are considered among the nutritious and digestible ingredients to generate feed intended for the aquaculture production of fish; the aforementioned activity continues in a trend of greater growth with respect to other sectors dedicated to food production (FAO, 2018).

Fish is a food that is sensitive to deterioration, so adequate hygienic manipulation is necessary due to its chemical composition, autolytic and microbial activities, it is also susceptible to contamination from the environment where it lives, along with fishing, harvesting, handling, processing, conservation, including stages of its commercialization which can have a negative impact on the quality and safety of these foods (Vázquez et al., 2018). Fish is considered a food frequently responsible for outbreaks of foodborne illnesses, being causative agents of chemical and biological origin (bacteria, viruses, parasites, and biotoxins) (Arias and Buelga, 2005; Quijada et al., 2005; Espinosa et al., 2014; Ortega et al., 2014; Soto et al., 2016; Vázquez et al., 2018). Among the contributing factors to these diseases are cross-contamination, handling and storage temperatures, contaminated feed, infected handlers, economic and sanitary fraud by extracting resources in prohibited areas, among others (Arias and Buelga, 2005; Quijada et al., 2005; Espinosa et al., 2014; Soto et al., 2016; Vázquez et al., 2018). Currently, as a result of the demand, production, and commercialization of aquatic food, the quality of the fish available in the market in terms of the degree of freshness, diversity, and safety is sought and demanded to protect the health of consumers (Anater et al., 2016; Castillo et al., 2017; FAO, 2019a).

**Fungi, mycotoxins, and mycotoxicosis**

Fungi are microorganisms widely distributed in nature, they can be eukaryotes, heterotrophs, unicellular and multicellular, acidophilic, aerobic, they can grow in a wide temperature range between 0 °C and 55 °C, have a filamentous mass called mycelium that, according to their function, can be vegetative or reproductive, its functional and structural unit is the hyphae, they have a cell wall but no chlorophyll so they do not perform photosynthesis, can tolerate high concentrations of solutes, as well as extreme pH conditions (2 to 8), the reproduction can be sexual or asexual through the generation of spores, while their nutrition can be saprophytic or parasites (optional or forced) generating various diseases to plants, animals and humans (Cortés and Mosqueda, 2013; Cortés et al., 2016).

As saprophytes, fungi contribute to the breakdown of organic matter, as well as to soil fertility, being responsible for food spoilage and generating mycotoxins, with negative health effects through poisonings called mycotoxicosis, derived from the consumption of contaminated foods (Gomez, 2007; Cortés and Mosqueda, 2013; Cortés et al., 2016).

Mycotoxins are secondary metabolites of chemical diversity produced by filamentous fungi at the end of the exponential phase or stationary phase of growth by contaminating food, feed, or raw materials intended for food processing (Robledo et al., 2012; Rojas et al., 2017; Gonçalves et al., 2018; Darwish, 2019).

These compounds can be found in their conjugated form, soluble or incorporated into other macromolecules in the food, they are heat stable so they are not destroyed by cooking or industrial processes, and can pass into the food chain through the consumption of meat, milk, and derivatives, and when consumed they generate toxicological disorders called mycotoxicosis affecting human and animal health (Tapia et al., 2010; Rojas et al., 2017; Assefa and Geremew, 2018).

The formation of mycotoxins depends on different factors such as the genetic ability of the fungus to produce them (the presence of the toxigenic fungus in a substrate does not necessarily imply the presence of mycotoxins), substrate composition, water activity, temperature (20 °C-25 °C), relative humidity (80% -90%), pH (4-8), redox potential, parasites, pests (insects), climatic conditions, and storage time (Murcia et al., 2010; Serrano and Cardona, 2015; Robledo et al., 2012; Rojas et al., 2017). Usually, the substrates affected by mycotoxins are corn, wheat, sorghum, cotton cake, barley, and peanuts (Table 1) (Murcia et al., 2010).

Approximately 300 mycotoxins have been described of which the ones of the greatest health and toxicological interest are aflatoxins, ochratoxins, trichothecenes, zearelenone, fumonisin, citrinin, patulin, and ergot alkaloids that present the greatest threat to human and animal health (Denli and Perez, 2006; Kumar et al., 2013; Serrano and Cardona, 2015; Rojas et al., 2017; Assefa and Geremew, 2018; Darwish, 2019).

