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Mycotoxin-detoxifying agents to counteract mycotoxins in livestock feeds

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Abstract
Mycotoxins are toxic secondary metabolites produced by fungi that can contaminate livestock feeds, posing a significant threat to animal health and productivity. Mycotoxin-detoxifying agents, such as binders and enzymes, have been developed to mitigate the negative effects of mycotoxin exposure in livestock. Binders, such as clays and activated charcoal, work by binding mycotoxins in the gut and preventing their absorption into the bloodstream. Enzymes, on the other hand, break down mycotoxins into non-toxic metabolites that can be excreted from the body. Several studies have demonstrated the efficacy of these detoxifying agents in reducing mycotoxin toxicity in livestock, with improvements observed in feed intake, weight gain, and immune function. However, further research is needed to optimize their use in different animal species and under different environmental conditions. Overall, mycotoxin-detoxifying agents represent a promising strategy for mitigating the negative impact of mycotoxins on animal health and productivity.

Keywords: Mycotoxins, livestock, binders, enzymes, detoxifying agents, feed intake, weight gain, immune function

Introduction
Livestock production plays an important role for the development of the national economy, and it provides high-quality food for human beings. Various factors *viz.* low-quality feed, naturally occurring toxic contamination in feed stuffs, poor management, diseases, climatic extremes etc. are the present threats that can adversely affect production performance and health of animals. Livestock feed is always at risk of contamination from microbes and insects. Fungal contamination of feed is one of the major problems as it reduces the nutritional value of feed and is responsible for mycotoxicosis (CAST, 1989; FAO, 2007) [4, 9]. Mycotoxins in livestock feed is of prime importance due to its deleterious effect on animal health and production apart from their presence in the food chain. The immunosuppressive properties of mycotoxins lead to decrease immunity which increases susceptibility to infections and vaccination failure consequently results in heavy economic losses for livestock industries.

Mycotoxins are toxic, chemically diverse secondary compounds or metabolites produced by a wide range of fungal species mainly *Aspergillus spp*., *Penicillum spp*. and *Fusarium spp*. (Akande *et al*., 2006) [2]. Factors that influence the mycotoxin production include temperature, moisture, oxygen, substrate aeration, inoculums concentration, microbial interaction, mechanical damage and insect infestation. Mycotoxins can cause damage to organ systems, reduce production and reproduction and increase incidences of diseases by suppressing immunity. Some mycotoxins are carcinogens, some target vital organs such as liver, kidney, digestive tract, or the reproductive system (Akande *et al*., 2006) [2]. The situations might be worsened by multiple contaminations because of the ability single mould species to produce several kinds of mycotoxins in a food ingredient. Thus, there is a possibility that several types of mycotoxins may be found in food or feed containing different contaminated ingredients or raw materials. The possibilities of simultaneous contamination may occur in animal feed since different raw ingredients can be used in feed preparation (Galvano *et al*., 2001) [11].

Major mycotoxins in livestock feed; their sources, potentially harmful level and toxicity in animals are shown in Table 1.

Table 1: Major mycotoxins in livestock feed, their sources, potentially harmful level and potential toxicities

Mycotoxin	Producing fungi	Potentially harmful level	Toxicity
Aflatoxin (B1, B2, G1 and G2)	*Aspergillus flavus A. parasiticus*	> 20 ppb	Hepatocarcinogen
Deoxynivalenol (DON)	*Fusarium graminearum*	6.0-12.0 ppm	Cytotoxin
Zearalenone	*Fusarium spp.*	4.0-7.0 ppm	Estrogenic
Trichothecene (T-2)	*Fusarium graminearum*	0.7-1.3 ppm	Cytotoxin
Ochratoxin A	*Aspergillus ochraceus*	5.0-9.0 ppm	Hepatotoxin
Citrinin	*Penicillum citrinum*	50-100 ppm	Nephrotoxin
Hydroxytryptamine (HT-2)	*Fusarium graminearum*	0.7-1.3 ppm	Cytotoxin
Diactoxyscirpenol (DAS)	*Fusarium* spp.	1.5-3.0 ppm	Cytotoxin
Fumonisin	*Fusarium spp.*	6.0-10.0 ppm	Cytotoxin
Patulin	*Penicillum expansum*	20-100 ppm	Neurotoxin
Rubratoxin	*Penicillium rubrum*	0.7-5.0 ppm	Hepatotoxin
Ergot alkaloids	*Claviceps* spp.	300-600 ppm	Neurotoxin

