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MYCOTOXINS IN FEED: AN OVERVIEW ON BIOLOGICAL EFFECTS AND DECONTAMINATION METHODS

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Abstract

Mycotoxins, secondary metabolites, produced by toxigenic fungi genera such as Penicillium, Aspergillus and Fusarium are widely known as one of the main causes for foodborne diseases. Not surprisingly, mycotoxins prevalence is higher with conditions such as climatic changes, lack of control systems and plentiful of suitable substrates, thus causing serious risks for both human and animal health. Since chemical and physical decontamination are not sufficiently effective, biological transformation is considered to be the most promising approach to reduce mycotoxin concentration. To detoxify mycotoxin-contaminated feed, the most frequent method for industrial purposes is the inclusion of sorbent materials that will remove toxins through selective adsorption, during passage through gastrointestinal tract. Another reliable approach is the addition of enzymes or microorganisms capable of detoxifying some mycotoxins. Although the process of identification and characterization of degrading enzymes is time consuming, it is necessary in order to understand the mechanism of degradation. The use of enzymes has some benefits in comparison with the use of microorganisms such as: reproducible performances, no risk of contamination and no safety concerns.

Key words*: mycotoxins, feed, degradation, enzymes, biotransformation.*

INTRODUCTION

Mycotoxins, secondary metabolites with low molecular weight are produced mainly by toxigenic fungi such as *Penicillium, Aspergillus* and *Fusarium* (Denli et al., 2015).

The term mycotoxin was used for the first time in history in 1962 after an unusually veterinary crisis near London during which nearly 100,000 turkey poults died. Turkey X disease was linked to a groundnut meal contaminated with secondary metabolites of *Aspergillus flavus* and that sensitized scientists to the possibility that other unknown mold metabolites might be deadly (Zain, 2011).

Not surprisingly, mycotoxins prevalence becomes higher with the available supporting conditions such as proper climate changes and lack of controls.

It is a well-known fact that the earth is warming at an unprecedented rate and therefore it is believed that the geographic distribution, as well as the phyllosphere microflora of crops are expected to be extremely affected by climate change. A good example in this case is the impact of climate change observed in Serbia, where no contamination with aflatoxins occurred prior, but extended hot and dry weather in 2012 followed in 69% of maize contaminated with aflatoxins (Liew & Mohd, 2018).

Human exposure can result from consumption of the plant-derived foods and animal products contaminated with toxins, or by exposure to contaminated air or dust, producing toxic effects referred to as mycotoxicosis (Bryden, 2012).

Currently, it is believed that a large number of mycotoxins exists, between 300 and 20,000 even 300,000 mycotoxins. Despite the huge number of secondary metabolites, scientist's attention is being drawn on those that have been proven to be of concern in public health (Lee & Ryu, 2017).

The most important mycotoxins that pose great potential risk in food and feed contaminants are: aflatoxins (AF), trichothecenes (TCT), fumonisins (F), zearalenone (ZEA) and ochratoxin A (OTA). Reports indicate that these toxins account for one billion dollars due to decrease of productivity and over 500 million dollars to reduce the damage produced

by only 3 mycotoxins: aflatoxins, fumonisins and trichothecenes (Dohlman, 2003).

Over the past years, there have been certified and used methods for the analysis of mycotoxins in food and feed such as: TLC (thin layer chromatography), HPLC (high performance liquid chromatography) coupled with FLD, UV or MS detection; GC (gas chromatography) coupled with ECD, FID or MS detection (Alshannaq & Yu, 2017).

Aflatoxins are produced by *Aspergillus flavus, A. parasiticus* (more frequently) *A. bombycis, A. ochraceoroseus, A. nomius , A. pseudotamari* and can be found as contaminants in maize and other cereals, peanuts, tree nuts and dried fruits (Peterson et al., 2001). There have been described 18 structurally related aflatoxins since the detection of aflatoxin B1 (AFB1) in 1960. The most important and studied among them are considered to be AFB1, aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), aflatoxin M1 (AFM1) and aflatoxin M2 (AFM2) (Aiko et al., 2015).

Aflatoxins have a massive impact on both human and animal population. Furthermore, they determine major effects on economy by agriculture loss.

Trichothecenes are a family that contains nearly 200 metabolites, divided into 4 functional groups: A (includes T2 and HT2 toxins), B (includes NIV and DON), C and D. They are produced by several fungal genera, of which approximately 40 are isolated from fungi that belong to *Fusarium* spp. (McCormick et al., 2011). Contamination with these secondary metabolites can be observed worldwide in commodities such as wheat, rye, maize, rice, barley and vegetables.

