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Review Article

Decontamination and Detoxification of Mycotoxins in Feed by Pre- and Post-Harvest Methods, including the Use of Microorganisms

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Abstract

Mycotoxins are secondary metabolites originating from various species of fungi that can contaminate feed, causing chronic or acute toxicity in animals. Several mechanisms of detoxification were investigated to remove mycotoxins. At pre-harvest level, agronomic methods (planning harvesting time, crop rotation, soil tillage, irrigation and fertilization), biological methods (use of microorganisms as fungal antagonists) and chemical procedures (fungicide and insecticides) were taken into account. At post-harvest level, storage management (control of temperature, humidity, and of O_2, CO_2 and N_2 concentrations), physical methods (sorting, separation, washing, solvent extraction, heating, irradiation, adsorption), chemical methods (alkaline treatment and use of ozone), biological methods (use of microorganisms for detoxification and of catabolizing enzymes and application of nutritional strategies). In this mini-review, the concern of mycotoxins feed contamination, causing important consequences for dairy livestocks health, is described, considering different methods to be used for mycotoxins decontamination, including microbiological methods.

Introduction

Mycotoxins are secondary metabolites produced by filamentous fungi and they are able to contaminate different substrates, including feed, posing consequent dangers for animal health, and eventually for food safety and human health [1]. Exposure to mycotoxins is a worldwide concern, with their occurrence that can be considered as an unavoidable phenomenon and can vary among geographical areas [2]. Mycotoxins can contaminate cereal plants, the latter constituting feed commodities, by fungi growing as pathogens on plants or growing on stored plants, in a saprophytic way. When mycotoxins are ingested by higher animals can induce a toxic response defined as mycotoxicosis [3]. Mycotoxins intake may induce long-term chronic effects resulting in teratogenic, carcinogenic, and oestrogenic or immune suppressive effects. There may be direct consequences of mycotoxin-contaminated animal feed ingestion, as a reduced feed intake, feed refusal, poor feed conversion, diminished body weight gain, suppression of immune defences and increase in diseases, and reduced reproductive capacities, all these aspects leading to economic losses [3]. Due to the important concerns mycotoxins can cause, with toxicological impact on human and animal health, these fungal secondary metabolites have been included among high priority compounds by the Food and Agriculture Organization of the United Nations (FAO) and by the World Health Organization (WHO) [4]. From the legislative point of view, limits for mycotoxins were introduced in about 100 countries, with formation of regional harmonisation for the European Union, Australia and New Zealand, the Gulf Cooperation Council and Mercado Común del Sur, defined MERCORSUR, including Argentina, Brazil, Paraguay, Uruguay, and Venezuela. In the European Union, maximum levels in feed are enforced for aflatoxins, deoxynivalenol, fumonisins, ochratoxins, zearalenone, and T-2 and HT-2 toxins. For aflatoxins have been defined legal limits, while for other toxins, there are national and international recommendations [5]. Mycotoxins can present a co-occurrence in feed, as it may be contaminated by several fungal species and mycotoxins at the same time, and the toxicological effects can be different according to the different combinations among mycotoxins [6].

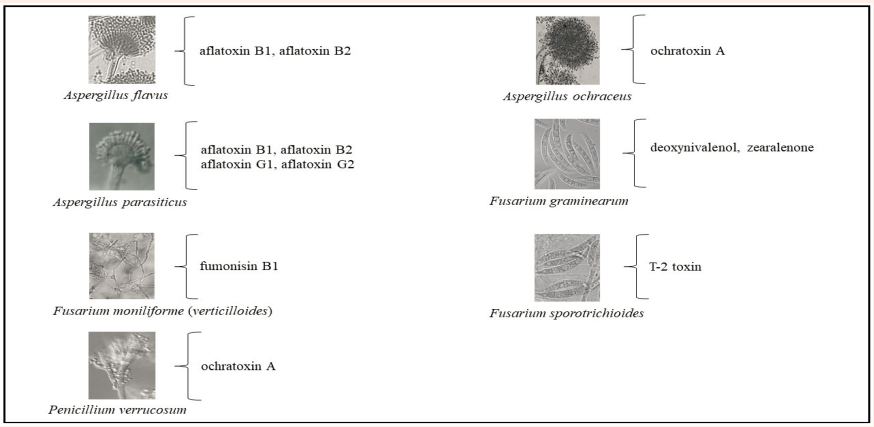

Figure 1: Mycotoxins produced of world-wide importance and examples of involved fungal species.

