# Review of mycotoxin-detoxifying agents used as feed additives: mode of action, efficacy and feed/food safety

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**SCIENTIFIC REPORT submitted to EFSA**

# Review of mycotoxin-detoxifying agents used as feed additives: mode of action, efficacy and feed/food safety [1]

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## Abstract

Mycotoxins are small and quite stable molecules which are extremely difficult to remove or eradicate, and which enter the feed chain while keeping their toxic properties.

The mycotoxins of major concern as feed contaminants that are potentially removable from feed, are mainly aflatoxins, ochratoxin A, *Fusarium* toxins (trichothecenes like deoxynivalenol, diacetoxyscirpenol, nivalenol, T2-toxin/HT2-toxin, zearalenone and fumonisins).

One of the strategies for reducing the exposure to mycotoxins is to decrease their bioavailability by including various mycotoxin-adsorbing agents in the compound feed, which leads to a reduction of mycotoxin uptake as well as distribution to the blood and target organs.

Another strategy is the degradation of mycotoxins into non-toxic metabolites by using biotransforming agents such as bacteria/fungi or enzymes.

The specific objectives of the project are to provide a critical and extensive review on mycotoxins detoxifying agents.

To this purpose, the consortium performed:

- The compilation and critical analysis of all published data available in the scientific literature ;
- The compilation and critical analysis of "grey literature" published worldwide (searches in patents databases) ;
- The audition of companies involved in the development and manufacture of mycotoxin-detoxifying agents, as well as stakeholders of feed manufacturer's;
- Comparison, validation and synthesis of the reviewed materials.

The report made an inventory of agents which may possibly reduce the impact of mycotoxins in feedstuffs by reviewing the literature and by collecting data from feed manufacturer's or from information published on the Internet.

The present report gives also the mechanism of action of the agents listed, by reviewing *in vitro* studies and identifies the relevant end-points to be studied when testing the efficacy of mycotoxin-detoxifying agents in *in vivo* trials.

Finally this document points out the benefits and risks associated with the potential uses of these agents and gives recommendations for the assessment of such products as feed additives.



## Key words

Mycotoxin, mycotoxin-detoxifying agents, adsorbing agents, biotransforming agents, poultry, pigs, ruminants, feed.

## Terms of reference

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## Glossary

AC: Activated Carbon
ADME: Absorption, Distribution, Metabolism, Excretion
ADTZ: Aflatoxin-detoxifizyme
AFB1: Aflatoxin B1
AFL: Aflatoxicol
AFM1: Aflatoxin M1
ALB: Albumin
ALP: Alkaline Phosphatase
ALT: Alanine Aminotransferase
AST: Aspartate Aminotransferase
BC50: Binding Concentration 50
BHT: Butylated hydroxytoluene
BW: Body weight
CAST: Council for Agricultural Science and Technology
CAT: Catalase
CEN: Comité européen de normalisation
CK: Creatinine Kinase
DAS: Diacetoxyscirpenol
DOM-1: deepoxy-deoxynivalenol
DON: Deoxynivalenol
EC50: Effective Concentration 50
ECEC: External surface Cation Exchange Capacity
EDTA: Ethylene diamine tetra acetic acid
EFSA: European Food Safety Agency
E-GM: Esterified Glucomannans
ELEM: Equine LeucoEncephaloMalacie
ELISA: Enzyme-linked immunosorbent assay
EU: European Union
FAO: Food and Agriculture Organisation
FB1: Fumonisin B1
FCR: Feed conversion ratio
FEFAC: European Feed Manufacturers' Federation
FEFANA: European Association of Feed Additives and premixtures operators
GGT: γ-Glutamyl-Transferase
GI: Gastro-intestinal
GLOB: Globin
GM: Glucomannan
GPx: Glutathione peroxidase
GRAS: Generally Recognized As Safe
HPLC: High Performance Liquid Chromatography
HSCAS: Hydrated sodium calcium aluminosilicate
IgA: Immunoglobulin A
IRMM: Institute for Reference Materials and Measurements
ISO: International Organization for Standardization)

LAB: Lactic Acid Bacteria
LD50: Lethal Dose, 50%
LDH: Lactate deshydro genase
LPO: Lipid peroxide
MMN: Modified montmorillonite nanocomposite
MN RBC: Micronucleated red blood cells
MPN: Mycotoxic porcine nephropathy
MWF: Micronized wheat fibres
NIV: Nivalenol
NSP: Nonstarch polysaccharide
OTA: Ochratoxin A
PAH: Polycyclic Atomatic Hydrocarbon
PMSF: Specific inhibitor of serine type-proteases
RSD: Relative Standard Deviation
SA: Sphinganine
SAC: SuperActivated Charcoal
SCOOP: Scientific Cooperation
SD: Standard Deviation
SO: Sphingosine
SOD: Superoxide dismutase
Stg: Sterigmatocystin
TIM: Gastrointestinal model
TP: Total protein
YCW: Yeast cell wall
ZN: Zeolite NaA
ZEA: Zearalenone
ZOL: Zearalenol

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# 1. BACKGROUND AND OBJECTIVES

## 1.1. Objectives

The specific objective of the project is to provide a critical and extensive review on mycotoxin-detoxifying agents, on their mode of action and on the benefits/risks associated with their potential use as feed additives. This review should help EFSA to develop guidelines for risk and efficacy assessment of mycotoxin-detoxifying agents in feeds. To this purpose, the project achieves the following results:

- The compilation and critical analysis of all published data available in the scientific literature ;
- The compilation and critical analysis of "grey literature" published worldwide, with a special focus on that published in the EU Member States. This includes searches in patents databases ;
- The hearing of industrials involved in the development and manufacture of mycotoxin-detoxifying agents, as well as stakeholders of feed manufacturer's and scientific experts if needed;
- Comparison, validation and synthesis of the reviewed materials with special emphasis on:
    - The precise definition of a detoxifying agent used as a feed additive and its categorization according to its mode of action or other scientific criteria;
    - An overview of the existing protocols and end-points for testing the efficacy and safety of mycotoxin-detoxifying agents.

One of the objectives of the final report is to define precisely subcategories of mycotoxin-adsorbing and biotransforming agents, based on their functional properties, their mode of action and their biological or physical and chemical characteristics. The most exhaustive list of feed additives is compiled. The report specifically deals with the toxicological and animal performance experimental studies implemented to demonstrate the innocuous properties of the absorbing or biotransforming agents.

## 1.2. Overview on the mycotoxin hazard and involvement in the feed chain

### 1.2.1. Assessment

Mycotoxins are secondary metabolic products from moulds belonging in particular to the *Aspergillus, Penicillium* and *Fusarium* genera. More than 300 secondary metabolites have been identified although only around 30 have true toxic properties which are of some concern. Toxinogenic moulds may develop under all climatic conditions on any solid or liquid supports as soon as nutritional substances and moisture (water activity $A_w$ over 0.6) are present, hence the wide variety of contaminated foodstuff substrates. These toxins are found as natural contaminants in many feedstuffs of plant origin, especially cereals but also fruits, hazelnuts, almonds, seeds, fodder and foods consisting of, or manufactured from, these products and intended for human or animal consumption.

Two groups of toxinogenic (mycotoxin producing) fungi can be distinguished. The first one consists of fungi (such as *Fusarium*) which invade their substrate and produce mycotoxins on the growing plants before harvesting: this is the category of field (pre-harvest) toxins. Aflatoxins and *Fusarium* toxins are included in this group. The other group contains fungi which produce toxins after

harvesting and during crop storage and transportation. These toxins are named storage (or post-harvest) toxins and ochratoxin A belongs to this group.

Mycotoxins are small and quite stable molecules which are extremely difficult to remove or eradicate, and which enter the feed chain while keeping their toxic properties.

#### 1.2.1.1. Mycotoxins of interest for farm animals

A list of mycotoxins of interest which are of some concern for the safety of animal feed in the European Union was published in an EU SCAN report (EU SCAN, 2003). It includes Aflatoxin B1 (AFB1) and Ergot sclerotia, which are subject to Commission Regulation (EC) No 1881/2006[2]. Zearalenone (ZEA), Deoxynivalenol (DON), Ochratoxin A (OTA) and Fumonisins (especially Fumonisin B1, FB1), the maximum levels of which are now recommended (Commission Recommendation 2006/576/EC[3]). This list also includes T-2 and HT-2 toxins. Mycotoxins such as mycophenolic acid, cyclopiazonic acid and moniliformin were considered as representing possible emerging risks for which occurrence and toxicological data are still limited and needed. Diacetoxyscirpenol (DAS) is another *Fusarium* mycotoxin subject to regulations in a lot of countries but not in the European Union (Commission Recommendation 2006/576/EC). Other mycotoxins such as patulin or other trichothecenes, like Nivalenol (NIV) are under scrutiny by feed operators and official regulating bodies: patulin as being able to contaminate beetroots pulps, NIV being a mycotoxin often co-occurring with DON or DAS in cereals depending on geographical areas (Canada for example) or meteorological conditions of the year.

#### 1.2.1.2. Occurrence of mycotoxins: the reality of co-contamination

In a recent paper, Binder reported a two-year survey programme undertaken to evaluate the incidence of mycotoxins in feed and feed raw materials in some of the major animal production regions by the feed additive producer Biomin®. A total of 2,753 assays were performed on 1,507 samples taken from European and Mediterranean markets for the determination of AFB1, OTA, DON, T2 toxin, ZEA and fumonisins. 52% of these samples were tested positive indicating that the incidence of mycotoxins is quite high in animal feed (Binder, 2007).

Multiple contaminations may also occur because of the ability of a given mould species to produce several kinds of mycotoxins in one type of food ingredient. Alternately, several types of mycotoxins may be found in the same food or feed containing different contaminated ingredients or raw materials.

A review of Galvano et al. mentioned several studies and surveys reporting the simultaneous contamination of corn by AFB1 and FB1 in China, AFB1, FB1, DON, and NIV in Vietnam, FB1, ZEA, DON, NIV and TCT in Korea, AFB1, FB1, and OTA in Korea also, AFB1, FB1, DON, NIV and ZEA in Indonesia, AFs, FB1 and ZEA in Brazil, AFs and FB1 in Ghana, moniliformin and FB1 in the USA, and also the simultaneous contamination of raw cereals and processed foods by both DON and fumonisins in Italy. These possibilities of simultaneous contamination may occur in animal feed since raw cereals can be used as raw materials in feed preparation (Galvano et al., 2006).

[2] Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs

[3] Commission Recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding (2006/576/EC)

### 1.2.1.3. Dietary exposure of farm animals to mycotoxins

Unlike for humans, the exposure of farm animals to mycotoxin is poorly described. In a recent report AFSSA investigated the exposure of cattle, pigs and poultry to mycotoxins using French cereal contamination data (Afssa, 2009).

The analytical data used for such an estimation were restricted to feeds for which the composition was clearly identified (excluding mixed meals...), the database being provided through official monitoring plans or data released by the feed industry. For all species, sub categories have been set according to gender stages of growth. For some species, a difference was made between summer and winter diets. The exposure of farm animals to mycotoxins was calculated after the mean contamination levels of the feed ration (expressed in µg/kg) at the 75e and 95e percentile and compared to the enforced (for aflatoxins) or recommended (DON, ZEA and OTA) maximum limits. Globally, for aflatoxins, the estimated mean feed contamination for pigs and poultry is far below the regulation enforced in the European Union. For DON and ZEA, when the 95e percentile of contamination was used, the calculation indicates that pigs could be exposed to higher levels than the recommended ones. For other mycotoxins and other animal species, the animal exposure, even with higly contaminated material, was below the recommended levels (AFSSA 2009).

The analysis was done for pigs and poultry but not for bovine for which the composition of feed rations was often not enough detailed. Nevertheless, we should keep in mind that silage can be contaminated by mycotoxins. Unfortunately this contamination is poorly described (Mansfield and Jones, 2008; Richard et al., 2007). However, a study on occurrence of mycotoxins in feedstuffs of dairy cows and estimation of total dietary intakes was performed in the Netherlands and considered any kind of feed ingredients. Silage and compound feed were the main diet ingredients, representing on average 67 and 23% of dry matter intake, respectively. DON, ZEA, roquefortine C, and mycophenolic acid were the mycotoxins with the highest incidence. DON and ZEA had a low incidence in forage samples and were not detected in ensiled by-product samples whereas roquefortine C and mycophenolic acid were only detected in silage and ensiled by-product samples. Because carry-over of DON, ZEA, roquefortine C, and mycophenolic acid into milk is negligible (see paragraph 1.2.1.4.), their occurrence in feedstuffs is not considered of significant concern with respect to the safety of dairy products for consumers (Driehuis et al., 2008).

The proportion of cereals in animal diet may depend on its going price, and consequently on the availability of cereals on the market. Also, due to the European policy of developing biofuel, more and more co-products of cereals can be found on the market with attractive going prices for animal feeding. All these kinds of co-products including food manufacture by-products (such as brewer's spent grain for instance) may be a source of mycotoxin exposure.

### 1.2.1.4. Residue of mycotoxins in animal products and dietary exposure of humans through the consumption of animal products

Generally, mycotoxins are not accumulated in muscles. For all animal species, the metabolism leads to excretion in urine and faeces, but also to eggs for poultry and milk for mammals. Regarding the occurrence of mycotoxin residues in eggs, very low AFB1 residue levels (around 0.3 µg/kg) can be found following a contaminated diet at a level as high as 10 000 µg AFB1/kg (10 mg/kg). This is also the case for other mycotoxins such as OTA, T-2, DON, ZEA and 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, lasting a few days, except for OTA in pig. OTA residue levels in a range of 4-71 µg/kg in pork kidneys can be found following contaminated diet at levels ranging from 0.1-1.4 mg/kg. In Denmark an action level has been set at a content of 25 µg OTA/kg of kidney: above this level, the whole pig carcass is rejected from the market based on the expectation of a level of around 10 µg OTA/kg of meat, while for levels in pig kidneys between 10 and 25 µg OTA/kg of kidney, only liver and kidneys are excluded from the market.

