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Reducing the Impact of Aflatoxins in Livestock and Poultry

Preventing Aflatoxin and Other Mycotoxin Contamination in Feedstuffs

Mycotoxin contamination of feed ingredients impart considerable economic costs stemming from cost of preventative and mitigation practices, reduced value of contaminated feeds, contamination of foods of animal origin and reduction in animal performance and health. Therefore, much research has sought to find methods to overcome these issues. Currently, the best procedure is to minimize mycotoxin production as well as animal exposure to mycotoxins. In line with this, good management practices (in the field, at harvest, at storage) may significantly reduce the contamination of feed and the intoxication of animals (Table 1).

However, even with excellent management there may be small amounts of mycotoxins which are unavoidable. These low concentrations are a constant concern for potential loss of feedstuffs, increased animal disease, reduced animal performance and residues in animal products. Importantly, many countries (such as is the case with aflatoxin in the U.S. per U.S. Food and Drug Administration) generally do not permit grains containing mycotoxins to be blended with uncontaminated grains, in order to reduce the mycotoxin content of the resulting mixture to levels acceptable for use as human food or animal feed. However, on occasion the FDA has relaxed its "no-blending" policy in response to widespread outbreaks of aflatoxin (AF) or to state-specific requests to address local outbreaks (as was allowed in Indiana during the 2012 harvest).

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Table 1. Some recommendations as good management practices (Whitlow, 2006)

In the field	• Avoid insect damage that may facilitate mold entry • Choose varieties that are adapted to the growing area and have resistance to fungal disease and insect damage • Apply crop rotation
At harvest	• Avoid lodged or fallen material at harvest, because contact with soil can increase mycotoxin contamination • Avoid delayed harvest, because mycotoxin contamination increases with late season rain and cool periods • Maintain harvesting equipment in good condition to avoid damage to grain kernels • Sort and clean broken and damaged kernels. Mycotoxin concentrations are greatest in the fines
At storage	• Store grains in cool and dry conditions • Protect feedstuffs from rain or other water sources • Clean silos and other storage facilities regularly to eliminate the source of inoculation

Several strategies have been suggested in order to decontaminate feed (Kabak et al., 2006). Among these strategies, ammoniation is an approved procedure prior to processing feed for the detoxification of aflatoxin-contaminated raw materials. It is the method of choice in some U.S. states as well as in Senegal, France and the United Kingdom. Nonetheless, the main drawbacks of this chemical method are the ineffectiveness against other mycotoxins and the possible deterioration of the animal health by excessive residual ammonia in feed (Grenier et al., 2012).

Overview of Potential Adsorbents for the Reduction of the Toxic Effects of AF

The high cost and limitations of physical and chemical treatments of contaminated feeds have prompted a search for other means to remedy mycotoxin contamination. One of the most recent approaches for the prevention of aflatoxicosis in livestock is the addition of adsorbents (also named binders or sequestering agents) that bind AF in the GIT and are capable of reducing its bioavailability (Huwig et al., 2001, Phillips et al., 2002). Substances used as mycotoxin binders include indigestible adsorbent materials such as silicates, activated carbons, complex carbohydrates and others. Presently, no adsorbent product is approved by the FDA for the prevention or treatment of aflatoxicoses or mycotoxicoses. Several of these adsorbent materials are recognized as safe feed additives (GRAS) and are used in diets for purposes such as flow agents and pellet binders.

Research with mycotoxin binders has been conducted for more than 20 years and has given strong evidence that some types of binders would be able to counteract the toxic effects of AF in animals. Table 2 gives an overview on the available and most studied AF adsorbents. This efficacy is based on a high affinity of the adsorbent for AF, resulting in the formation of a strong complex with little risk of dissociation. This adsorption is highly related to the physical structure (i.e., the total charge and charge distribution, the size of the pores and the accessible surface area) of the adsorbents, but also to the properties of the adsorbed molecules like polarity, solubility, size and shape of mycotoxins. These latter properties account for why some binders have little success in efficiently adsorbing other mycotoxins besides AF (Huwig et al., 2001, Kabak et al., 2006).