Fumonisins, represented by fumonisin B1 (FB1) and fumonisin B2 (FB2) are cancer-promoting secondary metabolites mainly produced by *Fusarium verticillioides* and *Fusarium proliferatum* that usually contaminate commodities such as maize, rice and wheat (Lerda et al., 2017).

Zearalenone is a mycotoxin produced by *Fusarium graminearum, F. culmorum, F. cerealis* and other *Fusarium* molds which normally contaminates wheat, maize, sorghum and rice. It is a non-steroidal oestrogen like compound with major implications in the affections of the reproductive tract on both human and animal organism (Da Rocha et al., 2014).

Ochratoxin A is a secondary metabolite produced mainly by fungi of the genera *Aspergillus* and *Penicillum*. Some of the most important species implicated OTA production includes *A. ochraceus, A. carbonarius, A. melleus* and *A. niger* (Liuzzi et al., 2017). OTA is a constant natural contaminant of many agricultural products such as cocoa beans, cereals, peanuts, tiger nuts, fish, dried fruits, grapes (Ramesh & Javagoudar, 2017).

INCIDENCE OF MYCOTOXINS IN ANIMAL FEED

In spite of the efforts made to control fungal contamination, both developed and under developed countries have been reporting extensively high mycotoxin incidence in animal feed. Being the main source of mycotoxin infestation of farm animals, the major concern regarding contaminated feed is referred to the impact on the economy, animal and human health (Alshannaq & Yu, 2017).

The most important mycotoxins that are subject to legal regulations are: aflatoxins (AF), deoxynivalenol (DON), zearalenone (ZEA), fumonisins (FB) and ochratoxin A (OTA).

Aflatoxins

According to recent surveys and literature data, aflatoxins continue to pose a significant problem with a global incidence contamination rate higher than 30%. AF's production occurs in nuts and cereals in areas of favourable climatic condition such as high temperatures and low rainfall. As a result, they are detected especially in Africa, South Asia, South America and Southern Europe. Highest level of AF detected in Africa was 1,642 µg/kg, while in Europe the highest level was of 33 µg/kg (Lee & Ryu, 2017).

The maximum limit for aflatoxin allowed in all feed materials is 0.02 mg/kg (Commision Directive 2003/100/EC).

In the actual context of climate change, an increase of temperature in cool and temperate climates may determine an increase of aflatoxin contamination in these areas (Pinotti et al., 2016).

Deoxynivalenol

As reported by recent studies, DON can be detected most commonly in wheat with a global incidence rate higher than 60%. Deoxynivalenol production is positively influenced by low temperatures and wet growing season. Therefore, it is mostly detected in North Asia, Northern Europe, North and South America (Pinotti et al., 2016).

DON, which is also known as vomitoxin induces feed refusal and emesis, alters the immune system and intestinal functions in pigs (Streit et al., 2012).

In consequence, the European Commission set maximum limits for DON in cereals and cereal products (8 mg/kg), maize by-products (12 mg/kg) and complementary and complete feeding stuff for pigs (0.9 mg/kg) (Commission Recommandation 2006/576/EC).

Zearalenone

Recent surveys show that the global incidence rate of ZEA is higher than 30%. Similar to DON, production of ZEA usually occurs at low temperatures and wet growing season (Pinotti et al., 2016).

The highest incidence of zearalenone contamination was detected in wheat and maize, in regions such as Central Europe, North and South America, North and South Asia (Lee & Ryu, 2017).

In Europe, guidance values for ZEA concentration has been issued by the European Commission as follows: for complementary and complete feeding stuffs for piglets and gilts (0.1 mg/kg), complementary and complete feeding stuffs for calves, dairy cattle, sheep and goats (0.5 mg/kg) and maize by products (3 mg/kg) (Commission Recommandation 2006/576/EC).

Fumonisins

The occurrence data from numerous reports reveals that fumonisins have a global incidence rate higher than 50% and they are usually developed in corn. Fumonisins have been detected in South America, Africa, Southern Europe and both North and South Asia. Europe showed the lowest incidence (39%) among the list, while in North and South America was reported the highest incidence rate (95%) (Lee & Ryu, 2017).

European guidance values for fumonisins are as follows: complementary and complete feeding stuffs for pigs, horses, rabbits and pet animals (5 mg/kg) and maize/maize products (60 mg/kg) (Commission Recommandation 2006/576/EC).

Ochratoxin A

Frequently found in cereals, can also contaminate soybeans and peanut, OTA is associated with insufficient drying or improper storage. According to numerous surveys it has been detected all over the world, having a 60% rate of global incidence contamination (Pinotti et al., 2016).

