EVALUATION OF MYCOTOXIN BINDERS

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Summary

Considerable research has been directed at finding methods to prevent toxicity of mycotoxins. Some of the approaches have included mycotoxin separation from contaminated feeds, detoxification and inactivation. Detoxification and inactivation methods include the use of binders or sequestering agents added to feed as an approach to reduce toxicity of mycotoxins by reducing reactivity of bound mycotoxins and reducing their intestinal absorption. Substances used as mycotoxin binders include indigestible adsorbent materials such as silicates, activated carbons, complex carbohydrates and others. The use of binders offers an approach to salvaging feeds with low levels of mycotoxins and to protecting animals from the background levels of mycotoxins that, although low in concentration, routinely occur and may cause chronic disease problems and losses in performance. A binder product that meets all the desirable characteristics is not available, but the potential currently exists for practical judicial use of mycotoxins binders for reducing mycotoxin exposure to animals. Various materials offer the potential to bind mycotoxins in feed. Silicates bind aflatoxin and some other mycotoxins, such as sterigmatocystin, which has a chemical structure similar to aflatoxin. There are many different silicates and they differ in mycotoxin binding. Chemically modification of silicates can increase binding to mycotoxins such as deoxynivalenol and zearalenone. Activated carbon (charcoal) has produced variable binding results, perhaps because of differences in physical properties of the test product. Aflatoxin binding by activated charcoal has been variable, but mostly positive. Charcoal may also bind zearalenone and deoxynivalenol. Complex indigestible carbohydrate polymers derived from yeast cell walls are shown effective in binding aflatoxin and restoring performance to animals consuming multiple mycotoxins (generally *Fusarium* produced). Bacterial cell walls also have potential to bind mycotoxins, but limited research has been conducted. Inorganic polymers such as cholestyramine and polyvinylpyrrolidone also have binding potential. No adsorbent product is approved by the U.S. Food and Drug Administration (FDA) for the prevention or treatment of 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.

Introduction

Molds are filamentous fungi that occur in many feedstuffs including grains (Russell *et al.*, 1991) and forages (Lacey, 1991). Molds can infect animals, especially during stressful periods when they are immune suppressed, causing a disease referred to as a mycosis. Molds also produce mycotoxins, which can cause a mycotoxicosis or toxic response in animals exposed primarily by consuming mycotoxin-contaminated feeds. The FAO has estimated that worldwide about 25% of crops are affected annually with mycotoxins (Jelinek *et al.*, 1989). Surveys reveal sufficiently high occurrences and concentrations of mycotoxins to suggest that they are a constant concern (Whitlow *et al.*, 1998).

Molds are present throughout the environment and mycotoxins can be formed on crops in the field, during harvest, or during storage, processing, or feeding. Mold spores are present in materials such as soil and plant debris, ready to infect the growing plant in the field. Field diseases are characterized by yield loss,

quality loss and mycotoxin contamination. Mold growth and the production of mycotoxins are usually associated with extremes in weather conditions, leading to plant stress or hydration of feedstuffs, to poor storage practices, low feedstuff quality and inadequate feeding conditions. While mold contamination often begins in the field, crops can become infected at any point of handling (CAST, 2003).

While it is generally accepted that the *Aspergillus*, *Fusarium* and *Penicillium* molds are among the most important in producing mycotoxins detrimental to animals, a wide range of different molds produce mycotoxins (CAST, 2003). The mycotoxins of greatest concern include: aflatoxin, which is generally produced by *Aspergillus*; deoxynivalenol, zearalenone, T-2 Toxin, and fumonisin, which are produced by *Fusarium*; and ochratoxin and PR toxin produced by *Penicillium*. Several other mycotoxins such as the ergots are toxic and may be prevalent at times in certain feedstuffs.