Worldwide, there is a diffuse incidence of mycotoxins contamination in feed and raw feed materials, mainly grains and grain co-products as well as other feed ingredients. The principal contaminating mycotoxins in feed include aflatoxins, deoxynivalenol, fumonisin B1, ochratoxin A, T-2 toxin, and zearalenone as reported in Figure 1, along with some examples of filamentous fungi producing mycotoxins [7,8]. Moreover, two important issues of great concern for feed safety reside in mycotoxin co-occurrence, and modified and emerging mycotoxins [9]. In this mini-review, a description of the most diffused mycotoxins is reported and a survey of methods used to counteract mycotoxin development, both at pre- and post-harvest perdiod, from field practices to storage and to the use of physical, chemical and biological methods is reported. A description of microorganisms involved in mycotoxin detoxifixation is moreover included.

Fungal Growth Conditions and Mycotoxin Production

Fungi that produce mycotoxins are very common pathogens, widespread in agricultural environments, in all regions of the world [10]. Those fungi can invade and grow on a wide range of substrates and are able to produce mycotoxins depending on diverse environmental conditions [11]. Primary fungal growth requires substrates as organic compounds to obtain synthesis of biomass and energy necessary to carry out chemical reactions and produce primary metabolites essential to growth. Secondary fungal growth, instead, starts after a period of maintained growth and can conduct to production of secondary metabolites. The latter including mycotoxis, with not significant effects on fungal growth, and maybe produced due to an excess primary metabolites precursors and serving to reduce their concentration in fungal cells [12]. The major factors that affect mycotoxin production are temperature, water activity, relative humidity, pH, type of fungal strain and substrate [13]. Concerning climate factors influencing fungal development, survival, distribution, colonization and frequency of mycotoxigenic fungi and their subsequent toxin accumulation activity, the most important are humidity and temperature, especially on the field [14]. If conditions of humidity and temperature are favorable, fungi can grow at different levels, either on the field or later during storage. During pre-harvest, on the field, fungi such as *Fusarium* dominate, due to the fact that they are hygrophilic fungal species requiring a relative humidity of 90% and above to germinate and grow. On the post-harvest phase, hygrophilic fungi disappear as mesophilic and xerophylic fungal species such as *Aspergillus* spp. and *Penicillium* spp., germinate, grow and produce mycotoxins at relative humidities of 80 to 90%, and 80% and less, respectively [11]. During storage, if the humidity in the surrounding environment exceeds the equilibrium relative humidity of the substrates, the latter increase in moisture and water activity (a_w) causing fungal growth and mycotoxin production [15]. Table 1 reports pre- and post-harvest mycotoxin development, examples of producing fungal species and optimal ranges of temperature and of water activity for mycotoxin production. Climate change can induce important environmental modifications as an increase in global temperature which is expected to rise from 1.5 °C to 4.5 °C at the end of the 21st century [16]. Moreover, increments in precipitation, heat waves and prolonged cold winter, flooding, and droughts are likely to occur. An increase of gases in the atmosphere including carbon dioxide (CO_2) is also expected within the next 25 to 50 years [17].

Climate change can also influence mycotoxins production indirectly by i) increasing insect populations and global spread; ii) anticipating maturing and ripening of crops; iii) decreasing plant resilience; iv) changing host pathology due the presence of CO_2 in the atmosphere [17]. Environmental changes accompanied by warm and humid weathers can heavily affect storage and cause unfavorable interactions between different factors leading to accelerated grain deterioration. Storage problems are mostly expected in developing countries especially if storage conditions are not well controlled. In warm climates fungi such as *Aspergillus flavus* are expected to become the most threatening since they survive in high temperatures and cause mycotoxin production such as aflatoxins, and in regions with high humidity and damp atmospheres, the risk of fungal invasion in stored grains also increases [18]. Important changes of environmental conditions can reduce grains resistance to fungal attacks, inducing an increase of fungal growth and mycotoxin production in storage. The formation of hotspots during storage is also expected, with increases in intragranular CO_2 and respiration in cereals, which cause higher a_w values in stored cereals [18]. It is thus highly crucial to store crops and grains in controlled storage facilities with the aim to maintain a safe temperature and humidity levels and provide proper aeration systems to maintain grain quality and protect it from fungal attacks and mycotoxin contamination [19,20].