The European Commission had issued guidance values for ochratoxin A as follows: 0.05 mg/kg for complementary and complete feeding stuffs for pigs, respectively 0.25 mg/kg for cereals and cereals products (Commission Recommandation 2006/576/EC).

IMPACT OF MYCOTOXINS ON ANIMAL HEALTH

Similar to all toxicological conditions, mycotoxicosis are categorized as acute or chronic.

As a result of the transplacental circulation of toxic metabolites, exposure to **aflatoxins** begins in the womb and continues during postnatal period through breastfeeding.

Acute aflatoxicosis generally appears with an obvious toxic response (haemorrhages, acute liver damage, edema), while chronic exposure to aflatoxins results in cancer and other irreversible effects on animal organisms. After ingestion of contaminated feed, AFB1 is metabolized in liver by specific cytochrome P450 enzymes into metabolites that may cause aflatoxicosis by binding to proteins, or cancer by attaching to DNA (Wild & Turner, 2002)

Consumption of contaminated feed in poultry and pigs is associated with reduced immunity, low growth performance due to poor feed utilization, causing major economic problems for the producers (Da Rocha et al., 2014). Feeding animals with contaminated feed, especially with AFB1, may results in finding the mycotoxins and/or their metabolites in milk (AFM1), meat and eggs.

AFB1, which was classified by the International Agency for Research on Cancer (IARC) as a Group 1 human carcinogen, acts synergistically with hepatitis B virus (HBV)

causing human primary liver cancer, mostly encountered in developing countries (Jemal et al., 2011).

Fumonisin B1, the most representative of the numerous fumonisin analogues is categorized by IARC in Group 2B carcinogen (possibly carcinogenic in humans).

Fumonisins are hydrophilic substances which enhances their toxic effect. In animals, their toxic effects vary from pulmonary edema and hydrotorax in swine, to leukoencephalomalacia in equine and hepatocellular carcinoma in rats.

These secondary metabolites, whom toxic action is explained by the inhibitory effect on the uptake of folic acid via the folate receptor, where found to be associated with an epidemic of neural tube defects that occurred along Texas-Mexico border and China. Also, they have been implicated in an incident of acute food-borne disease located in India in which the manifestation of borborygmi, abdominal pain and diarrhoea was connected with the oral ingestion of contaminated maize (Ortiz et al., 2015).

Regarding **trichothecenes**, T-2 toxin and DON are the most important mycotoxins that causing toxicity to both human and animals through oral contamination. They are well-known compounds with inhibitory effect on protein synthesis via binding of peptidyl-transferaze (Yang et al., 2017).

When ingested in high dosage, deoxynivalenol or vomitoxin, causes nausea and vomiting in animal organism, while small doses can cause refusal to eat and weight loss (Da Rocha et al., 2014).

Furthermore, DON has been associated with scabby grain toxicosis that occurred in USA, China, Australia and Japan (Etzel & Ryu, 2014).

Over the years, it has been suggested that trichothecenes were used as biological warfare agents; for example, in Cambodia an investigation conducted between 1978-1981 concluded DON, DAS, T2-toxin were isolated from water around affected areas (Etzel & Ryu, 2014).

Ochratoxin A is considered to be the most economically important and prevalent secondary metabolite in the group of ochratoxins. OTA develops an immunosuppressive, teratogenic and nephrotoxic potential (Russo et al., 2016). Researches revealed that OTA is strongly linked to kidney affections (Ladeira et al., 2017).

Ingestion of contaminated feed with OTA induces negative effects on the digestive system which are expressed by the reduction of nutrient absorption, cell apoptosis and disruption of intestinal permeability (Pfohl-Leszkowicz, 2009).

Zearalenoneis non-steroidal compound with oestrogen-like activity that is mainly implicated in hyperoestrogenic conditions in humans. ZEA is involved in the affections of the reproductive system of farm animals like swine and cattle. These effects are explained by the structure of ZEA which allows it to link to the mammalian oestrogen receptor. ZEA has also proven to be hepatotoxic, immunotoxic and genotoxic (Fink-Gremmels & Malekinejad, 2007).

ZEA has been associated alongside DON with scabby grain disease toxicosis encountered in Japan, China and Australia, manifested with symptoms such as nausea, vomiting and diarrhoea (Etzel & Ryu, 2014).