Special attention should be paid to polygastric lactating animals regarding the possible excretion of 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 only be found in cow's milk when high quantities (several milligrams) of toxins have been experimentally administered to animals (Yiannikouris and Jouany, 2002). Consequently, based on these data, only the occurrence of AFM1 (the "milk aflatoxin" from AFB1 metabolism) in milk is a matter of concern with regard to the transfer of mycotoxins in the dairy food chain. This is the reason why some regulatory limits have been set not only for AFM1 in milk but also for AFB1 in complete and complementary feeds for dairy animals by several countries as well as the European Union (see below). The EFSA opinion concluded that the current maximum levels of AFB1 in animal feeds (**Annex 1**) "*seem to successfully prevent undesirable concentrations of aflatoxin M1 in milk*" (EFSA, 2004).

Estimates of human exposure to mycotoxins have been made in a lot of countries: in the European Union, several SCOOP tasks identified the key food products contributing to the mycotoxin load in humans (Leblanc et al., 2005). In these studies, it was shown that food products deriving from animals contribute only a very small part of the total mycotoxin intake by humans in Europe.

#### 1.2.1.5. Toxicity of mycotoxins for farm animals

- General considerations

Historically, mycotoxins were "discovered" following a sudden and fatal outbreak which occurred in 1960 on turkey farms in Great Britain (Asao et al., 1963). This acute case led to the identification of aflatoxins and consecutively the relationship between moulds, their toxins and mycotoxicosis.
Similarly, many cases of nephropathy in pigs were reported a few years later in Denmark due to barley being naturally contaminated by OTA and have led to the identification and characterisation of chronic adverse effects in animals related to the contamination of their feed (Krogh et al., 1973). Other diseases associated with the ingestion of mycotoxin-contaminated feed by farm animals were observed and described a long time ago such as the common feed refusal when feed are contaminated by mycotoxins or the oestrogenic syndrome in pork; other syndromes such as the Equine LeucoEncephaloMalacie (ELEM) in horses due to fumonisins-contaminated-oats were only discovered in the late nineteen eighties (FAO, 2007; Gelderblom et al., 1988) (**Annex 2**). Nowadays acute mycotoxicosis episodes in livestock are very rare in Europe.

It is well known that mycotoxins can also cause chronic diseases in animals and can have different effects (hepatotoxicity, genotoxicity, nephrotoxicity, neurotoxicity, reprotoxicity, immunotoxicity, etc) (**Annexes 3 and 4**).

It should be noted that toxicity may vary considerably within a structural group of mycotoxins and that the danger may not always be due to the toxin itself but to its metabolites.

Chronic intoxication can adversely affect animal health, leading to problems with reproduction, increased susceptibility to infectious diseases, and also altered zootechnical performance. This latter parameter has been widely used as a bio- indicator to detect adverse effects of mycotoxins. Data on mycotoxin levels in mycotoxin contamination causing a reduction in zootechnical performance in farm animals (ruminants, pigs and poultry) were compiled in the AFSSA report (Afssa, 2009).

- Toxic effect of mycotoxins when present at concentrations below the regulatory level

The maximum levels of mycotoxins in the Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs and in the Commission Recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding (**Annexes 5 and 6**) were set to protect animal and human health. Therefore, anticipate logical conclusion is that the toxic effect of mycotoxins when present below those levels should be very low. Indeed very few studies have investigated the effect of mycotoxins when present at these levels. Some publications indicate that fumonisin, when present at the maximum level recommended by the EU, does not induce a toxic effect in duck, which is a very sensitive avian species (Tardieu et al., 2008; Tardieu et al., 2009).

To the best of our knowledge, there are no scientific papers describing toxic effects of mycotoxin when present at very low levels. Among the thousands of studies dealing with the effect of mycotoxins on farm animals, we should however mention two studies.

The first one indicates that feed contaminated with 1 mg/kg DON and 260 µg/kg ZEA, decreases total protein, albumin, and globulin in the sera of exposed piglets while at the same time increasing the serum enzyme activities of γ-glutamyltransferase (GGT), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). When the animals received this feed for 6 weeks, a modulation of the immune response was observed as measured by antibody titers specific for anticlassical swine (significant increase at day 14, significant decrease at day 28, no statistical difference at day 42). Some histopathological alterations were also observed in the liver, spleen, lymph nodes, uterus, and kidneys. The maximum levels of DON and ZEA recommended by the EU for feed for complementary and complete feedgstuffs for pigs are 900 µg/kg and 100 µg/kg, respectively (Chen et al., 2008).
The second study indicates that DON at 600 µg/kg in feed significantly increases the concentration of immunoglobulin A (IgA) in the serum of piglets (Drochner et al., 2004). The level of IgA in the serum of pig has been measured in several studies (Etienne and Wache, 2008) but most of them were using feed contaminated with higher levels of toxin (1 to 6.8 mg/kg). Some other studies have also demonstrated that plasmatic IgA concentrations remained unchanged when piglets were fed a diet contaminated with 4 mg/kg of DON for up to 12 weeks (Bergsjo et al., 1992; Dänicke et al., 2004).

- Toxic effect of mycotoxin combinations

As mentioned above, mycotoxins are often present simultaneously in raw material. Moreover complete feed is made of different raw materials. Thus, animals are generally not exposed to one mycotoxin but to several toxins at the same time. When mycotoxins are present simultaneously, interactive effects can be classified as additive, antagonistic or synergistic. Several *in vitro* studies have characterized the interaction between mycotoxins (Bernhoft et al., 2004; Tajima et al., 2002). The last CAST report reviews 33 studies on mycotoxin interactions in farm animals published in referred journals. The results of the review indicated that additive or antagonist effects were the predominant interactions observed (78%). It should be mentioned that many of these studies involved acute or subacute levels of mycotoxins. Moreover, the studies cited do not consider more subtle

changes such as for example those occurring within the immune system (CAST, 2003).

- Toxic effect of masked/bound mycotoxins

Mycotoxins may also occur in conjugated form, either soluble (masked mycotoxins) or incorporated into/associated with/attached to macromolecules (bound mycotoxins). These conjugated mycotoxins can emerge after metabolization by living plants, fungi and mammals or after food processing (Berthiller et al., 2009).

In general, masked mycotoxins show lower toxicity than the original, non-conjugated, more toxic parent compounds (Gareis, 1994). For example, thermal degradation of DON under alkaline conditions gives a mixture of compounds, which can be isolated such as norDON A, norDON B, and norDON C, 9-hydroxymethyl DON lactone, norDON D, norDON E, and norDON F. Cell culture experiments using IHKE cells showed that these compounds are less cytotoxic than DON (Bretz et al., 2006). Similarly, fumonisins are converted to their hydrolyzed analogues by alkaline cooking (nixtamalization) and hydrolyzed fumonisins are less toxic than the parental toxin (Howard et al., 2002; Voss et al., 2009). It should be pointed out that most of the comparative analyses between masked mycotoxins and the parental compound have been conducted *in vitro*. A few data are available using laboratory animals but more knowledge of the toxic effects of these derivates on farm animals is sorely needed.

Hydrolysis in the digestive tract of mammals may transform masked mycotoxin back to the original, non-conjugated, parent compounds. For example, zearalenone-4-beta-D-glucopyranoside is cleaved during digestion in the intestinal tract of swine, releasing the estrogenic aglycone (Gareis et al., 1990). When assessing the toxicity of masked mycotoxin it is important to determine the percentage of masked mycotoxin hydrolyzed in the intestinal tract. To the best of our knowledge, such data are lacking for most masked toxins.

### 1.2.2. Existing risk management measures

To avoid not only direct adverse effects, both acute and chronic, for livestock and their economic consequences, but also indirect effects due to the presence of toxin residues in animal products and their sanitary consequences for humans, several types of management measures have been developed such as regulations or recommendations to limit the mycotoxin content of feed, official monitoring plans to control the compliance of feed with regulations, development of standardised analytical methods to determine the mycotoxin content of a feed lot and decontamination processing of feed contaminated by mycotoxins when allowed.

#### 1.2.2.1. Analytical tools

Numerous analytical methods have been developed for analysing mycotoxins in feed and feedstuffs (cereals and composed feed) but only a few of them have been fully validated (intra- and inter-laboratory validation) and standardized in the European Union by CEN as the standardization body (http://www.cenorm.be). The CEN Technical Committee 275 (CEN/TC 275) has already standardized specific protocols for the analysis of mycotoxins in human food. Another CEN Committee, the CEN/TC 327, is specifically devoted to the standardization of validated methods in feeding-stuffs, including for mycotoxins: one protocol (EN ISO 17375:2006) has been standardized and published for the determination of AFB1 in feeding-stuffs; another method (ISO 14718:1998) which uses High-Performance Liquid Chromatography (HPLC) for the determination of AFB1 content in mixed feeding-stuffs is under approval.

Other standards are under development:

- Determination of DON in animal feed - HPLC method with immunoaffinity column clean-up (pr EN 15791:2009),
- Determination of ZEA in animal feed - HPLC method with fluorescence detection and immunoaffinity column clean-up (pr EN 15792:2009),
- Determination of the Sum of FB1 and FB2 in compound animal feed with immunoaffinity clean-up and RP-HPLC with fluorescence detection after pre- or post-column derivatisation (prEN 16006),
- Determination of OTA in animal feed by immunoaffinity column clean-up and HPLC with fluorescence detection (prEN 16007).

Standardized methods are the most accurate and reliable ones recommended for monitoring food and feed (including for raw materials) at industrial levels and for official controls. However, those protocols are time-consuming and costly. Rapid ELISA-type techniques can also be used, even though the analytical results may only be indicative (for a review of commercial or non-commercial rapid tests and kits see www.mycotoxins.org).

Certified reference materials are distributed by IRMM, (Geel, B, http://www.irmm.jrc.be) but only two of them are available for mycotoxins in feed (BCR 375 and 376 for Aflatoxins in compound feed (blank and high level). Others are available for cereals: Aflatoxins in peanut meals (BCR 262 to 264), DON in maize (BCR 377) and wheat (BCR 396) flours, OTA in wheat (BCR 471) and ZEA in maize (ERM-BC716 and 717).

As already mentioned, mycotoxins may also occur in a masked form. Awareness of such altered forms of mycotoxins is increasing, but reliable analytical methods, measurement standards and occurrence and toxicity data are still lacking (Berthiller et al., 2009).

More details are reported in **Annex 7**.

#### 1.2.2.2. European regulations and recommendations

The European Commission has introduced a Regulation for aflatoxins and recommendations concerning other key mycotoxins in animal feeds (**Annexes 5 and 6**) (Directive 2002/32/EC[4], Commission Directive 2003/100/EC[5], Commission Recommendation 2006/576/EC). Moreover, European or national regulations for animal-derived products have been imposed, especially for AFM1 in milk and OTA in pork kidneys (**Annex 6**) (Commission Regulation (EC) No 1881/2006), (FAO, 2003) which in particular implies a strict control of the AFB1 content in feed for dairy cattle and of OTA in feed for pigs.

#### 1.2.2.3. Measures for limiting mycotoxins in feed

Depending on the meteorological conditions of the year, cereals and by-products used by the feed industry may be contaminated above the regulated or recommended limits for mycotoxins. To avoid discarding too many provisions, decontamination of cereals in order to reduce the mycotoxin content or to counter their potential toxicity for animals may be tolerated. For example, a list of stategies or detoxification of mycotoxin in maize is provided in **Annex 8** (excerpt from FAO report, 1999).

[4] Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed

[5] Commission Directive 2003/100/EC of 31 October 2003 amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed

In the European Union, decontamination process involving any treatments but physical separation of moldy and contaminated grains from healthy ones are strictly forbidden for food intended for humans. However, cereals and cereal products for animal feeding may be treated for providing no harmful molecules for animals: for aflatoxins, the directive 2002/32/EC states that "*Proper handling and drying practices can keep the aflatoxin levels in the different feed materials low, and efficient decontamination procedures exist to reduce levels of the aflatoxin B1*". Indeed, several specific decontamination procedures for reducing aflatoxin content in feed have been tolerated by many countries including the European Union. For example, the ammoniation process is currently used at the industrial scale for detoxifying the carcinogen aflatoxins for peanut meals chiefly destined to dairy cattle. Briefly, the lactone ring of AFB1 is irreversibly hydrolyzed by the ammonia process and the subsequent loss of the cyclopentenone ring results in a non-toxic compound. This process originally developed and patented by Frayssinet and Lafarge was demonstrated to reduce AFM1 also in milk (Frayssinet and Lafarge, 1972; Fremy et al., 1988; Fremy and Quillardet, 1985; Hoogenboom et al., 2001) and consequently approved by several international bodies (FAO, Codex Alimentarius) and several countries including in the European Union and the United States of America. Recently, AFSSA also released an opinion concluding that formaldehyde-ammoniation process could safely be used to reduce aflatoxins content in animal feed (Afssa, 2007).

Nevertheless, it should be recalled that field and storage good practices as described by FAO or *Codex Alimentarius* should be first carefully followed to produce raw commodities safe to mycotoxin hazard. As pointed out above, it should also be stressed that mycotoxin exposition of farm animals to levels below the EU regulatory or recommended levels will not represent a significant health concern as far as the compliance of feed lots to regulatory or recommended limits for mycotoxins is respected.