Mycotoxins Prevention

The prevention of mycotoxin formation in the field is supported by proper crop rotation and fungicide administration at the right time. In case of toxin manifestation, specifical measures against certain types and groups of toxins are required for detoxification approaches to mitigate mycotoxin contamination of feedstuffs and compound feed [27]. Proper field practices are necessary as the majority of fungi are considered as phytophathogens as they infect the plants on the field, so management of contamination during pre-harvest is extremely important since it presents the first route of mycotoxin introduction into food. Generally, fungi that dominate on the field are species of *Fusarium* spp., *Cladosporium* spp., and *Alternaria* spp. On the other hand, *Aspergillus* spp. and *Penicillium* spp. can be found on field but at low rates and in general, the extent of contamination is expected to be higher wherever climatic conditions are in principle favorable to mycotoxin contamination [28]. While it is impossible to completely prevent mycotoxin development at preharvest, it is still extremely important to develop strategies that aim at reducing contamination during this phase. Those strategies should be of a high priority since decreasing the inoculum concentration at pre-harvest is considered crucial to the quality of the subsequent product. Therefore, in order to adopt proper strategies, a sufficient understanding of the toxigenic fungi, type of crops, field management, and harvesting practices should be applied [29]. In the field, many factors can contribute to mycotoxins presence such as drought stress, insect infestation, heat, poor soil fertility, and delayed harvesting. Proper field practices include field preparation and management before planting, and field and crop management after planting. Field preparation before planting is crucial to control fungal attack and mycotoxin contamination and it includes; tilling and deep plowing, crop rotation, timing the production cycle, and the use of high-quality seeds or disease-resistant cultivars. Tilling and deep plowing are essential to remove any

Table 1: Production of mycotoxins during different periods and optimum of temperature and water activity.

Period of Onset	Mycotoxins	Examples of Mycotoxin Producing Fungal Species	Contaminated Feed Commodities	Optimum Temperature (°C) for Mycotoxin Production	Optimum Water Activity (a_w) for Mycotoxin Production	References
pre-harvest	deoxynivalenol	*Fusarium graminearum*	corn, wheat, barley, oats	25 - 30	0.98 - 0.99	[21]
pre-harvest	fumonisin B1	*Fusarium moniliforme* (*verticilloides*)	corn, wheat, sorghum, barley, oats	15 - 30	0.90 - 0.995	[22]
pre-harvest	T-2 toxin	*Fusarium sporotrichioides*	wheat, barley, corn, rye, millet, oats	20 - 30	0.98 - 0.995	[23,13]
pre-harvest	zearalenone	*Fusarium graminearum*	maize, wheat, barley, rye	25 - 30	0.96 - 0.98	[21]
post-harvest	aflatoxins B1, B2	*Aspergillus flavus*	corn, wheat	28 - 35	0.95 - 0.99	[24,21]
post-harvest	aflatoxins B1, B2, G1, G2	*Aspergillus parasiticus*	corn, wheat	33	0.95 - 0.99	[21]
post-harvest	ochratoxin A	*Aspergillus ochraceus*	wheat, maize, barley, oats	25 - 30	0.96 - 0.98	[24-26]
post-harvest	ochratoxin A	*Penicillium verrucosum*	wheat, maize, barley, oats	25	0.95	[24,21]