There are hundreds of different mycotoxins, which are diverse in their chemistry and effects on animals. This diversity in chemistry can also affect binding. It is likely that moldy, mycotoxin-contaminated feeds will contain more than one mycotoxin and animals may be exposed to multiple mycotoxins. Mycotoxins can also reduce animal performance and health as a result of toxic effects that can impact on most every bodily system (CAST, 2003). Effects are dependent on the specific mycotoxin(s), the amount, the duration of exposure, and animal factors such as age, sex, and level of stress. Diagnosis of a mycotoxicosis has been complicated by a disregard or unawareness of mycotoxin effects, acceptance of chronic low productivity and disease, lack of feed samples, sampling difficulties, analytical complications, species specific responses, nonspecific effects on various bodily systems, universal symptoms that may be similar to other disorders, and a cascading of animal responses (as seen with immune suppression) resulting in disorders not thought to be associated with mycotoxins. Mycotoxin toxicity and management have been reviewed (CAST, 2003, Whitlow and Hagler, 2005).

Economic costs of mycotoxins include the cost of preventative and mitigation practices, the reduced value of contaminated feeds, the contamination of foods of animal origin and the reduction in animal performance and health. Milk is the food product of animal origin for which the greatest concern exists and which is easily contaminated with aflatoxin when feed ingredient concentrations exceed regulatory limits (GAO, 1991; Van Egmond, 1989). The FDA provides regulatory advice on mycotoxins (Wood and Trucksess, 1998).

Management of mycotoxins includes prevention, regulation, monitoring, avoidance, decontamination, detoxification and animal treatments. Safe levels of mycotoxins are difficult to determine (Hamilton, 1984; Whittaker, 1991). Even with excellent management, there may be unavoidably low levels of mycotoxins as a constant concern for potential loss of feedstuffs, increased animal disease, reduced animal performance, and food residues. The use of mycotoxin binders, or adsorbents, may have the greatest application for routine avoidance of this constant exposure to low levels of multiple mycotoxins. The use of adsorbents to prevent effects of mycotoxins has been actively researched for over 20 years. A number of binder products have been shown effective and their use offers one of the greatest potentials for preventing animal toxicity.

Prevention of Mycotoxins

Field and harvest

Efforts to prevent mycotoxin formation are crucial since there are few ways to completely overcome problems once mycotoxins are present. Drought and insect damage are most important in instigating molding and mycotoxin formation in the field. Field production of mycotoxins can be reduced by choosing varieties that are adapted to the growing area, have resistance to fungal disease, and have resistance to insect damage such as with Bt hybrids. Mycotoxin formation in the field can also be reduced by proper irrigation and fertilization. Because contact with soil can increase mycotoxins, lodged or fallen material should be avoided at harvest. Harvest should be timely, avoiding delayed harvest because mycotoxins increase with late season rain and cool periods. Maintain harvesting equipment in good

condition to avoid damage to grain kernels. Broken and damaged kernels support more mold growth and mycotoxin concentrations are greatest in the fines. Thus, cleaning can greatly reduce mycotoxin concentrations in the feedstuff.

Feed storage

Storage management is critical in preventing mold growth and mycotoxin production in harvested feedstuffs. Grain should be stored below 15% moisture and preferably to <13% to reduce spots of higher moisture and to preserve feeds in hotter climates. Storage should prevent moisture migration, moisture condensation, and leaks. Grain stored for more than two weeks should be kept aerated and cool. Commodity sheds should protect feedstuffs from rain or other water sources and be constructed with a vapor barrier in the floor. Bins, silos, and other storage facilities should be routinely cleaned to eliminate sources of inoculation. Silage should be made by following accepted practices aimed at preventing deterioration. Generally accepted silage making practices include harvesting at the proper moisture content, uniform chopping at the proper length, rapid filling of the silo, sufficient packing of the silage, use of an effective fermentation aid, and covering to eliminate air and water. Infiltration of air after ensiling allows growth of acid tolerant microorganisms, an increase in pH, and increased mold growth. Additives that reduce pH rapidly after ensiling can reduce mold growth and mycotoxin formation. Various silage additives are shown to be at least partially effective at inhibiting mold growth. Spoilage should not be fed and feed bunks should be cleaned regularly. Wet feeds, such as wet byproduct feeds, must be stored at proper moisture contents in a well maintained structure and managed well to prevent mold. Wet feeds must be handled in quantities that allow them to be fed out quickly. Organic acids can be helpful in preventing mold in feeds and can extend storage life.

