Invited review: Remediation strategies for mycotoxin control in feed

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Abstract
Mycotoxins are secondary metabolites of different species of fungi. Aflatoxin B1 (AFB1), deoxynivalenol (DON), zearalenone (ZEN) and fumonisin B1 (FB1) are the main mycotoxins contaminating animal feedstuffs. These mycotoxins can primarily induce hepatotoxicity, immunotoxicity, neurotoxicity and nephrotoxicity, consequently cause adverse effects on the health and performance of animals. Therefore, physical, chemical, biological and nutritional regulation approaches have been developed as primary strategies for the decontamination and detoxification of these mycotoxins in the feed industry. Meanwhile, each of these techniques has its drawbacks, including inefficient, costly, or impractically applied on large scale. This review summarized the advantages and disadvantages of the different remediation strategies, as well as updates of the research progress of these strategies for AFB1, DON, ZEN and FB1 control in the feed industry.

Keywords: Animal health, Feed, Mycotoxin, Performance, Remediation strategies

Introduction
Mycotoxins are secondary metabolites of various species of fungi that can cause chronic or acute toxicity in animals. Although over 500 mycotoxins have been identified, those of importance in feed safety are primarily produced by the five fungal genera Aspergillus, Fusarium, Penicillium, Claviceps and Alternaria [1–5]. Aflatoxin B1 (AFB1), deoxynivalenol (DON), zearalenone (ZEN) and fumonisin B1 (FB1) are well-known as the main mycotoxins contaminating animal feedstuffs, such as corn, barley, wheat, peanuts, peas, nuts, millet, forage, and their by-products [3–6]. The toxicity of these mycotoxins varies depending on their chemical structure (Fig. 1). The most toxic mycotoxin is AFB1, mainly produced by Aspergillus, which is classified as a Group one carcinogen [7]. It displays hepatotoxic, immunotoxic, mutagenic, carcinogenic and teratogenic characteristics in many animal species [8–11]. Notably, all of DON, ZEN and FB1 are primarily produced by Fusarium molds [5, 12]. DON, a type B trichothecene, can induce anorexia, vomiting, and endanger intestinal and immune functions in different animals by inhibiting the synthesis of nucleic acids and proteins [13–16]. ZEN has a similar structure to estrogen and thus competing with 17 β-estradiol for estrogen receptor binding, consequently leading to fertility and reproductive disorders in livestock [16–19]. FB1 is the most plentiful fumonisins, which can cause hepatotoxicity, neurotoxicity, nephrotoxicity, immunotoxicity, developmental toxicity and cancer in humans and animals [20].

Mycotoxins have been proven to have significant effects on animal health, performance, as well as quality and safety of products, this led to intensive studies over the past few decades on counteracting methods for mycotoxins control in feedstuffs and feed. Generally, physical, chemical, biological and nutritional regulation approaches have been developed as the main strategies for the detoxification of mycotoxins in the feed industry.
Nevertheless, many techniques have been proven to be inefficiency, costly, or impractically applied on large scale [21, 22]. The purpose of this review was to summarize the advantages and disadvantages of the various detoxification strategies, as well as update the research progress of these strategies for AFB\(_1\), DON, ZEN and FB\(_1\) control in the feed industry.

### The strategies of mycotoxin reduction and detoxification

#### Physical methods

Decontamination of mycotoxin by physical techniques mainly includes sorting and separation, washing, solvent extraction, heating, irradiation, and adsorption [27, 28]. The commonly used methods of physical detoxification of mycotoxins are summarized in Table 1.

![Fig. 1 Structural diversity of AFB\(_1\), DON, ZEN and FB\(_1\). AFB\(_1\): Aflatoxin B\(_1\); DON: deoxynivalenol; ZEN: zearalenone; FB\(_1\): fumonisin B\(_1\).](image)

| Methods                           | Commonly used measures and reagents                                    | Decontamination efficiency                                                                 | References |
|-----------------------------------|------------------------------------------------------------------------|-------------------------------------------------------------------------------------------|------------|
| Sorting and separation            | Sieving, aspiration, gravity separation, photoelectric separation, image processing | Removed at least 51%, 63%, 93% of AFs, trichothecenes and fumonisins from the shelled white maize. | [27]       |
| Washing and solvent extraction    | Washing, solvent extraction (methanol, ethanol, hexane, acetonitrile, isopropanol and aqueous acetone etc.) | Removed aflatoxins, trichothecenes, ZEN and fumonisins by 51-72%, 64-69%, 2-61% and 73-74% from the grains through floating and washing with water. | [25, 27, 29] |
| Heating                           | High temperature, high voltage                                         | Decomposed 78.88% of AFB\(_1\) in rice by cooking with pressure (0.10 MPa) at 160 °C for 20 min. Destroyed 90% of DON or ZEN in barely power at 220 °C in 11 or 85min. Reduced 80% FB\(_1\) while cooking rice at 100 °C for 10 min. | [30–32]    |
| Irradiation                       | X-rays, γ-rays and electron beam, ultraviolet rays, infrared and microwave | Reduced 22.0-90.7% of AFB\(_1\) by irradiation. Decomposed 17.2-100% of DON by irradiation. Decontaminated 25.0-86.0% and 60.0-100% of ZEN by γ-rays and ultraviolet rays. FB\(_1\) was inactivated by 63.5-100%, 58.1% and 93.3% by γ-rays, electron beam and microwave in feedstuffs. | [33–41]    |

\(\text{AFs} \): Aflatoxins; \(\text{AFB}_1\): Aflatoxin \(\text{B}_1\); \(\text{DON}\): deoxynivalenol; \(\text{ZEN}\): zearalenone; \(\text{FB}_1\): fumonisin \(\text{B}_1\).
**Sorting and separation** The mycotoxins are not uniformly distributed in grains, which mainly appeared in the moldy, broken and discolored parts [42, 43]. Meanwhile, the specific gravity of the mycotoxins-contaminated cereals is relatively lower than the normal ones. These characteristics enable sieving, aspiration, gravity separation, photoelectric separation, image processing techniques to be used to isolate the mycotoxins-contaminated feedstuffs [27, 44]. Specifically, Matumba et al. [27] reported that flotation, dehulling and hand sorting alone can remove at least 51%, 63%, 93% of aflatoxins (AFs), trichothecenes and fumonisins, respectively, from the shelled white maize, while 98% of these mycotoxins can be removed when combining three of these methods. However, these techniques are costly and only suitable for small-scale applications. Aspiration and gravity separation methods can reduce the DON in wheat, while it reduced the yield of harvested grain [21]. Additionally, near-infrared spectroscopy and optical visual sorting strategies can be used to detect the moldy maize and wheat kernels with more than 92% level of accuracy [22–28, 42–46].