**CONCLUDING REMARKS**

- Main mycotoxins occurring in feed and feedstuffs are AFB1, ZEA, DON, OTA, fumonisins, alcaloids of Ergot sclerotia and T2/HT2-toxins. Other mycotoxins of less concern are mycophenolic acid, cyclopiazonic acid, moniliformin, diacetoxyscirpenol, patulin and some other trichothecenes like NIV.

- Maximal limits have been set through a regulation specific to aflatoxins and through recommendations for some other major mycotoxins.

- Dietary exposure of farm animals to mycotoxins (and also to some of their metabolites) may result in many kinds of heavy acute or chronic health troubles. Human exposure through animal-derived products contaminated by mycotoxins is considered of low concern, except the well known AFM1 contamination of milk.

- Multiple contamination of same feed by several kinds of mycotoxins is commonly observed questioning the overall toxicity of a feed lot.

- Very few data are available on the effect of mycotoxins below the regulatory levels. It should be highlighted that "mycotoxin-detoxifying agents" are claimed to be used on feed lots contaminated below the regulatory levels.

- Synergistic effects of mycotoxins have been observed but data concern only acute toxicity.

- "Masked-mycotoxins" are an emerging concern but both analytical problems, and lack of *in vivo* toxicological data limit an accurate evaluation of their importance.

- In the EU, risk management in regards to the mycotoxin concern in feed is currently based on official regulation/recommendation, information and contribution of feed operators, validated and standardized analytical methods for mycotoxins, sampling and monitoring plans, tolerated mycotoxin-detoxification measures or protocols for feed.

# 2. DESCRIPTION OF SUBCATEGORIES OF MYCOTOXIN DETOXIFYING AGENTS

## 2.1. Definition of mycotoxin-detoxifying agents subcategories

The Commission regulation (EC) No 386/2009 of 12 May 2009[6] defines a new functional group of feed additives as "*substances for reduction of the contamination of feed by mycotoxins: 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 them or transforming them into less toxic metabolites.

Therefore, we can define at least two main categories:

- **Adsorbing agents**

One of the strategies for reducing the exposure to mycotoxins is to decrease their bioavailability by including various **mycotoxin adsorbing agents** in the compound feed, which leads to a reduction of mycotoxin uptake as well as distribution to the blood and target organs.

Adsorbing agents are also called binding agents, adsorbents, binders…

- **Biotransforming agents**

Another strategy is the degradation of mycotoxins into non-toxic metabolites by using **biotransforming agents** such as **bacteria/fungi** or **enzymes.**

Substances which do not directly interact with mycotoxins, i.e. antioxidant agents, immunostimulatory agents, have been reviewed but are not considered *sensu stricto* as mycotoxin-detoxifying agents. However, we are aware that such compounds may be very efficient for reducing the toxicity of mycotoxins.

## 2.2. Inventory of agents which may possibly reduce the impact of mycotoxins in feedstuffs: review of the literature

### 2.2.1. Mycotoxin-adsorbing agents

Mycotoxin-adsorbing agents are large molecular weight compounds that should be able to bind the mycotoxins in contaminated feed without dissociating in the gastrointestinal tract of the animal. In this way the toxin-adsorbing agent complex passes through the animal and is eliminated via the faeces. This prevents or minimizes exposure of animals to mycotoxins.

Mycotoxin-adsorbing agents can be silica-based inorganic compounds or carbon-based organic polymers. The inorganic adsorbing agents currently on the market include natural clay products as well as synthetic polymers. Based on a literature review (from the late 1990s to 2009), the inventory of mycotoxin-adsorbing agents and the mycotoxins they are targeting in feedingstuffs is summarized in **Table 1**.

[6] Amending Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the establishment of a new functional group of feed additives

#### 2.2.1.1. Aluminosilicates

Silicate minerals are the largest class of mycotoxin sequestering agents and most studies on the alleviation of mycotoxicosis by the use of adsorbing agents have focused on aluminosilicates.
Within this group, there are 2 important subclasses: the phyllosilicate subclass and the tectosilicate subclass. Phyllosilicates include bentonites, montmorillonites, smectites, kaolinites, illites. The tectosilicates include zeolites.

- Bentonites

Bentonites are originally created from the weathering of volcanic ash in situ (Ramos et al., 1996). 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).

- Montmorillonites

Montmorillonite is a layered silicate which adsorbs organic substances either on its external surfaces or within its interlaminar spaces (Ramos et al., 1996).

Modified montmorillonite nanocomposite (MMN) is a new sorptive additive. Developped with nano-modification techniques, MMN has a sizable surface area, higher porosity, and stronger cation exchange activities along with more active sites, which make its nanoparticle effect easy to exert, and as a result, its adsorption efficacy is greatly enhanced.

- Zeolite

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 $SiO_4$ and $AlO_4^-$ (Kabak et al., 2006; Ramos and Hernandez, 1997).

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).
Clinoptilolite is a natural zeolite whose main application is the adsorption of heavy metals from aqueous solutions (Kleiner et al., 2001).

- HSCAS (Hydrated sodium calcium aluminosilicate)

HSCAS is perhaps the most studied mycotoxin-sequestering agent among the mineral clays (Galvano et al., 2001);(Diaz and Smith, 2005; Kabak et al., 2006).

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).

#### 2.2.1.2. 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).

AC is known as one of the most effective and non-toxic 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 19$^{th}$ century (Huwig et al., 2001).

The sequestrant properties of AC depend on many factors including pore size, surface area, structure of the mycotoxin and doses. Superactivated 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 superactivated charcoals (Ramos et al., 1996).

#### 2.2.1.3. Yeast cell walls

Cell walls derived from the *Saccharomyces cerevisiae* yeast are also used as a dietary mycotoxin-adsorbing agent.

Yeast cell walls consist almost entirely of proteins and carbohydrates. The carbohydrate fraction is composed primarily of glucose, mannose, and N-acetyglucosamine. Glucans and mannans, the two main sugars, are found in about equal concentrations in *Saccharomyces cerevisiae*. Yeast mannan chains of various sizes are exposed on the external surface and are linked to cell wall proteins (Evans and Dawson, 2000). The cell walls harboring polysaccharides, proteins and lipids exhibit numerous different and easy 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).

#### 2.2.1.4. Micronized fibers

Micronized fibers can be obtained from different plant materials such as cereals (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).

#### 2.2.1.5. Bacteria

Lactic acid bacteria (LAB) are a group of gram-positive, acid-tolerant, generally non-sporulating bacteria that have common metabolic and physiological characteristics. These bacteria, usually found in decomposing plants and lactic products, produce lactic acid as the major metabolic end-product of carbohydrate fermentation. The main strains that comprise the LAB are *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*, and *Streptococcus* as well as the more peripheral *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Oenococcus*, *Sporolactobacillus*...

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 detoxify mycotoxins (El-Nezami et al., 1998).

#### 2.2.1.6. Polymers

- Cholestyramine

Cholestyramine is an insoluble, quaternary ammonium anion exchange resins which strongly binds anionic compounds (Underhill et al., 1995). It has been used as medicine in human for absorbing bile acids in the gastrointestinal tract in order to reduce cholesterol (Diaz and Smith, 2005).

- Polyvinylpyrrolidone

Polyvinylpyrrolidone is a highly polar amphoteric polymer (Celik et al., 2000).

### 2.2.2. Mycotoxin-biotransforming agents

Some studies have shown that some microorganisms have the ability to degrade mycotoxins. Biotransforming agents include bacteria, yeasts, fungi, and enzymes. The concept is for each mycotoxin, or class of mycotoxins, to use enzyme that specifically degrade the toxin into a non-toxic compound. Such enzymes have been described in bacteria, yeast or fungi. Purified enzymes have also been tested for this purpose. Based on a literature review, the inventory of mycotoxin biotransforming agents and the mycotoxins they are targeting in feedingstuffs is summarized in **Table 2**.

#### 2.2.2.1. Bacteria

- Gram-positive, anaerobic bacteria

The bacterial strain BBSH 797, a microbial feed additive was isolated from rumen fluid. It is a Gram-positive, non-spore-forming irregular rod living strictly anaerobic. It is 0.2±0.4 1±1.5 mm and occurs singly and in long chains up to 100 mm (Fuchs et al., 2002). Analysis of the 16s rRNA and the guanine/cytosine content together with the specialized nutritional demands and other physiological characteristics indicates a new species of the genus *Eubacterium* (Binder and Binder, 1998).

- Gram-positive, aerobic bacteria

*Nocardia asteroides* is a species of *Nocardia,* which is a genus of weakly-staining Gram-positive, catalase-positive, rod-shaped bacteria. It forms partially acid-fast beaded branching filaments. *Nocardia asteroides* are pathogenic: they can cause nocardiosis, a severe pulmonary infection in immunocompromised hosts. *Nocardia* are found worldwide in soil that is rich with organic matter (Wu et al., 2009).

*Corynebacterium* is a genus of gram-positive, catalase positive, non-spore-forming, non-motile, rod-shaped bacteria that are straight or slightly curved. Their size falls between 2-6 µm in length and 0.5 µm in diameter. They are characterised by high G:C content, with close philogenetic relationship to *Arthrobacter*, *Mycobacterium*, *Nocardia*, and *Streptomyces*.
They are widely distributed in nature and are mostly innocuous. Some are useful in industrial settings such as *C. glutamicum,* while others cause human disease such as *C. diphtheriae*, the pathogen responsible for diphtheria.
*Corynebacterium rubrum*, named in this way due to the brilliant red color of its colonies, is a nonacid-fast bacterium capable of producing large quantities of lipids (Wu et al., 2009).

*Mycobacteria* are aerobic and nonmotile bacteria that are characteristically acid-alcohol fast. Mycobacteria are usually classified as Gram-positive due to their lack of an outer cell membrane. *Mycobacterium* strain DSM 44556T was isolated with fluoranthene as the single carbon source from soil of a former coal gas plant, polluted with polycyclic aromatic hydrocarbons. The physiological properties, fatty acid pattern, and the 16S ribosomal RNA gene sequence indicated membership to the genus *Mycobacterium,* but were different from all type strains of *Mycobacterium* species. This strain represented a new species, for which the name *Mycobacterium fluoranthenivorans* sp. Nov was proposed (Wu et al., 2009).

*Rhodococcus erythropolis* is an aerobic, gram-positive, nonmotile, catalase-positive actinomycete that forms rods to extensively branched vegetative mycelium. It is an opportunistic pathogen in immunocompromised patients.

*Curtobacterium* sp. strain 114-2 belongs to the genus of *Curtobacterium*. They are bacteria of the order Actinomycetales. They are Gram-positive soil organisms (Ueno et al., 1983).

- Gram-negative, aerobic bacteria

*Flavobacterium aurantiacum* (NRRL B-184) is a species of *Flavobacterium* which is a genus of Gram-negative, non-motile, rod-shaped bacteria consisting of about ten recognized species. Flavobacteria are found in soil and fresh water in a variety of environments. Several species are known to cause disease in freshwater fish (Wu et al., 2009).

*Pseudomonas fluorescens* is a common Gram-negative, rod-shaped bacterium. It belongs to the *Pseudomonas* genus. *P. fluorescens* has multiple flagella. It has an extremely versatile metabolism, and can be found in the soil and in water. It is an obligate aerobe but certain strains are capable of using nitrate instead of oxygen as a final electron acceptor during cellular respiration (Megharaj et al., 1997).

*Alcaligenes* is a genus of Gram-negative, aerobic, rod-shaped bacteria. *Alcaligenes* species have been used for the industrial production of non-standard amino acids (Megharaj et al., 1997).

*Flavobacterium, Pseudomonas, Alcaligenes* and *Bacillus* can be used in a mixture.

#### 2.2.2.2. Fungi

- *Aspergillus*

*Aspergillus* species are highly aerobic and are found in almost all oxygen-rich environments, where they commonly grow as molds on the surface of a substrate, as a result of the high oxygen tension. Commonly, fungi grow on carbon-rich substrates such as monosaccharides and polysaccharides. *Aspergillus niger* is a fungus and one of the most common species of the genus *Aspergillus*. It is a common contaminant of food. It is ubiquitous in soil and is commonly reported from indoor environments.
*Aspergillus flavus* is a common mold in the environment and can cause storage problems in stored grains. *A. flavus* is particularly common on corn and peanuts, as well as water damaged carpets. It can also be a human pathogen, associated with aspergillosis of the lungs and sometimes causing corneal, otomycotic, and nasoorbital infections. Many strains produce significant quantities of aflatoxin. *A. flavus* spores are allergenic (Nakazato et al., 1990).
*Aspergillus candidus* belongs to the genus Aspergillus and can be found in warm soils, grain and in the secondary decay of vegetation (Wu et al., 2009).
*Aspergillus parasiticus* is a mold known to produce aflatoxin and sometimes found on black olives.

- *Eurotium herbariorum*

Eurotium has a moderately rapid growth rate with downy to powdery colonies. The genus Eurotium is generally found in tropical and subtropical zones. Eurotium is a xerophilic fungus and is isolated primarily from soil, plants, stored grains, and house dust (Nakazato et al., 1990).

- *Rhizopus sp*.

Rhizopus is a genus of molds that includes cosmopolitan filamentous fungi found in soil, decaying fruit and vegetables, animal faeces, and old bread.
Rhizopus grows well on general fungal media. Rhizopus species may overgrow and inhibit other fungi. Some structures are visible to the naked eye, i.e. sporangia appear macroscopically as black dots in the midst of white, cottony mycelia (Nakazato et al., 1990).

- *Penicillium raistricki*

*Penicillium raistricki* belongs to *Penicillium* which is a genus of ascomycetous fungi of major importance in the environment, food and drug production. The mycelium typically consists of a highly branched network of multinucleate, septate, usually colorless hyphae (Wu et al., 2009).