Mycotoxin decontamination

If unacceptably high levels of mycotoxins occur, removal or dilution of the contaminated feed is preferable; however, it is sometimes impossible to replace contaminated feeds. Detoxifications methods are limited. Ammoniation of grains can destroy some mycotoxins, but there is no practical method to detoxify affected forages. Most mycotoxins are tolerant to low amounts of heating, so that roasting or extrusion destroys very little of the mycotoxin. Cleaning grains can be helpful, and the handling and movement of grains during processing may decrease mycotoxins through loss of the fines. Chemical, physical, and biological methods are reviewed along with other methods for management and detoxification of mycotoxins (Lopez-Garcia and Park, 1998; Sinha, 1998). An enzyme from a pure bacterial strain has been isolated that can de-epoxidize some trichothecenes (Binder *et al.*, 2000). A feed additive with this enzyme has been evaluated, but to date, results are not persuasive (Doll and Danike, 2004).

Nutrient interactions

Dietary strategies to counteract the effects of mycotoxins have been reviewed and include the effects of nutrients, antioxidants, and plant extracts (Galvano *et al.*, 2001). Increased dietary levels of protein, energy, and antioxidant nutrients have been valuable in some situations as has been the use of mycotoxin binders.

Mycotoxin binders

Reviews of mycotoxin binders have been published (Avantaggiato *et al.*, 2005;Ramos *et al.*, 1996a; Ramos and Hernandez, 1997; Huwig *et al.* 2001).

The addition of mycotoxin binders to contaminated diets has been considered the most promising dietary approach to reduce effects of mycotoxins (Galvano *et al.*, 2001). The theory is that the binder decontaminates mycotoxins in the feed by binding them strongly enough to prevent toxic interactions with the consuming animal and to prevent mycotoxin absorption across the digestive tract. Therefore, this approach is seen as prevention rather than therapy.

Potential absorbent materials include activated carbon, aluminosilicates (clay, bentonite, montmorillonite, zeolite, phyllosilicates, etc.), complex indigestible carbohydrates (cellulose, polysaccharides in the cell walls of yeast and bacteria such as glucomannans, peptidoglycans, and others), and synthetic polymers such as cholestryamine and polyvinylpyrrolidone and derivatives.

Binder Evaluations and Concerns

Research with mycotoxin binders has been conducted for over 20 years, and yet products are not yet approved for the marketplace and information for producers is limited. Mycotoxin binders have been evaluated using both *in vitro* and *in vivo* systems. *In vitro* evaluations have been useful as a screening method for potential binder products, providing an idea of binding affinity and capacity. *In vitro* methods are not standardized and therefore are not comparable across all laboratories. The *in vitro* techniques have not produced results that correlate well with *in vivo* results. Therefore, *in vitro* data should not be used to make decisions about products to use in practice (Doll *et al.*, 2004; Diaz *et al.*, 2004; Garcia *et al.*, 2003). *In vivo* studies have generally used performance responses or biological markers such as tissue residues or changes in biochemical parameters to determine effectiveness of binders. These measurements can only estimate binding indirectly. Because many factors and conditions of the study affect results, binders need to be evaluated with different inclusion rates; with different mycotoxins; across animal species, ages, and genders; and under different environmental conditions. However, if comparisons are to be made across studies, experimental criteria must be standardized. Even with standardization, products may vary significantly by lots, resulting in different results *in vitro* or *in vivo* and from study to study (Bailey *et al.* 1998). Any negative effects of the binder should also be evaluated. Gathering the definitive information is complex, expensive, time-consuming, and thus frustrating to an industry waiting for solutions.