Mycotoxin-detoxifying agents

To counteract toxic effects of these mycotoxins effectively, mycotoxin-detoxifying agents can be used as feed additives in livestock feed. The Commission regulation (EC) No. 386/2009 defines a new mycotoxin-detoxifying agents group of feed additives as "*substances for reduction of the contamination of feed by mycotoxins or substances that can suppress or reduce the absorption, promote the excretion of mycotoxins or modify their mode of action*". Depending on their mode of action, these feed additives may act by reducing the bioavailability of the mycotoxins or by degrading or transforming to fewer toxic metabolites. Therefore, mycotoxin- detoxifying agents are described in two main categories *viz*. adsorbing agents and bio- transforming agents.

Mycotoxin adsorbing agents

Mycotoxin adsorbing agents are large molecular weight compounds also called as binder that should be able to bind the mycotoxins in contaminated feed without dissociating in the gastrointestinal tract of the animal. Thus, the toxin-adsorbing agent complex passes through the gastrointestinal tract of the animal and eliminated via the faeces causing reduction of mycotoxin uptake as well as distribution to the blood and target organs. This prevents or minimizes exposure of animals to mycotoxins. Mycotoxin adsorbing agents can be silica based inorganic compounds or carbon-based organic polymers.

Aluminosilicates

Silicate minerals are the major class of mycotoxin sequestering agents and studies on the alleviation of mycotoxicosis using adsorbing agents have mainly focused on aluminosilicates. Within this group, there are two important subclasses *viz*. phyllosilicate and tectosilicate. Phyllosilicates include bentonites, montmorillonites, smectites, kaolinites, illites. The tectosilicates include zeolites.

a. Bentonites are originally created from the weathering of volcanic ash in situ (Ramos *et al*., 1996) [22]. They belong to the phyllosilicate group and are adsorbing agents with a layered crystalline microstructure and variable composition. Bentonites are generally impure clay consisting mostly of montmorillonite. Due to their montmorillonite content, bentonites swell and form thixotropic gels (Diaz and Smith, 2005) [6].
b. Montmorillonite is a layered silicate which absorbs organic substances either on its external surfaces or within its interlaminar spaces (Ramos *et al*., 1996) [22]. Modified montmorillonite nanocomposite (MMN) is a new adsorptive additive. Developed with nano-modification techniques, MMN has a sizable surface area, higher porosity and stronger cation exchange activities apart from more active sites, which make its nano- particle effect easy to exert and as a result, its adsorption efficacy is greatly enhanced.
c. Hydrated sodium calcium aluminosilicate (HSCAS) is perhaps the most studied mycotoxin-sequestering agent among mineral clays (Galvano *et al*., 2001; Kabak *et al*., 2006) [11, 14]. It is a naturally occurring and heat processed calcium montmorillonite that is commonly used as an anticaking additive in animal feed (Wang *et al*., 2008) [27]. Zeolites are crystalline hydrated aluminosilicates of alkali and alkaline hearth cations characterized by an infinite three-dimensional structure. Zeolites are a group of silicates consisting of interlocking tetrahedrons of SiO4 and AlO4 (Kabak *et al*., 2006) [14]. Zeolites have large pores that provide space for large cations such as sodium, potassium, calcium. They are characterized by their ability to lose and absorb water and exchange constituent cations without damage to the crystalline structure (Diaz and Smith, 2005; Papaioannou *et al*., 2002) [6, 20]. Clinoptilolite is a natural zeolite whose main application is the adsorption of heavy metals from aqueous solutions (Kleiner *et al*., 2001) [15].