BIOLOGICAL CONTROL OF THE MYCOTOXIN-PRODUCING FUNGI

The prime control strategy of the mycotoxin-producing fungi is developed on the use as fungicides, which are also considered to be efficient in controlling postharvest diseases. Recently, due to public perception that pesticides are a threaten to both human and environmental safety and the increasing demand for produce food free of synthetic fungicides, it has been requested by the public the replacement of the chemicals with a safer and cleaner alternative approach. As a result, the use of antagonistic microorganisms to manage storage fungi and mycotoxin production has become a promising alternative (Korsten, 2006).

Even though *in vitro* were discovered numerous useful biological control agents, because of the environmental conditions there have been reported no correlations between *in vitro* tests and field performance. Currently the number of biological control agents is increasing and it is evaluated at 1% of

agricultural chemical sales with high expectancies to grow in future years (Medeiros et al., 2012).

Regarding the sources of biological antagonists against mycotoxigenicmolds there have been tested different species of yeasts, fungi and bacteria (Fravel, 2005).

According to Wiesniewski (1994), the most important characteristics of an ideal antagonist are: genetic stability, efficacy at low concentrations against a wide range of pathogens, simple nutritional requirements, high survival rate in adverse conditions of environment, lack of pathogenicity for the host plant, resistance the most used pesticides, compatibility with other chemical treatments and no production of potentially toxic metabolites to humans.

Yeasts inherent features such as fast development, capacity of depriving nutrients from pathogens and fruit surface colonization have placed them as one of the most appropriate biocontrol agents. As a result, medical researches have been focused on their selection, isolation and potential use for mycotoxin-producing fungi (Abraham et al., 2010).

Mycotoxin-producing microorganisms may be "field fungi" that invade before harvest and "storage fungi" that develop especially during storage. Therefore, antagonists can be delivered by two main ways: at pre or post-harvest. Pre-harvest delivery main purpose is to prevent the growth of the fungi and to reduce their mycotoxin production and it is based on the competition between toxigenic and nontoxigenic strains of *Aspergillus flavus* (Medeiros at al., 2012).

There are two products that have been registered as biopesticides used in aflatoxin control: AF-36 (aflatoxin control on cottonseed) and Afla-Guard (aflatoxin control on corn and peanuts). Also, it has been brought to attention the effect of *Saccharomyces cerevisiae* RC008 and RC016 strains selected based on their mycotoxins binding ability and beneficial properties against *A. carbonarius* and *P. graminearum* (Mayfield et al., 2012). Both biological control agents have proven to be effective to reduce growth and production of OTA, ZEA, FB1 and DON.

Bacteria also have been widely used as biocontrol agent against toxigenic fungi being developed into commercial product. Certain strains of *Bacillus subtilis* are used as an inhibitor of growth and aflatoxin production, while strains of *Lactobacillus*, *Bifidobacteria* and *Steptococcus* are used as biological agents to control production of OTA. Also, *Bacillus amyloliquefaciens* and *Enterobacter hormaechei* were proven to have antagonist effects on *Fusarium verticilloides* development and secondary metabolites production (Medeiros et al., 2012).

CHEMICAL METHODS FOR MYCOTOXIN DECONTAMINATION

There are a few chemical treatments that can convert toxic metabolites of fungi into non-toxic substances. The ones who pose a great amount of interest will be presented in the following paragraphs.

Oxidation

Ozon and hydrogen peroxide, both oxidizing agents that can react with numerous functional groups, have been used to render mycotoxin contaminated feed providing no harm to it. For example, the biological activities of trichothecenes were changed by oxidation, ZEA was degraded by 83.9% using 10% H_2O_2 at 80°C, for 16 hours (Zinedine et al., 2007).

McKenzie et al (1998) proved that the treatment with electrochemically produced ozone on contaminated corn provided protection against AFB1 in young turkey poults. Regarding OTA contamination, "Oxigreen" process using ozone reduced microbiological and mycotoxin contamination, but determined side-effects such as DNA adducts in wheat.

Reduction

Ascorbic acid, $NaHSO_3$, $Na_2S_2O_5$ are reducing agents that decrease mycotoxin levels, especially AFB1 and DON. Transformation of DON to DON-sulfonate (less toxic compound) is reported as an effective tool for overpowering the depressive effects of it on piglets. Nonetheless, the reaction at 65°C, for 48 hours, of FB1 with d-glucose/d-fructose (reducing sugars) blocks the primary amino group and may prevent FB1 induced toxicity on

cell tissue cultures of both rats and swine (Jard et al., 2011).

Ammoniation

Applied for several years, ammoniation, it is considered to be an efficient procedure used to detoxify mainly aflatoxin-contaminated feed. When carried out at high temperature and pressure, this process can lead to the transformation of AFB1 in AFD1 (less toxic substance). This expensive treatment is inefficient against other mycotoxins and may lead to damaged food quality because of the extreme ammonia level involved (Park et al., 1988).

