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Mycotoxin detoxication of animal feed by different adsorbents

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Abstract

The contamination of animal feed with mycotoxins represents a worldwide problem for farmers. These toxins originate from molds whose growth on living and stored plants is almost unavoidable particularly under moist conditions. Mycotoxin-containing feed can cause serious diseases in farm animals resulting in suffering and even death and thus can cause substantial economic losses. The most applied method for protecting animals against mycotoxicosis is the utilization of adsorbents mixed with the feed which are supposed to bind the mycotoxins efficiently in the gastro-intestinal tract. Aluminosilicates are the preferred adsorbents, followed by activated charcoal and special polymers. The efficiency of mycotoxin binders, however, differs considerably depending mainly on the chemical structure of both the adsorbent and the toxin. This review describes the most important types of adsorbents and the respective mechanisms of adsorption. Data of the in vitro and in vivo efficacy of detoxication are given.

Keywords: Review; Mycotoxin detoxication; Activated charcoal; Aluminosilicate; Clay; Zeolite

1. Introduction

Mycotoxins are produced by several fungi, particularly by many species of *Aspergillus*, *Fusarium*, *Penicillium*, *Claviceps*, and *Alternaria*. They comprise a group of several hundreds of chemically different toxic compounds (William, 1989; Moss, 1996; Rotter et al., 1996; Sweeney and Dobson, 1998). The most common mycotoxins are aflatoxins, ochratoxin A, trichothecenes, zearalenone, and fumonisins.

Cereal plants may be contaminated by mycotoxins in two ways. First, there are fungi growing as pathogens on plants; secondly, there are fungi growing saprophytically on stored plants. In this context, it has to be considered that not all of these fungi form mycotoxins, i.e. the detection of fungi is not the same as the detection of mycotoxins because many fungi are not able to produce mycotoxins or produce them in different amounts depending on the substrate on which they are growing. However, high incidence rates of con-

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tamination of cereal grains and animal feed have been reported worldwide (Placinta et al., 1999; Spahr et al., 1999), so that the contamination of diets by mycotoxins and the carry-over of mycotoxin related compounds through the food chain (Ramos and Hernandez, 1996) have to be accurately controlled. Although in terms of acute toxicity even the most poisonous of the mycotoxins is far less toxic than the botulinum toxin (Moss, 1996), the consumption of mycotoxin contaminated diet may induce acute and long-term chronic effects resulting in a teratogenic, carcinogenic (mainly for liver and kidney), oestrogenic, or immunsuppressive impact not only on animals but also on man whereas animals usually suffer more due to grain of lower quality (D'Mello et al., 1999; Steyn and Stander, 1999; Casteel and Rottinghouse, 2000). In addition to the toxic effects, a mycotoxin contaminated diet may lead to other consequences like feed refusal, poor feed conversion, diminished body weight gain, increased disease incidence due to immune suppression, and interference with reproductive capacities (CAST, 1989; Lindemann et al., 1993; Kubena et al., 1998a) which are responsible for great economical losses.

In order to avoid mycotoxicosis, several strategies have been investigated (Doyle et al., 1982; Park, 1993; Bauer, 1994; Ramos and Hernandez, 1997) which can be divided into pre- and post-harvest technologies and into biological, chemical, and physical methods.

The best procedure to prevent the effect of mycotoxins is the minimizing of the mycotoxin production itself (Miedaner and Reinbrecht, 1999), e.g. by harvesting the grain at maturity and low moisture and storing it at cool and dry conditions which is difficult to perform in countries with a warm and humid climate. Furthermore, the growth of fungi and therefore the production of mycotoxins is limited by the use of propionic acid or ammonium isobutyrate. Feed additives like antioxidants, sulphur-containing amino acids, vitamins, and trace elements can be useful as detoxicants (Nahm, 1995).

Biological methods are not yet used in practice though the number of corresponding patents increases continously (Erber, 1996; Duvick and Rood, 2000). These methods include fermentation procedures with microorganisms. One example is the conversion of aflatoxin B_1 (particularly by *Flavobacterium auranticum*) to harmless degradation products. The conversions, however, are generally slow and incomplete (Sweeney and Dobson, 1998; Arici, 1999; Bata and Lásztity, 1999; Karlovsky, 1999).