The negative health effects from exposure to these toxic metabolites may be carcinogenesis, teratogenesis, immunosuppression and clinical symptoms of neurotoxicity, nephrotoxicity, hepatotoxicity, dermotoxic, immunotoxic, myelotoxicity, and pulmonary or endocrine toxicity (Tolosa et al., 2013; Kumar et al., 2013; Serrano and Cardona, 2015; Assefa and Geremew, 2018). The mycotoxins' mechanisms of action vary and depend on their ability to inhibit proteins, inhibit DNA and RNA synthesis, induce lipid oxidation, cell death programming, and alter the structure and function of cell membranes (Pinton et al., 2019; Darwish, 2019).

The main mycotoxin-producing fungi belong to the *Ascomycetes* of the genus: *Aspergillus, Penicillium, Fusarium, Claviceps,* and *Alternaria,* which are spread by spores that are highly resistant to extreme conditions of temperature and pressure, and they germinate when conditions are appropriate (Lopez, 2012; Escórcio et al., 2015; Santillan et al., 2017; Assefa and Geremew, 2018).

**Table 1.** Different mycotoxins, fungi producers and generally contaminated substrates or food (Denli and Perez, 2006; Abrunhosa et al., 2012; Dinolfo and Stenglein, 2014; Cortés et al., 2016; Santillan et al., 2017; Rojas et al., 2017; ELIKA, 2018; Pinton et al., 2019)

| Producer fungi | Micotoxin | Common affected foods |
|---|---|---|
| *Aspergillus flavus*<br>*Aspergillus parasiticus*<br>*Aspergillus niger*<br>*Aspergillus ruber*<br>*Aspergillus fumigatus*<br>*Aspergillus terreus*<br>*Aspergillus candidus*<br>*Penicillium frecuentans*<br>*Penicillium variable*<br>*Penicillium puberulum* | Aflatoxins | Rice, corn, wheat, peanut, sorghum, coffee, pistachio, almond, nuts, figs, cotton seeds, spices, vegetable oils, milk and dairy products. |
| *Aspergillus ochraceus*<br>*Aspergillus carbonarius*<br>*Aspergillus alliaceus*<br>*Aspergillus melleus*<br>*Aspergillus ostianus*<br>*Aspergillus petrakii*<br>*Aspergillus sclerotiorum*<br>*Penicillium aurantiogriseum*<br>*Penicillium cyclopium*<br>*Penicillium chrysogenum*<br>*Penicillium expansum*<br>*Penicillium variabile*<br>*Penicillium purpurescens*<br>*Penicillium verrucosum* | Ochratoxins | Cereals, dry fruit, wine, grapes, coffee, cocoa and cheese. |
| *Fusarium culmorum*<br>*Fusarium graminearum*<br>*Fusarium poae*<br>*Fusarium sporotrichioides*<br>*Fusarium equiseti*<br>*Fusarium cerealis*<br>*Fusarium verticiilloides* | Zearalenone<br>Tricothecenes | Corn, wheat, rye, barley, oats, vegetable oils |
| *Fusarium proliferatum*<br>*Fusarium verticillioides*<br>*Fusarium anthophillum*<br>*Fusarium globosum*<br>*Fusarium liseola* | Fumonisins | Corn, wheat, barley, sorghum and sub-products. |
| *Fusarium poae* | Enniatin Beauvericin | Cereals and products. |
| *Aspergillus flavus*<br>*Aspergillus oryzae*<br>*Aspergillus caelatus*<br>*Penicillium griseofulvum*<br>*Penicillium chrysogenum* | Cyclopiazonic acid | Corn and peanut. |
| *Aspergillus terreus*<br>*Penicillium citrinum*<br>*Penicillium expansum*<br>*Monascus ruber* | Citrinin | Rice, rye and derivates, fruits. |
| *Penicillium expansum*<br>*Penicillium claviforme*<br>*Penicillium patulum*<br>*Penicillium vulpinum*<br>*Penicillium carneum*<br>*Aspergillus clavatus* | Patulin | Apples, apple juice and concentrates. |
| *Fusarium moniliforme*<br>*Fusarium acuminatum*<br>*Fusarium avenaceum*<br>*Fusarium poae* | Moniliformin | Cereals and derived products. |

| | | |
|---|---|---|
| *Acremonium coenophialum*<br>*Claviceps purpurea*<br>*Claviceps africana*<br>*Alternaria alternata*<br>*Alternata solani*<br>*Alternaria citri* | Ergot alkaloids | Rye, barley, wheat, oats, triticale, oilseeds. |