Alkalization

Mycotoxin structure can vary under alkaline conditions; for instance, the 12-13-epoxy group of DON can be opened (Jard et al., 2011).

Acidification

It was discovered that treatments using strong acids can destroy the biological activity of AFB1, transforming it into the hemi-acetal form. A method using HCl has been proven to be effective in reducing AFB1 levels by 19.3% in 24 hours (Jard et al., 2011).

PHISICAL METHODS FOR MYCOTOXIN DECONTAMINATION

Sorting and washing

In case of a heterogeneous contamination, the removal of the contaminated fraction can reduce the level of mycotoxins in the final product. Contaminated grain does not have the same colour as safe grain, so it can be sorted depending on its colour and even its density (Jard et al., 2011).

Also, washing food and grain may reduce the level of contamination with mycotoxins. For example, the first step in the production of spaghetti is washing the wheat, which is proved to remove 23% of DON. It is important to mention that both methods (washing and sorting) are not specific and generally exhaustive (Jard et al., 2011).

Thermal treatment

Some of the mycotoxins are substances with a remarkable stability at high temperatures, therefore, are rarely degraded using thermic methods. In normal cooking conditions (frying, boiling), little or no reduction in mycotoxin level occurs. For instance, DON is stable at 120°C, moderately stable at 180°C and partially stable at 210°C. Fumonisins are totally degraded at 220°C, while thermic treatment of ZEA was proved to be ineffective (Kabak, 2009).

An important role in toxin degradation has the initial level of contamination, type and concentration of the mycotoxin, heating temperature, time period, moisture content, pH and ionic strength (Jard et al., 2011).

Degradation by extrusion

This method implies protein denaturation and inhibition of enzymatic activity and enables the removal of AF, DON, ZEA and FB1 from maize (Cazzaniga et al., 2001).

Radiation

A major part of mycotoxins possesses complex molecular structures, as a result they are not affected by irradiation. Gamma- radiation can reduce microorganisms, while radiation of AFB1 (sensitive to UV, X, Gamma rays) reduces the level of contamination. Also, micro-waves have the ability to reduce aflatoxins in peanuts and trichothecenes in maize (Jard et al., 2011).

MICROORGANISMS USED FOR MYCOTOXIN DECONTAMINATION

Flavobacterium aurantiacum, also known as *Nocardiacoryne bacteriodes* was one of the first bacteria investigated for its capacity of removing AFB1 (Hao et al., 1989). Using the crude extract, the scientists were able to transform AFB1 into a less toxic compound, involving an intracellular enzyme. *Stenotrophonas maltophilia* 35-3 was isolated on a selective medium that contained coumarin (source of carbon), which is a chemical component of the nucleus of AFB1. The culture supernatant was able to reduce AFB1 by 84.8% after incubation at pH 8 (Guan et al., 2008).

Also, certain strains of *Bacillus subtilis* are able to detoxify AFB1 contaminated food/feed and even to facilitate animal growth rate. *Mycobacterium fluoranthenivorans* was proven effective in the removal of AFB1 from the contaminated food and feed (Teniola et al., 2005).

OTA can be transformed into ochratoxin alfa (less toxic compound) by numerous biological such as bacteria, moulds, plants and yeasts.