remaining plant material. Previous crop residues that persist on the ground eventually deteriorate and harbor soil-borne fungi increasing their readiness to invade any new crops. So plowing buries the debris underground making them inaccessible to fungal inhabitation [30]. Tilling may also increase water availability to crops by minimizing the compressed layers of soil [31]. On the other hand, crop rotation prevents fungal species build-up and it has been shown that mycotoxin contamination is higher in plots where the same crops are grown over consecutive years since molds that might be well-established on a plant can prevail from a year to another if the same kind was planted continuously. Planning the dates of planting and timing the production cycle are crucial as well to achieving vigorous crops at harvest. It is specifically critical to plan this cycle ahead of time to prevent early or late maturing of the plant and avoid the harvest at a time of rainfall or high relative humidity [30,32]. Seeds used for planting are of extreme importance too, since they are the foundation of any new crop.

Therefore, good seed quality contributes to the growth of healthy plants that can withstand fungal attacks. Seeds must be inspected to ensure the absence of any disease or pest attack, otherwise, they will not germinate or they will be prone to fungal invasions that will successively increase the risk of mycotoxin contamination. Using resistant cultivars, on the other hand, may present a successful approach to prevent disease and control toxin contamination. At present, there are not totally resistant varieties, but partially resistant ones exist that can be used, but those do not provide protection against all genera of fungi. Partially resistant seeds are also mostly effective in cooler temperature climates, while, the resistance is needed to a bigger extent in tropical and sub-tropical regions where fungal infections are more frequent [30]. After planting, facilitating the growth of healthy plants by implementing proper field practices and reducing stress on the crops minimizes fungal growth and mycotoxins production. This stage includes the use of fertilizers, appropriate irrigation methods, weed and insect control, chemical control, and biological control [33]. The use of fertilizers improves plant health and maintains its resistance towards disease and fungi. Nutrient availability is very important for plant vigor and lack of proper plant nutrition leads to breaking in the stem of the plant making it more exposed to fungal invasion. So, in case nutrients were deficient in the soil, fertilizers can be used to increase soil fertility. Appropriate irrigation can also prevent mycotoxins accumulation and it includes two main aspects; irrigation timing and method. Proper timing can prevent drought stress that results in plant cracking and facilitates fungal spores' entrance. The irrigation method, in which splashing is controlled is also essential to prevent fungal spreading [30]. Weed and insect control is also crucial to prevent disease in crops and further fungal invasion. Weeds contribute to contamination by acting as reservoirs of fungal inoculum and by competing for water and nutrients with the crops hence rendering them weak. Therefore, weed removal should be continuously practiced. Insects, on the other hand, can cause fungal dissemination and make the grains more vulnerable to infection by causing physical damage. Hence, it is important to keep the area clean from plant debris, since removing any residual plants or vegetable matters makes food unavailable for rodents and reduces pest attack possibilities. The application of insecticides at appropriate doses, as well, can help control the frequency of attack.

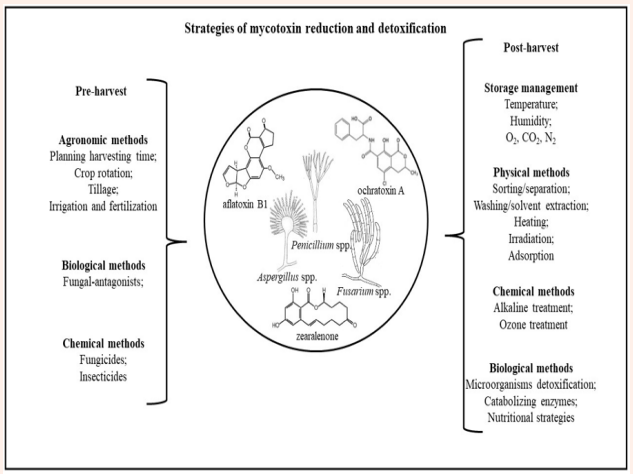

Figure 2: Strategies of mycotoxins detoxification and reduction both at pre- and post-harvest levels, see text for explanations.