Activated carbons

Activated carbon (AC) is a non soluble powder formed by pyrolysis of several organic compounds and manufactured by activation processes aimed at developing a highly porous structure (Galvano *et al*., 2001) [11]. AC is known as one of the most effective and nontoxic group of sorbents and has been shown to be a tenacious adsorbing agent of a wide variety of drugs and toxic agents. It has been commonly used as a medical treatment for severe intoxications since the 19th century (Huwig *et al*., 2001) [13]. The sequestrant properties of AC depend on many factors including pore size, surface area, structure of the mycotoxin and doses. Super activated charcoal differs from AC in that the particle size is reduced thereby increasing surface area. The specific surface area of AC indeed varies from 500 m²/g to 3500 m²/g for super activated charcoals (Ramos *et al*., 1996) [22].

Yeast cell walls

Cell walls which are derived from the *Saccharomyces cerevisiae* yeast are also used as a mycotoxin adsorbing agent. Cell walls of yeast entirely consist of proteins and carbohydrates. The carbohydrate fraction is composed primarily of glucose, mannose and N-acetyglucosamine (NAG). Glucans and mannans are the two main sugars that are found in equal concentrations. Mannan chains of various sizes are exposed on the external surface and are linked to cell

wall proteins (Evans and Dawson, 2000) [8]. The cell walls harboring polysaccharides, proteins and lipids exhibit numerous different and easily accessible adsorption centers. It has been suggested that cell wall peptidoglycans and polysaccharides are the two most important elements responsible for binding by lactic acid bacteria (Kabak *et al*., 2006) [14].

Bacteria

Specific bacterial strains have been tested to adsorb mycotoxins in feed and can be beneficial to remove mycotoxins from feed. Strains of lactic acid bacteria such as *Lactobacillus rhamnosus* strain GG and *Lactobacillus rhamnosus* strain LC-705 are used to remove mycotoxins. *Streptococcus thermophilus* NG40Z and C5 have also been tested for their ability to absorb mycotoxins (El-Nezami *et al*., 1998).

Micronized fibers

Micronized fibers can be obtained from different plant materials such as wheat, barley, oat, pea hulls, apple, bamboo etc. They are constituted mainly of cellulose, hemicelluloses and lignin and can be obtained in ultrafine (<100 µ) or less fine (>100µ) fractions (Aoudia *et al*., 2009) [3].

Polymers

a. Cholestyramine is an insoluble, quaternary ammonium anion exchange resins which strongly binds anionic compounds (Underhill *et al*., 1995) [26]. It has been used as medicine in humans for absorbing bile acids in the gastrointestinal tract in order to reduce cholesterol (Diaz and Smith, 2005) [6].
b. Polyvinylpyrrolidone is a highly polar amphoteric polymer (Celik *et al*., 2000) [5] and has potential to bind mycotoxin effectively.

Mycotoxin-bio-transforming agents

Another strategy is the degradation of mycotoxins into non-toxic metabolites by using bio- transforming agents such as bacteria, yeasts, fungi and enzymes. Some microorganisms and specific enzymes have been shown to have the ability to degrade mycotoxins. The idea is for each mycotoxin or say class of mycotoxins, to use enzyme that specifically degrade the toxin into a nontoxic compound. Such enzymes are obtained either from bacteria, yeast, fungi or may be artificially synthesized. Purified enzymes have also been tested for this purpose. Studies conducted on specific microorganisms and enzymes targeting individual or group of mycotoxins are shown in Table 2.