**Washing and solvent extraction** According to the water-soluble or fat-soluble properties of mycotoxin, it could be decontaminated by washing with water or extraction with organic solvent [47]. Floating and washing with water can remove AFs, trichothecenes, ZEN and fumonisins by 51–72%, 64-69%, 2-61% and 73-74%, respectively, from the grains [25, 27, 29]. Notably, floating and washing with a water solution consists of 10-30% NaCl, 30% sucrose, or 1 mol/L sodium carbonate can increase the removal rate of fumonisins from the corn and wheat [25, 48]. A combination of washing and hand sorting technologies together can reduce 84% of fumonisins [49]. The solvents, including methanol, ethanol, hexane, acetonitrile, isopropanol and aqueous acetone, are most commonly used for mycotoxin extraction. Previous studies showed that hexane-aqueous acetone-water (56%:42%:2%) and dimethyl ether can eliminate over 98% of AFs in oil crops [50, 51]. However, these methods have major disadvantages as they result in loss of nutrients, and costly due to drying and toxic extracts disposal, which limit their large-scale application.

**Heating** Thermal treatment has been applied for the decontamination of mycotoxins in feed for many years. The efficiency of this method depends on the chemical structure and concentration of mycotoxins, temperature, duration, moisture content, pH and ionic concentration during the thermal treatment [52]. AFB$_1$, DON, ZEN and FB$_1$ are heat-stable compounds with decomposition temperatures more than 237, 175, 220, 150 °C, respectively [30, 53, 54], which makes it difficult to eliminate them by conventional thermal processing. Conventional hydrothermal treatment (cooking) with pressure (0.10 MPa) at 160 °C for 20 min can decompose AFB$_1$ by 78-88% in rice [31], as well as pressure heating (0.10 MPa) at 120 °C for 4 h can degrade AFB$_1$ by 95% in moist peanut powder [55]. Yumbe-Guevara et al. [30] reported that 90% of DON or ZEN in barley powder can be destroyed at 220 °C for 11 or 85 min. Frying chips at 190 °C for 15 min or drying rice from 150 to 200 °C for 40 min resulted in a loss of 67-70% of FB$_1$, while cooking rice at 100 °C for 10 min reduced 80% of FB$_1$ [32, 56]. Nevertheless, thermal treatments use an excessive amount of energy, also high temperature-induced Maillard reaction would reduce the nutritional values of feed ingredients. This led to a restriction in the application of heat treatments in the feed industry [33].

**Irradiation** Irradiation might be a feasible technology for removing mycotoxins from the feed on an industrial scale. It can be classified into ionizing (x-rays, γ-rays and electron beam) and non-ionizing radiations (ultraviolet rays, infrared and microwave) [57, 58]. The action of irradiation on feedstuffs can induce physical, chemical and biological effects, which reduce or eliminate the mycotoxins [59, 60]. Specifically, AFB$_1$ can be reduced by 43.0-87.8%, 65.7-71.5%, 22.0-100%, 90.7% by γ-rays, electron beam, ultraviolet rays and microwave, respectively, in different cereals [33–35]. DON can be decomposed by 37.0-82.4%, 17.2-56.3%, 83.4-100% by γ-rays, electron beam and ultraviolet rays, respectively, in feedstuffs [36–39]. ZEN can be decontaminated by 25.0-86.0% and 60.0-100% by γ-rays and ultraviolet rays, respectively, in grains [34, 36–38]. FB$_1$ was inactivated by 63.5-100%, 58.1% and 93.3% by γ-rays, electron beam and microwave, respectively, in feedstuffs [35, 40, 41]. These different decomposition efficiencies of irradiation depend on the variation in the treatment condition, including doses and time of irradiation, the shape and composition of feedstuffs [61, 62]. Although irradiations can be considered as a potentially promising approach to decontaminate mycotoxins in feedstuffs, their safety issues such as mutagenesis that generates harmful microorganisms and damage the nutritional values of feedstuffs require a declaration and further studies.

**Adsorption** Adsorption binders can form a complex with mycotoxins, thus prevent mycotoxins passage from the gastrointestinal tract into the blood and organs of animals. In the past decades, numerous binders from different origins have been investigated for their capacity to adsorb mycotoxins [52, 63]. Therefore, the adsorbent detoxification treatment is currently well understood and widely used to detoxify mycotoxins in the feed industry. In general, any ideal mycotoxin absorbent should
possess these following properties, including high adsorption capacity against either range of mycotoxins (especially mycotoxins with low hydrophobicity), low non-specific binding to nutrients, as well as high safety, stability and palatability [52]. Table 2 shows lists of current patents related to adsorbing mycotoxin including AFB, DON, ZEN and FB control in the feed.

