



Prevention of mycotoxins' effects — from field to table

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ABSTRACT

It is assumed that mycotoxins have been present in feed and food since the beginning of eukaryotic fungi's life on Earth. With the recognition of the symptoms of the first intoxications, the so-called mycotoxicosis, there was a desire to find strategies in the fight against secondary metabolites of different types of fungi. The mycotoxins that most commonly contaminate feed are aflatoxin B1 (AFB1), deoxynivalenol (DON), zearalenone (ZEN) and fumonisin B1 (FB1). These mycotoxins can primarily cause hepatotoxicity, immunotoxicity, neurotoxicity and nephrotoxicity, and consequently cause adverse effects on animal health and performance. Today, in the 21st century, the need to find a multidisciplinary and integrated plan in the fight against mycotoxins has grown with the realization that mycotoxins cause large-scale damage in livestock. Physical, chemical, biological and nutritional strategies have been developed to combat mycotoxins in the feed industry. Meanwhile, the use of each of these strategies achieves benefits, but also has drawbacks, including being expensive or impractical to apply on a large scale.

1. Introduction

Mycotoxins are secondary metabolites of different species of fungi that can cause chronic or acute toxicity in animals. Although a large number of mycotoxins have been identified, those of feed safety importance are primarily produced by the five fungal genera *Aspergillus*, *Fusarium*, *Penicillium*, *Claviceps* and *Alternaria*. Aflatoxin B1 (AFB1), deoxynivalenol (DON), zearalenone (ZEN), and fumonisin B1 (FB1) are well known as the major mycotoxins that contaminate feed, such as corn, barley, wheat, and their by-products (Zhao *et al.*, 2021). The most toxic mycotoxin is AFB1, mainly produced by *Aspergillus*, which is classified as a group 1 carcinogen (Zhang *et al.*, 2019). It shows hepatotoxic, immunotoxic, mutagenic, carcinogen-

ic and teratogenic properties in many animal species. DON and trichothecene type B cause anorexia and vomiting and can compromise intestinal and immune functions in all animal species by inhibiting nucleic acid and protein synthesis. ZEN has a similar structure to oestrogen and, therefore, competes with 17- β -oestradiol for binding to oestrogen receptors, which consequently leads to disruption of the reproductive capacity of animals. FB1 is the most abundant fumonisin, and which can cause hepatotoxicity, neurotoxicity, nephrotoxicity, immunotoxicity, developmental toxicity, and cancer in humans and animals (Chen *et al.*, 2021).

It has been proven that mycotoxins have a significant negative impact on animal health and performance, as well as on the quality and safety of food of animal origin, which has led to a great chal-

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lenge for the scientific and professional public. Many mycotoxins, especially *Fusarium* toxins (zearalenone, trichothecenes), can be synthesized during different stages of plant development. That process is often forced by unfavourable conditions for plant development, mechanical damage to plants, nutritional imbalances, temperatures unusual for the season, and frequent rainfall. As a preventive measure, it is necessary to implement good agricultural practices. After grain storage, physical, chemical and biological strategies have been developed to detoxify mycotoxins. The time between storage and potential application of the selected treatments should be as short as possible considering the very short time required for the synthesis of mycotoxins (sometimes only 2–3 days). When designing recipes, there is also a nutritional approach to reducing the harmful effects of mycotoxins (Liu *et al.*, 2020). However, many techniques have been shown to be ineffective, expensive, or impractical to apply on a large scale.

2. Physical methods

Decontamination of mycotoxins by physical techniques in practical conditions is mostly cellar washing processes or solvent extraction, sorting or heating of grain.

According to the properties of water- or fat-soluble mycotoxins, cereals can be decontaminated by washing with water or by extraction with organic solvents. Flotation and water washing can remove 51–72%, 64–69%, 2–61% and 73–74%, respectively, of aflatoxin, trichothecenes, ZEN and fumonisins from grains (Matumba *et al.*, 2015). Flotation and rinsing with an aqueous solution consisting of 10–30% NaCl, 30% sucrose, or 1 mol/L sodium carbonate can increase the rate of fumonisin removal from corn and wheat. A combination of washing technology and manual sorting together can reduce 84% of fumonisins (Westhuizen *et al.*, 2011). Solvents commonly used for mycotoxin extraction include methanol, ethanol, hexane, acetonitrile, isopropanol, and aqueous acetone. However, these methods have major drawbacks, as they lead to loss of nutrients and are expensive due to drying and disposal of toxic extracts, which limits their large-scale application.

Mycotoxins are not evenly distributed in the grain and mostly occur in mouldy, broken and discoloured parts. Meanwhile, the specific gravity of grains contaminated with mycotoxins is relatively lower than normal. These characteristics allow sieving, aspiration, and gravity separation to be used to isolate

grains contaminated with mycotoxins (Tibola *et al.*, 2016). Aspiration and gravity separation methods can reduce DON in wheat (Salgado *et al.*, 2011).