Charcoal or activated carbon

Activated carbon is a general adsorptive material with a large surface area and excellent adsorptive capacity. It has been recommended as a general toxin adsorbing agent and is routinely recommended for various digestive toxicities (The Merck Veterinary Manual, Eighth Edition, Merck & Co., Inc., Whitehouse Station, NJ). In one of the first studies to test the concept of mycotoxin binding, activated charcoal at a high dosage was shown to reduce aflatoxicosis in goats (Hatch *et al.*, 1982). In subsequent studies, the effects of activated charcoal have been variable. Galvano *et al.* (1996) showed reduced aflatoxin residues in milk of cows consuming different sources of charcoal, but responses to charcoal did not exceed that seen with a clay based binder (a hydrated sodium calcium aluminosilicate or HSCAS). Likewise, Diaz *et al.* (2004) showed that low levels (45 g/cow daily) of activated carbon did not significantly reduce milk aflatoxin residues, whereas clay type binders (225 g/cow daily) or an organic polymer of esterified glucan (10 g/cow daily) significantly reduced milk aflatoxin. Responses to charcoal with broilers (Edrington *et al.*, 1997), turkey poults (Edrington *et al.*, 1996), rats (Abdel-Wahhab *et al.*, 1999) and mink (Bonna *et al.*, 1991) also suggest that charcoal may not as effective in binding aflatoxin as are clay based binders.

Activated charcoal may be important in binding zearalenone and/or deoxynivalenol (Doll *et al.*, 2004; Dante *et al.*, 2005; Bueno *et al.*, 2005). In an *in vitro* gastrointestinal model, activated carbon reduced availability of deoxynivalenol and nivalenol (Avantaggiato *et al.*, 2004).

Silicate binders

Silicates are divided into subclasses, not by their chemistries, but by their structures. Minerals in different subclasses may have similar chemistries. The silicate subclasses include neosilicates (single tetrahedrons), sorosilicates (double tetrahedrons), inosilicates (single and double chains), cyclosilicates (rings), phyllosilicates (sheets), and tectocilicates (frameworks). Silicates investigated as adsorbent materials are classified primarily as phyllosilicates and tectosilicates. Perhaps the most extensively studied of these materials is one designated a hydrated sodium calcium aluminosilicate (**HSCAS**). Several reviews are

available (Bingham *et al.*, 2003; Kubena *et al.*, 1987; Phillips, 1999; Phillips *et al.*, 1991; Ramos and Hernandez, 1997). It is suggested that this specific silicate minerals can bind with aflatoxin by chelating the β-dicarbonyl moiety in aflatoxin with uncoordinated metal ions in the clay materials (Phillips *et al.*, 1991). Other silicates that have been studied include bentonites, zeolites, clinoptilolites and various others that are often not completely characterized. The clay group is a subcategory of the phyllosilicates. Bentonite is a general clay material originating from volcanic ash and containing primarily montmorillonite as the main constituent. Montmorillonite clay is a hydrated sodium calcium aluminum magnesium silicate hydroxide. Clays are silica sheets that are similar to other phyllosilicates but contain a high concentration of water. Zeolites are classified as tectosilicates consisting of interlocking tetrahedrons. The zeolite structure provides vacant spaces that form channels of various sizes allowing movement of molecules into and out of the structure. Zeolites can lose and absorb water without damage to their crystal structures. A reference to minerals may be found at Amethyst Galleries, Inc. (http://mineral.galleries.com/minerals/silicate/class.htm).

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. Phillips *et al.* (1988) screened a large number of silicates and selected one of the better materials for further study. That specific HSCAS included at 0.5% to 2.0% of the diet is well documented to adsorb aflatoxin and to prevent aflatoxicosis across species, including chickens (Huff *et al.*, 1992, Kubena *et al.*, 1987, 1990a,b, 1992, 1993; Ledoux *et al.*, 1999; Phillips *et al.*, 1988; Scheideler, 1993), turkeys (Kubena *et al.*, 1991), swine (Colvin *et al.*, 1989; Harvey *et al.*, 1989; Lindemann *et al.*, 1993), lambs (Harvey *et al.*, 1991a), dairy cows (Harvey *et al.*, 1991b), dairy goats (Smith *et al.*, 1994) and mink (Bonna *et al.*, 1991). Responses to HSCAS appear to be dose dependent (Smith *et al.*, 1994).