Aureobasidium pullulans has been used as a control agent in wine, preventing OTA accumulation in grapes (De Felice et al., 2008).
Trichosporon mycotoxinivorans has been developed into a product that can detoxify OTA in animal feed in less than 2 hours and a half (Molnar et al., 2004).
The two main metabolites of DON are epoxidized DON and 3-keto-DON (both less toxic than DON). The *Eubacterium* sp. strain BBSH 797 developed into a commercial product has been proven to be effective in detoxifying trichothecenes in animal feed (Jard et al., 2011).
The metabolism of T2 toxin was revealed in 1988, it contains a sequence of steps: first, T-2 toxin is transformed into HT-2 toxin (deacetylation), then it is converted to T-2 triol (20 times less toxic than T-2) and finally into T-2 tetraol. Bacteria isolated by enrichment from contaminated soil/water complete the sequence of transformation into less toxic compounds (Rood et al., 1988).
It is a well-known fact that FB1 toxicity resides in its primary amine, therefore, the deamination of this molecule reduces its negative effects. The main microorganism which was proven capable of degrading FB1 is *Exophialaspinifera* (black yeast). The transformation of FB1 into AP1 is possible due to the intervention of an extracellular carboxylesterase. This enzyme was cloned and has been used with positive effects on transgenic maize, as it became resistant to fumonisins contamination (Duvick et al., 2003).
Bacteria, yeasts, plants and molds can transform ZEA into oxidised compounds (zearalanone), hydroxyl compounds (alfa, beta-zearalenol), methyl compounds, gluco or sufo-conjugates and hydrolysed compounds. It was brought to attention that the transformation of ZEA into alfa-zearalenol is not a detoxifying action since the oestrogenic effects are even higher than ZEA's (Jard et al., 2011). *Trichosporon mycotoxinivorans*, *Rhodococcus* and *Nocardia* spp. have the ability to decarboxylate ZEA. Recently, using an MCF-7 cell line, it was shown that ZEA sulfonating leads to a reduction in oestrogenic toxicity (Duvick et al., 2003).
Nevertheless, it has not been proven that sulfonating or glycosylation leads effectively to detoxification insofar as hydrolysis of this conjugate could occur in the digestive tract.
Strains of soil *Pseudomonas* sp. were able to remove ZEA, even though the product was not identified but was assumed to be less toxic than ZEA. Also, an unidentified bacteria isolated from pig intestine was also able to remove ZEA. Furthermore, it was identified a concentration of 26% ZEA-sulphate and 18% of alfa-zearalenol after a ZEA transformation by *Rhyzopus* (Jard et al., 2011)

ENZYMATIC BIOTRANSFORMATION OF MYCOTOXINS

The process of identification and characterization of degrading enzymes is a time consuming, but necessary action to understand the mechanism of degradation. Enzymes deliver reproducible performances, no risk of contamination and no safety concerns compared to the use of microorganisms.
Currently, there are a few available commercial biotransforming feed additives, such as Mycofix, Biomin, BBSH 797 and FUMzyme.
The majority of enzymes for degrading **aflatoxins** belong to oxidoreductase class. In 1998, aflatoxin-oxidase was isolated from Chinese edible mushroom *Armillariella tabescens* and has the capacity of oxidising the furofuran ring and releasing H_2O_2 (Loi et al., 2017). Regarding the toxicity of this enzyme it was shown to have minimal impact on liver health and to reduce genotoxicity on chicken embryos. AFB1 (0.05 µg/mL) was degraded 100% after incubating at 28°C at pH=6 (Liu et al., 1998).
Three peroxidases with high degrading capacity on AFB1 were extracted and purified from *Armoracia rusticana* (42.2% *in vitro* degradation/ 41% in real matrix), *Phanerochaete sordida* YK-624 (86% *in vitro* degradation) and *Pleurotus ostreatus* (90% *in vitro* degradation). Laccases produced by *Trametes versicolor* (87% *in vitro* degradation) and *Streptomyces coelicor* (100 % *in vitro* degradation), have been proposed for ZEA and AF's biotransformation (Chitrangada et al., 2000.)
F420H2-dependent reductases isolated from *Mycobacterium smegmatis* were used against AFB1, AFB2, AFG1 and AFG2 (Taylor et al., 2010).

The most important path of **ochratoxin A** detoxification is represented by the hydrolysis of the amide bond between phenylalanine and the isocoumarin residue followed by the formation of ochratoxin alpha which has no toxicity. OTA degradation has been associated with two classes of carboxypeptidase: CPA (carboxypeptidase A), isolated from bovine pancreas and CPY (carboxypeptidase Y) isolated from *Sacchraromyces cerevisiae* (Loi et al., 2017). CPA uses one zinc ion for hydrolysis within the protein and it is able to perform the degrading reaction with Km= 1.5ˆ10´4 M at a temperature of 25°C, while CPY which is a serine-type carboxypeptidase has a very low specific activity of 52% after incubating for five days at 37°C and pH=5.6 (Pitout, 1969). Most of carboxypeptidases have optimal reaction temperatures of 30°C or higher. Lipases, amidases and commercial neutral proteases are also able to perform OTA hydrolysis (Loi et al., 2017).

Fumonisin B1 is degraded by the consecutive action of a carboxylesterase, an aminotransferase and a fumonisinesterase. These enzymes were isolated from *Sphingomonas* spp. (ATCC5552) and *Sphingopyxis* spp. (MTA 144). ATCC5552 has proven 100% efficacy over FB1 (1000 µg/mL) after an overnight incubation at 37°C, while MTA degraded FB1 (3.6 µg/mL) after 2 hours of incubation at 37°C (Loi et al., 2017).

The esterase MTA 144 produced by a genetically modified strain has been included in a patented formulation FUMzyme. It partially degrades FB1 and related fumonisins by cleavage of the diester bonds and release of the tricarballylic acid (Heinl et al., 2009).