Table 2. Overview of some binders suggested counteracting the adverse effects of aflatoxins (AF)

	General points & features	Binding mechanism	Efficacy other than AF[a]	Studies[b]	Major drawbacks
Clays or inorganic adsorbents					
HSCAS	Most effective adsorbent for use against aflatoxicosis; initially sold as an anti-caking additive for animal feed	β-carbonyl system of AF interacts with uncoordinated edge site aluminium ions	Little success	++++++++	“AF-selective clay,” not a good adsorbent of other mycotoxins
Bentonite	Originating from volcanic ash and containing primarily montmorillonite; extensively used in the clarification of beverages and discoloration of oils	Adsorption depends on the interchangeable cations (Na^+, K^+, Ca^{++}, Mg^{++}) present in the layers	Little success	+++++	Adsorption ability may vary from one geological deposit to another
Zeolite/Clinoptilolite	Formed where volcanic rocks and ash layers react with alkaline groundwater; widely used for water purification and production of laundry detergent	Similar to molecular sieves as well as ion exchange resins	OTA, ZEA	+++++	Conflicting results depending on the type of zeolite (natural, synthetic, modified by ion exchange)
Kaolin	Its main constituent is kaolinite; commonly used as a palliative for diarrhea and digestive problems in humans	Cation exchange capacity	No data	++	Insufficient information
Diatomaceous earth	Could be extracted from quarry	High cationic interchange capacity	OTA	+	Insufficient information
Miscellaneous or organic adsorbents					
Activated charcoal	Formed by pyrolysis of organic materials; used as an antidote against poisoning since the 19th century	Hydrogen bonding	T-2 toxin, DON, ZEA	++++	Unspecific adsorbent which may also adsorb essential nutrients; black color of the feed
Polyvinylpyrrolidone	Synthetic water soluble polymer which attracts polar particles	Attracts polar particles	No data	++	Cost is a limiting factor for a current use
Humic acid	Produced by biodegradation of dead organic matter	Affinity to bind various substances	No data	+	Insufficient information
Yeast cell walls	Polysaccharides such as mannans and glucans; excellent nutritional value	Hydrogen and van der Waals bonds, ionic, or hydrophobic interaction	Multi-contam. feed	++++	Inconsistent results
Lactic acid bacteria	Cell wall peptidoglycans and polysaccharides responsible for binding	Related to the cell surface hydrophobicity	No data	+	Numerous studies in vitro by opposite to in vivo data

DON = Deoxynivalenol (vomitoxin); HSCAS = hydrated sodium calcium aluminosilicates; OTA = Ochratoxin A; ZEA = Zearalenone.
[a]Demonstrated in vivo; [b]Number of studies investigating the in vivo efficacy of the binder.

Ideally, for an industrial use the binder incorporated into animal feed should or must meet the following guidelines (Table 3).

Table 3. The "ideal" sequestering agent

DO'S		DON'TS
Prevent the intestinal absorption of mycotoxin	--►	Be digestible
Form a stable complex with the toxin in the "hostile" conditions of the GIT	--►	Release the toxin in the GIT
Demonstrate its efficacy in in vivo experiments	--►	Draw conclusions on in vitro results
Be able to target multiple mycotoxins at the same time	--►	Be unspecific and decrease the bioavailability of nutrients
Be safe, free of impurities, off-flavors and odors	--►	Be contaminated at the extraction source or during the production
Take as little space in the diet as possible	--►	Possess a low binding capacity and reach rapidly saturation
Biodegradable	--►	Accumulate in the environment after being excreted by animals

Among these recommendations, the major point concerns the need for the product to demonstrate efficacy in vivo (i.e., in the animal). Indeed, many sorbents have shown a high binding capacity for mycotoxins in in vitro systems, but they eventually fail to neutralize the toxic effects of AF when fed to animals. This is especially true for activated charcoal.

Therefore, in vitro data should not be used to make decisions about products to use in practice. In line with that, the European Food Safety Authority (EFSA) stated that toxicokinetic studies have to be performed for the investigation of the bioavailability (urine, blood serum or plasma concentration of the toxin and its metabolite[s]) and the absorption/excretion of mycotoxins in combination with detoxifying agents (Anonymous, 2009).

Moreover, trials have to be performed with respect to the recommended maximum levels for the toxins in animal feed. However, few data are available so far on the clearance of mycotoxins from blood when combined to a sequestering agent, and therefore this report provides mostly data on the potential of these products to eliminate the toxic effects of AF in animals (Table 4).