- *Rhinocladiella atrovirens*

Colonies are restricted, velvety or lanose, often slightly mucoid at the centre. Conidiogenous cells are cylindrical, intercalary or free, 9-19 x 1.6-2.2 µm; denticulate rachis up to 15 µm long, with crowded, flat or butt-shaped, unpigmented conidial denticles.
*Rhinocladiella* contains 6-8 species, with two species of medical interest; *R. atrovirens* and *R. aquaspersa* (Blackwell et al., 1999).

#### 2.2.2.3. Yeast

- *Trichosporon mycotoxinivorans*

*Trichosporon mycotoxinivorans* belongs to the genus *Trichosporon,* which is characterized by the production of arthroconidia. 35 species have been described in the genus until now. This genus is monophyletic, on the basis of 18S and 26S rDNA sequences. *T. mycotoxinivorans* is a yeast strain isolated from the hindgut of the lower termite *Mastotermes darwiniensis* (Mastotermitidae). The name of this recently isolated strain refers to an important characteristic of *T. mycotoxinivorans* to detoxify mycotoxins such as OTA and ZEA (Molnar et al., 2004).

- *Phaffia rhodozyma* and *Xanthophyllomyces dendrorhous* isolates

*Phaffia rhodozyma* was isolated in the 1960s. At least two species appear to exist, including the anamorph *Phaffia rhodozyma* and the teleomorph *Xanthophyllomyces dendrorhous*. The yeast has attracted considerable biotechnological interest because of its ability to synthesize the carotenoid astaxanthin as its major pigment. This property has stimulated research on the biology of the yeast as well as development of the yeast as an industrial microorganism for astaxanthin production by fermentation (Peteri et al., 2007).

#### 2.2.2.4. Enzymes

- Protease A

Proteases are enzymes which break down proteins (proteolysis), by hydrolysis of the peptide bonds linking amino acids together in the polypeptide chain. Proteases work best in acidic conditions.
Protease A is obtained through fermentation processes from selected *Aspergillus niger* strains (Abrunhosa et al., 2006).

- Pancreatin

Pancreatin is a mixture of several pancreatic enzymes produced by the exocrine cells of the pancreas. It is composed of trypsin, amylase, lipase and protease (Abrunhosa et al., 2006).

- Carboxypeptidase A

Carboxypeptidase A usually refers to the pancreatic exopeptidase which hydrolyzes peptide bonds of C-terminal residues with aromatic or aliphatic side chains. Most scientists in the field now refer to this enzyme as *CPA1* (Schatzmayr et al., 2006).

- Epoxidase

Epoxidases are enzymes which are able to biotransform epoxide groups into diene groups (Schatzmayr et al., 2006).

- Lactonohydrolase

Lactonohydrolases are enzymes which catalyse the hydrolysis of lactone rings (intramolecular cyclic esters) to produce a hydroxyl group and a carboxyl group (Takahashi-Ando et al., 2002).

**Table 1: Review of adsorbing agents based on available literature**

| Categories of adsorbing agents | Product | Company | Physico-chemical properties | Targeted mycotoxin | Studies |
|---|---|---|---|---|---|
| ***Aluminosilicates*** | | | | | |
| Bentonite | Astra Ben 20® (sodium bentonite) | Prince Agri-products | | AFB1, AFM1 | (Diaz et al., 2004) |
| | Red Crown® (calcium bentonite) | Prince Agri-products | | AFB1, AFM1 | (Diaz et al., 2004) |
| | Flow Guard® (sodium bentonite) | Laporte Biochem, Inc. | | AFB1, AFM1 | (Diaz et al., 2004) |
| | Microsorb® (sodium bentonite) | American Colloid Co. | • White colour<br>• Not soluble in water | AFB1, AFM1 | (Diaz et al., 2004) |
| | Volclay FD-181 (sodium bentonite) | Volclay International Pty Ltd | • Powder<br>• pH: 8.0 - 10.5 at 6% solids<br>• Chemical composition: 63.02% $SiO_2$, 21.08% $Al_2O_3$, 3.25% $Fe_2O_3$, 0.35% FeO, 2.67% MgO, 2.57% $Na_2O$, 0.65% CaO | AFB1 | (Ellis et al., 2000), (Schell et al., 1993), (Marroquin-Cardona et al., 2009) |
| | Sodium bentonite | - | • Mean particle size: 53 µm<br>• Chemical composition: 54.91% SiO2, 21.41% Al2O3, traces of Fe2O3, 0.01% MnO, 0.1% TiO2, traces of CaO, 2.81% MgO, 1.70% Na2O, 0.16% K2O, traces of SO3, 0.05% P2O5, 5.59% H2O<br>• pH: 8.5 | AFB1, FB1 | (Miazzo et al., 2005), (Eraslan et al., 2004), (Rosa et al., 2001) |
| | ATOX® (Natural combination of smectite and sepiolite (E-558 and E-562) of high purity) | Tolsa | • Light cream colour<br>• Fluid powder | | |

| Categories of adsorbing agents | Product | Company | Physico-chemical properties | Targeted mycotoxin | Studies |
|---|---|---|---|---|---|
| | Bentonite | Sigma chemical | | FB1 | (Ramos et al., 1996), (Solfrizzo et al., 2000) |
| Montmorillonite | Sodium calcium montmorillonite | Engelhard Chemical corp. | | AFB1, ZEA | (Desheng et al., 2005), (Lemke et al., 1998) |
| | Organophil modified montmorillonite | Sud-Chemie | • Chemical composition: 54.8% SiO2, 15.6% Al2O3, 4.2% Fe2O3, 2.0% CaO, 3.5% MgO, 1.5% K2O, 3.5% Na2O | DON, ZEA | (Döll et al., 2005) |
| | Montmorillonite | Aldrich-Chemie | • Powder<br>• Surface area: 20-40 m²/g<br>• Bulk density: 800-850 g/l<br>• Average particle size < 1µm | Aflatoxin | (Ramos and Hernandez, 1996) |
| | Modified montmorillonite nanocomposite | Feed science institute, China | • Particle size: 10-60 nm | Aflatoxin | (Shi et al., 2006) |
| | Milbond-TX®: inert montmorillonite clay-based adsorbing agent | Milwhite Inc | • Chemical composition: 54.6-65.6% SiO2, 14.5-19.7% Al2O3, 4.05-5.02% Fe2O3, 0.64-0.97% CaO, 0.94-2.08% MgO, 0.6-1.19% K2O, 0.54-1.37%Na2O, 0.63-0.77% TiO2 | AFB1 | (Marroquin-Cardona et al., 2009) |
| | Swy-2: wyoming sodium montmorillonite | Source Clay Repository of the Clay Minerals Society | • Average particle size < 2µm | | (Wiles et al., 2004) |
| Zeolites | Octadecyldimethyl benzy ammonium exchanged-clinoptilolite-heulandite tuff | | | OTA<br>NIV, DAS, T-2 toxin, ZEA<br>AFB1 | (Dakovic et al., 2003)<br>(Curtui, 2000)<br>(Tomasevic-Canovic et al., 2002) |

| Categories of adsorbing agents | Product | Company | Physico-chemical properties | Targeted mycotoxin | Studies |
|---|---|---|---|---|---|
| | Clinoptilolite | Engelhard Chemical corporation | • Average diameter of particles: 2.68 µM<br>• Specific surface: 1.35 m²/g | AFB1 (+ other aflatoxins) | (Kleiner et al., 2001), (Kyriakys et al., 2002), (Mayura et al., 1998), (Oguz and Kurtoglu, 2000), (Oguz et al., 2000), (Ortatatli and Oguz, 2001), (Ortatatli et al., 2005) |
| | Calcium/potassium/sodium hydrated aluminosilicate | Silver and Baryte Ores Mining Co. | • Size < 1 mm<br>• Chemical composition: 68.26% SiO2, 13.30% Al2O3, 0.08% Fe2O3, 4.34% CaO, 1.05% MgO, 0.94% K2O, 0.26 Na2O, 11.6% L.O.I | | (Papaioannou et al., 2002) |
| HSCAS | NovaSil™ | Engelhard Chemical corporation | • Off-white- tan colour<br>• Powder | AFB1, AFM1 | (Galvano et al., 1996), (Harvey et al., 1994), (Jaynes et al., 2007), (Lemke et al., 2001), (Moschini et al., 2008), (Nageswara and Chopra, 2001), (Pimpukdee et al., 2004), (Afriyie-Gyawu et al., 2008), (Wang et al., 2008), (Wiles et al., 2004) |
| | Myco-Ad®<br>Zeolex® | Special Nutrients | • Cream-coloured<br>• Fine powder | T-2 toxin | (Diaz et al., 2005) |
| ***Activated carbon*** | Activated carbon | Sigma F.I.S. | Very porous non-soluble powder with a high surface to mass ratio | ZEA, FB1, FB2, OTA, DON, AFB1, AFM1 | (Avantaggiato et al., 2003), (Avantaggiato et al., 2007), (Galvano et al., 1996), (Galvano et al., 1998), (Nageswara and Chopra, 2001) |
| | Filtrasorb 400 | Calgon carbon corporation | • High specific area<br>• Slightly alkaline character<br>• Granular form<br>• High density<br>• Average size : 0.55-00.75 mm | Aflatoxin | (Di Natale et al., 2009) |

| Categories of adsorbing agents | Product | Company | Physico-chemical properties | Targeted mycotoxin | Studies |
|---|---|---|---|---|---|
| | Aquacarb™ 207EA | Waterlink Sutcliffe carbon | • High specific area<br>• Slightly alkaline character<br>• pH 7-8<br>• Surface Area : 950-1100 m2g-1 | Aflatoxin | (Di Natale et al., 2009) |
| | GCN 1240 | Norit | • High specific area<br>• Slightly alkaline character | Aflatoxin | (Di Natale et al., 2009) |
| | Nuchar® SA-20 | Westvaco | • Powder<br>• pH 4-6<br>• Surface area : 1400-1800 m²/g | AFB1, AFM1 | (Diaz et al., 2004) |
| | Darco KB-B | Aldrich Chemical Co. | • 100 – 325 mesh<br>• Commercial powder form<br>• Diameter < 45 mm | AFB1, OTA | (Lemke et al., 2001), (Rotter et al., 1989) |
| | Superactivated charcoal | Requa, Inc. | • Surface area : 2000 m²/g<br>• Granulated form | Aflatoxin, T-2 toxin | (Edrington, 1997) |
| | Activated carbon | Carlo Erba | | FB1, FB2 | (Solfrizzo et al., 2000) |
| | SORBOPOR MV 125 | Camel Environment S.r.L | • Origin : various wood sources<br>• Surface area : 1116 m²/g | FB1 | (Piva et al., 2005) |
| ***Yeast cell wall*** | Yeast cell wall | Alltech Lesaffre Group | | Aflatoxin, OTA, ZEA, T-2 toxin | (Aoudia et al., 2008), (Aravind et al., 2003), (Oguz et al., 2001), (Santin et al., 2003), (Yiannikouris et al., 2003), (Yiannikouris et al., 2004) |
| | MTB-100® (polymeric glucomannan adsorbing agent extracted from the cell wall of yeast) | Alltech | • Light brown colour<br>• Slightly soluble in water | OTA, FB1, Monilifor min, ZEA, AFB1, AFM1, T-2 toxin, DAS, fusaric acid | (Bursian, 2004), (Chowdhury and Smith, 2005), (Diaz et al., 2004), (Kogan and Kocher, 2007), (Meissonnier et al., 2009), (Swamy et al., 2002), (Swamy et al., 2002), (Yegani et al., 2006) |

| Categories of adsorbing agents | Product | Company | Physico-chemical properties | Targeted mycotoxin | Studies |
|---|---|---|---|---|---|
| | Mycosorb™ (mycotoxin adsorbing agent based on yeast glucan): polymeric glucomannan | Alltech | • Fine pale brown powder<br>• Insoluble in water | FB1, ZEA, DON, NIV, T-2 toxin, Aflatoxin | (Avantaggiato et al., 2005), (Diaz et al., 2005), (Diaz-Llano and Smith, 2006), (Dvorska, 2003), (Dvorska, 2007), (Karaman et al., 2005), (Moschini et al., 2008), (Smith et al., 2008), (Swamy et al., 2003) , (Swamy et al., 2004), (Volkl and Karlovsky, 1998) |
| | Esterified glucomannan (product name not specified) | Alltech | | AFB1, OTA, T-2 toxin | (Raju and Devegowda, 2000) |
| | EX16 (vinasse containing 16% liquid yeast cell walls) | Lesaffre (Bio-Springer) | | OTA | (Ringot et al., 2005), (Ringot et al., 2007) |
| | BETA (dried purified beta-glucans fraction of cell walls) | Lesaffre (Bio-Springer) | | OTA | (Ringot et al., 2005), (Ringot et al., 2007) |
| | LEC (dry yeast cell wall fraction) | Lesaffre (Bio-Springer) | | OTA | (Ringot et al., 2005), (Ringot et al., 2007) |
| ***Bacteria*** | *Lactobacillus rhamnosus strain GG*<br>*Lactobacillus helveticus 46 and 72*<br>*Lactobacillus jugurti 63*<br>*Lactobacillus lactis 170*<br>*Lactobacillus casei spp. Casei C3*<br>*Streptococcus thermophilus NG40Z and C5*<br>*Lactobacillus paraplantarum* | Promochem INRA Thivernal-Grignon | | DON, FB1, FB2, ZEA | (Niderkorn et al., 2006), (Niderkorn et al., 2007), (Niderkorn et al., 2008), (Niderkorn et al., 2009) |
| | *Lactobacillus rhamnosus strain GG*<br>*Lactobacillus rhamnosus strain LC-705* | Valio Ltd. | | AFB1, ZEA, | (El-Nezami et al., 1998), (El-Nezami et al., 2002), , (Gratz et al., 2007), (Haskard et al., 2000), (Haskard et al., 2001) |
| | *B. longum*<br>*L. acidophilus*<br>*S. typhimurium* | | | AFB1 | Bolognani 1997 |

| Categories of adsorbing agents | Product | Company | Physico-chemical properties | Targeted mycotoxin | Studies |
|---|---|---|---|---|---|
| ***Micronized fibers*** | ADFIMAX® | REALDYME | Ultrafine fraction < 100µ | OTA | Tangni 2003, (Tangni et al., 2005), (Aoudia et al., 2008), (Aoudia et al., 2009) |
| ***Polymers*** | Cholestyramine | Bristol-Myers<br><br>Sigma Chemical | | ZEA<br>FB1, FB2<br>OTA | (Avantaggiato et al., 2003), (Kerkadi et al., 1998), (Madhyastha et al., 1992), (Ramos et al., 1996), (Underhill et al., 1995), (Solfrizzo et al., 2000) |
| | Antitox Vana (Polyvinylpolypyrrodilone) | Qualitech Products Inc. | | DON | (Friend, 1984) |
| | Polyvinylpolypyrrodilone | Sigma Chemical | • Physical State: Solid<br>• Colour: Very faintly beige<br>• Form: Powder | Aflatoxin, ZEA | (Ramos et al., 1996), (Celik et al., 2000) |
| Ocra-Tox | Additive resulting from the modification and activation of diatomaceous earth, which is a natural material extracted from a quarry with a maximum of 70% silicon dioxide | - | | OTA | (Denli et al., 2008) |