The mycotoxigenic fungi in field can be counteracted by biological systems based on strategies that can compete in field environments. Several microorganisms have been reported as bio-control agents of *A. flavus* and aflatoxin in pre-harvest conditions. Treatment of soil with non-toxic strains of *A. flavus* and *A. parasiticus* significantly reduced aflatoxin contamination. Soil treatment with atoxic F. verticillioides evidenced beneficial effect avoiding growth of fungal strains producing fumonisin B1. Moreover, a reduction of saprophytic colonization and sporulation of toxic strains of the species *F. verticillioides* and *F. proliferatum* in maize residues by non-pathogenic *Fusarium* species was observed. Fungal antagonists evidenced the ability to cope with toxic fungi and this is related to macro- and micro-climatic conditions in the antagonist–pathogen interaction. Optimal results for mycotoxin bio-control depends on the ability of non toxic agents to colonize the target substrate and to be active under different environments in the field [19,20]. Figure 2 reports several examples of pre-harvest mycotoxin treatment.

Strategies to Reduce and Detoxify Mycotoxins

Mycotoxins control can be regulated by various detoxification strategies, including physical and chemical methods, nutritional procedures, and biological interventions, the latter focusing on the use of both prokaryotic and eukaryotic microorganisms. Figure 2 includes several examples of post-harvest mycotoxins treatments. Physical methods allowing mycotoxin detoxification can include sorting and separation that are processes based on the not uniform distribution of mycotoxins and on the isolation of mycotoxins-contaminated feed. In this context, the interventions of flotation, decortication and manual sorting are effective in the removal of high percentages of mycotoxins from feed. Aspiration and gravity separation in wheat can reduce the deoxynivalenol content [34]. Contaminated maize and wheat can be dectected by using near-infrared spectroscopy and optical visual sorting [34]. Depending on hydrophilicity and lipophilicity, mycotoxins can be separated by washing with water or extracted by using organic solvents [35]. Procedures of floating and washing with water can remove aflatoxins, zearalenone, fumonisin and tricothecene [35]. The use of solvents as hexane-aqueous acetone-water and dimethyl ether can eliminate almost the total content of aflatoxins in oil crops [34]. A physical method consisting in thermal treatment has been used for mycotoxin decontamination in feed for many years. Nevertheless, the efficiency of the thermal treatments depends on the chemical structure of mycotoxins. Aflatoxin B1, deoxynilvalenone, zearalenone and fumonisin B1 are heat-stable compounds with decomposition temperatures higher than 150 °C, thus needing excessive amounts of energy and very high temperatures that could reduce the nutritional values of feed commodities [36]. Thinking on an industrial scale, an advantageous method for mycotoxin removal is represented by irradiation, divided into ionizing radiations as x-rays, γ-rays, electron beam, and non-ionizing radiations represented by ultraviolet rays, infrared and microwave [37]. Irradiations on feed and feed commodities can reduce or eliminate mycotoxins by inducing physical, chemical and biological effects [37]. Aflatoxin B1, deoxynivalenol, zearalenone, fumonisin B1 can be decomposed, reduced or inactivated by irradiation [38]. The physical process of adsorption is a very important approach that is based on the use of adsorption binders forming complexes with mycotoxins. The largest group of mycotoxin adsorbents are represented by aluminosilicate minerals, including bentonite, montmorrilonite, zeolite, hydrated sodium calcium aluminosilicate, kaolin, etc. [39]. Efficiency of bindind depends on the surface area, charge distribution, and pore size of adsorption binders and on the charge distribution, polarity and shape of each mycotoxin. As an example, the ionic charge of aflatoxins allows clay minerals such as bentonite, illite, zeolite and kaolin to remove them from feed with a high efficiency [40]. In ruminant feed, bentonite or montmorillonite allow depletion of aflatoxins and zearalenone. Bentonites represent promising adsorbents for high-efficient removal of mycotoxins from animal feed, and the are eco-friendly, low-cost and highly efficient as mycotoxins adsorbents [41]. The one dioctahedral bentonite (1m588) is an adsorbent authorized since 2009 as an anti-aflatoxin additive by the EU Regulation [42]. Tri-octahedral bentonite could adsorb more than 90% of zearalenone and fumonisin B1 [43]. Increasing of adsorbing properties of raw montmorillonite transforming to modified montmorillonite, improves aflatoxins adsorption from 0.51 mg g^{-1} to 2.78 mg g^{-1} and zearalenone adsorption from 0.00 mg g^{-1} to 8.54 mg g^{-1} [44]. The binder Amdetox™ is comprised of hydrated sodium calcium alumino-silicate modified by cethypyridinium chloride and intercalation with β-glucan, and this modification increases the surface area of hydrated sodium calcium aluminosilicate, maximizing the binding of mycotoxins and avoiding nutrients adsorption [45].