Chemically, some mycotoxins can be destroyed with calcium hydroxide monoethylamine (Bauer, 1994), ozone (McKenzie et al., 1997; Lemke et al., 1999) or ammonia (Park, 1993). Particularly the ammoniation is an approved procedure for the detoxication of aflatoxin-contaminated feed in some U.S. states as well as in Senegal, France, and the UK. The average ammoniation costs vary between 5 and 20% of the value of the commodity (Coker, 1998). Main drawbacks of this kind of chemical detoxication are the ineffectiveness against other mycotoxins and the possible deterioration of the animals health by excessive residual ammonia in the feed.

The physical methods are focused on the removal of mycotoxins by different adsorbents added to mycotoxin-contaminated diets (Ramos et al., 1996a) with the hope of being effective in the gastro-intestinal tract more in a prophylactic rather than in a therapeutic manner. At present, however, the utilization of mycotoxin-binding adsorbents is the most applied way of protecting animals against the harmful effects of decontaminated feed.

2. Efficacy of different adsorbents for the binding of mycotoxins

Herein, the adsorbents are discussed particularly concerning efficacy, specificity, and the mechanism of the adsorption process. The latter is similar to a chemical reaction and therefore, the release of free energy (ΔG) is the driving force of every adsorption. The most important feature of the adsorption is the physical structure of the adsorbent, i.e. the total charge and charge distribution, the size of the pores and the accessible surface area. On the other hand, the properties of the adsorbate molecules, the mycotoxins, like po-

larity, solubility, size, shape and — in case of ionized compounds — charge distribution and dissociation constants play a significant role, too. Therefore, the efficacy of every adsorption process has to be investigated in regard to the particular properties of the adsorbate.

2.1. Activated charcoal

Activated charcoal which is formed by pyrolysis of organic materials is a very porous non-soluble powder with a high surface to mass ratio (500–3500 m^2/g). Since the 19th century it has been used as an antidote against poisoning. Therefore, it might also inactivate mycotoxins. In aqueous solution, it can adsorb most of the mycotoxins efficiently (Table 1) whereas different activated charcoals have less or even no effects against mycotoxicosis (Table 2). This might be due to the fact that activated charcoal is a relatively unspecific adsorbent and, hence, essential nutrients are also adsorbed particularly if their concentrations in the feed are much higher compared to those of the mycotoxin. In other trials with goats, however, it was shown that high doses of activated charcoal are beneficial in an acute poisoning situation concerning the intake of high amounts of aflatoxins (Hatch et al., 1982).

2.2. Aluminosilicates (zeolites, HSCAS, clays)

Most studies related to the alleviation of mycotoxicosis by the use of adsorbents are focused on aluminosilicates, mainly zeolites and hydrated sodium calcium aluminosilicates (HSCAS), and aluminosilicate-containing clays, all consisting of aluminates, silicates and some interchangeable ions, mainly alkali metal and alkaline earth metal ions (Barrer, 1989; Mumpton, 1999). Clay minerals are primarily layered silicates with the common chemical formula $[Si_2O_5^{2-}]_{xy}$, e.g. kaolin $Al_4(OH)_8Si_2O_5$. Zeolites are composed of tetrahedrons of SiO_4 and AlO_4 as the two fundamental building blocks with the metal atom at the center of each tetrahedron. The common chemical formula is $[AlSi_3O_8^-]_{xyz}$, e.g. orthoklas $KAlSi_3O_8$, zeolite A $\{Na_{12}[Al_{12}Si_{12}O_{48}]\cdot 27\ H_2O\}_8$. While the SiO_4-unit is electrically neutral, the AlO_4-unit carries one negative charge which has to be compensated by positive charges, usually sodium ions as in zeolite A. Zeolites are similar to molecular sieves as well as to ion exchange resins and are suitable for the distinction of different molecules by size, shape, and charge. HSCAS contain calcium ions and protons which are exchanged against the naturally occuring sodium ions. They are a type of montmorillonite belonging to phyllosilicates which are composed of layers of aluminium and silicon connected in a 1:1 or 2:1 arrangement.