**Table 2: Review of biotransforming agents based on available literature**

| Categories of biotransforming agents | Description | Company / lab | Origin | Targeted mycotoxin | Studies |
|---|---|---|---|---|---|
| **Bacteria** | Anaerobic bacteria *Eubacterium* s.p. BBSH 797 | Biomin | Rumen fluid | T-2 toxin, HT-2 toxin, T-2 tetraol, T-2 triol, scirpentriol | (Fuchs et al., 2002) |
| | *Nocardia asteroides Mycobacterium fluoranthenivorans* sp. nov. *Rhodococcus erythropolis* | | | AFB1 | (Wu et al., 2009) |
| | Mixed culture (*Alcaligenes*, *Bacillus*, *Achromobacter*, *Flavobacterium*, and *Pseudomonas* | | | ZEA | (Megharaj et al., 1997) |
| | *Curtobacterium* sp. strain 114-2 | | | T-2 toxin | (Ueno et al., 1983) |
| **Fungi** | *Aspergillus niger*, *Eurotium herbariorum*, *Rhizopus sp*., and non-aflatoxin (AF)-producing *A. flavus* | | | AFB1, Aflatoxiol | (Nakazato et al., 1990) |
| | *A. parasiticus* NRRL 2999 and NRRL 3000 | | | AFB1 | (Wu et al., 2009) |
| **Yeast** | *Trichosporon mycotoxinivorans* | | | OTA, ZEA, DON | (Molnar et al., 2004), (Schatzmayr et al., 2006) |
| | Phaffia rhodozyma and Xanthophyllomyces dendrorhous isolates | | | OTA | (Peteri et al., 2007) |
| | Mycotox® (Oxicinol, tymol, micronized yeast) | | | Aflatoxin | (Sehu et al., 2005) |
| | Mycofix® Plus (Toxin deactivator containing the yeast *Trichosporon mycotoxinivorans* and showing adsorbing properties, upgraded by the addition of epoxidase and lactonase activities) | Biomin | | FB1, ZEA, DON, NIV, DAS, T-2 toxin, OTA | (Avantaggiato et al., 2005), (Dänicke et al., 2002), (Dänicke et al., 2002), (Dänicke, 2002), (Dänicke et al., 2003), (Diaz et al., 2002), (Diaz et al., 2005), (Hanif et al., 2008), (Politis et al., 2005) |
| **Bacteria + yeast** | Combination of *Eubacterium* BBSH 797 and *Trichosporon mycotoxinivorans* | Biomin | | OTA, ZEA | (Hofstetter et al., 2006) |
| **Enzymes** | Protease A | Amano Inc. | *Aspergillus Niger* | OTA | (Abrunhosa et al., 2006) |
| | Pancreatin | Biocatalysts | Porcine pancreas | OTA | (Abrunhosa et al., 2006) |
| | Epoxidase from *Eubacterium* BBSH 797 | | | ZEA, OTA, DON | (Schatzmayr et al., 2006) |
| | Aflatoxin-detoxifizyme (ADTZ) | | *Armillariella tabescens* | AFB1 | (Liu et al., 2001) |
| | Lactonohydrolase | | *Clonostachys rosea* IFO 7063 | ZEA | (Takahashi-Ando et al., 2002) |

We could notice that the literature is more abundant when dealing with adsorbing agents that when dealing with biotransforming agents. Most of adsorbing agents, especially aluminosilicates, have been tested for their ability to bind aflatoxins. The mycotoxins targeted by yeast cell wall, bacteria and other adsorbing agents are more diverse. Mycotoxin-biotransforming agents, as anticipated by their mode of action, show narrower spectrum in term of targeted mycotoxins.

## 2.3. Comprehensive list of mycotoxin-detoxifying agents (marketed or on way of development), indications for use and composition: request from companies

### 2.3.1. Request from companies

After an in-depth review of the available literature to gather information on the different categories of mycotoxin-adsorbing or biotransforming agents, it was important to draw up a comprehensive list of agents that are currently being developed or already available on the market for other purposes or in other countries. This was done by contacting the companies manufacturing or using these types of products.

A list of companies involved in animal feed had previously been made along with the details of who to contact. There were about 60 companies likely to be concerned by our review (**Annex 9**).

As mentioned in the answer to the call from EFSA, two questionnaires were prepared to be sent to the companies listed (**Annex 10**).
The first questionnaire was addressed to manufacturers of mycotoxin-detoxifying agents and raised several questions about the nature of the agents developed, the authorisation to use these products, their efficacy, and the performing of *in vitro* and *in vivo* tests, etc.
The second one was aimed at users of mycotoxin-detoxifying agents. The questions dealt with the conditions under which their products were used, their interest and the adverse effects they may have observed.

The questionnaires were sent mid-July to the different companies listed, along with a covering letter explaining the background to the project and its objectives. The companies were asked to answer by the end of August, in order to leave time for us to collect and compile the information provided.

### 2.3.2. Answers collected from companies

We have received feedback from 13 companies, which means a response rate of about 20%.
Some companies contacted us and asked questions about the project, in particular about confidentiality issues and respect for their ownership rights to their data.
Ten companies completed questionnaire 1, giving more or less detailed answers. Two companies were not concerned by the questionnaires, as they were neither producers nor users of mycotoxin-detoxifying agents. Two companies asked questions but have not yet sent back the completed questionnaires. One company completed questionnaire 2 and gave information about the use of a product combining bentonite and yeast cell wall.
This low response rate can be explained by the fact that the deadlines for companies to answer our questionnaires were too short and fell in the summer vacation period. Indeed, questionnaires were sent mid-July and companies were asked to reply by the end of August. Because of the short deadline of the call, we could not contact the companies again.

As the companies did not participate as expected, it is not sure that the table represents an exhaustive list of the main products that have the potential to be used in Europe as mycotoxin-detoxifying agents in contaminated feed.

It should be noted that most of the companies which answered are aware that products belonging to the functional class of detoxifying agents are yet not registered in Europe.

## 2.4. Collection of information on mycotoxin-detoxifying agents which has been published on the Internet

In parallel with the collection of information from industrial stakeholders, another task consisted in searching on the Internet for information related to mycotoxin-detoxifying agents.
This was done by visiting the website of each company listed and searching for information on mycotoxin-detoxifying agents.

The name of the product and its description are given in **Table 3**. The products were classified into 2 categories: adsorbing agents or biotransforming agents. All information related to the physical (appearance for example) and chemical (chemical composition) properties were retrieved. The table also mentions information about the marketing and regulation of these agents. *In vitro* and/or *in vivo* studies, as well as targeted mycotoxins, are also mentioned.

This table includes about 35 products and some of them are clearly described as mycotoxin-detoxifying agents on the companies' websites.

This review was performed from July to October 2009 based on available information, the table is therefore not considered as exhaustive.

**Table 3: Table gathering information on different mycotoxin-detoxifying agents (Internet Research)**

| Product name | Company | Category<br><br>Description | Physico-chemical properties | Comments | Studies Targeted mycotoxins |
|---|---|---|---|---|---|
| ***ToxTrap***™ | ABAC<br><br>www.abac.ch | Adsorbing agent<br><br>Binder based on plant fibre | • Powder | - | -<br>Aflatoxins, ZEA, T-2 toxin |
| ***SORBATOX*** | Kiotechagil<br><br>www.agil.com | Adsorbing agent<br><br>Complexed hydrated aluminium silicate, with kaolinite, feldspartz, quartz, carbonaceous material | • White powder<br>• Acid pH (4.9-5.4)<br>• Miscible with water<br>• Melting point > 1200°C | Hydrated aluminium silicate: E559 | -<br>Primarily designed to bind aflatoxins Excellent activity against *Fusarium* toxins such as T2, DON or ZEA |
| ***Agrabond*** | Agranco Corp<br><br>www.agranco.com | Adsorbing agent<br><br>Calcium and sodium based alumino-silicate | • Chemical composition: 63.3% SiO2, 21.4% Al2O3, 3.8% Fe2O3, 0.3% K2O, 0.2% MgO, 0.7% CaO, 2.7% NaO<br>• Particle size: 2.2 µm<br>• Powder<br>• Light grey<br>• pH: 9.0 | - | -<br><br>Aflatoxin and ZEA |
| ***EMBI-100*** | Agri-growth International Inc.<br><br>www.agriorganics.com | Adsorbing agent<br><br>Contains natural montmorillonite, humidified vegetable carbons, mannan and fructose oligosaccharides | - | Status ingredients: GRAS status, comply with the American Association of Feed Control Officials | -<br>Aflatoxin |
| ***FLO-BOND*** | Agri-Tec<br><br>www.agritectx.com | Adsorbing agent<br><br>Hydrated Sodium Calcium Aluminosilicate | • Buff-coloured<br>• pH 7.4<br>• Chemical composition: 69.10% SiO2, 18.9% Al2O3, 5.8% Fe2O3, 2.90% MgO, 1.0% K2O, 1.2% CaO, 0.70% TiO2, 0.5% NaO, 5.70% H2O | GRAS listed | Lab and field studies (Avantaggiato et al., 2005)<br><br>AFB1, T-2 toxin, DON, OTA, FB1, ZEA |
| ***FLO-BOND PLUS*** | Agri-Tec<br><br>www.agritectx.com | Adsorbing agent<br><br>FLO-BOND + buffered propionic acid | • Buff-coloured<br>• pH 6.5<br>• Chemical composition: 62.80% SiO2, 17.2% Al2O3, 5.3% Fe2O3, 2.60% MgO, 0.90% K2O, | GRAS listed | Lab and field studies<br><br>AFB1, T-2 toxin, DON, OTA, FB1, ZEA |

| Product name | Company | Category<br><br>Description | Physico-chemical properties | Comments | Studies Targeted mycotoxins |
|---|---|---|---|---|---|
| | | | 1% CaO, 0.60% TiO2, 0.4% NaO, 5.70% H2O | | |
| ***Agrotox*** | Agromed<br><br>www.agromed.at | Adsorbing agent<br><br>Binds mycotoxins through certain minerals and components of yeast | • Crystalline structure | | ZEA<br>DON |
| ***MTB-100®*** | Alltech<br><br>www.alltech.com | Adsorbing agent<br><br>Non-viable dried yeast of *Saccharomyces cerevisiae* | • Light-brown powder<br>• Partially soluble in water | - | (Bursian, 2004), (Chowdhury and Smith, 2005), (Diaz et al., 2004), (Kogan and Kocher, 2007), (Meissonnier et al., 2009), (Swamy et al., 2002), (Swamy et al., 2002) , (Yegani et al., 2006) |
| ***Mycosorb®*** | Alltech<br><br>www.alltech.com | Adsorbing agent<br><br>Composed of glucomannan molecules extracted from the yeast cell wall of *Saccharomyces cerevisiae,* sodium calcium aluminosilicate and calcium carbonate | • Fine pale brown powder<br>• Insoluble in water | Available in Europe, Latin America and Asia Pacific. Not available in the USA or Canada. | (Avantaggiato et al., 2005), (Diaz et al., 2005), (Diaz-Llano and Smith, 2006), (Dvorska, 2003), (Dvorska, 2007), (Karaman et al., 2005), (Moschini et al., 2008), (Smith et al., 2008), (Swamy et al., 2003), (Swamy et al., 2004), (Volkl and Karlovsky, 1998) |
| ***Azomite®*** | Azomite Mineral Products, Inc<br><br>www.azomite.com | Adsorbing agent<br><br>Hydrated Sodium Calcium Aluminosilicate | • Melting point > 1000°C<br>• Appearance: tan to pink<br>• Solubility in water < 1%<br>• Unstable in acid | HSCAS listed in the U.S. Code of Federal Regulations (21 CFR 582.2729) as an anti-caking agent, and generally recognized as safe (GRAS) by the FDA | - |
| ***Mycofix® Plus*** | Biomin<br><br>www.biomin.net | Adsorbing and biotransforming agent | - | Mycofix is not available in the US and Canada | (Avantaggiato et al., 2005), (Dänicke et al., |