Second generation adsorbents have been originated from the cell wall components of microorganisms. A common adsorbent that cannot be used by gut microorganisms is represented by glucomannan that strongly adsorbs toxic substances and in particular esterified glucomannan is a broad-spectrum absorbing mycotoxins evidencing a

high efficiency in mycotoxins adsorption corresponding to 95%, 75%, 59% and 12% for aflatoxins, zearalenone, fumonisin B1 and deoxynivalenol, respectively [46]. An effective zearalenone inactivation was evidenced in the presence of the adsorbent β-D-glucan chains obtained from yeast cell walls [47]. Inactivated yeast cell walls decrease aflatoxins and deoxynivalenol synthesis of 82% and 93%, respectively [48]. Another general absorbent is represented by activated charcoal, a general adsorbent very active in liquid moieties. In presence of mycotoxins, activated charcoal evidenced reduction of aflatoxins, zearalenone and deoxynivalenol. In an *in vitro* experiment, activated charcoal addition of 2% decreases zearalenone availability from 32% to 5% [49]. Polyvinylpyrrolidone evidences good adsorption *in vitro* conditions, with adsorption of 0.3 mg g^{-1} of zearalenone [50]. Acid treated durian peel, an agricultural waste with adsorption properties, absorbs aflatoxin B1, zearalenone both at concentrations of 98.4%, and fumonisin B1 at 86.1% [51]. Magnetic materials as magnetic carbon nanocomposites remove mycotoxin wuth the 90% of aflatoxin B1 removed in 180 minutes [52]. An interesting substrate to be used to remove mycotoxins is represented by cross-linked chitosan polymers that was able to absorb 73% of aflatoxin B1, 94% of zearalenone and 99% of fumonisin [53].

Microbial absorbents were moreover investigated, with lactic acid bacteria and as the yeasts the most representatives. Bacterial cells of the species *Lactobacillus casei* evidenced an important reduction of aflatoxin in the intestinal tract [54]. *Lactobacillus plantarum* strain F22 depleted aflatoxin B1 by an adsorption rate of 56.8% [55]. Mixed cultures of different lactic acid bacterial strains evidenced a higher efficiency in decontaminate from aflatoxins, as respect to the activity of a bacterial monoculture [56]. Chemical methods for mycotoxin transformation in less or non toxic compounds includes alkaline treatment, ozone treatment and the use of other chemical compounds. Alkaline treatments use alkaline chemicals as ammonia, sodium hydroxide, potassium hydroxide, and others, that are able to destroy mycotoxins developed in feedstuff [57]. The chemical pathway in case of aflatoxin B1 allows alkaline chemicals to open base hydrolysis the lactone ring structure of the mycotoxin molecule by basic hydrolysis, producing coumarin sodium salt that can be elimnated by washing with water [34]. Aflatoxin B1 is currently removed from feed by using ammoniation and hydroxide salts treatments, with a removal percentage higher than 95% [58]. Toxicity of deoxynivalenol resides in the epoxide at C-12 and C-13, the latter can be destroyed by alkaline treatment [59]. Deoxynivalenol can be reduced by 83.9% in feed commodities [34]. Ozone treatment is an effective detoxification procedure able to change mycotoxins structures. The ozone is used along with other oxidizers such as hydrogen peroxide, sodium and calcium hypochlorite, chlorine and others [60]. Aflatoxins, deoxynivalenol, zearalenone, fumonisin B1, all can be degraded by ozone [61,62]. Aflatoxins B1, B2, G1, and G2 were all degraded by ozone [63]. Ozone treatment in corn revealed a reduction of aflatoxins equal to a value from 92% to 95%, of deoxynivalenol by values in a range from 70% to 90% and a depletion of 90.7% of zearalenone [64]. Several other oxidizers resulted effective in degrading mycotoxins, as in the case of sodium hypochlorite [65] and hydrogen peroxide [66].