The applicability of aluminosilicates for the adsorption of mycotoxins (Table 1) has been studied for more than 20 years (clays: Masimango et al., 1978; zeolites: Mumpton and Fishman, 1977; HSCAS: Davidson et al., 1987; Ramos and Hernandez, 1997). Phillips et al. (1988) analyzed the in vitro binding capacities of different adsorbents which were representative for the major chemical classes of aluminas, silicas, and aluminosilicates and selected HSCAS as a possible suitable candidate for in vivo trials concerning the prevention of aflatoxicosis in chicken. HSCAS was shown to have a high affinity for aflatoxin B_1 forming a complex which was stable at temperatures of 25 and 37°C, in a pH range of 2–10, and in an eluotropic series of solvents. When HSCAS was added in a concentration of 0.5% to chicken diets containing 7.5 mg/kg aflatoxin B_1, the growth inhibitory effects were significantly decreased. In this study, the adsorption of HSCAS was thought to be chemisorption including the formation of strong bonds. Two years later, Phillips et al. (1990a) interpreted the binding mechanism as the formation of a complex by the β-carbonyl system of the aflatoxin with 'uncoordinated edge site' aluminium ions. Thus, HSCAS can be used as an 'inorganic sponge' sequestering aflatoxins in the gastro-intestinal tract of farm animals. Ramos et al., 1996b investigated the adsorption of aflatoxins to montmorillonite according to Freundlich and Langmuir isotherm calculations. They obtained a better fit of their adsorption data employing the Freundlich isotherm and suggested therefore the presence of a heterogeneous surface with different adsorption centers having different affinities for the adsorbate or the co-existence of

different adsorption mechanisms or both. The use of aluminosilicates for the adsorption of other mycotoxins was also tested, but with little success (Bauer, 1994; Ramos et al., 1996b; Lemke et al., 1998) except of a chemically modified montmorillonite with a binding capacity for zearalenone of 108 mg/g (Lemke et al., 1998). This clay was derivatized with cetylpyridinium or hexadecyltrimethylammonium resulting in an increased hydrophobicity of the clay surface following a high affinity to the hydrophobic zearalenone. In contrast, a closely related organophilic phyllosilicate showed a significantly lower binding capacity (Schall et al., 2000). A surprisingly high binding

Table 1
In vitro adsorption of mycotoxins by different adsorbents

Adsorbent	Mycotoxin	Adsorption capacity (mg/g)	Reference
Activated charcoal			
Activated charcoal	afl	10.0	Decker and Corby, 1980
Activated charcoal	afl/fum	120/11.0	Galvano et al., 1997
Activated charcoal	och	100.0	Bauer, 1994
Activated charcoal	och/tri	124/9.9	Galvano et al., 1998
Aluminosilicates			
Aluminosilicates	afl	<0.02	Flores et al., 1999
HSCAS (Milbond-TX®)	afl	2.5	Ledoux et al., 1999
HSCAS	afl	86.0	Phillips et al., 1988
HSCAS	afl	62.4–72.4	Phillips et al., 1990b
Montmorillonite	afl	1.9	Ramos and Hernandez, 1996
Aluminosilicates (Ethacal®, Novasil™, perlite, zeobrite)	afl	0.06–0.80 μg/g	Scheideler, 1993
Phyllosilicates, Bentonite	afl/och/zea	0.03–0.44	Schall et al., 2000
Diatomaceous earth	afl/och/zea/tri	0.5–1.5	Natour and Yousef, 1998
Montmorillonite[a]	zea	108	Lemke et al., 1998
Montmorillonite	zea	0.19	Ramos et al., 1996b
Bentonite	zea	0.11	Ramos et al., 1996b
Sepiolite	zea	0.07	Ramos et al., 1996b
Mg trisilicate	zea	0.02	Ramos et al., 1996b
Bentonite	och	1.5–9.0	Bauer, 1994
HSCAS	och	0–2.2	Bauer, 1994
Acidic clay	cpa	0.74	Dwyer et al., 1997
Neutral clay	cpa	0.28	Dwyer et al., 1997
Clinoptilolite	cpa	0.08	Dwyer et al., 1997
Montmorillonite	erg	290	Huebner et al., 1999
Miscellaneous			
Yeast[b]	och	1.2–8.6	Grünkemeier, 1990; Bauer, 1994
Yeast cell walls (Mycosorb™)	zea	2.7	Völkl and Karlovsky, 1998
Modified yeast cell walls extract	afl/och/zea/tri	0.2–1.9	Howes and Newman, 2000
Cholestyramine	och	9.6	Bauer, 1994
Cholestyramine	zea	>0.3	Ramos et al., 1996b
Crospovidone	zea	0.3	Ramos et al., 1996b
Cross-linked polyvinylpyrrolidone	zea	0.5–2.1	Alegakis et al., 1999