| Product name | Company | Category<br><br>Description | Physico-chemical properties | Comments | Studies Targeted mycotoxins |
|---|---|---|---|---|---|
| | | Composed of a mix of algae and plant extracts and specific enzymes | | | 2002); (Dänicke 2002b, Dänicke 2002a, Dänicke 2003)<br><br>Aflatoxin, Fumonisin, OTA, DON, T-2 toxin, ZEA |
| ***Mycosil*** | Dresen<br><br>www.dresen.com.mx | Adsorbing agent<br><br>Hydrated sodium and calcium aluminosilicate | • Colour: green<br>• Chemical composition: aluminium, silicon, sodium, calcium, potassium, iron oxides | Use doses:<br>2.0 Kg/MT preventive<br>4.0 Kg/MT corrective | (Marroquin-Cardona et al., 2009)<br><br>AFB1 |
| ***Elitox®*** | Impextraco<br>www.impextraco.com | Adsorbing and biotransforming agent<br><br>Synergistic combination of toxin inactivating enzymes, toxin binding silicates and biopolymers | - | - | - |
| ***Moldstop® Myco Plus*** | Impextraco<br><br>www.impextraco.com | Adsorbing agent<br><br>Selected mineral carriers with mycotoxin binding activity | - | - | - |
| ***Ecocell®*** | Impextraco<br><br>www.impextraco.com | Adsorbing agent<br><br>Balanced prebiotic based on mannanoligosaccharides and β-glucans extracted from purified yeast cell walls (S. cerevisiae) | - | - | - |
| ***Toxfin® Brand Toxin binder*** | Kemin Europa<br><br>www.kemin.com | Adsorbing agent<br><br>Formulation of several activated clays | - | Available in all geographies except the United States | *In vivo* and *in vitro* trials |
| ***LUCTMOLD*** | Lucta | Preservative used to prevent mycotoxin | - | - | - |

| Product name | Company | Category<br><br>Description | Physico-chemical properties | Comments | Studies Targeted mycotoxins |
|---|---|---|---|---|---|
| | www.lucta.com | contamination | | | |
| ***Mexsil®*** | Mexsil<br><br>www.mexsil.com | Adsorbing agent | • Powder | - | AFB1 |
| ***CAPTURA ™ AF*** | Novus<br><br>www.novusint.com | Adsorbing agent<br><br>Free flowing mycotoxin binder<br>Composition: bentonite montmorillonite, clinoptilolite, propionic acid, ammonium propionate, silica | • White powder | Bentonite montmorillonite: E558<br>Clinoptilolite: E567<br>Propionic acid: E280<br>Ammonium propionate: E284<br>Silica: E551a | -<br><br>Aflatoxins |
| ***Duotek®*** | Nutek | Adsorbing agent<br><br>Organo-aluminosilicate | • Chemical composition: 4.3% $K_2O$, 1.8% $Na_2O$, 5.2% CaO | | Aflatoxin, ZEA |
| ***Zeotek®*** | Nutek<br><br>www.grupoidisa.com.mx | Adsorbing agent<br><br>Adsorbing organoaluminosilicate | • Chemical composition: 45-50% $SiO_2$, 13-15% $Al_2O_3$, 1-4% $Fe_2O_3$, 1-2.6% MgO, 0.1-0.4% CaO, 0.01-0.5% $Na_2O$, 0.03-0.3% $K_2O$ | | Designed against ZEA, T2-toxin, OTA, cyclopiazonic acid, FB1, Aflatoxins |
| ***Calibrin-A*** | Oil-Dri<br><br>www.oildri.com | Adsorbing agent<br><br>Highly-refined montmorillonite sorbent mineral | - | This Product is not for sale in the USA or Canada | *In vitro* and *in vivo* testings<br><br>Aflatoxin |
| ***Calibrin-Z*** | Oil-Dri<br><br>www.oildri.com | Adsorbing agent<br><br>Highly-refined montmorillonite sorbent mineral | - | This Product is not for sale in the USA or Canada | *In vitro* and *in vivo* testings<br><br>Zearalenone |
| ***Amadeite®*** | Olmix<br><br>www.olmix.com | Adsorbing agent<br><br>Hybrid composite material (nanoclay) combining activated montmorillonite and seaweed extracts | - | - | - |
| ***Mycobond*** | Optivite<br><br>www.optivite.co.uk | Adsorbing agent<br><br>Silicon and aluminium in their | - | - | - |

| Product name | Company | Category<br><br>Description | Physico-chemical properties | Comments | Studies Targeted mycotoxins |
|---|---|---|---|---|---|
| | | oxide forms | | | |
| ***Zetox*** | Optivite<br><br>www.optivite.co.uk | Adsorbing agent<br><br>Combination of Moldgard (for mould and yeast control) and Mycobond (for mycotoxin binding capabilities) | - | - | - |
| ***ADFIMAX*** | Realdyme<br><br>www.realdyme.com | Adsorbing agent<br><br>Binder based on plant fibers | Powder | | Tangni 2003, Tangni 2005, Aoudia 2008, Aoudia 2009 |
| ***Myco-Ad®*** | Special Nutrients<br><br>www.specialnutrients.com | Adsorbing agent<br><br>Hydrated sodium/calcium aluminosilicate | • Cream-coloured<br>• Fine powder | - | (Diaz et al., 2005)<br><br>Aflatoxin, OTA, T2-toxin |
| ***Myco-Ad Az*** | Special Nutrients<br><br>www.specialnutrients.com | Adsorbing agent<br><br>Hydrated sodium/calcium aluminosilicate | - | - | (Avantaggiato et al., 2005)<br><br>ZEA, fumonisin, trichothecenes |
| ***BIONIT®S and FENA®-MIN*** | Süd-Chemie AG<br><br>www.sud-chemie.com | Adsorbing agent<br><br>Surface active clay minerals (bentonite, montmorillonite) Bentonite, alkaline activated | • Beige-grey powder<br>• Particle size: 85-75% < 63 µm<br>• pH value : 9.0-10.0<br>• Chemical composition: 58.0% SiO2, 3.5% MgO, 20.0% Al2O3, 2.0% Na2O, 6.0% Fe2O3, 1.0% K2O, 2.5% CaO<br>• Loss on ignition 7.5 % | - | - |
| ***TOXISORB® Classic*** | Süd-Chemie AG<br><br>www.sud-chemie.com | Adsorbing agent<br><br>Partially modified aluminosilicate with high and selective surface area Bentonite, alkaline activated | • Beige-grey powder<br>• Particle size: min 60% < 63 µm<br>• pH value: 9.5-11.0<br>• Chemical composition : 59.0% SiO2, 4.5% MgO, 16.8% Al2O3, 3.1% Na2O, 4.3% Fe2O3, 0.8% K2O, 2.4% CaO<br>• Loss on ignition 8.0 % | No Mycotoxin claims are made in the USA, EU and Canada | *In vitro* studies using AFB1, OTA, ZEA, T-2 toxin, Fumonisin<br><br>Effective against AFB1 |

| Product name | Company | Category<br><br>Description | Physico-chemical properties | Comments | Studies Targeted mycotoxins |
|---|---|---|---|---|---|
| ***TOXISORB ® Premium*** | Süd-Chemie AG<br><br>www.sud-chemie.com | Adsorbing agent<br><br>Partially modified aluminosilicate with high and selective surface area<br>Organic modified bentonite | • Beige-grey powder<br>• Particle size: min 60% < 63 µm<br>• pH value: 9.5-11.0<br>• Chemical composition : 57.5% SiO2, 4.0% MgO, 16.0% Al2O3, 2.7% Na2O, 4.1% Fe2O3, 0.7% K2O, 2% CaO<br>• Loss on ignition 12.0 % | No Mycotoxin claims are made in the USA, EU and Canada | Effective against AFB1, OTA, ZEA, T2-toxin, FB1 |
| ***FIXAT®*** | Süd-Chemie AG<br><br>www.sud-chemie.com | Selected Aluminosilicate with a high binding characteristics for aflatoxins | • Beige to grey powder<br>• Grain size: 70 % < 63 µg<br>• pH value: 8.5 – 10.5 (80 g/ l H2O)<br>• Chemical composition: 50-65% $SiO_2$, 3-6% MgO, 15-25% $Al_2O_3$, 3-6% $Fe_2O3$, 3-6% CaO, $Na_2O/K_2O$ < 5% | | (Marroquin-Cardona et al., 2009)<br>*In vitro* studies using AFB1, OTA, ZEA, T-2 toxin, Fumonisin |
| ***ATOX*** | Tolsa<br><br>www.tolsa.com | Adsorbing agent<br><br>Natural combination of smectite and sepiolite of high purity | • Light cream colour<br>• Fluid powder | Smectite: E558<br>Sepiolite: E562 | (Moschini et al., 2008)<br><br>Aflatoxin |
| ***UT-Aflatrol*** | Ultra-Biologics Inc.<br><br>www.ublcorp.com | Adsorbing agent<br><br>Contains natural montmorillonite layer silicate mineral clays and, humidified vegetable carbons, mannan and fructose oligosaccharides | - | UT-Aflatrol is registered in Taiwan and many parts of Asia, Latin America, North America under private label and/or license agreement | -<br><br>Aflatoxin |
| ***ZAR-MIN*** | Zeo Inc.<br><br>www.zeoinc.com | Adsorbing agent<br><br>100% natural zeolite: clinoptilolite, zeolite, hydrated sodium potassium calcium aluminosilicate | • Off-white colour<br>• Melting point > 1200°C | FDA approved for use as an anti-caking agent. | Research studies are available on the website<br><br>Binds large spectrum of mycotoxin |

## CONCLUDING REMARKS

- Two main categories of feed additives can be defined:

**- Adsorbing agents**: one of the strategies for reducing the exposure to mycotoxins is to decrease their bioavailability by including various mycotoxin adsorbing agents in the compound feed, which leads to a reduction of mycotoxin uptake as well as distribution to the blood and the target organs.

**- Biotransforming agents:** another strategy is the degradation of mycotoxins into non-toxic metabolites by using biotransforming agents such as bacteria/fungi or enzymes.

- Both review of literature and Internet research are more abundant when dealing with adsorbing agents than with biotransforming agents.

- Most of adsorbing agents, especially aluminosilicates, have been tested for their ability to bind aflatoxins. Adsorbing agents such as yeast cell wall, bacteria and others can target a larger spectrum of mycotoxins. Concerning mycotoxin biotransforming agents, as anticipated by their mode of action, their spectrum in term of targeted mycotoxins, are narrower.

# 3. MECHANISMS OF ACTION OF MYCOTOXIN-DETOXIFYING AGENTS STUDIED *IN VITRO*

## 3.1. *In vitro* mechanisms of action of adsorbing agents

Adsorbing agents can be classified, among others, on the basis of:

- Origin: mineral, biological, synthetic, etc.
- Mechanism and type of interactions involved: hydrophobic, electrostatic, molecular recognition, etc.

Besides these qualitative differences, quantitative aspects such as affinity, capacity and selectivity are of course vitally important. As far as possible they should be measured and expressed in a way that allows for comparison of adsorbing agents and prediction of their behaviour in different circumstances.

This chapter discusses these different points, with more emphasis on the quantitative aspects and on how data from the literature can be compared and interpreted quantitatively.

The distribution coefficients $K_d$ and the "Binding Concentration 50" $BC_{50}$, defined below, have been selected as parameters to enable quantitative comparison of experimental results obtained under widely variable conditions and a pragmatically useful interpretation in terms of adsorbing agent working concentration.

Data from the literature have been reviewed, translated into $K_d$ values whenever possible, and compiled in database-like tables. These tables reveal some general trends for affinity, capacity and selectivity and show the relevance of parameters such as the presence of a food matrix.

### 3.1.1. Origins

- Mineral: aluminosilicates (clays)
- Activated coals
- Biological :
    - Yeast and bacterial cell walls
    - Vegetal fibers (e.g. apple pumice, micronized vegetal fibers)
- Synthetic :
    - Modified natural clays (e.g. grafting of quaternary ammonium groups)
    - Synthetic resins (e.g. polyvinylpyrrolidone, cholestyramine)

### 3.1.2. Mechanisms and types of interactions

Several aspects should be taken into account when attempting a classification of adsorbing agents on the basis of binding mechanisms.

- It must be recalled that when any molecule is adsorbed onto a particle's surface, a number of solute-solvent and surface-solvent bonds are replaced by solute-surface and solvent-solvent bonds. Different types of intermolecular interactions can be involved in the same adsorption process: hydrogen binding, Van der Waals forces, electrostatic attraction or repulsion, etc. A convenient and commonly used concept such as "hydrophobic binding" thus actually designates a complex process, involving more than one type of bond, but in which lipophilic/hydrophilic balance is the most relevant feature and allows for qualitative and quantitative prediction based for instance on the octanol-water distribution coefficient ('Pow') of the molecules. In the same way, 'electrostatic binding' designates a

process in which a dominant feature is the long-distance electrostatic attraction between an ionised molecule and an adsorbing agent (e.g. a weak acid, under deprotonated form above a certain pH, strongly attracted by a polycationic adsorbing agent). Of course electrostatic binding will often be pH-dependent, and even hydrophobic binding may be indirectly affected by pH: if both the molecule and the adsorbing agent are anionic above a certain pH, electrostatic repulsion will prevent the hydrophobic binding that would be possible at a lower pH.

- Besides their type(s) and strength(s), the number of bonds formed between the molecule and the adsorbing agent is of course also crucial. This explains shape effects such as the enhanced adsorption of planar molecules by planar adsorbing agents (e.g. the two closely related herbicides simazine and atrazine: due to its higher log Pow, atrazine is more strongly bound than simazine by an alkyl-grafted silica, but, due to its planar shape, simazine is more strongly bound than atrazine by planar adsorbing agents such as graphitized carbon black; another example is the much stronger adsorption of dioxins (planar) than of PCBs by particles with planar surfaces).