This HSCAS is characterized as an "aflatoxin-selective clay," is not a good adsorbent of other mycotoxins (Phillips *et al.*, 1999), and therefore, is not expected to be protective against feeds containing multiple mycotoxins. Cyclopiazonic acid, which has co-occurred with aflatoxin, is not adsorbed by HSCAS (Dwyer *et al.*, 1997). Garcia *et al.* (2003), using a silicate material, failed to show reduced ochratoxin toxicity but did demonstrate reduced T-2 toxicity. Huff *et al.* (1992) also failed to see a benefit to adding HSCAS to diets containing ochratoxin. Watts *et al.* (2003) showed that 1% HSCAS was not protective to chicks and poults receiving diets containing 1 mg deoxynivalenol, 5 mg moniliformin, 5 mg fumonisin B1, 100 μg aflatoxin B11 mg zearalenone and 0.5 mg ochratoxin per kg of diet. HSCAS was protective to mink against zearalenone (Bursian *et al.*, 1992). Patterson and Young (1993) failed to see a benefit to the addition of HSCAS in pig diets containing deoxynivalenol. Huebner *et al.* (1999) and Chestnut *et al.* (1992) found that clays bind well with ergotamine *in vitro*; however, *in vivo* studies with sheep showed that an HSCAS added at 2% of the diet did not reduce fescue toxicity (Chestnut *et al.*, 1992).

Abdel-Wahhab *et al.* (2005) showed that montmorillonite binds well with sterigmatocystin, a mycotoxin chemically similar to aflatoxin. A clinoptilolite was effective in reducing the effects of aflatoxin in quail (Parlat *et al.*, 1999). Various clay products including a calcium bentonite were similar in effectiveness to a HSCAS in restoring performance to pigs consuming aflatoxin (Schell *et al.*, 1993a; Schell *et al.*, 1993b). Diaz *et al.* (2004) studied the efficacy of several clay types to reduce aflatoxin residues in milk of dairy cows. Several products were effective; however, a sodium bentonite reduced milk aflatoxin more than a similar amount of calcium bentonite. Diatomaceous earth has shown the potential *in vitro* to bind aflatoxin, sterigmatocystin, T-2 toxin, zearalenone and ochratoxin (Natour and Yousef, 1998). Zeolites have not proven to reduce the toxicity of T-2 toxin (Kubena *et al.*, 1998; Devorska and Surai, 2001).

A number of studies have examined chemically modified silicates. Doll *et al.* (2004) examined a chemically modified aluminosilicate that showed good binding with zearalenone *in vitro* confirming previous work (Lemke *et al.*, 1998; Tomasevic-Canovic *et al.*, 2003). Others have shown that chemical modifications have increased the binding of HSCAS with zearalenone (Pimpukdee *et al.*, 2004). Dakovic, *et al.* (2005) demonstrated adsorption of aflatoxin, ochratoxin and zearalenone by an octadecyldimethylbenzyl ammonium treated zeolite.

Organic polymers as binders

Some complex indigestible carbohydrates (cellulose, polysaccharides in the cell walls of yeast and bacteria such as glucomannans, and peptidoglycans, and others) and synthetic polymers such as cholestryamine and polyvinylpyrrolidone can adsorb mycotoxins.

Undigestible dietary fiber has adsorbance potential for mycotoxins. Alfalfa fiber has reduced the effects of zearalenone (James and Smith, 1982; Stangroom and Smith, 1984) in rats and swine and T-2 toxin in rats (Carson and Smith, 1983).

Saccharomyces cerevisiae live yeast was shown to reduce the detrimental effects of aflatoxin in broiler diets (Stanley *et al.*, 1993). The aflatoxin protective effect of live yeast was confirmed in rats, but thermolysed yeast was shown ineffective (Babtista *et al.*, 2002). Fibrous material from the yeast cell wall was shown to have a potential to bind several mycotoxins (Devegowda *et al.* 1998). Esterified glucomannan polymer extracted from the yeast cell wall was shown to bind with aflatoxin, ochratoxin and T-2 toxin, individually and combined (Raju and Devegowda 2000). Additions of esterified glucomannan at 0.5 or 1.0 g/kg to diets supplying 2 mg of total aflatoxin resulted in dose dependent responses in broiler chicks (Basmacioglu *et al.* 2005). Addition of esterified glucan polymer to aflatoxin contaminated diets of dairy cows has significantly reduced milk aflatoxin residues (Diaz *et al.*, 2004).