afl, aflatoxin; och, ochratoxin A; zea, zearalenone; tri, trichothecenes; fum, fumonsins; cpa, cyclopiazonic acid; erg, ergotamine.
[a] Derivatized with long-chain quarternary ammonium residues.
[b] 40% sterilized yeast, 60% fermentation residua of beer production.

Table 2
In vivo adsorption of mycotoxins by different adsorbents

Adsorbent	Concentration (%)	Mycotoxin	Effects observed	Reference
Activated charcoal				
Activated charcoal	0.5	afl	Decreased excretion of afl M_1, no protective effects against aflatoxicosis	Edrington et al., 1996
Super-activated charcoal	0.5	afl	Significant increase in body weight gains	Edrington et al., 1997
Activated charcoal	High	afl	100% survival of goats given a lethal dose	Hatch et al., 1982
Activated charcoal	0.5	afl	No effect	Kubena et al., 1988
Super-activated charcoal	0.5	tri	No effect	Edrington et al., 1997
Activated charcoal	10.0	och	Significant reduction of the och concentration in blood, bile, tissues of pigs	Bauer, 1994
Aluminosilicates				
HSCAS, Bentonite	0.5	afl	Growth inhibitory effects on pregnant rats significantly diminished; ability of reproduction warranted	Abdel-Wahhab et al., 1999
Bentonite	0.5/1.0	afl	Growth inhibitory effects of broiler chickens diminished by 64 and 84%	Araba and Wyatt, 1991
Ethacal®	0.5/1.0	afl	No significant effect (broiler chickens); ethacal® alone reduced feed intake and body weight and increased water consumption	Araba and Wyatt, 1991
HSCAS	0.5/1.0	afl	Growth inhibitory effects on broiler chickens diminished by 38 and 84%	Araba and Wyatt, 1991
HSCAS	0.1/0.5	afl	Reduction of bioavailability of aflatoxins in the liver and blood of chickens in a dose-dependent manner	Davidson et al., 1987
HSCAS	0.0–1.0	afl	Growth inhibitory effects on chickens diminished by 50–100%; feed conversions improved in a dose-dependent fashion; no full protection against liver or spleen weight changes by afl	Doerr, 1989
HSCAS	0.5	afl	Significant decrease of urinary excretion of afl M_1 in turkey poults when HSCAS simultaneously dosed with afl	Edrington et al., 1996
HSCAS	0.5	afl	Growth inhibitory effects on chickens diminished by 55–100%	Kubena et al., 1988
HSCAS	0.5	afl	68% decrease in mortality of growing male turkey poults	Kubena et al., 1991
HSCAS	0.5	afl	Growth inhibitory effects on chickens diminished by 39–68% (2.5 mg afl/kg feed) and by 46–88% (5 mg afl/kg feed)	Kubena et al., 1993b
HSCAS (Milbond-TX®)	1.0	afl	Growth inhibitory effects on broiler chicks completely prevented	Ledoux et al., 1999
Bentonite	0.5	afl	Growth inhibitory effects on pigs diminished by 87–89%	Lindemann et al., 1993
HSCAS	0.5	afl	Growth inhibitory effects on pigs diminished by 80%	Lindemann et al., 1993
HSCAS	0.5	afl	Decrease of growth inhibitory effects, protective effects on gross hepatic changes	Phillips et al., 1988
Aluminosilicates (Ethacal®, NovaSil™, perlite, zeobrite)	1.0	afl	Growth inhibitory effects on chickens diminished by 85–100%	Scheideler, 1993
HSCAS	0.5	afl	Growth inhibitory effects on average daily gain of pigs diminished by 82%	Schell et al., 1993a