Drying, as a treatment with the main goal of lowering the moisture in cereal grains, is still the most widely used method. Thermal treatment has been applied for the decontamination of mycotoxins in animal feed for many years. The effectiveness of this method depends on the chemical structure and concentration of mycotoxins, temperature, duration, moisture content, pH and ion concentration during heat treatment. AFB1, DON, ZEN and FB1 are thermally stable compounds with decomposition temperatures higher than 237, 175, 220, 150°C, respectively, which makes elimination by conventional heat treatment difficult. Conventional hydrothermal treatment (cooking) under pressure (0.10 MPa) at 160°C for 20 minutes can degrade AFB1 by 78–88% in rice, while heating under pressure (0.10 MPa) at 120°C for 4 hours can degrade AFB1 by 95% in wet peanut powder (Fan, 2003). However, thermal treatments use an excessive amount of energy, so the Maillard reaction caused by high temperature reduces the nutritional value of feed ingredients. This has led to limitations in the application of heat treatments in the feed industry.

3. Chemical methods

Chemical techniques can destroy the structure of mycotoxins, creating mildly toxic or non-toxic products. Decontamination of mycotoxins by chemical techniques primarily involves treatment with acids and bases, as well as other treatments with chemical agents (Jalili & Son, 2011).

Preventing the growth and development of mould is mainly based on the use of chemical agents that maintain low water activity (*a_w*) in the substrate. It is imperative that none of the chemicals used be toxic to animals. Organic acids, such as sorbic, benzoic, propionic, acetic and formic, are very often used as preservatives, especially for stored grain foods.

Given that salts are more soluble in water, suitable salts of potassium, sodium (sodium sorbate) or calcium (calcium propionate) are generally used. The mechanism of action of preservatives is based on inhibition of enzymatic activity in fungal cells (propionic and sorbic acid) or on damage to mould membranes (natamycin), but they cannot reduce the content of mycotoxins already present in feed. Propionic acid, which has pronounced antigerminative properties, is most often used in the animal feed industry. Alkaline chemicals, including ammo-

nia, sodium hydroxide, potassium hydroxide, and sodium carbonate, have been used to destroy various mycotoxins in mouldy feed (Fang et al., 2020). Although the application of these chemical agents almost completely destroys mycotoxins, some agents lead to significant nutritional losses and a negative impact on palatability, which consequently causes worse feed consumption.

4. Biological methods

Screening and isolation of naturally occurring microorganisms that show the ability to biotransform against specific mycotoxins is a modern strategy to combat this problem. Mycotoxin biodegradation technology is a process by which the toxic group of mycotoxin molecules is broken down and destroyed by secondary metabolites produced by microorganisms or their secreted intracellular and extracellular enzymes, while non-toxic or less toxic degradation products are produced.

A number of different fungi have been shown to detoxify AFB1. Certain fungal strains such as *Saccharomyces cerevisiae* degrade AFB1 at levels of 69.0% (Chlebicz & Śliżewska, 2020). Similarly, some studies reported that different strains of *Aspergillus niger* showed the ability to degrade AFB1 at levels of between 88.6% and 98.7% (Fang et al., 2020). Bacteria degraded aflatoxin mainly by secreting extracellular enzymes. Some strains of *Nocardia corynebacterioides*, *Flavobacterium aurantiacum* and *Bacillus* have been shown to degrade AFB1. Microorganisms metabolize ZEN mainly through conversion or degradation into α -zearalenol, β -zearalenol, sulphate, and other secondary metabolites with low toxicity. *Bacillus natto* and *Bacillus subtilis* strains have been shown to remove ZEN from liquid media: more than 75% of ZEN can be biodegraded after incubation. Some fungal and bacterial microorganisms have been reported to be able to degrade fumonisins. Styriak et al. (2001) examined two strains of preserved laboratory yeast that were able to significantly degrade fumonisins in the culture medium. One is *Saccharomyces cerevisiae* IS1/1, which can degrade 45% of FB1 and 50% of a mixture of FB1 and FB2 in the culture medium, and the other is *Saccharomyces cerevisiae* SC82, which also degrades FB1 and a mixture of FB1 and FB2; the degradation rates were 22% and 25%, respectively. Together with the use of biotechnology, the activity of modern preparations in the fight against mycotoxins is based on these principles.