Aluminosilicate minerals, as the largest class of mycotoxin adsorbents, are the most widely applied and studied minerals in the decontamination of mycotoxin. Such adsorption binders mainly include bentonite, montmorillonite, zeolite, hydrated sodium calcium aluminosilicate, kaolin, illite, etc. [63]. The binding efficacy of mineral adsorbents is associated with the structures of both the binders and the mycotoxins. The binding efficiency depends significantly on the surface area, charge distribution and pore size of adsorption binders and the charge distribution, polarity and shape of the mycotoxins [52]. Some mycotoxins such as AFs have an ionic charge, thus clay minerals such as bentonite, illite, zeolite and kaolin are effective at removing them from the feed with more than 90% efficiency [87]. Numerous

Table 2 Summary of adsorbents with mycotoxins mitigation effects

| Adsorbent                        | Mycotoxins | Binding efficiency                                      | Reference |
|----------------------------------|------------|--------------------------------------------------------|-----------|
| Zeolite                          | AFB        | Decreased AFB residue in duck meat by 65% significantly and numerically decreased AFB residue in liver and egg. | [64]      |
| Bentonite clay                   | AFB        | Decreased liver AFB residue by 41-87% when broilers fed AFB in diet. | [65]      |
| Sodium bentonite                 | AFB        | Decreased liver AFB residue by 62.5% when broilers fed AFB in diet. | [66]      |
| Modified maifanite               | ZEN        | Decreased ZEN residue in liver and muscle by 54.96% and 42.41% respectively at the dose of 1% when pig fed 1.11 mg/kg AFB in diet. | [67]      |
| Bentonite or montmorillonite     | AFB, ZEN   | Decreased rumen concentration of AFB and ZEN, decreased AFM in milk and ZEN in feces. | [68]      |
| Organo-clay composites           | AFB        | Decreased AFB concentrations in liver, kidney and plasma significantly in chickens. | [69]      |
| Tri-octahedral bentonite         | DON, ZEN   | Adsorbed more than 90% of ZEN and FB, while the adsorption dose up to 0.2% w/v. | [70]      |
| Pillared montmorillonite         | DON        | Adsorbed 14.7-23.4% and 21.8-27.4% of DON at pH 2.0 and pH 6.8. | [71]      |
| Nonionic surfactant octylphenol polyoxyethylene ether modified montmorillonites | AFB, ZEN | The adsorption capacities of modified montmorillonites to AFB and ZEN increased up to 2.78 and 8.54 mg/g respectively from 0.51 and 0.00 mg/g by the raw montmorillonite. | [72]      |
| Hydrated sodium calcium alumino silicate | AFB, FB | Adsorbed AFB and FB, in an aqueous solution, and the adsorption ratio ranged from 95.3% to 99.1% and 84.7% to 92.4%, respectively. | [73]      |
| Modified Hydrated sodium calcium alumino silicate | DON | Reduced the toxicity of DON in weanling piglets. | [16]      |
| Esterified glucomannan           | AFs, ZEN, DON | Adsorbed 95%, 80% and 12% of aflatoxin, ZEN and DON. | [73, 74] |
| Inactivated yeast cell wall and low Yeast fermenting volatile organic compound | AFs, DON | Decreased AFs and DON synthesis by 82% and 93% respectively. | [75]      |
| Distillers' wet grain, distillers' dried grains and distillers' dried grain with solubles | DON, ZEN | Adsorbed 48.9% and 67.9% of DON and ZEN (1 ppm each) using 5 g/L of micronized (20 mkm) yeast mass at 37 °C for 1h. | [76]      |
| Yeast cell wall extract          | ZEN        | Adsorbed 40% of the total ZEN content in the intestines in monogastric animals. | [77, 78] |
| Activated charcoal               | AFB, ZEN   | Reduced the toxicity of AFB, on broilers and decreased the absorption rate of ZEN in small intestine from 32% to 5% when adding 2%. | [79, 80] |
| Cholesteryamine                  | ZEN        | Decreased the absorption rate of ZEN in small intestine from 32% to 16%. | [80]      |
| Magnetic carbon nanocomposites   | AFB        | Adsorbed nearly 90% of AFB within 180 min at pH 7.0. | [81]      |
| Cross-lined chitosan polymers    | AFB, ZEN, FB, DON | Adsorbed 73% of AFB, 94% ZEN and 99% FB, but the adsorption ratio of DON less than 30%. | [82]      |
| Polyvinylpyrrolidone             | ZEN        | Adsorbed 2.1 mg/g of ZEN. | [83]      |
| Lactobacillus casei              | AFB        | Reduced the absorption of aflatoxin in the intestinal tract significantly. | [84]      |
| Lactobacillus plantarum F22      | AFB        | Adsorbed 56.8% of AFB. | [85]      |
| Lactobacillus plantarum B7       | FB         | Adsorbed 52.9% of FB. | [86]      |
| Lactobacillus pentosus X8        | FB         | Adsorbed 58% of FB. | [86]      |