Mycotoxins are produced and located in many feed and foods, especially those of plant origin such as cereals during pre-harvest, post-harvest, transport, processing and storage (Assefa and Geremew, 2018; Gonçalves et al., 2018; Darwish et al., 2019).

The importance and economic impact of mycotoxin contamination can be reflected in various sectors of production and consumption due to the loss of food and feed, agricultural activities, analysis costs, and regulatory programs to minimize risks to human and animal health (Castillo and Duran, 2006; Gomez, 2007; Hernández et al., 2009; Assefa and Geremew, 2018; Gonçalves et al., 2018).

In addition to its importance in human and animal health and food safety, the transfer of mycotoxins through the food chain can be through different routes: I. Directly through unprocessed foods (cereals, legumes, oilseeds, fruits, spices), or processed (cereal products, wine, coffee, juices, beer, and porridges) from contaminated crops that cause primary mycotoxicosis, and II. Indirectly through food from animals that have consumed contaminated feed such as meat, eggs, and milk, generating secondary mycotoxicosis (Gomez, 2007; ELIKA, 2018).

**Aquaculture and mycotoxins**

Aquaculture globally contributes about half of the total foodstuffs of aquatic origin destined mainly for human consumption, being, therefore, an important part of the food industry (Anater et al., 2016; FAO, 2018).

In the hygiene and sanity of the fish, the sanitary conditions of ponds influence animal health, and consequently the public health, due to meat consumption; some factors such as overcrowding, malnutrition, and poor water quality are those that mainly favor diseases in farmed fish; within the diet, the presence of toxic substances such as mycotoxins in feed are factors that generate stress and diseases in fish, so controlling the contamination of fungi and mycotoxins is relevant (Tolosa et al., 2013; Anater et al., 2016; Millan et al., 2017; Gonçalves et al., 2018). Mycotoxins in aquaculture have a significant negative economic and health impact after exposure to a contaminated diet, which results in growth abnormalities, immunological, physiological and histological imbalances that also result in decreased yield and profitability of production (Villarreal et al., 2014; Escórcio et al., 2015; Anater et al., 2016). Frequent mycotoxicosis in fish is caused by aflatoxins, ochratoxins, zearalenone, and trichothecenes (Tolosa et al., 2013; Gonçalves et al., 2018). The presence of mycotoxins in aquatic species is mainly due to the ingestion of contaminated foods that have been made with material of plant origin (cereals, legumes, or by-products) used as food ingredients that are excellent substrates for fungal growth, specifically in favorable conditions of humidity and temperature (Tolosa et al., 2013; Escórcio et al., 2015; Gonçalves et al., 2018). Different toxins such as aflatoxins, deoxynivalenol, zearalenone, fumonisins, ochratoxins A, and T-2 / HT-2 toxins have been commonly reported in animal feed (Huerta et al., 2016).

**Effect of mycotoxins in fish**

The effects of mycotoxins vary depending on the type, concentration, and period of exposure of the animal to these substances, as well as the species, sex, and age of it (Anater et al., 2016). The effects may include gastrointestinal disorders, reproductive and growth disorders, suppression of the immune system, renal damage, liver damage, carcinogenic, genotoxic effects, bioaccumulation phenomena (with the derivative risk to human health due to meat consumption), and in severe cases, the exposure leads to fish mortality with the consequent economic and production losses (Tolosa et al., 2013; Anater et al., 2016; Gonçalves et al., 2018).