5. Nutritional approach to mitigating the effects of mycotoxins

It is possible to prevent the harmful effects of mycotoxins with an adequate correction of the feed recipe. Detoxification systems, including CIP450, ketoreductase and α -glutathione transferase, can degrade mycotoxins (Zhang et al., 2016). Therefore, any nutrient that can promote the normal functioning of one of the above detoxification enzyme systems can be used as a strategy. Glutamate, cysteine and glycine can be used as substrates for glutathione synthesis. On the other hand, mycotoxins can reduce nutrient intake, so adding critical nutrients is one way to mitigate the adverse effects of mycotoxins (Liu et al., 2020). Oxidative stress is an important mechanism of mycotoxin-induced cytotoxicity (Zhang et al., 2016). Addition of antioxidants to feed contaminated with mycotoxins can improve the antioxidant capacity of the body and increase the resistance of animals to mycotoxins. Selenium and vitamins A, C and E and their precursors have pronounced antioxidant properties. Since most mycotoxins negatively affect the digestibility of proteins, and inhibit protein synthesis, as one of the mitigation methods recommended is to use feed with 1–2% more protein than usual levels. Andretta et al. (2012) suggested that methionine can moderate the adverse effects caused by DON in growing pigs. Dietary supplementation of glutamic acid, arginine, aspartate and lysine had positive effects on remission of DON-induced visceral disease, increase in antioxidant capacity and improvement of physiological and biochemical indices in the blood of fattening animals.

In practice, the most widely applied method of mitigating or eliminating the harmful effects of mycotoxins is the use of adsorbents. Adsorbents are substances that are not resorbed from the intestines, and have the ability to physically bind certain chemical substances, thus preventing their resorption. Any ideal mycotoxin adsorbent should possess at least the following properties: high adsorption capacity against mycotoxins (especially mycotoxins with low hydrophobicity), low nonspecific binding to nutrients, as well as high safety, stability, and palatability (Daković et al., 2007).

Medicinal charcoal is a carbon-containing substance obtained by pyrolysis of organic matter that is then subjected to activation processes in order to obtain a highly porous structure. Activated medicinal charcoal has high mycotoxin-adsorbent properties, being especially effective against AFB1 and ochratoxin A. The negative side of using medicinal char-

coal is the variable degree of adsorption of nutrients (vitamins and microelements), colouring of feed, but also a high proportion (>1%) in feed mixtures that significantly reduces the energy and nutritional value. Bentonites are adsorbents that have a lamellar crystal microstructure and a different chemical composition, and the adsorptive capacity depends on the presence of exchangeable cations (Na^+ , K^+ , Ca^{+2} , Mg^{+2}) in the lattice. Bentonites bind AFB1 and moderate the toxic effects of the T-2 mycotoxin group.

Zeolites are crystalline, hydrated aluminosilicates of alkaline and alkaline earth cations. They have an infinite three-dimensional crystal structure. They have the ability to lose and receive water without major structural changes and to exchange some of their constitutional cations. Zeolites, precisely because of their structures, are applied in the adsorption of mycotoxins, since these crystalline compounds are used as molecular sieves and cation exchangers (Daković *et al.*, 2007). Zeolites are formed from an aluminosilicate network $(\text{SiO}_4)_4$ and $(\text{AlO}_4)_4$, in which the basic building unit is a tetrahedral structure (TO_4) with silicon or aluminium atoms at the centre, and oxygen atoms at the corners. The tetrahedra connect to each other in various ways, making the zeolite structure rich in channels and cavities. In this way, molecules are separated according to the molecular sieve system, a characteristic feature of most zeolite minerals. If the pore size is compatible with the mycotoxin molecule, a high degree of adsorption is observed. For natural zeolites to be effective in feed, a relatively high proportion in feed (about 1%) is needed, which means zeolites significantly negatively affect the amount of nutrients in the feed.

Latest-generation adsorbents have been developed from the cell wall components of microorganisms. Glucmannan is a common adsorbent that

cannot be used by gut microbes. Mycotoxins can be adsorbed by esterified glucmannan, which is a type of broad-spectrum mycotoxin adsorbent with an effective binding capacity for aflatoxin, ZEN, fumonisin and DON of 95%, 75%, 59% and 12%, respectively. It has been proven that esterified glucmannan somewhat mitigates the harmful effects of mycotoxins when it comes to performance, immunity, haematological and biochemical indicators in the blood of broilers (Vila-Donat *et al.*, 2020). At the beginning of the era of microbial adsorbents, bacteria were shown to adsorb mycotoxins to form a complex and then excrete them together with the toxins, thereby reducing the hazard (Liu *et al.*, 2020). Besides yeasts, lactic acid bacteria are the most studied microbial adsorbents. *Lactobacillus casei* can significantly reduce the absorption of aflatoxin in the intestinal tract. Zeng *et al.* (2009) reported that *Lactobacillus plantarum* F22 had a strong adsorption capacity for AFB1 and that the adsorption rate could reach 56.8%.

6. Conclusion

The occurrence of mycotoxins is a major concern and an unavoidable problem in the feed industry worldwide. Mycotoxins also threaten human health through the cycle of the food chain. This review summarizes a number of strategies for reducing mycotoxin contamination that are most commonly applied in terms of physical detoxification, chemical treatments, biological detoxification methods, and nutritional strategies. However, with growing awareness of environmental protection as well as feed and food safety, there is a growing expectation for more green and innovative technologies to control mycotoxin contamination.

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