*AFs Aflatoxins, AFB, Aflatoxin B, DON deoxynivalenol, ZEN: zearalenone; FB:B: fumonisin B*
studies reported that zeolite, bentonite clay and sodium bentonite decreased AFB$_1$ residues in the liver by 41-87% and numerically decreased AFB$_1$ residue in the meat and egg when broilers or ducks fed AFB$_1$ contaminated diet [64-66]. Chen et al. [67] reported that ZEN residue in liver and muscle of pigs were decreased by 55.0% and 42.4%, respectively, when supplemented with 1.0% modified maifanite in diet included 1.11 mg/kg ZEN. In ruminant feed, bentonite or montmorillonite decreased rumen concentration of AFB$_1$ and ZEN and also decreased AFM$_1$ in the milk and ZEN in the feces in goats [68]. Tzou et al. [69] prepared organo-clay composites by mixing bentonite-enriched clay with nonionic surfactants (Brij 30 and Igepal CO-890) and added organo-clay composites to feed. After chickens had consumed amended feed for 11 weeks, AFB$_1$ concentrations in the liver, kidney, and plasma were significantly lower than the AFB$_1$ control dietary treatment. Although many aluminosilicate adsorbents can adsorb strongly polar toxins, such as AFB$_1$, FB$_1$, etc. as supported by many studies, they appear to be ineffective at absorbing other non-aflatoxin mycotoxins including DON and ZEN [88, 89]. Bentonites have been considered as promising adsorbents for high-efficient removal of mycotoxins from the animal feed as they are eco-friendly, low-cost and highly efficient in adsorption of mycotoxins, modifying clays also could help to increase their adsorptive ability to non-polar mycotoxins [90-92]. To date, only one di-octahedral bentonite (1m588) was authorized as an anti-aflatoxin additive by the EU Regulation in 2009 [93]. Vila-Donat et al. [70] reported that tri-octahedral bentonite could adsorb more than 90% of ZEN and FB$_1$ while the adsorption dose up to 0.20% (w/v). Nonionic surfactant octylphenol polyoxyethylene ether and modified montmorillonites, as mycotoxins adsorbent, were used for adsorption of AFB$_1$ and weak polar ZEN in both single and binary-contaminate systems by simulating the conditions of the gastrointestinal tract. Modified montmorillonites increased the adsorption capacities to AFB$_1$ from 0.51 mg/g of raw montmorillonite to 2.78 mg/g and ZEN from 0.00 mg/g of raw montmorillonite to 8.54 mg/g [72]. Adsorption of DON by pillared montmorillonite modified with aluminum, iron and titanium was investigated using UPLC-MSMS (at pH 2.0 and 6.8) and the results demonstrated that the adsorption ratios were 14.7-23.4% at pH 2.0 and 21.8-27.4% at pH 6.8 [71]. The commercially hydrated sodium calcium aluminosilicate has an excellent capability of adsorbing AFB$_1$ and FB$_1$ in an aqueous solution, and the adsorption ratio ranged from 95.3-99.1% and 84.7-92.4% of the available AFB$_1$ and FB$_1$, respectively [73]. Mineral adsorbents have been modified with quaternary long-chain alkyl/aryl amines to improve the adsorption of non-aflatoxin mycotoxins [74]. The binder Amadetox™ is mainly comprised of hydrated sodium calcium aluminosilicate that has been modified by cetlypyridinium chloride and intercalation with β-glucan [94]; these modifications increase the surface area of hydrated sodium calcium aluminosilicate, which maximizes the binding of mycotoxins with minimal adsorption of nutrients. Zhang et al. [16] reported that a modified hydrated sodium calcium aluminosilicate adsorbent could reduce the toxicity of DON in weaning piglets [16]. Furthermore, it must be noted that these adsorbents can adsorb micronutrients and have negative effects on the bioavailability of trace minerals and vitamins.

Second generation adsorbents have been developed originating from the cell wall component of microorganisms. Gluconannan is a common adsorbent that cannot be used by gut microbes and strongly adsorbed toxic substances and harmful pathogenic bacteria in animals. Mycotoxins can be adsorbed by esterified glucomannan, which is a kind of broad-spectrum mycotoxin adsorbent with an effective binding ability for AFs, ZEN, FBs and DON by 95%, 75%, 59% and 12%, respectively [73, 74]. Esterified glucomannan has been proved to improve the adverse consequences of mycotoxins on the performance, immunity, blood haematological and biochemical indices of chickens [70, 76, 78, 94]. The β-D-glucan chains of yeast cell walls have been demonstrated to effectively inactivate ZEN [77, 95]. Zeidan et al. [75] reported that inactivated yeast cell walls and low yeast fermenting (L. thermotolerans) volatile organic compounds could decrease AFs and DON synthesis by 82% and 93%, respectively, in vitro. A combination of mineral clay and yeast cell walls showed a considerably enhanced binding capacity of AFs, ZEN and fumonisins in an in vitro study; however, the adsorption abilities toward DON, ochratoxin A and T-2 toxin were low (< 60%) [96]. The yeast biomass obtained from distillers’ wet grain, distillers’ dried grains and distillers’ dried grain with solubles have the ability to bind various mycotoxins and adsorbed 48.9% and 67.9% of DON and ZEN (1.0 mg/kg each), respectively, using 5.0 g/L micronized yeast mass at 37 °C for 1 h [76]. In addition, the yeast cell walls extract adsorbed ZEN in the gastrointestinal tracts of monogastrics [77] and was able to adsorb 40% of the total ZEN contents in the intestines [78].

Activated charcoal, as a general adsorbent, has a large surface area and excellent adsorption capabilities in aqueous environments. Activated charcoal has demonstrated the ability to reduce AFs, ZEN, DON due to its porous structure in several studies [97, 98]. The partial protection induced by activated charcoal in lowering mycotoxin residues in the liver of broilers has been observed previously [65, 99]. The addition of 0.1% activated carbon to feed containing 10 mg/kg AFB$_1$ was able to reduce the detrimental effects of AFB$_1$ on broilers [79].
Avantaggiato et al. [80] found that the absorption rate of ZEN in the small intestine decreased from 32% to 5% when activated carbon was added at 2.0% in an in vitro gastrointestinal model. Cholestyramine is an anion exchange resin. The addition of cholestyramine decreased the absorption rate of ZEN in the small intestine from 32% to 16% using a laboratory model. The adsorption effect of multiple lactic acid bacteria was more effective than a single strain.

**Chemical methods**

Chemical techniques can destroy the structure of the mycotoxins, which generate mildly toxic or nontoxic products. Decontamination of mycotoxins by chemical techniques primarily includes alkaline and ozone treatments, as well as other chemical agent treatments [104, 105]. The commonly used methods of chemical detoxification of mycotoxins are summarized in Table 3.

**Alkaline treatment** Alkaline chemicals, including ammonia, sodium hydroxide, potassium hydroxide and sodium carbonate, etc., have been used for the destruction of various mycotoxins in the moldy feedstuffs [104, 105]. The lactone ring structure of AFB1 can be opened by base hydrolysis to produce coumarin sodium salt and then further be eliminated by washing with water [120]. Ammoniation and hydroxide salts treatments are the common approach that has been used to remove AFB1 from feed ingredients, with more than 95% removal rate in various cereals [107–110]. An epoxide at C-12 and C-13, essential for the toxicity of DON, can be destructed under alkaline conditions [28]. Sodium carbonate and hydroxide salts treatments can reduce DON by 83.9–100% in different feedstuffs [111, 112]. Although these treatments could nearly reduce the complete concentration of mycotoxins, the possible transformation of mycotoxins to other forms such as masked mycotoxins, along with the harmful side effects on the environment and food (changes in nutritional quality, texture, or flavor), the quality and safety assessments of chemically treated products are necessary [104, 105].