Table 2 (*Continued*)

Adsorbent	Concentration (%)	Mycotoxin	Effects observed	Reference
Clay	1.0	afl	3-Phase study: nursery, growing, metabolism phase; performance and liver function were enhanced, but not all functions restored	Schell et al., 1993a
Calcium bentonite	0.25–2.0	afl	Growth inhibitory effects on average daily gain of pigs diminished by 64–82%	Schell et al., 1993b
Clinoptilote	5.0	afl	Decreased food consumption of quail chicks diminished by 57%, growth inhibitory effects diminished by 70%	Parlat et al., 1999
HSCAS	0.5	afl/och	Growth inhibitory effects on chickens diminished by 65%, no effect against toxicity of och, little effect against toxicity of combined toxins	Huff et al., 1992
Inorganic	0.5	afl/tri	Growth inhibitory effects on broiler chickens diminished by 25%; no protective effect against T-2	Bailey et al., 1998
HSCAS	0.5	afl/tri	Growth inhibitory effects on turkey poults diminished by 55–100% only for afl, no effect against T-2 induced toxicity	Kubena et al., 1990
HSCAS	0.5	afl/tri	Growth inhibitory effects on chickens diminished by 85% (afl), 76% (afl+tri), 3% (tri)	Kubena et al., 1993a
HSCAS	0.25/0.375/0.8	afl/tri	Growth inhibitory effects on young broiler chickens diminished by 43%; no significant effect against tri toxicosis	Kubena et al., 1998b
HSCAS	1.0	och	No significant effect (pigs)	Bauer, 1994
Bentonite	1.0/10.0	och	No significant effect (pigs)	Bauer, 1994
HSCAS	0.5	zea	Reproductive effect of zea alleviated; protection against increase in gestation length, decrease in litter size and increase in kit mortality of mink	Bursian et al., 1992
HSCAS	0.5/1.0	tri	No significant effect (pigs)	Patterson and Young, 1993
Acidic clay	1.0	cpa	No significant effect (broilers)	Dwyer et al., 1997
Neutral clay	1.0	cpa	No significant effect (broilers)	Dwyer et al., 1997
Clinoptilolite	1.0	cpa	No significant effect (broilers)	Dwyer et al., 1997
Miscellaneous				
Yeast[a]	5.0	och	No reduction of the och concentration in blood, bile, tissues of pigs	Bauer, 1994
Cholestyramine	1.0	och	No reduction of the och concentration in blood, bile, tissues of pigs	Bauer, 1994

afl, aflatoxin; och, ochratoxin A; zea, zearalenone; tri, trichothecenes; fum, fumonsins; cpa, cyclopiazonic acid. The efficacy of each adsorbent was estimated by the effects on, for instance, the animal performance, clinical chemistry parameters, or body weight gain. As far as possible, it was calculated as percentage of the decrease of growth inhibitory effects.

[a] 40% sterilized yeast, 60% fermentation residua of beer production.

capacity of 290 mg/g for the alkaloid ergotamine was achieved with calcium montmorillonite (Huebner et al., 1999).

Related to in vivo trials, the amount of an adsorbed mycotoxin is difficult to calculate. Therefore, the efficacy of adsorption has to be determined by the animal performance, e.g. body weight gain, feed intake, mortality, concentrations of the corresponding mycotoxin in blood, tissues, and organs. The results from such feeding trials are presented in Table 2.