- Specific (non-planar) shape effects can also occur, such as in molecular recognition between antibodies and antigens.

- In some cases shape effects may even lead to "cooperativity": binding of a first molecule induces conformational changes in the adsorbing agent which result in enhanced affinity for the next molecules bound. The paradigm for this mechanism is the binding of oxygen by hemoglobin. The result is a finer regulation of the activity of hemoglobin, with a sharp increase of binding above a certain "trigger" level of oxygen.

Thus it would be useful to be able to state:

- the dominant type(s) of interaction, as a function of pH;
- the type of shape effects, if any.

However there may be often a lack of interpretable and reliable experimental data on these aspects, so that any classification on this basis will be provisional, since it is largely based on hypotheses which may be disproven by further research.

### 3.1.3. Quantitative aspects

From a pragmatic point of view, the nature of the bonds discussed above is less important than the possibility of comparing and predicting how many different adsorbing agents will bind different toxins in different environments. Comparison on the basis of literature may appear to be difficult because of the different types of experiments performed (from single-concentration measurements to elaborate gastro-intestinal models) and the different models, formalisms and units used for evaluating and expressing the results (Freundlich, Langmuir, Lineweaver-Burke, Hill, etc; µg/g, mol/kg etc). However for affinity the simple and "assumption-free" parameter $K_d$ (distribution coefficients) can most often be estimated from published data and provides a good basis for comparison of adsorbing agents on a relative scale. Also, as we shall see, this parameter can be interpreted in such a way as to provide a very pragmatic and operational parameter: the "$BC_{50}$" or binder concentration necessary to bind half of the mycotoxins present.

#### 3.1.3.1. Types of experimental studies

*In vitro* analysis of mycotoxin adsorption is a powerful tool for screening potential mycotoxin-detoxifying agents. If a sequestering agent does not adsorb a mycotoxin *in vitro*, it has little or no chance to do so *in vivo*. These laboratory techniques can be very useful in identifying and ranking

potential mycotoxin-detoxifying agents and in helping to determine the mechanisms and conditions favorable for adsorption to occur (Diaz and Smith, 2005).

The experimental studies published range from single-concentration studies to classical isotherm studies (binder concentration fixed, toxin concentration increasing) and beyond to more elaborate set-ups (gastro-intestinal tract models; variable loading binding experiments etc).

- Single-concentration studies

The single-concentration method is the simpler to perform, less wasteful of toxin and most widely used *in vitro* method. It measures adsorption of purified toxin preparations in aqueous medium, where a known amount of mycotoxin is reacted with a known amount of test product in an aqueous solution. The results are usually reported as "%ads", the fraction of toxin bound to the adsorbing agent. This parameter is strongly dependent on adsorbing agent loading (g/L). Provided adsorbing agent loading is known, the distribution coefficient $K_d$ may be calculated from the %ads.

- Adsorption isotherms

Adsorption isotherms have been efficiently used to evaluate mycotoxin-detoxifying agents (Grant and Phillips, 1998; Ramos and Hernandez, 1996). The amount of mycotoxin adsorbed per unit of weight is plotted against the concentration of the mycotoxin in solution at a constant temperature and under stable conditions. This system takes into account that sequestering of mycotoxins is a reversible process that can be characterized as a chemical equilibrium. The results from isotherm studies are usually interpreted by curve-fitting using one or more models such as Freundlich, Langmuir, etc., discussed below.

Unless totally unusual as in the case of S-shaped curves suggesting cooperative binding, the isotherm curves in themselves provide information on affinity and capacity only. However the comparison of isotherms obtained with adsorbing agents and/or toxins differing in structure and properties may provide information about the type of adsorption process. The influence of temperature may be studied in order to calculate the enthalpy of adsorption. Some authors have investigated the stability of the complexes in organic solvents and measured the "chemisorption index", defined as the amount of toxin remaining adsorbed after extraction in organic solvent, divided by the total amount of toxin initially present (i.e. before the adsorption step in aqueous suspension).

- Adsorption isotherms in the presence of a food matrix

Modified isotherms can also be performed in order to compare mycotoxin adsorption in the presence and absence of a feed matrix. The results of these studies are usually examined to assess whether a matrix commonly associated with mycotoxin contamination can affect adsorption efficiency. Experimentally, modified isotherms are obtained as usual; the only difference is the addition of a feed matrix to the test tubes. Data of mycotoxin adsorption are plotted and fitted by the standard mathematical models, Freundlich, Langmuir, etc. Of course, in performing theses studies, the feed matrix should be analyzed for the absence of mycotoxins themselves. Unspecific binding of mycotoxins to the matrix has also to be assessed by carrying out a positive control with the buffered solution of mycotoxins in the presence of a matrix and in the absence of the mycotoxin-detoxifying agent.

- Static and dynamic gastro-intestinal experimental models

*In vitro* studies for the assessment of efficiency of mycotoxin-detoxifying agents in binding mycotoxins can be subjected to a simulated gastrointestinal model, which is useful for identifying physiological conditions that are important to the binding. Several *in vitro* approaches, in so called "static" and "dynamic gastro-intestinal models", have been developed to test the efficacy of mycotoxin-detoxifying agents.

Using a "static gastro-intestinal model", Vekiru et al. showed that bentonites generally become less efficient when gastro-intestinal conditions are simulated. This means that the efficacy of mycotoxin-adsorbing agents may depend on the actual conditions during passage through the gastro-intestinal tract (Vekiru et al., 2007).

However, such static gastro-intestinal models are too far from the *in vivo* conditions. In the *in vivo* gastro-intestinal tract, small molecular weight compounds are transported across the intestinal epithelium into the body, thereby keeping the unbound compound concentration low in the chyme in the intestine. In beakers or batch agitated vessels, the compounds are not removed from the chyme during simulated digestion. Therefore, the binding capacity may be overestimated when saturation of the compound occurs in the chyme in the beaker (Versantvoort et al., 2005). In addition, the above mentioned static *in vitro* methods do not really mimic the kinetic physiological conditions of the animal gastro-intestinal tract, including secretion of saliva, gastric juice, bile and pancreatic juice in combination with peristaltic mixing and transit, and absorption of ingested compounds. An exception is the dynamic, multi-compartmental, computer-controlled *in vitro* gastro-intestinal model (TIM) reported by Avantaggiato et al. (Avantaggiato et al., 2003; Avantaggiato et al., 2004; Avantaggiato et al., 2007). The TIM system, comprising four compartments connected by peristaltic valves, simulates the kinetic digestive processes in respectively the stomach, duodenum, jejunum and ileum of humans and mono-gastric animals, e.g. pigs. Parameters include the secretion of saliva, gastric juice, pancreatic juice and bile for the simulation of realistic pH values, electrolyte concentrations, and digestive enzyme activities as well as, body temperature and peristaltic movements for mixing and gastrointestinal transit. Hollow-fiber semi-permeable membranes are connected to the jejunum and ileum compartments for continuous dialysis of the digested and released compounds (the bioaccessible fraction) and absorption of water. Because mycotoxins are most likely absorbed by passive diffusion, the dialysis system is a suitable way of studying the bioaccessibility of mycotoxins and the efficacy of adsorbing agents (Avantaggiato et al., 2007).

In contrast to static *in vitro* methods, studies with TIM on the bioaccessibility of mycotoxins, under simulation of the gastro-intestinal conditions of pigs, have a high predictive quality: results are in accordance with *in vivo* studies obtained in pigs or other animal studies (Avantaggiato et al., 2004; Avantaggiato et al., 2007; Blanquet et al., 2004).

This model has been successfully used to assess the efficacy of mycotoxin-detoxifying agents in sequestering mycotoxins. The addition of activated carbon or cholestyramine to DON, NIV and ZEA contaminated pig feed significantly reduced the intestinal absorption of these mycotoxins as compared to the contaminated diet without adsorbing agents (Avantaggiato et al., 2003; Avantaggiato et al., 2004). The bentonite adsorbing agent Mg-smectite added to contaminated feed at levels from 0.2 to 1% showed a significant reduction in the bioaccessibility of ZEA and AFB1, up to 50 and 60%, respectively (Blanquet et al., 2004). The efficacy of a carbon/aluminosilicate based product (0.3 to 2%) was tested in TIM simulating the gastro-intestinal conditions of pigs, using multi-mycotoxin contaminated pig feeds. The results showed a dose-effect relation in reducing the bioaccessibility of mycotoxins (Avantaggiato et al., 2007). AFB1 bioaccessibility was reduced by 88%, ZEA by 44%,

FB1 and B2 by 29% and OTA by 20%. However, the carbon/ aluminosilicate based product was not effective for DON.

In conclusion, gastro-intestinal models can be considered rapid and physiologically relevant methods for assaying the efficacy of adsorbing materials in binding mycotoxins and can be used in pre-screening studies to select the most promising adsorbing materials as potential mycotoxin-detoxifying agents.

- Conclusions on the different types of *in vitro* studies

In general, all the above *in vitro* studies, ranging from simple single-concentration studies to complex studies simulating gastro-intestinal conditions, should be considered as the key elements of an optimal prescreen strategy to select and rank promising adsorbing materials. Such a multi-tiered approach is highly desirable as it can limit the number of animal studies.

### 3.1.3.2. Models in use for isotherm studies

Three of the most important models referred to in the literature are Freundlich, Langmuir and Hill. Several tranforms and slight modifications of these models have also been applied to mycotoxin adsorbing agents by e.g.(Grant and Phillips, 1998),

The Freundlich equation

$$K_F = C_{ads} / C_{aq}^{\,1/n}$$

(where $C_{ads}$ is the concentration adsorbed, in µg/g,
$C_{aq}$ the concentration in solution, in µg/mL
$K_F$ is the Freundlich distribution coefficient)

is designed for a situation in which only a small fraction of the adsorption sites are occupied ($C_{ads} << C_{sat}$ , where $C_{sat}$ is the saturation concentration), but the average quality of these adsorption sites slowly decreases with $C_{ads}$ (this is the role of the exponent 1/n) . This is typically the case for soils, in which the very great number of slightly different adsorption sites in slightly different local environments provides a quasi-continuous distribution of sites with slightly different adsorption enthalpies.

The Langmuir equation

$$C_{ads} = C_{sat}.K_L.C_{aq} /(1 + K_L.C_{aq})$$

is designed for a situation in which a significant fraction of the adsorption sites is occupied ($C_{ads} \sim C_{sat}$), so that the availability (concentration) of free sites may no longer be considered constant. This is typically the case for the adsorption of an inert gas on a surface, during measurements of the specific surface of solids. In the above equation the quality of the bonds remains constant, unlike the Freundlich model. Sometimes the two models are combined by adding an exponent to the factors ($K_L.C_{aq}$) and ($1+K_L.C_{aq}$) in Langmuir's equation to account for decreasing quality of the binding sites.

The Hill equation

$$\theta = C_{aq}^{\,n} /(K_d + C_{aq}^{\,n})$$

(where $\theta = C_{ads} / C_{sat}$ is the fraction of binding sites occupied a molecule

n is a measure of cooperativity (n=2.8-3 for hemoglobin-oxygen, =1 in the absence of cooperativity)

is designed for the very specific case of an increased binding rate of oxygen binding by hemoglobin, due to cooperativity in the conformational change of the 4 identical sub-units of the molecule. It has been used by Yiannikouris et al. to fit the unusual (sigmoid) shape of their isotherm experiment results (Yiannikouris et al., 2003).

The Freundlich equation (with exponent = 1) may thus be regarded as a special case of the Langmuir equation, valid only for the first part of the curve, where $C_{ads}$ is sufficiently small relative to $C_{sat}$ for the concentration of free sites to be considered constant (in Langmuir's equation it appears through $\theta$). It can easily be shown that the product $K_L.C_{sat}$ is equal to the $K_F$ that can be estimated from the first part of the curve. In contrast, Hill's equation is quite different and gives a sigmoid shape to the $C_{ads}=f(C_{aq})$ function that can not be obtained with either Freundlich's or Langmuir's equation.