**Ozone treatment** Mycotoxin oxidizing agent treatment is an effective detoxification method through changing the molecular structure of mycotoxins. The oxidizers commonly used are ozone, hydrogen peroxide, sodium and calcium hypochlorite, chlorine and other oxidizers [106, 121]. AFs, DON, ZEN and FB1 have been shown to be effectively degraded by ozone [122–124]. Agiropoulou et al. [125] has found that ozone has the ability to degrade AFs (AFB1, AFB2, AFG1 and AFG2). Trombete et al. [126] reported that ozone concentration, form and exposure time influenced positively the reduction of DON, AFs and fungal count. AFs can be reduced by 92-95% in corn and by 91% or 78% in cottonseed or peanut meal, respectively, by ozone [113, 127, 128]. DON can be reduced by 70-90% in corn and by 20-80% in wheat by ozone [112, 114–116]. The degradation of ZEN in corn can reach 90.7% through the ozone treatment with 100 mg/L ozone for 180 min [117]. Furthermore, there are other oxidizing agents such as sodium hypochlorite and hydrogen peroxide that can effectively degrade mycotoxins [118, 119, 129, 130].
Although the ozone treatment can result in a complete reduction in the mycotoxin concentration, it can cause changes in the physical and chemical composition of the feed, such as changes in starch structure, lipid oxidation, protein denaturation, color change and processing properties [106, 113, 126]. Moreover, these treatments may produce some harmful chemicals to the health of animals [106, 113, 126].

**Biological methods**

Although many physical and chemical decontamination strategies have been developed to reduce or eliminate mycotoxins in feed ingredients or complete feed, few techniques met the requirements of practical application owing to their limitation of binding efficiency, bio-safety or cost-effectiveness. Therefore, as a promising strategy, owing to their limitation of binding efficiency, bio-safety techniques met the requirements of practical application.

**Microorganisms with detoxification activities**

Biology-based detoxification methods are widely recognized as specific, efficient and environment-friendly. The nutritive and sensory characteristics like color and flavor are reserved without involving harmful chemicals. Screening and isolating naturally existing microorganisms that show biotransformation capabilities against specific mycotoxins have been a popular strategy. Mycotoxin biodegradation technology is the process by which the toxic group of the mycotoxin molecules is broken down and destroyed by the secondary metabolites produced by microorganisms or their secreted intracellular and extracellular enzymes, while producing non-toxic or less toxic degradation products.

A number of different fungal have been shown to detoxify AFB1. Fungal strains such as *S. cerevisiae* LOCK 0119 has been shown to degrade AFB1 at levels of 69.0% [131]. Similarly, some studies reported that the ability of various *Aspergillus* strains such as *A. niger* RAF106 have shown the ability to degrade AFB1 to levels between 88.6% and 98.7% [132, 133]. Bacteria degraded AFs mainly by secreting extracellular enzymes. Some strains of *Nocardia corynebacterioides*, *Flavobacterium aurantiacum* and *Bacillus* have been shown to degrade AFB1. Smiley and Draughon reported that the degradation efficiency of AFB1 by *Nocardia corynebacterioides* reached 74.5% in 24 h [159]. *Flavobacterium aurantiacum* could degrade AFB1 efficiently and its crude protein extract could degrade 74.5% of AFB, [160, 161]. *Bacillus* is an important class of bacteria capable of degrading AFB1. Farzaneh et al. [141] isolated *Bacillus subtilis* UTBS1 from Iranian pistachio nut and the degradation rate of AFB1 reached 78.4–95.0%. *Bacillus subtilis* ANS806 isolated from the fish intestine could degrade 81.5% of AFB1 within 72 h [162]. In addition, other *Bacillus* such as *Bacillus licheniformis* CFR1, *Bacillus velezensis* DY3108, *Bacillus subtilis* JSW-1 and *Bacillus shackletonii* L7 have been able to degrade AFB1 to levels between 67.2–94.7% [136–139]. Other bacteria such as *Pseudomonas putida*, *Escherichia coli* CG1061 and *Stenotrophomonas* sp. CW117 also showed very efficient biodegradation rates up to 90% or more for AFB1 [134, 135, 140].

*Devsosia insulae* A16, Strain E3-39, *Bacterial consortium* C20, *Pseudomonas* sp. Y1 and *Lysobacter* sp. S1 isolated from soil samples can convert DON to 3-ketoDON or 3-epi-DON, a less toxic derivative [142, 144, 145, 150]. Several studies have revealed that these strains resulted in 74-100% reduction of DON [142, 144, 145, 150]. From a different point of view, *Bacterial isolates* LS100 and SS3, *Bacterial strain* BBSH 797 and *Eggerthella* sp. DII-9 presented a high biotransformation activity of converting DON to diepoxy-deoxynivalenol [146, 148, 149]. Strains isolated from the intestine of donkeys and soil samples, namely *Bacillus subtilis* ASAG 216 and *Aspergillus* (NJA-1) have shown to decrease DON concentration by 81.1% and 94.4% [143, 147].