The use of biological methods to remove mycotoxins from feed and feed commodities highlights evident advantages and represents an environment-friendly approach. Microorganisms act against mycotoxins by degrading their toxic groups thus producing less toxic or non toxic products. Biological transformation approaches by microorganisms for the detoxification of aflatoxin B1 were recovered in filamentous fungi *Aspergillus niger* strains FS10 and RAF106, with aflatoxin B1 detoxification efficiencies corresponding to 98.65% and 88.59%, respectively [67,68]. *Aspergillus* sp. strain (NJA-1) showed the capability to degrade deoxynivalenol with a biotransformation efficiency of 94.40% [69]. Fungal strains of *Aspergillus* spp., *Rhyzopus* spp. and *Penicillium* spp. are particularly effective for ochratoxin A removal [70]. The yeast *Saccharomyces cerevisiae* strain ŁOCK 0119 highlighted the capability to degrade aflatoxin B1 with a biotransformation efficiency of 69.00% [71] and *S. cerevisiae* strains IS1/1 and SC82 evidenced biotransformation of fumonisin B1 with efficiencies of 22% and 50%, respectively [72]. The concentration of the T2-toxin after 24 h of incubation with *S. cerevisiae* strains was reduced by 61% of the initial concentration [73]. Another yeast strain assigned to the species *Trichosporon mycotoxinivorants* showed the capability to degrade ochratoxin A and zearalenone in feed [74]. Bacteria of the species *Bacillus subtilis* strains JSW-1 and UTBSP1, were able to degrade aflatoxin B1 with efficiecies of 67.20% [75] and 95.00% [76], respectively; *B. subtilis* strain ASAG 216 evidenced an efficiency of 81.10% for deoxynivalenol degradation [77]; a *B. subtilis* strain and *B. subtilis* strain ANSB01G evidenced the 100.00% and the 88.65% degradation of zearalenone, respectively [78]. Other strains of the genus *Bacillus* evidenced the capability to biotransform aflatoxin B1, including *B. velezensis* strain DY3108 with a biotransformation efficiency of 91.50% [79]; *B. shackletonii* strain L7 92.10% [80]; *B. licheniformis* strain CFR1 with a biotransformation efficiency of 94.70% [81]. A strain of the species *B. natto* was able to degrade zearalenone with an efficiency of 87.00% [82]; *B. pumilus* strain ES-21 and *B. amyloliquefaciens* ZDS-1 evidenced both a zearalenone degradation capability of 95.70% [83,84]. Fumonisin B1 was biotransformed by the three strains *Bacillus* spp. S9, S10 and S69, with an efficiency of biotransformation from 43% to 83% [85]. Strains *Bacillus* spp. showed significant T-2 toxin biodegrading capacity, leading to an 88% decontamination in substrates artificially contaminated with mycotoxin [86]. A strain of the species *Pseudomonas putida* showed an aflatoxin B1 biotransformation efficiency of 90.00% and strain *Pseudomonas* sp. Y1 evidenced the 100.00% removal of deoxynivalenol. Aflatoxin B1 was removed by *Stenotrophomonas* sp. strain CW117 with an efficiency of 100.00% and by *Escherichia coli* strain CG1061 at 93.70%. Deoxynivalenol was biotransformed by *Devisia insulae* strain A16 and by *Eggerthella* sp. strain DII-9, with efficiences of 88.00% and 100.00%, respectively. *Flavobacterium aurantiacum* strain B-184 was able to irreversibly remove aflatoxins from solutions [87]. *Desulfitobacterium* sp. strain PGC-3-9 strain completely de-epoxidated HT-2, deoxynivalenol, nivalenol and 15-acetyl deoxynivalenol, and efficiently eliminated deoxynivalenol in wheat grains under aerobic and anaerobic conditions. This bacterial strain may be used for the development of detoxification agents in the agriculture and feed industries and the isolation of de-epoxidation enzymes [88].