Both hydroxylation and glycosylation of **trichothecenes** results in less toxic derivates. These substances usually generate a reverse reaction in the digestive tract of humans and animals; therefore, the use of these enzymes is limited in feed-related applications

Recently it has been shown that DON, NIV and 3-acetyl DON are hydroxylated by the bacterial cytochrome P450 isolated from *Sphingomonas* sp. strain KSM1, DON's catabolic product being used by this strain as carbon source. *In vitro* degradation of DON (99.86 µg/mL) was proved to be efficient after 3 days of incubation, while NIV (105.25 µg/mL) was totally degraded after 5 days. Besides that, it was identified a UDP-glycotransferase produced by *Arabidopsis thaliana* with the ability to catalyze the transfer of glucose from UDP-glucose to hydroxyl group at C3 of DON creating 3-O-glucopyranosyl-4-DON (Loi et al., 2017)

Commercial products such as BBSH 797 that contains a pure culture of *Eubacterium* (isolated from bovine rumen fluid) capable of transforming DON into its de-epoxy form DOM-1 have already developed and patented. Nonetheless, purified enzymes that dispose a specific trichothecenes degrading activity has not been brought to public attention yet (Loi et al., 2017).

The main purpose of the detoxification strategy of **zearalenone** is to disrupt its oestrogenic activity by an irreversible reaction.

A lactonohydrolase from the fungus *Clonostachys rosea* is capable of degrading ZEA. Degrading conditions for this enzyme include incubation at 27°C for 4h or at 32°C degree for 2 h with 100% efficacy. In addition to this, the enzyme was purified, cloned and characterized by heterologous expression in *S. cerevisiae* and *E. coli* (Takahashi-Ando et al., 2005).

Apart from their activity towards AFs, mentioned above, laccases produced by *Streptomyces coelicolor* (incubation at 37°C for 24 h inducing a 100% efficacy) and *Trametes versicolor* (incubation at 30°C for 4 h with only 58% efficacy) have also the capacity to degrade ZEA (Azam et al., 2019).

An additional ZEA degrading oxidoreductase is 2-cis peroxiredoxin (Prx), produced by *Acinetobacter* sp. SM04. It is important to mention that nearly 90% of the toxin was degraded in contaminated corn samples treated at 30°C for 6 h with purified Prx and 0.09% H_2O_2.

Compulsory requirements for enzyme applications in food and feed industry are represented by: safety, effectiveness, stability at wide ranges of temperatures/pH/organic solvents and low cost of both production and purification (Tang et al., 2013).

DECREASING BIOAVAILABILITY OF MYCOTOXINS USING ADSORBANTS

Mineral and organic adsorbants

The most commonly procedure used for decreasing exposure to mycotoxins is to reduce their bioavailability by the inclusion of various binding agents or adsorbents, which reduce mycotoxin uptake and subsequent distribution to the blood and target organs.

These adsorbents show effectiveness only if the complex is stable in the animal's digestive tract, so that bound mycotoxins are eliminated via urine and faeces. Mineral adsorbents are usually effective against AFB1 but their efficacy varies for other mycotoxins. Furthermore, in some cases, the addition of clay increases the effects of mycotoxicosis.

a. Activated charcoal

Due to its large surface area, its excellent adsorptive capacity and porosity, activated charcoal is a widely used adsorptive material that has positive effects on the main mycotoxins in an aqueous environment. One of the first conducted studies in which was tested the concept of mycotoxin binding revealed that activated charcoal could efficiently adsorb AFB1. Subsequent researches have shown that charcoal may not be as effective in binding aflatoxin as clay-based binders such as HSCAS (hydrated sodium calcium aluminosilicate). Charcoal can be used as a binder for both ZEA and DON (Huwig et al., 2001).

In an *in vitro* gastrointestinal model it was shown that activated carbon significantly decreased the availability of DON, NIV and ZEA. For OTA and FB1 binding, there were no positive effects reported. Concerning T-2 toxin and HT-2, responses were variable depending on the tested animal. For instance, the addition of charcoal in contaminated feed elevated the rate of survival in mice from 50% to 90 %, while in chicken no positive effects were reported (Jard et al., 2011).

b. Silicate binders

They are divided into subclasses based on their structure. Phyllosilicate group is characterized by a sheet-type framework; the most extensively studied material in this group is HSCAS which was shown to have adsorptive effects *in vitro* on 80% of AFB1 (Jard et al., 2011).