Table 2: Bio transforming agents as feed additives to counteract mycotoxins

Bio transforming agent	Mycotoxin targeted	Studies conducted
Bacteria		
Eubacterium s.p. BBSH 797	T-2 & HT-2 toxin	(Fuchs *et al.*, 2002) [10].
Nocardia asteroides Mycobacterium fluoranthenivorans sp. nov. *Rhodococcus erythropolis*	Aflatoxin B1	(Wu *et al.*, 2009) [28].
Mixed culture (*Alcaligenes*, *Bacillus*, *Achromobacter*, *Flavobacterium*, and *Pseudomonas*	Zearalenone	(Megharaj *et al.*, 1997) [17].
Fungi		
A. parasiticus NRRL 2999 and NRRL 3000	Aflatoxin B1	(Wu *et al.*, 2009) [28].
Aspergillus niger, *Eurotium herbariorum*, *Rhizopus spp*	Aflatoxin B1	(Nakazato *et al.*, 1990) [19]
Yeast		
Trichosporon mycotoxinivorans	Ochratoxin, Zearalenone, DON	(Molnar *et al.*, 2004; Schatzmayr *et al.*, 2006) [18, 12].
Phaffia rhodozyma and	Ochratoxin	(Peteri *et al.*, 2007)
Xanthophyllomyces dendrorhous isolates		
micronized yeast	Aflatoxin	(Sehu *et al.*, 2005) [24].
Bacteria + Yeast		
Eubacterium BBSH 797 and *Trichosporon mycotoxinivorans*	Ochratoxin, Zearalenone,	(Hofstetter *et al.*, 2006) [12].
Enzymes		
Protease A	Ochratoxin	(Abrunhosa *et al.*, 2006) [1].
Pancreatin	Ochratoxin	(Abrunhosa *et al.*, 2006) [1].
Epoxidase	Ochratoxin, Zearalenone, DON	(Schatzmayr *et al.*, 2006) [12].
Aflatoxin-detoxifizyme	Aflatoxin	(Liu *et al.*, 2001) [16].
Lactonohydrolase	Zearalenone	(Takahashi-Ando *et al.*, 2002) [25].

Human Nutrition Perceptive

From human nutrition point of view, it has been observed that mycotoxins do not accumulate in muscles. In animals the metabolism of mycotoxins in the body leads to excretion not only in urine and faeces, but also in animal products such as eggs in poultry and milk in mammals. Regarding the occurrence of mycotoxin residues in poultry eggs, very low residue levels (~ 0.3 µg/kg) can be detected by consuming diet containing levels of Aflatoxin B1 (AFB1) as high as 10 mg/kg. This can also be applicable to other mycotoxins such as Ochratoxin A (OTA), T-2, Deoxynivalenol (DON), Zearalenone (ZEA) and Fumonisin B1 (FB1) for which no significant carry-over rate in a range of 0.6-0.001% in eggs has been observed.

The half-life of most mycotoxins or their metabolites is short and may be lasting for a few days except for OTA in pig. Residue of OTA levels in a range of 4-71 µg/kg in kidneys of pigs can be found after consumption of contaminated diet at levels ranging from 0.1-1.4 mg/kg. In European countries a level has been set at a content of 25 µg OTA/kg of kidney and above this level, the pig carcass is rejected from the market based on the expectation of a level of around 10 µg OTA/kg of meat. Special attention should be given to large ruminant's animals (lactating) regarding the possibility of excretion of mycotoxin metabolites in milk. The mean rate of carry- over in milk varies according to the mycotoxins from 0.3-2.2% for AFB1 to 0.05% for FB1 and T2-toxin. OTA and DON residues can be found in cow milk when high quantities of

toxins have been experimentally administered to animals (Yiannikouris and Jouany, 2002). Consequently, based on these data, only the occurrence of Aflatoxin M1, milk aflatoxin from AFB1 metabolism, in milk is a matter of concern about the transfer of mycotoxins in food chain.

Conclusion

Proper handling and drying practices can keep the fungal toxin levels low in the different feed ingredients and efficient decontamination procedures do exist to reduce levels of the aflatoxin. There is considerable potential for the use of mycotoxin detoxifying agents as feed additives in livestock feed to counter mycotoxins. Most of the mycotoxin detoxifying agents confer total protection against individual mycotoxin, however their efficacies against other mycotoxins are very limited. If they do bind to multiple mycotoxins, they do not have equal affinities and capacities. Also, they could incur significant hidden risks due to interactions with critical nutrients in the diet. Therefore, mycotoxin detoxifying agents should be adequately tested not only for their *in vitro* binding capabilities, but also for its *in vivo* efficacy to prove their practical applicability in livestock feed industry.

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