Microorganisms metabolize ZEN mainly through conversion or degradation to α-zearalenol, β-zearalenol, sulfate and other secondary metabolites with low or

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**Table 3** Summary of physical methods for mycotoxins detoxification.

| Methods          | Measures and reagents                                      | Detoxification efficiency                                                                 | Reference |
|------------------|-----------------------------------------------------------|-------------------------------------------------------------------------------------------|-----------|
| Alkaline treatment | Ammonia, sodium hydroxide, potassium hydroxide and sodium carbonate etc. | Removed 95% of AFB1 in various cereals by ammoniation and hydroxide salts treatments. Reduced DON by 83.9-100% in different feedstuffs through sodium carbonate and hydroxide salts treatments. | [106–111] |
| Ozone treatment  | Ozone, hydrogen peroxide, chlorine, sodium and calcium hypochlorite etc. | Reduced 92-95%, 91% and 78% of AFBs in corn, cottonseed and peanut meal respectively by ozone. DON can be reduced 70-90% in corn and 20-80% in wheat by ozone. The degradation of ZEN in corn can reach 90.7% through the ozone treatment with 100 mg/L ozone for 180 min. | [112–119] |

*AFB1; Aflatoxin B1, DON deoxynivalenol, ZEN zearalenone, FB1 fumonisin B1*
non-toxicity. *Bacillus natto* and *Bacillus subtilis* strains were shown to remove ZEN from the liquid medium: more than 75% ZEN could be biodegraded after incubation. In another study, up to 99% of ZEN was degraded by *B. subtilis* strain [151]. Lei et al. [154] isolated *Bacillus subtilis* ANSB01G from broiler intestinal chyme, and the degradation rate of ZEN by this strain in a liquid medium, natural mold corn, distillers' dried grain with solubles and a complete pig feed were 88.7%, 84.6%, 66.3% and 83.0%, respectively. *Bacillus pumilus* ES-21 and *Bacillus amyloliquefaciens* ZDS-1, isolated from soil samples, showed 95.7% reduction of ZEN [152, 153].

Some fungal and bacterial microorganisms have been reported to be able to degrade fumonisins. Styriak et al. [157] screened two strains of preserved yeast from the laboratory 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 the mixture FB1 and FB2 in the culture medium, the other one is *Saccharomyces cerevisiae* SC82, which also degrade FB1 and the mixture FB1 and FB2, the degradation rates were 22% and 25%, respectively [157]. Camilo et al. [158] screened three strains such as *Bacillus* spp. S9, S10 and S69, that degraded 43%, 48% and 83% FB1, respectively. Strain NCB 1492, isolated from soil samples, can completely degrade FB1 under 25°C, after 24 h [156]. Notably, another study reported that the degradation rate of FB1 by *Bacterial consortium* SAAS79 can reach 100% [155].

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**Table 4:** Biological biotransformation approaches by microorganisms for the detoxification of mycotoxins.

| Mycotoxins | Microorganisms | Biotransformation efficiency | Reference |
|------------|----------------|-------------------------------|-----------|
| AFB1       | Aspergillus niger FS10 | 98.65%                        | [133]     |
|            | Aspergillus niger RAF106 | 88.59%                        | [132]     |
|            | Stenotrophomonas sp. CW117 | 100.00%                      | [134]     |
|            | *S. cerevisiae* LOCK 0119 | 69.00%                        | [131]     |
|            | *Escherichia coli* CG1061 | 93.70%                        | [135]     |
|            | *Bacillus velezensis* DY3108 | 91.50%                      | [136]     |
|            | *Bacillus subtilis* JSW-1 | 67.20%                        | [137]     |
|            | *Bacillus stackeletii* L7 | 92.10%                        | [138]     |
|            | *Bacillus licheniformis* CFR1 | 94.70%                      | [139]     |
|            | *Pseudomonas putida*     | 90.00%                        | [140]     |
|            | *Bacillus subtilis* UTBSP1 | 95.00%                        | [141]     |
| DON        | *Bacterial consortium* C20 | 74.29%                      | [142]     |
|            | *Bacillus subtilis* ASAG 216 | 81.10%                      | [143]     |
|            | *Deovisia insulare* A16  | 88.00%                        | [144]     |
|            | *Pseudomonas* sp. Y1 and *Lysobacter* sp. S1 | 100.00% | [145] |
|            | Eggerthella sp. DII-9       | 100.00%                      | [146]     |
|            | Aspergillus (NJA-1)           | 94.40%                        | [147]     |
|            | *Bacterial isolates* LS100 & SS3 | 100.00%       | [148]     |
|            | *Bacterial strain* BBSH 797  | -                             | [149]     |
|            | Strain E3-39                | 100.00%                      | [150]     |
| ZEN        | *Bacillus subtilis*         | 100.00%                      | [151]     |
|            | *Bacillus natto*            | 87.00%                        | [151]     |
|            | *Bacillus pumilus* ES-21    | 95.70%                        | [152]     |
|            | *Bacillus amyloliquefaciens* ZDS-1 | 95.70% | [153] |
|            | *Bacillus subtilis* ANSB01G | 88.65%                        | [154]     |
| FB1        | *Bacterial consortium* SAAS79 | 100.00%                      | [155]     |
|            | Strain NCB 1492             | 100.00%                      | [156]     |
|            | *Saccharomyces cerevisiae* IS1/1 and SC82 | 22%-50% | [157] |
|            | *Bacillus spp.* S9, S10 and S69 | 43%-83% | [158] |

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*a AFB1, Aflatoxin B1, DON deoxynivalenol, ZEN zearalenone, FB, fumonisin B1.

-means the biotransformation efficiency did not reported.
The usage of catabolizing enzymes Although some microorganisms are highly active in biodegrading mycotoxins, some of them might secrete harmful metabolites or cannot survive in the gastrointestinal tract of the animals [163, 164]. Therefore, screening the enzymes from these microorganisms might be the promising strategy to solve the issues. Recently, there are many researches that have focused on the isolation of the enzymes that can biodegrade AFB₁, DON, ZEN and FB₁. The enzymes for the biodegradation of AFB₁, DON, ZEN and FB₁ in the feed are presented in Table 5.

The main fungal enzymes known to have degradation activity against AFB₁ are laccase and oxidase [163]. The enzyme for AFB₁ detoxification designated as aflatoxin-detoxifyzyme was reported [164]. The gene was identified and cloned from an Armillariella tabescens. The recombinant aflatoxin-detoxifyzyme was able to detoxify AFB₁ and significantly reduce its mutagenic effects. Manganese peroxidase (1.5 U/mL) can degrade 90% AFB₁ after 48 h of reaction [165]. Alberts et al. [167] recombinantly expressed the laccase gene by gene cloning and its degradation rate of AFB₁ was 55%. Bacillus aflatoxin-degrading enzyme and myxobacteria aflatoxin degradation enzyme secreted by Bacillus shackletonii L7 and Myxococcus fulvus ANSM068 are also efficient in degrading AFB₁ [138, 166].