In the case that the use of microorganisms for mycotoxin detoxification is critical because microorganisms features dealing unsuccesful processes or damages to animals, the use of purified enzymes for mycotoxins biodegradation is suggested [34]. Two genes capable of synthesizing enzymes for the degradation of fumonisin B1 by the bacterium *Sphingopyxis* sp. have been isolated and expressed in heterological terms, highlighting the effect of these gene-encoded enzymes to detoxify fumonisin B1 [89,27]. Fungi are able to produce enzymes as laccase and oxidase with a high potential for aflatoxin B1 degrading capability [90]. Moreover, a specific enzyme designated for aflatoxin B1 detoxification named aflatoxin-detoxifizyme, was obtained from the fungus *Armillariella tabescens* and evidenced the capability to degrade aflatoxin B1 and consistently reduce its mutagenic effect [91,92]. Cloning and recombinant expression of the gene of laccase evidenced a 55.00% degradation of aflatoxin B1 [93]. A high efficiency in aflatoxin B1 degradation was also evidenced both in an aflatoxin degrading enzyme from *Bacillus shackletonii* strain L7 and in the enzyme aflatoxin degrading from *Myxococcus fulvus* strain ANSM068 [80,94]. Peroxidase such as manganese peroxidase and lignin peroxidase pointed out the potential for deoxynivalenol degradation [95,96]. The enzyme laccase has the capability to degrade zearalenone [97]. The enzyme fumonisin carboxylesterase FumD can degrade fumonisin B1[98]. Nutritional strategies help mitigation of the mycotoxicoses. Glutamate, cysteine and glycine can be used for the synthesis of glutathione that is involved in the detoxification processes [34]. Mycotoxins are able to cause oxidative stress, a serious mechanism of cytotoxicity. Antioxidants added to mycotoxin-contaminated feed increase the resistance of livestock to mycotoxins. Superoxide anion, the dangerous product of oxidative stress, can be scavenged by antioxidant substrates as selenium, precursors and vitamins A, C, E. These substances were used against mycotoxins toxicity. Selenium addition in animal diet allow protection against aflatoxin B1 toxicity [99]. Selenium counteracts the immunosuppressive activity of deoxynivalenol. Dietary supplement of vitamin B1 reduced the toxicity of *Fusarium* spp. strains. Vitamin E counteracts fumonisin B1 negative effects and ascorbic acid reduced damages to DNA caused by ochratoxin A and zearalenone. Carotenoids are excellent antioxidants, including carotene and xanthophylls, have the capability to counteract damages of aflatoxin B1 [100]. Silymarin and curcumin alleviate mycotoxins effect [101]. Butylated hydroxytoluene is a dietary antioxidant reducing the toxic effects of aflatoxin B1 by inducing glutathione and inhibiting cytochrome P450 [102]. Quercetin counteract toxicity of aflatoxin B1 and resveratrol increase the antioxidant activity [103]. Methionine can alleviate the deoxynivalenol effects and other compounds as soybean isoflavone extract efficiently protects against fumonisin B1 [104,34].

Conclusions

Mycotoxins originated by filamentous fungi contaminating feed and feed commodities are a serious concern for human and animal health and can cause economic loss. Production of mycotoxins as secondary metabolites depends on temperature and humidity. Climate change likely can increase mycotoxins productions in the next years. Pre- and post-harvest interventions can represent valid supports to detoxify different substrates from mycotoxins. Physical, chemical and biological processes can be used to deplete mycotoxins concentrations. Among biological interventions, microorganisms, including bacteria, yeasts and multicellular fungi, can represent sustainable and effective methods for mycotoxin removal. Nevertheless, the most advantageous procedure is represented by combination different approaches, as interventions by agronomic methods, physical and chemical processes and by using biological methods, focusing on microorganisms and on the eventual use of purified

enzymes of microbial origin. It thus emerges that a multidisciplinary approach could give important results to obtain mycotoxins reduction and detoxification in feed and feed components. As future research and insights, further investigations could be carried out on processes using new autochthonous microorganisms capable of degrading mycotoxins.

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