**Tilapia**

Tilapia are those fish originating from Africa, belonging to the family *Cichlidae* genus *Tilapia* and *Oreochromis*, having relevance in the production of animal protein in temperate, tropical, and subtropical waters globally as a source of sustainable, economical, and high-quality protein to meet the demand derived from the constant increase of the human population (Wicki and Gromenida, 1998; Bautista and Ruiz, 2011; INP, 2018; Jacome et al., 2019). The species of the genus *Oreochromis* are those of greater acceptance in commercial cultivation, highlighting *O. niloticus*, *O. aureus, O. mossambicus,* and *Oreochromis* spp., due to factors such as the versatility of eating habits (including formulated food), rapid growth, high density and ability to tolerate adverse conditions in culture (Wicki and Gromenida, 1998; Bautista and Ruiz, 2011; Jacome et al., 2019). Tilapia is one of the most popular species and aquaculture production, being China, the main producing country contributing 10% worldwide in 2016 (Magouz et al., 2018; FAO, 2018). Once in cultivation when reaching its commercial weight, the tilapia harvest for human consumption is generally in its entire fresh form or fillet (Balbuena, 2014; Jacome et al., 2019).

The use and consumption of feed at a global level in aquaculture activities is increasing due to availability and profitability, and where tilapia production is no exception (FAO, 2018). And the tilapia culture is prone to mycotoxin exposure through its diet with negative effects on aquaculture production, health, and safety (Deng et al., 2019).

Different studies around the world have been reported tilapia and their exposure to mycotoxins and have revealed the negative impact on the animal health of these compounds. For example, Anater et al. (2016) studied the effects on Nile tilapia and tilapia (*O. niloticus*) of aflatoxin (AFB1) in periods ranging from 5, 10, 15, and 20 weeks through feeding with doses of 5, 29, 100, 200, 245, 793, 1641, 2500 µg / kg, being among the different negative effects growth reduction, weight reduction, yellow or black coloration on the body surface, abnormal behavior, hematocrit involvement and growth performance, mortality (16.7%) (200 µg / kg) and reduction of survival up to 67% (5-38.62 µg / kg); while for exposure to fumonisins by *Oreochromis niloticus* at concentrations of 150,000 µg / kg for 4 weeks through feeding, the effects developed were weight reduction and hematocrit.

Magouz *et al*. (2018) stated that exposure to aflatoxins B1 (150ppb) for 16 weeks through feeding and showed a negative effect on the growth and survival of 13.5g of *O. niloticus*. Meanwhile, Deng *et al*. (2019) noted that in *O. niloticus* culture of 15 g of weight exposed for 20 days to diets with trichocethenes (T-2) (4.8, 7.2, 10.8, 16.2, and 24.3 mg / kg) there were mycotoxins produced by *Fusarium* species that infect cereal crops (corn, wheat, and soy); the survival rate, weight gain, and hepatosomatic index, induced damage of liver cells and myofibers, an increase of T-2 residues in the liver and fish muscle were reduced.

**Control and prevention of mycotoxins in food and feed**

Mycotoxin-producing fungi are widespread throughout nature, can contaminate crop plants (corn, wheat, rye, among others) and produce toxins during growth, post-harvest, storage, transportation, processing, and use as feed in the farm, as well as being present in foods such as milk and eggs that are indirectly contaminated when producing cattle are fed contaminated cereals (Denli and Perez, 2006; Hernández et al., 2009; Lopez, 2012; Anater et al., 2016).

Mycotoxins are heat-stable compounds and it is difficult to eliminate them once they have been synthesized; therefore, food control is through the prevention of fungal growth and contamination or once these molecules are present by detoxification or decontamination of food through physical, chemical, and biological methods, the latter only used in animal feed (Tapia et al., 2010; Cortés et al., 2016; ELIKA, 2018; Darwish, 2019).

It has been determined that the implementation of good hygiene practices in the entire food production chain (cultivation, collection, storage, processing, packaging, transport, and storage of food), hazard analysis and control of critical points (HACCP), as well as maximum concentration limits present, are effective measures to reduce the risk of exposure to mycotoxins through food in human and animal health (Gomez, 2007; Lopez, 2012; Espinosa et al., 2014; Cortés et al., 2016; ELIKA, 2018; Assefa and Geremew, 2018).

Meanwhile, and regarding the activities for aquaculture food production, some good hygiene practices have been established and implemented, in which are included some focused on cultivation conditions according to the species, water quality, facilities, transport, and animal feed (CXC 54-2004; MAAMA, 2014; Zahran et al., 2020). Currently, to guarantee the safety of aquaculture products, animal welfare, and health, various certification standards have been developed based on good hygiene practices and implementation of HACCP plans such as Global GAP, Accredited Fish Farm Scheme, Bio Suisse, Krav, Safe Quality Food (SQF), ISO 22000 among others (MAAMA, 2014).