The esterified glucan polymer may have the capability to bind several mycotoxins. Yiannikouris, *et al.* (2004) demonstrated the mechanism of binding with zearalenone. A glucan polymer bound both T-2 toxin and zearalenone *in vitro* (Freimund *et al.*, 2003). The glucan polymer product was protective against depression in antioxidant activities resulting from T-2 toxin consumed by growing quail (Dvorska and Surai, 2001). A glucan polymer product has protected swine (Swamy *et al.*, 2002), broilers (Swamy *et al.*, 2004) and hens (Chowdhury and Smith, 2004) against some of the detrimental effects of multiple mycotoxins, but without restoring growth rate. Aravind *et al.* (2003) using dietary additions of 0.5% esterified glucomannan, alleviated growth depression in broilers associated with naturally contaminated diets containing aflatoxin, ochratoxin, zearalenone and T-2 toxin. A glucan polymer product was effective in preventing aurofusarin toxicity in quail (Dvorska *et al.*, 2003). A glucan polymer product did not alleviate the toxic effects on mink consuming diets contaminated with fumonisin, ochratoxin, moniliformin and zearalenone (Bursian *et al.*, 2004).

Certain bacteria, particularly strains of lactic acid bacteria, propionibacteria and bifidobacteria, appear to have the capacity to bind mycotoxins, including aflatoxin and some Fusarium produced mycotoxins (El-Nezami *et al.*, 2000, 2002a, 2002b; Haskard *et al.*, 2001 and Oatley *et al.*, 2000; Yoon *et al.*, 1999). The binding appears to be physical with deoxynivalenol, diacetoxyscerpenol, nivalenol, and other mycotoxins associated with hydrophobic pockets on the bacterial surface. Research reports on the subject are limited.

A synthetic water soluble polymer, polyvinlypyrrolidone (PVP) has been investigated as a binder of mycotoxins. Insufficient information is available to make any recommendations. PVP is reported to bind with aflatoxin and zearalenone *in vitro* (Alegakis *et al.*, 1999). PVP did not alleviate the toxicity of deoxynivalenol seen in pigs (Friend *et al.*, 1984).

Cholestyramine resin is used in human medicine for the reduction of cholesterol and functions through adsorption of bile acids. Cholestyramine has proven to adsorb zearalenone (Ramos *et al.*, 1996b; Doll *et al.*, 2004) and fumonisins (Solfrizzo *et al.*, 2001). In rats consuming ochratoxin, cholestyramine reduced plasma ochratoxin and increased fecal ochratoxin excretion (Kerkadi *et al.*, 1998). In another *in vivo* study, cholestyramine did not bind ochratoxin (Bauer, 1994). Because of cost, cholestyramine use is questionable.

Desirable Characteristics of a Binder

A binder must be effective at sequestering the mycotoxin(s) of interest. In some cases, it may be of value to bind one specific mycotoxin and in others, to bind multiple mycotoxins. A binder should significantly

prevent animal toxicity. There should not be serious detrimental effects on the animal, or at least detrimental effects should not outweigh the benefits. Costs should render its use practical and profitable. Animal/product residues of mycotoxins should not increase. There should be no detrimental effects on the animal food product. Mycotoxins in feeds should not be masked such that feed contamination cannot be verified. The binder should be physically usable in commercial feed manufacturing situations. Binder use and efficacy should be verifiable.

Conclusions

There is an excellent potential for binders to help manage the mycotoxin problem. Various materials can bind mycotoxins in feed and thus reduce toxic exposure to consuming animals. No product currently meets all the characteristics for a desirable binder. Mycotoxin control measures may require many approaches. In addition to binders or multiple binders, diets may be treated with other decontamination products. Animals may also be supplemented with antioxidants and other beneficial substances.

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