Although there are early reports on an NADH-dependent bacterial cytochrome P450 system that transforms DON into 16-hydroxy-DON, no efficient DON biotransformation enzymes are patented yet [172]. Peroxidase such as manganese peroxidase and lignin peroxidase showed the potential for significant DON degradation [168, 171]. Aldo-keto reductase DepA and DepB can transfer DON to 3-keto-DON and 3-epi-DON which have lower toxicity than DON [170]. A quinone-dependent dehydrogenase and two NADPH-dependent aldo/keto reductases (AKR13B2 and AKR6D1) can detoxify deoxynivalenol in wheat via epimerization in a Devisoa strain [169].

Laccases are copper-containing oxidases have high potential in degrading the heat-stable mycotoxin ZEN, which involved in many industrial application [176, 177]. A novel ZEN-specific lactonohydrolase was developed previously as a producer of different hydrolytic enzymes for feed biorefinery. The recombinant ZEN-specific lactonohydrolase secreted by the transformed fungal clones into the culture liquid was shown to remove ZEN [173]. A recombinant fusion enzyme by combining two single genes named ZEN-specific lactonohydrolase and carboxypeptidase have demonstrated that can completely degrade ZEN to the non-toxic product in 2 h at an optimum pH of 7 and a temperature of 35 °C [174].

The fumonisin carboxylesterase FumD can degrade FB₁ to its less toxic metabolite the hydrolyzed FB₁ in the gastrointestinal tract of turkeys and pigs [175]. Within 2 h of incubation with FumD, FB₁ was completely degraded to hydrolyzed FB₁ in the duodenum and jejunum in an ex vivo pig model [175].

### Nutritional strategies
It is well accepted that none of the physical, chemical or biological strategies can totally decontaminate the mycotoxin in feed, considering that even a low consumption level of a mycotoxin can cause chronic toxicity including a reduction of the performance and immunosuppression in animals [45], therefore, development of nutritional

| Mycotoxins | Degrading enzyme | Origin | Reference |
|------------|------------------|--------|-----------|
| AFB₁       | Bacillus aflatoxin-degrading enzyme | Bacillus shackletonii L7 | [138] |
|            | Manganese peroxidase | Pleuratus ostreatus | [165] |
|            | Afattoxin-Oxidase | Armillariella tabescens | [163] |
| DON        | Myxobacteria aflatoxin degradation enzyme | Myxococcus fulvus ANSM068 | [166] |
|            | Laccase | White rot fungi | [167] |
|            | Manganese peroxide and Lignin peroxidase | Spent Mushroom Substrate | [168] |
|            | Quinone-dependent dehydrogenase, NADPH-dependent aldo/keto reductases | Devosia sp. D6-9 | [169] |
|            | Aldo-keto reductase DepA/DepB | Devosia mutans 17-2-E-8 | [170] |
|            | Peroxidase | Rice bran | [171] |
|            | Cytochrome P450 system | Sphingomonas sp. strain KSM1 | [172] |
| ZEN        | ZEN-specific lactonohydrolase | Recombinant enzymes | [173] |
|            | A fusion enzyme by combining ZEN-specific lactonohydrolase and carboxypeptidase | Clonostachys rosea strain IFO7063 and Bacillus amyloliquefaciens strain ASAG1 | [174] |
| FB₁        | Fumonisin carboxylesterase FumD | Recombinant enzymes | [175] |

*aAFB₁, Afatoxin B₁, DON deoxynivalenol, ZEN zearalenone, FB₁, fumonisin B₁*
Strategies to help mitigation of the mycotoxicoses is also important. Some nutritional strategies that have been disclosed are presented in Table 6.

It is feasible to modulate the mycotoxin detoxification system through nutritional measures. On the one hand, detoxification systems in animals including CYP450s, ketoreductase, α-glutathione transferase, etc. can degrade mycotoxins [9, 10]. Therefore, any nutrient that can promote the normal functioning of one of the above detoxification enzyme systems can be used as a nutritional regulator. Glutamate, cysteine and glycine can be used as substrates for the synthesis of glutathione and participate in the detoxification process by forming glutathione. On the other hand, mycotoxins can reduce nutrient uptake, so adding critical nutrients is one of the ways to mitigate the harmful effects of mycotoxins [13–15].

Oxidative stress is an important mechanism of cyto-toxicity caused by mycotoxins [9, 10]. Adding antioxidants to mycotoxin-contaminated feed can improve the antioxidant capacity of the organism and increase the resistance of livestock and poultry to mycotoxins. Selenium, some vitamins A, C and E, and their precursors have marked antioxidant properties that act as superoxide anion scavengers. For these reasons, these substances have been investigated as protecting agents against toxic effects of mycotoxins. Selenium is an essential trace element for humans and animals as it plays an important role in antioxidant defense, anticancer, immunity, and detoxification [181, 182]. Previous studies have shown that dietary selenium supplementation can help to protect against AFB1-induced hepatotoxicity, immunotoxicity, and genotoxicity in chicks, which is mainly associated with regulation of redox/inflammation/apoptotic signaling and CYP450 isozymes [11]. Selenium has the potential to counteract DON-induced immunosuppression in piglets by increased the expression levels of IL-2, IL-10, IFN-γ, IgG, and IgM mRNA and protein in piglet splenic lymphocyte [190]. Selenium, vitamins C and E could be used as antioxidants to protect the spleen and brain cell membranes from DON toxicity and against DNA damage in liver caused by DON [191]. Nagaraj et al. [183] reported that dietary supplemented vitamin B1 reduced the toxicity of fumaric acid and alpha-tocopherol reduced DNA adducts in the kidney and liver of mice exposed to ochratoxin A and ZEN from 70-90%. Carotenoids (carotene and xanthophylls) are excellent antioxidants with antimutagenic and anticarcinogenic properties, which have been demonstrated can inhibit AFB1-induced liver DNA damage in rats [178].