In the matter of prevention and control of negative effects due to the presence of mycotoxins in feedstuffs in aquaculture feeding, studies with favorable results have been carried out involving the reduction of negative effects on animal health and increasing the defense capacity of fish and metabolism against toxins, which is why natural additives (phenolic compounds, selenium, vitamins) or synthetic antioxidants (BHA or BHT) have been recommended in diets during feeding, and thus improving food safety (Agouz et al., 2011; Rahman et al., 2017; Magouz et al., 2018; Tewodros et al., 2018; Deng et al., 2019) or by applying adsorbent substances to detoxify the food (Huwing et al., 2001; Tapia et al., 2010; Tewodros et al., 2018; Zahran et al., 2020). In aquaculture feeding, inherent costs are an important point in production, so products that improve feed efficiency and animal health are of special interest (Tewodros et al., 2018).

**Analysis of mycotoxins in the laboratory**

The presence or contamination by mycotoxins in food and feedstuffs is often unavoidable, producing toxicity in foods at minimum concentrations; therefore, it is necessary to have sensitive and reliable methods for their detection (Chauhan et al., 2016). Food analysis in search of mycotoxins is part of the mechanisms of control and prevention of poisoning, and involves a series of steps that cover sampling, preparation, extraction, cleaning, and detection through various techniques such as: Enzyme-Linked Immunosorbent Assay (ELISA), analysis with biosensors and instrumental methods such as Thin Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC) in its different adaptations such as coupled with ultraviolet (UV), diode array (DAD), HPLC- FLD (HPLC coupled with fluorescence) and HPLC-MS / MS (HPLC coupled with mass spectrometry), gas chromatography (GC) coupled with electron capture (ECD), flame ionization (FID) or MS detectors (Stark, 2009; Arroyo et al., 2014; Chauhan et al., 2016; Assefa and Geremew, 2018; Gonçalves et al., 2018). Molecular techniques have also been developed in the detection of mycotoxin-producing fungi, indicating the potential hazard in food (Fungaro et al., 2004; Stark, 2009; Gong et al., 2015).

An important point in the analysis of mycotoxins in foods is the collection of the sample since the distribution and concentration of mycotoxins can be heterogeneous and the concentration in analyzed material can be determined erroneously (Lopez, 2012; Cortés et al., 2016). According to the above, the following recommendations can be taken in the sampling phase: 1. Collect a greater weight and consider a greater number of sampling points; 2. Subsampling with an increase in the weight of the subsample, or by reducing particles due to milling processes; 3. An analysis of a larger number of samples; 4. The sample should be collected as close as possible to the place where the intoxicated animal was fed; 5. Obtaining and analyzing blood samples and testing of animal organs facilitates a retrospective analysis of some contamination, mainly in situations where food availability is involved. At the end of the sample collection, it must be sent to the laboratory as soon as possible for analysis, not exceeding 48 hours (Cortés et al., 2016). Likewise, in the context of food sampling for the detection of mycotoxins, sampling plans have been developed for their detection through regulation N$^{o}$ 401/2006 of the European Communities Commission and for the particular case of aflatoxins in cereals such as corn and peanuts the Food and Agriculture Organization (FAO) has developed a sampling plan for the efficacy and precision in the detection of toxic compounds (FAO,1993).

**Sanitary regulation of mycotoxins in human food and animal feed**

Around the world, there is a need to ensure food safety, human, and animal health through regulations that set limits on the concentration of mycotoxins in food and feed (Darwish, 2019). Through the sanitary regulation, several countries have established the permissible limits of mycotoxins in agricultural products, mainly of aflatoxins; for example, in the United States of America, 20 µg / kg (B1 + B2 + G1 + G2) are allowed in food, 0.5 µg / kg of aflatoxin M1 in milk, and table 2 shows the limits for other foods and feed; meanwhile, in the European Union, raw cereals and derivatives with 5 µg / kg and 3 µg / kg of ochratoxin A are allowed, and the sanitary regulations of the food of the Republic of Chile indicate that only up to 5 µg / kg of aflatoxins B1 should be allowed, B2, G1 and/or G2 in food, and up to 0.05 µg / kg of M1 in milk (Murphy et al., 2006;Martinez et al., 2013; Serrano et al., 2015).