Use of Inorganic Adsorbents (the clay group)

Clays are natural adsorbents chemically made of silicates or aluminosilicates. They include a large range of products such as hydrated sodium calcium aluminosilicates (HSCAS), phyllosilicates (of which montmorillonite or magnesium hydrated HSCAS is one of the major compounds in this group), bentonite and zeolite (the latter two are clays of volcanic origin).

Clearly much of the pioneering work with mycotoxin binders was done with silicates and specifically with the HSCAS material studied at Texas A&M University by the Phillips' research group. These binders have the property of adsorbing organic substances either on their external surfaces or within their inter-laminar spaces, by the interaction with/or substitution of the exchanged cations within these spaces. Therefore, mycotoxins can be adsorbed into this porous structure and be trapped by elementary, electric charges. However, clay and zeolitic minerals, which comprise a broad family of diverse aluminosilicates, are not produced equally and, thus, do not possess the same physical properties.

As previously mentioned, the most extensively studied of these materials is HSCAS, which have a high affinity for AF forming a stable complex at temperatures of 25°C and 37°C, in a pH range of 2-10. That specific HSCAS included at 0.5-2 percent of the diet is well documented to adsorb AF and prevent aflatoxicosis (up to 7.5 mg/kg -or- ppm of feed) across species, including chicken, turkey, swine, lamb, dairy cow, dairy goat and mink (Table 4). However, this adsorbent seems to fail in the adsorption of other mycotoxins, thus HSCAS is not expected to be protective against feeds containing multiple mycotoxins.

Alternatively, bentonites and zeolites are commonly used in the adsorption of AF. Two types of bentonites have been mostly used in animal experiments — sodium and calcium bentonite — referring to the cations present in the layers.

Although less studied than HSCAS, evidence suggests that bentonite is useful to counteract the AF effects (Table 4). With regard to zeolites, these compounds have normally less capacity than HSCAS or bentonites to adsorb AF in vitro, but show some in vivo efficacy under practical conditions, especially with the natural zeolite, clinoptilolite (Table 4).

Use of Organic Adsorbents (miscellaneous)

Substances investigated as potential organic mycotoxin-binding agents include activated charcoal, synthetic polymers, yeast cell walls and components thereof, and bacterial cells.

Activated carbon is a general adsorptive material with a high surface to mass ratio (500-3500 m^2/g). It has been recommended as a general toxin adsorbing agent and is routinely recommended for various digestive toxicities (Whitlow, 2006). However, the effects of activated charcoal have been variable (Table 4), and responses to charcoal with poultry also suggest that charcoal may not be as effective in binding AF as are clay-based binders.

Yeast cell walls, particularly the cell wall of Saccharomyces cerevisiae, are an environmentally friendly alternative to inorganic adsorbents, which are not extensively biodegradable and are associated with the risk of contaminants. In addition to the beneficial effects observed in counteracting AF (Table 4), these organic binders would be efficient against a large range of mycotoxins, which make them more adapted to the most frequent cases of multi-contaminated feed (Jans et al., 2012). Bacterial cell walls also have potential to bind AF, but limited research has been conducted.

Effect of Sequestering Agents on the Carryover of AF Residues into Milk

Milk is the food product of animal origin for which the greatest concern exists and which is easily contaminated with AFM_1 (metabolite of AFB_1) when feed ingredient concentrations exceed regulatory limits. Due to the high consumption of milk and milk products by humans, especially children, the regulatory limit that can be fed to dairy animals is 20 ppb (parts per billion = μg/kg) by the FDA.

Accordingly, several studies focused on the efficacy of adsorbents in reducing the transfer of AFM_1 into milk. Conclusions of these experiments were in agreement concerning HSCAS and bentonites, which significantly diminished the secretion of AFM_1 into milk (Table 4), whereas inconsistent outcomes were reported on activated charcoal and yeast cell extracts (Diaz et al., 2004, Kabak et al., 2006, Jans et al., 2012).

Limits and Conclusions on the Decontamination Through Feed Additives

The binding capacity of adsorbents has raised many controversial questions regarding their influence on the utilization of nutrients such as carbohydrates, proteins, vitamins and minerals. Adsorbents, such as activated charcoal, which is unspecific with high binding capacity, should be subjected to determination of efficacy in the target animal while also determining influence on nutrient and energy use by the animal. In line with that, aluminosilicates have been investigated and HSCAS do not impair phytate, phosphorus, riboflavin, vitamin A or manganese utilization. Similarly, bentonites and zeolites do not affect vitamin uptake or mineral metabolism. However, the studies investigating this issue are relatively scarce and are therefore difficult to draw substantive conclusions.