Regarding the applicability of aluminosilicates for the binding of mycotoxins, it can be concluded that they are very effective in preventing aflatoxicosis, but their efficacy against zearalenone, ochratoxin, and trichothecenes is limited. In addition to the narrow binding range concerning different mycotoxins, aluminosilicates have the disadvantage of showing high inclusion rates for vitamins and minerals.

2.3. Miscellaneous adsorbents

2.3.1. Polymers

Cholestyramine is an anion exchange resin which is used for the binding of bile acids in the gastro-intestinal tract and for the reduction of low density lipoproteins and cholesterol. The in vitro binding capacity of this resin for ochratoxin A and zearalenone was 9.6 mg/g (Bauer, 1994) and more than 0.3 mg/g (Ramos et al., 1996b), respectively, but in vivo, cholestyramine had only a very small effect on the reduction of the ochratoxin concentration in blood, bile, and tissues.

Another adsorbent is crospovidone (polyvinylpyrrolidone), a highly polar amphoteric polymer the in vitro adsorbance of which was measured as 0.3 mg/g for zearalenone by Ramos et al. (1996b). Up to now, this polymer has not been tested in vivo. An improved cryogel of cross-linked polyvinylpyrrolidone recently showed increased values up to 2.1 mg/g (Alegakis et al. 1999).

2.3.2. Yeast and products from yeast

Besides its excellent nutritional value, yeast or yeast cell walls can also be used as adsorbents for mycotoxins (Grünkemeier, 1990; Bauer, 1994). The in vitro adsorption of ochratoxin by yeast (consisting of 40% sterilized yeast and 60% fermentation residua of yeasts used for beer production) is dependent on the pH being at maximum in acidic solutions (at pH 3: 8.6 mg/g, at pH 8: 1.2 mg/g). However, in trials with pigs employing a feed supplement of 5% of yeast, only a slight reduction of the ochratoxin A concentration in blood plasma, bile, and tissues was achieved. By the use only of yeast cell walls instead of whole cells, the adsorption of mycotoxins can be enhanced. The cell walls harboring polysaccharides (glucan, mannan), proteins, and lipids exhibit numerous different and easy accessible adsorption centers including different adsorption mechanisms, e.g. hydrogen bonding, ionic, or hydrophobic interaction. Therefore, it was possible to bind 2.7 mg zearalenone per gram of cell walls. The binding was rapid and reached equilibrium after only 10 min, which is superior to commercial available clay-based toxin binders (Völkl and Karlovsky, 1998, 1999).

In another context, it was shown that yeast killer toxins were adsorbed by the polysaccharides and not by the proteins or fatty acids of yeast cell walls (Radler and Schmitt, 1987) and that this adsorption was not unspecific because cellulose and glycogen were not able to bind killer toxins.

3. Conclusion

The applicability of different binders for the adsorption of mycotoxins was first investigated by in vitro experiments demonstrating that most of the mycotoxins were sufficiently bound by at least one adsorbent (Phillips et al., 1988, 1990b; Bauer, 1994; Galvano et al., 1997, 1998; Huebner et al., 1999), which was possibly derivatized, e.g. employing cetylpyridinium or hexadecyltrimethylammonium (Lemke et al., 1998). Adsorbents exhibiting high binding capacities in vitro were further tested in lifestock and it was shown that some adsorbents are suitable to alleviate the toxic effects of specific mycotoxins. The addition of HSCAS for example resulted in almost total protection against aflatoxicosis (Kubena et al., 1988; Doerr, 1989; Ramos and Hernandez, 1996), but

its efficacy against zearalenone and ochratoxin was very limited (Bursian et al., 1992; Huff et al., 1992; Bauer, 1994) and against trichothecenes practically zero (Kubena et al., 1990, 1993a; Patterson and Young, 1993; Kubena et al., 1998b). So far, no single adsorbent was tested to be effective against most types of mycotoxins. However, the addition of different adsorbents or of very promising derivatized adsorbents to animal feed provides versatile tools of preventing mycotoxicosis.

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