Silymarin is a potent antihepatotoxic agent provide protection against the negative effects of AFB1 on performance of broiler chicks [184]. Curcumin alleviates AFB1 toxicity through downregulating CYP450 enzymes, promoting ATPase activities in chickens [185]. Pretreatment with silymarin, curcumin enhanced the viability of cells exposed to the mycotoxins and attenuated reactive oxygen species formation by DON, partially reduced ROS formation by FB1 [180]. Curcumin significantly decreased apoptosis in cells exposed to DON, whereas silymarin was able to prevent apoptosis exposed to FB1 and DON in PK-15 cells [180]. Gao et al. [17] reported that dietary silymarin supplementation protected rats from ZEN-induced hepatotoxicity and reproductive toxicity through improvement in the antioxidant capacity and regulation in the genes related to ZEN metabolism, hormone synthesis, protein synthesis, and ABC transporters in the tissues.

Butylated hydroxytoluene, a dietary antioxidant in mammals, has been shown to lessen the toxic effects of AFB1 by inducing the activity of glutathione sulfotransferase and inhibiting the activity of cytochrome P450 1A5 [198]. Li et al. [186] reported that alpha lipoic acid improved the growth performance and alleviated the

| Mycotoxins | Nutritional strategies | Mechanisms | Reference |
|------------|-----------------------|------------|-----------|
| AFB1       | Selenium, vitamins C, vitamins E, vitamin B1, carotenoids, silymarin, curcumin, butylated hydroxytoluene, alpha lipoic acid, quercetin, resveratrol, rhamnoides oil | Mainly by improving antioxidant capacity and detoxification enzyme activities to alleviate the harm of AFB1 to livestock and poultry | [11, 178–180] |
| DON        | Selenium, vitamins C, vitamins E, silymarin, curcumin, functional amino acid (methionine, glutamic acid, arginine, aspartate and lysine), antimicrobial peptide, astragalus | Primarily through enhancement of antioxidant capacity and immune functions to improve the resistance to DON in livestock and poultry. | [179, 180, 189–194] |
| ZEN        | Retinol, ascorbic acid, alpha-tocopherol, silymarin, soybean isoflavone | Alleviated the toxic effects of ZEN by improving the antioxidant capacity and inhibiting the estrogenic toxicity of ZEN. | [17, 190, 195] |
| FB1        | Vitamin E, silymarin, curcumin, soybean isoflavone | Mainly via counteracting the oxidative stress caused by FB1 to livestock. | [180, 196, 197] |

*AFB1, Aflatoxin B1, DON deoxynivalenol, ZEN zearalenone, FB1, fumonisin B1*
liver damage associated with improved the antioxidant capacity in the broilers exposed to AFB1. Quercetin exerted its beneficial effects by depressing the bioactivation of AFB1 and counterbalancing its pro-oxidant effects in a bovine mammary epithelial cell line [187]. Resveratrol, a polyphenol derived from red grapes, berries and peanuts, exerted anti-inflammatory and antioxidant effects. Dietary supplementation of resveratrol helped in increasing the activities of the oxidative enzymes and in improving the plasma total antioxidant capacity and total protein in broilers fed with AFB1 [188]. Solcan et al. [199] reported that *rhamnoides* oil had a potent hepatoprotective activity, reduced the concentration of AFs in the liver and diminished their adverse effects in broilers.

Andretta et al. [192] suggested that methionine can alleviate the DON induced adverse effects in growing pigs. Supplementing glutamic acid, arginine, aspartate and lysine to a diet had positive effects on remission of visceral disease induced by DON, enhancement of antioxidant ability and improvement of blood physiological and biochemical indexes of fattening pigs [200]. Dietary supplementation of 2.0% glutamic acid could mitigate DON induced negative effects on the growth performance and intestinal injury in the weaned piglets [193]. Xiao et al. [179, 194] found that an antimicrobial peptide complex composed of lactoferrin peptide, plant defensin and active yeast effectively improved the adverse effects of DON on production performance, autoimmunity and intestinal functions of weaned piglets. Astragalus played an important role in the reduction of immunosuppression and organ damages of the liver and kidney induced by DON and can improve the immunofunction significantly in mice [189]. Wang et al. [190] suggested that soybean isoflavone added to diets at 600 mg/kg could reduce the harmful effects induced by 2.0 mg/kg ZEN on the reproductive organs in prepubertal gilts during the growth phase. In an in vivo study on rats, Lu [197] reported that soybean isoflavone extract has a marked protective action against FB1 hepatotoxicity by the suppression of FB1-stimulated prostaglandin production.

### Conclusion and perspectives

The occurrence of mycotoxins in the feed is of a great concern and an unavoidable problem in the feed industry around the world. Mycotoxins also endanger human health through the cycle of the food chain. This review summarizes a number of strategies to reduce mycotoxin contamination in terms of physical detoxification (separation, washing, heating, irradiation and adsorption), chemical treatments (bases and oxidizing agents), biological detoxification methods (microorganisms and enzymes), and nutritional regulation strategies. Each of these approaches can be practically used while along with their own advantages and disadvantages. However, with the 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.

### Abbreviations

AFs: Aflatoxins; AFB1: Aflatoxin B1; DON: Deoxynivalenol; ZEN: Zearalenone; FB1: Fumonisin B1.

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### Authors’ contributions

LHS conceptualized and designed this review. ML, LZ, GXG, LZ, LS, JFD, YMH, and YYW collected the data. ML and LHS wrote the manuscript. MMK and JFD have revised the grammar of the manuscript. All authors have read and approved the final manuscript.

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### Availability of data and materials

The datasets used and/or analyzed during the current study are publicly available.

### Declarations

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

All authors have approved the final manuscript.

**Competing interests**

The authors declare no conflict of interest.

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