The European Union legislation establishes a set of rules to ensure that food and feed are safe and healthy through the agri-food chain, among which the regulation (EU) 2017/625, regarding controls and other official activities carried out to ensure the application of food and feed legislation, and of the rules on animal health and welfare, plant health and plant protection products stands out. Directive 96/23/EC relates to the applicable control measures regarding certain substances and their residues in live animals and their products. Among these substances, the mycotoxins are considered. Commission Regulation (EC) 1881/2006 sets the maximum content of certain contaminants in food products for human consumption based on cereals, dairy products, and fruits including mycotoxins (Aflatoxins, Ochratoxin A, Patulin, Deoxynivalenol, Zearalenone, and Fumonisins). Commission Recommendation 2006/583/EC refers to the prevention and reduction of Fusarium toxins in cereals and cereal products. Corresponding to undesirable substances in animal feed about feed, they are regulated in the European Union through Directive 2002/32/EC. Commission Recommendation 2006/576/EC establishes maximum limits of deoxynivalenol, zearalenone, ochratoxin A, toxins T-2 and HT-2, and fumonisins in products intended for animal feed, including guideline values of fumonisins in the fish feed (Table 3). And the Commission Regulation 884/2014, where special conditions are imposed on the importation of feed and food that may be contaminated by aflatoxins.

International organizations such as The Food and Agriculture Organization (FAO) a specialized agency of the United Nations, through Codex Alimentarius, have issued different codes of hygiene practices to prevent mycotoxins contamination in specific foods or susceptible ones to the presence of these compounds. Some of these codes are the code of practice to prevent and reduce contamination of mycotoxins cereals (CXC 51-2003), code of hygiene practices for foods with low moisture content (CXC 75-2015), code of hygiene practices for spices and dried aromatic herbs (CAC / RCP 42 -1995), and code of practice for the prevention and reduction of mycotoxins contamination in spices (CXC 78-2017).

**Table 2.** Regulation and permissible limits of the United States of America about mycotoxins in different foods (Murphy et al., 2006)

| Micotoxin | Food or feed | Maximum allowable limit |
|---|---|---|
| Patulin | Apple juice and derivates | 50ppb |
| Deoxynivalenol DON | Wheat products | 1ppm |
| Aflatoxins B1+B2+G1+G2 | Peanuts destined to process | 15ppb |
| Fumonisin B1+B2+B3 | Dry grounded corn products | 2ppm |
| | Maize and sub-products destined to: Pigs and catfish | 20ppm |
| Aflatoxins B1+B2+G1+G2 | Maize, peanut products, and other animal feed and food ingredients, except cottonseed meal, intended for immature animals | 100ppb |
| | Maize, peanut products, cottonseed meal, and other animal feed ingredients for dairy animals, animal species or when the intended use is unknown | 20ppb |

*ppm: parts per million; ppb: parts per billion.

Contributing to the prevention of mycotoxins contamination in 2003, the FAO developed a manual on the application of the Hazard Analysis and Critical Control Point (HACCP) system in the prevention and control of mycotoxins, which is applied by the food industry and official authorities for the control of hazards associated with food such as pathogenic microorganisms and toxic chemicals (FAO,2003).

In the approach to animal nutrition and health, the World Health Organization (WHO) since 1999 has produced a technical report that exposes and explains the different hazards (mycotoxins) of food associated with aquaculture products and strategies to guarantee their safety, like good aquaculture practices which cover aspects of breeding and feeding, as well as the implementation of HACCP system (WHO, 1999).

In 2003, FAO developed technical guidelines for suitable procedures in the manufacture of food for aquaculture so that producers of these foods can supply quality products (FAO, 2003a). The code of practice on good animal nutrition (CXC 54-2004) and general standard for contaminants and toxins present in food and feed (CODEX STAN 193-1995), which indicates the use of good agricultural practices, and manufacturing for hazard reduction, maximum levels, and related sampling plans for pollutants and natural toxic substances (mycotoxins) found in food and feed and that should be applied to internationally marketed products to reduce the risk to public health.