Similarly, care must be taken to not generalize any conclusions for a given adsorbent group, as all products under a specific classification will not always have the same composition or efficacy. For example, clays vary in structure and mineral composition while yeast used for production of “yeast cell wall products” differ in cell wall composition. In either case, the physico-chemical properties that determine sequestrant efficacy can vary markedly among products in the same category. In addition, possible dioxin contamination may be a risk factor for using natural clays in case of forest and trash fire near their source.

To conclude, a binder product that meets all the desirable characteristics is not available today. However, the potential currently exists for practical judicious use of mycotoxin binders for reducing mycotoxin exposure to animals.

Table 4. Conclusions on the most studied binders in the neutralization of aflatoxin's (AF) effects (reviewed by Huwig et al., 2001; Jans et al., 2012) – Aluminosilicates, activated charcoal, and yeast cell walls

Binders (# of studies[a])	AF dose	Binder inclusion	Effects of the addition of the binder in animals fed AF-contaminated feed	Additional information
HSCAS (n = 15)	Range: 0.5-7.5 mg/kg Average: 4 mg/kg	Range: 0.25-2% Average: 0.5%	• Improved body weight gain and feed intake, reduced mortality • Reduced secretion of AFM_1 into milk for lactating dairy cows and goats • Restored the hematological and biochemical values • Positive effects on organ weight, decline in the severity of lesions and improvement of liver functions • Neutralized the immunotoxic effects of AF on lymphocyte proliferation, macrophage activity and function -► demonstrated in chicken, turkey, pig, lamb, trout, mink, rat	HSCAS is the most studied binder for 20 years; A lot of studies conducted by the group of T.D Phillips; Most animal trials reported beneficial effects of HSCAS use
Bentonite (n = 7)	Range: 0.2-5 mg/kg Average: 2.5 mg/kg	Range: 0.25-0.5% Average: 0.5%	• Improved body weight gain and feed intake • Reduced secretion of AFM_1 into milk for lactating dairy cows • Prevention of maternal and developmental effects of AF • Restored the biochemical values • Better protection against Newcastle disease — improved HI titres -► demonstrated in chicken, pig, rat	Bentonite known to reduce the transit time of digesta through the GIT; Some reports showed better results of local bentonites compared to commercial products, efficacy may vary depending on bentonite origin and type
Zeolite/ Clinoptilolite (n = 7)	Range: 0.1-3.5 mg/kg Average: 2.5 mg/kg	Range: 0.5-5% Average: 1%	• Improved body weight gain and feed intake • Decline in the severity of lesions in liver and kidney, reduced the increase in liver lipid concentration caused by AF • Inconsistent results in the prevention of maternal and developmental toxicity • Ameliorations on humoral immunity -► demonstrated in chicken, quail, rat	There are about 45 naturally occurring minerals that are recognized as members of the zeolite group; A comparative study reported differences in the ability of five tested zeolites to alleviate AF effects
Activated charcoal (n = 5)	Range: 0.1-4 mg/kg Average: 2 mg/kg	Range: 0.5-1% Average: 0.5%	• Inconsistent results in the improvement of performance, reduced mortality • Decline in the severity of lesions in liver • Reduced carryover of AFM_1 into milk for lactating dairy cows -► demonstrated in chicken, turkey, mink	Activated charcoal is one of the most effective sorbents with a large surface area and excellent adsorptive capacity; A superactivated charcoal may be more effective in reducing toxicity caused by AF
Yeast cell walls (n = 5)	Range: 0.3-2.5 mg/kg Average: 2 mg/kg	Range: 0.05-0.2% Average: 0.1%	• Improved body weight gain and feed intake • Positive effect on hatchability and egg production • Restored the biochemical values • Decline in the severity of lesions in liver • Prevent the disruption of the vaccinal immune response -► demonstrated in chicken, pig, fish	Studies conducted either with β-glucan or glucomannan; Live yeast (Saccharomyces cerevisiae) culture may provide protection as well; Yeast cell walls may display immune-stimulant properties

[a]Refers to the number of studies (as n = X) investigating the binder and used to draw the conclusions in the table
HI, Haemagglutination Inhibition; HSCAS = hydrated sodium calcium aluminosilicates

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