It was shown that HSCASs adsorb selectively aflatoxins during the digestive process, providing unavailable aflatoxin for absorption. Also, it is important to mention that responses to HSCASs seem to be dose - dependent. Usually, HSCAS have low affinity for OTA, T-2 toxin and DON and variable affinity for ZEA. Nevertheless, they were effectively used for a mixture of AFB1 and FB1 with an adsorption rate of 95% and 85% respectively (Jard et al., 2011).

The other intensively studied silicate include zeolites, bentonites and clinoptilolites. Originated from volcanic ash and containing montmorillonite as main constituent, bentonite is a widely known clay material. Similar to phyllosilicates, clays are silica sheets with high concentrations of water. Due to their structure, zeolites present a large specific surface (1000 m^2/g) that provides vacant spaces to form channels allowing movement of molecules in and out of it (Colella et al., 2011).

OTA can be adsorbed in wine by bentonite and montmorillonite, the rate of adsorption may vary from 40% to 100%. Regarding, ZEA and T-2 toxin adsorption no positive effects were discovered. Beyond that, studies revealed that the addition of montmorillonite in ZEA contaminated feed increased oestrogenic toxicity. Zeolites have been proven to be very efficient in bovine rumen juice, with AFB1 adsorption rate of 100%, but there were no positive findings on T-2 toxin binding capacity (Jard et al., 2011)

c. Other mineral silicates

Cholestryamine and polyvinylpyrrolidone, which are synthetic polymers can adsorb mycotoxins, while non-digestible dietary fibers have shown binding activity as well. Both fibre and cholestyramine can reduce the negative effects of ZEA; cholestyramine has been effective on FB1 and partially OTA contaminated feed, while non-digestible fibers shown binding activity toward T-2 toxin (Boudergue et al., 2009).

Polyvinlypyrrolidone (PVP), which is a synthetic water-soluble polymer, has been investigated as a binder for mycotoxins; positive effects were reported on AFB1 and ZEA *in vitro* binding, while the toxicity of DON in pigs was not diminished (Jard et al., 2011).

Biological adsorbants

a. Yeast and /or yeast extracts

Saccharomyces cerevisiae has the capacity to bind AFB1 and reduce its negative effects on broilers and rats. While the effect against aflatoxin when using live yeasts was confirmed in rats, thermalysed yeast were proven to be ineffective on other mycotoxins, but it was discovered that some thermalysed yeast cell walls successfully adsorbed ZEA. For example, esterified glucomannan polymer, originating from yeast cell wall, proved to have binding capacity on AFB1, OTA and T2-toxin when used in combination or individually. Moreover, the addition of esterified glucan polymer to contaminated cow feed induced a massive decrease of AFM1 residues and, when added in equine's diet body weight and biochemical recovered (Whitlow, 2006).

Aravind et al. (2003), adding 0.5% esterified glucomannan in their diet, reduced growth depression in broilers associated with contaminated diets containing AFB1, OTA, ZEA and T-2 toxin. Nonetheless, a glucan polymer product did not reduce the toxic effects of FB1, OTA and ZEA in minks. In pigs, the addition of glucomannan in their diet resulted in the reduction of the negative effects of both ZEA and DON, as the biochemical and immunological parameters significantly decreased. FB1 adsorption by yeast and/or cell wall yeast is deficient, while HT-2 toxin binding responses depended on the yeast modified glucan.

b. Lactic acid bacteria

Propionibacteria and bifidobacteria, both groups of lactic acid bacteria can be used with success in the mycotoxins-binding process. This process, which efficiency depends on the used strain, appears to be based on physical DON, NIV and other mycotoxins linkage to the hydrophobic pockets on the bacterial surface (Sicuia et al., 2014).

AFB1 and AFM1 adsorption performed with both living and dead bacterial strains was proved to be reversible. Furthermore, inactivation of the bacteria, using acid or heat, increased the intensity of the adsorption. Positive effects were observed in binding OTA, and ZEA at pH between 4 and 8 (Haskard et al., 2001).

Adsorption of FB1 and DON by *Lactobacillus rhamnosus* was not very efficient due to weak hydrophobic interactions involved in the process (Jard et al., 2011).

CONCLUSIONS

Mycotoxins, secondary metabolites mainly produced by toxigenic fungi such as *Penicillium*, *Aspergillus* and *Fusarium* are a major threat on economy and on both human and animal health.

Their prevalence is higher with favourable conditions such as climatic changes, lack of control systems and plentiful of suitable substrates, thus causing serious risks for both human and animal health.

Since chemical and physical decontamination are not sufficiently effective, biological transformation is considered to be the most promising approach to reduce mycotoxin concentration.

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