In Latin American countries such as Mexico, food safety legislation is through the "NOM-247-SSA1-2008" standard that refers to sanitary specifications for aflatoxins (20 µg / kg maximum limit) in food for human consumption such as cereals, cereal flours, semola, as well as food-based on: cereals, edible seeds, flour, semola or their mixtures. Meanwhile, the "NOM-243-SSA1-2010" standard establishes the sanitary specifications for products of animal origin such as milk, milk formula, combined dairy product, and dairy products for human consumption in the field of aflatoxins M1 (0.5 µg / L maximum limit). In both standards, it does not refer to other mycotoxins, which leads to a review and update of them.

**Table 3.** Mycotoxin regulations on feed in the European Union (Directive 2002/32 / EC; Commission Regulation 574/2011; Commission Recommendation 2006/576 /EC)

| Micotoxin | Products destined to animal feed | Maximum content in mg / kg (ppm) in feed calculated on the basis of a content of 12% humidity |
|---|---|---|
| **Aflatoxin B1** | All feed materials | 0.02 |
| | Complementary feedingstuffs | 0.02 |
| **Rye Ergot (*Claviceps purpurea*)** | • All feedingstuffs containing unground cereals | 1000 |
| | • Compound feed for cattle (except dairy cattle and calves), sheep (except dairy sheep and lambs), goats (except dairy goats and kids), pigs (except piglets) and poultry (except young animals). | 0.02 |
| **Deoxynivalenol** | Raw materials for feed: | |
| | • Cereals and cereal products, except for maize by products | 8 |
| | • Maize by-products | 12 |
| **Zearalenone** | Feed materials | |
| | • Cereals and cereal products, except for maize by-products | 2 |
| | • Maize by-products | 3 |
| **Ochratoxin A** | Feed materials: | |
| | • Cereals and cereal products | 0.25 |
| **Fumonisins B1 + B2** | Feed materials: | |
| | • Maize and maize products | 60 |
| | Complementary and complete feed for: | |
| | • Pigs, horses (equidae), rabbits, and pets | 5 |
| | • Fish | 10 |
| | • Poultry, calves (under 4 months), lambs, and kids | 20 |
| | • Ruminants older than four months and minks | 50 |

* General data. For specifications, check complete regulations.

On the other hand, and regarding the animal health and production in the context of food intended for animal consumption, the "NOM-061-ZOO-1999" standard establishes the zoo sanitary specifications of food products including mycotoxin levels. Meanwhile, "NOM-188-SSA1-2002" establishes the control of aflatoxins in cereals for human and animal consumption. It claims that concentrations below 20 µg / kg of total aflatoxins in cereals are tolerated for human consumption, and from 21 to 300 µg / kg, the cereal may only be used for animal consumption. In aspects of production, health, and food safety in aquaculture activities, manuals of good practices have been established contemplating aspects of hygiene and handling of food for animal consumption (SENASICA, 2019), and specifically for aquaculture production of tilapia fish (García and Calvario, 2008).

## CONCLUSION

Food safety is the guarantee that an ingested food negatively affects the health of the consumer, and this characteristic is considered a basic, mandatory, and non-negotiable concept in the entire food production chain.

Fish is a food of frequent and varied consumption worldwide due to its nutritional value, being the main source for humans through fishing and aquaculture activities.

Aquaculture worldwide is reported (FAO) to supply almost half of the fish for human consumption and other various uses such as the generation of animal feed and is considered one of the main economic and food-generating activities.

Aquaculture activities include feeding and handling food for fish; these activities are therefore essential in the risk of fish exposure to different hazards such as mycotoxins, thus compromising animal health, favoring the transfer of these pollutants in the food chain and in turn generating a risk to human health.

Within the activities of surveillance, prevention, and control of contamination by mycotoxins in the food chain; specifically in fish and aquaculture activities, there are the establishment and implementation of sanitary regulations in the matter of animal feeding and limits in the concentration of the different potentially contaminating mycotoxins, implementation of procedures of good hygiene practices (with emphasis on animal feeding and animal health), and the Hazard Analysis and Critical Control Points (HACCP) system, the latter being accepted worldwide as actions to achieve food safety, protect human and animal health.

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