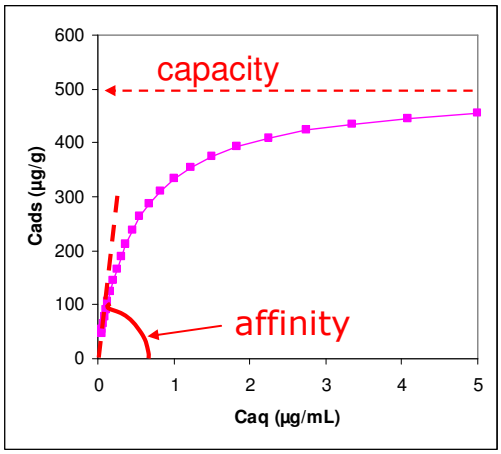


**Figure 1: Ideal full-range adsorption isotherm curve, showing the differences between the two regions of the curve, dominated respectively by affinity and capacity**

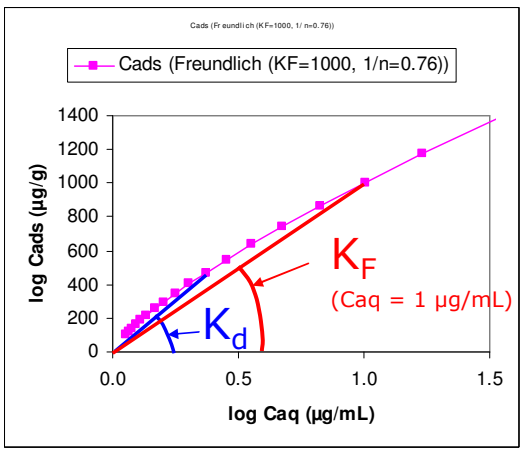


**Figure 2: Calculation of Kd in the first part of the curve (far below saturation)**

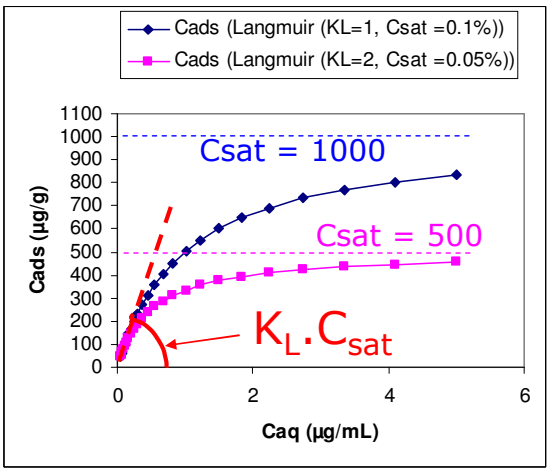


**Figure 3: Application of the Langmuir model to the whole curve**

The OECD guideline on the testing of adsorption of chemicals on soils prescribes only the reporting of the "distribution coefficients"

$$K_d = C_{ads} / C_{aq}$$

and the use of the Freundlich model. The distribution coefficients $K_d$ are purely descriptive and do not rely on any model assumptions. An isotherm experiment generates a series of $K_d$ values, one for each substance concentration tested. These values often slightly decrease with increasing substance concentration. The aim of the Freundlich model is nothing more than reducing this series of $K_d$ values to two parameters, $K_F$ and (1/n). The equation is re-written in logarithm form as:

$$\log(C_{ads}) = \log(K_F) + (1/n).\log(C_{aq})$$

In a Freundlich plot, $\log(C_{ads})$ is plotted against $\log(C_{aq})$ so that the intercept with the y-axis (equal to $K_d$ at $C_{aq}$ = 1 µg/mL) gives $\log(K_F)$ and the slope gives (1/n). The OECD guideline does not address saturation, which is hardly relevant for its purpose, i.e. the prediction of the migration of traces of substances in soils. (It does address hysteresis effects. The differences between adsorption and desorption equilibrium may be very relevant for soils but, in the case of mycotoxin-adsorbing agents, they are probably negligible in comparison to the differences between *in vitro* and *in vivo* behaviour, which is a much more important issue).

### 3.1.3.3. Further discussion on the meaning of the distribution coefficient $K_d$ and the $BC_{50}$

As stated above, the distribution coefficients $K_d$ are purely descriptive and do not rely on any underlying model or assumptions. Therefore $K_d$ are not constants, except in ideal cases, i.e. cases giving Freundlich curves with exponent 1/n equal to 1. In a more realistic case with e.g. 1/n=0.9 and log KF=3, the log $K_d$ will decrease from 3.2 at 0.01 µg/mL toxin to 3 (=log $K_F$) at 1 ug/mL.

Not forgetting this approximative character (and the restriction to adsorbing agent/toxin high enough to avoid saturation), the numerical values of $K_d$ can, in practice, give two meanings. The first is that it is the amount of toxin (in µg) that 1 g of adsorbing agent will be able to bind when in equilibrium

with a 1 µg/mL solution. But, as stated above, the distribution coefficient can also be interpreted in such a way as to have a much more concrete and pragmatic meaning:

$$K_d = 1/BC_{50}$$

where $BC_{50}$ = "Binding Concentration 50" is the binder concentration necessary to ensure binding of 50% of the toxin

This can easily be shown by re-arranging the definition of $K_d$ in the following way:

$$\begin{aligned} K_d &= C_{ads}(\mu g/g)/C_{aq}(\mu g/mL) \\ &= (C_{ads}(\mu g/mL)/C_{binder}(g/mL))/C_{aq}(\mu g/mL) \\ &= (C_{ads}(\mu g/mL)/C_{aq}(\mu g/mL))/C_{binder}(g/mL) \\ &= (\%_{ads}/\%_{aq})/C_{binder}(g/mL) \end{aligned}$$

where $C_{binder}$ is the binder concentration (g/mL)

$\%_{ads}$ is the percentage of the molecule that is bound to the adsorbing agent

$\%_{aq}$ is the percentage of the molecule remaining free in solution

and whence, at 50% binding of the molecule :

$$(\%_{ads} = \%_{aq}) \Leftrightarrow C_{binder}(g/mL) = 1/K_d$$

This rearrangement of the $K_d$ definition allows for useful approximate predictions. For example, $K_d$ = 1000 means that

- 1 g binder per liter (=0.001 g/mL) will adsorb 50% of the toxin present
- 10 g binder per liter will be necessary to adsorb 91% of the toxin
- 0.1 g binder per liter will only adsorb 9% of the toxin

**Annex 11** gives two detailed examples, taken from the literature (Ramos and Hernandez, 1996) and (Döll et al., 2004) showing the validity and the usefulness of the $BC_{50}$ concept, and compares it to the closely related "C50" of Döll et al. (Döll et al., 2004).

The $BC_{50}$ value may also be helpful for designing *in vitro* or *in vivo* experiments, by indicating the minimum adsorbing agent concentration, under which significant binding or a significant effect on the animal is not expected to occur.

A further advantage of the $BC_{50}$ concept is that it allows for easy and straightforward comparison with the results of *in vivo* studies, where the effects observed can usually be expressed in terms of $EC_{50}$.

### 3.1.4. Literature review

#### 3.1.4.1. Explanation of the comparative tables

A number of results from the literature have been summarized in the tables below, on the basis of the principles outlined above.

One table is devoted to the results from single-concentration experiments. Since these results are obtained at one concentration only and are often in the 90-100% range (or inversely in the <20% range), they are generally less reliable quantitatively than the results from isotherm studies, summarized in another table. The approximate $K_d$ values have been estimated by:

$$K_d = (\%_{ads} / \%_{aq}) / C_{binder}(g / mL)$$

and the corresponding approximate $BC_{50}$ values by

$$BC_{50} = 1/ K_d$$

Many publications report very large series of single-concentration results. These results have been grouped whenever there was no significant differences between them (e.g. in table 1 of (Avantaggiato et al., 2005), 19 out of the 21 adsorbing agents tested bound less than 15% of the DON present, at two DON concentrations and at 2 pH : for each adsorbing agent these 4 results have been summed up as one line). A small number of results were regarded as outliers and ignored (e.g. in table 1 of (Avantaggiato et al., 2005) again, the anion-exchange resin Dowex Marathon MSA was reported to bind 33%-22%-0% of the FB1 present, at pH 3-7-8 respectively : the "0%" was regarded as most likely an outlier and ignored).

For isotherms studies, the results as reported by the authors ($K_F$, $K_L$, $C_{sat}$ …) are given, but also, for the sake of comparability and whenever it was possible to do so, the $K_d$ as estimated from the initial slopes of the adsorption curves. The capacities at saturation are reported only when they can be considered reliable. For example, (Ramos and Hernandez, 1996) concluded that their data could be fitted successfully by Freundlich's but not by Langmuir's equation (and indeed the plots show that saturation was far from being reached): accordingly the estimated $K_F$ values were taken into the table but not the estimated $C_{sat}$ values. In some cases where the $C_{sat}$ were not reported by the authors, they have been estimated from the plateau reached.

'Modified isotherms', carried out in the presence of gastric juices or of food matrix, were treated the same way as the other isotherms.

Also 'dose-response studies', in which the variable is not toxin but adsorbing agent concentrations, have been included in the same table. With such studies, the $BC_{50}$ is given directly by the X-axis of the plot rather than calculated from the slope.

A third table is devoted to the more elaborate experiments in which the gastro-intestinal tract was modeled, with a succession of "digestion" steps in different environments (pH, reconstructed gastric juices...).

### 3.1.4.2. Trends observed

- Aflatoxins

Isotherm studies

Aflatoxins are relatively hydrophilic (log Pow = c. 1.2) aromatic planar molecules, with a very strong tendency to adsorb on planar surfaces. Furthermore their beta-dicarbonyl system allows for the formation of coordination bonds with metallic cations (Al3+ or others) present in clays (Phillips et al., 1995). As a consequence, they exhibit very high affinities for planar clays: the log $K_F$ estimated by Grant and Phillips for HSCAS (Grant and Phillips, 1998) and the log $K_d$ estimated from the isotherms

of Lemke et al. (Lemke et al., 2001) for HSCAS and from those of Jaynes et al. (Jaynes et al., 2007) for sepiolite, HSCAS and other clays are all above 4.5, higher than for any other toxin-adsorbing agent combination (excepted activated carbons, discussed below). The corresponding $BC_{50}$ values are lower than 0.03 g/L. The capacities were typically around 200 mg/g. Therefore, even with an aflatoxin concentration as high as 5 mg/L, a suspension of these clays as diluted as 0.03 g/L will bind at least 50% of the toxin without themselves being half-saturated. This are several orders of magnitude lower than the $BC_{50}$ of most other toxin-adsorbing agent combinations, which are often in the 1-10 g/L range.

The importance of the planar shapes of both toxin and adsorbing agent is shown by the much lower affinities observed with zeolites, which are clays with cage-like structures rather than plane surfaces: e.g. log $K_d$ < 3 for clinoptilolite (Lemke et al., 2001). Also the importance of the surface area is shown by the much lower log $K_d$ (c. 3.6) and $C_{sat}$ (18 mg/g) observed with "collapsed" HSCAS (with specific area decreased from 848 to 77 $m^2$/g) (Grant and Phillips, 1998).

The affinities of aflatoxin for some activated carbons are also very high, with log $K_d$ in the 5->6 range. The main mechanism here is probably hydrophobic binding, although shape effects will also be important with activated carbons presenting planar surfaces (e.g. graphitized carbon black).

Tomasevic-Canovic et al. have carried out isotherm studies on zeolites modified by exchange of 20-50% of their ECEC (external surface cation exchange capacity) with an organic cation: the log $K_d$ estimated from the slopes of these isotherms are c. 3.5 (Tomasevic-Canovic et al., 2002).

Modified isotherms (presence of food matrix)

All the affinities discussed so far, being measured in water or buffers, are "best-cases" because in actual practice the food matrix will be present and will influence binding, which underlines the importance of selectivity. A low selectivity will be detrimental in two ways: the adsorbing agent may bind essential nutrients such as vitamins, and the food matrix components may reduce the binding of toxin by competing for the adsorption sites and partly saturating the adsorbing agent. This competition has been studied in a few publications:

- Jaynes et al. repeated their isotherms in the presence of corn meal suspension (0.5 g/mL) and observed marked decreases of binding. The log $K_d$ estimated from isotherm slopes decreased from 5.5 in water to 3.1 in corn meal suspension for HSCAS, from 4.9 to 4.1 for sepiolite, from >6 to 3 and 2.3 for two other clays, and from >6 to 2.3 for activated carbon. As a result, the $BC_{50}$ are much higher (up to 5 g/L) in the presence of a food matrix than in water (<0.03 g/L). Expressed relative to food matrix (0.5 g/mL), the $BC_{50}$ of activated carbon was found to be as high as 10 g/kg (Jaynes et al., 2007).
- Lemke et al. have tested the effect of a much lower food matrix concentration (maize, 4 g/L) on activated carbon. The log $K_d$ decrease less than in the previous experiment (from c. 5.95 to c. 4.85). However, even though the corresponding $BC_{50}$ is still very low if expressed in g/L (c. 0.015 g/L), it rises to 4 g/kg if expressed relative to the food matrix (Lemke et al., 2001).
- Di Natale et al. have studied the adsorption of AFM1 in milk, in a dose-response study (5 to 50 g/L adsorbing agent). The $BC_{50}$ experimentally measured were between 10 and 20 g/L, corresponding to log $K_d$ between 1.7 and 2. The log $K_d$ estimated from the single-concentration studies were slightly higher (2.05 for bentonite, and 2.3-2.6 for the activated carbons, emphasizing the lower reliability of the log $K_d$ estimated from results close to 100% binding. Clinoptilolite, other zeolites and a carbon showed very low $K_d$ values (<1) in these screening single-concentration tests (Di Natale et al., 2009).

Note that Di Natale et al. also studied the influence of the adsorbing agents on milk quality, and concluded that the adsorbing agents reduced the concentration of nutritional factors proportionally to the sorbent loading, with more pronounced effects for activated carbons (Di Natale et al., 2009). However, with the only exception of one activated carbon, a sorbent loading as high as 35 g/kg preserved the levels of proteins, chlorides, and organic acids concentrations within acceptance limits.

Vekiru et al. showed that at 2 g/L activated carbon adsorbed, in addition to 100% of the AFB1, 99% of the vitamin B12 and 78% of the vitamin H (all present at 4 mg/L). HSCAS and bentonites adsorbed 92%-100% of the aflatoxin but only 7%-47% of the vitamin B12 and 3% of the vitamin H (log Pow 3.6 and 0.4 respectively) (Vekiru et al., 2007). This confirms the very low selectivity of activated carbons and the relative selectivity of clays for aflatoxins.

Single-concentration experiments

In a comparative series of single-concentration measurements, Vekiru et al. have shown that the apparent affinities of charcoal, HSCAS and a series of bentonites for AFB1 strongly decreased in the presence of swine gastric juice. The percentages adsorbed dropped from 88% down to 35% (log $K_d$ from 4.6 down to 3.4) for activated charcoal, from 98% to 72% (log $K_d$ from 5.4 to 4.1) for HSCAS, and generally by more than 15% for a series of bentonites. The chemisorption indeces, measured for 20 adsorbing agents which had bound more than 63% of the toxin at pH 5, ranged from 0.81 to 0.92 (Vekiru et al., 2007).

Most single-concentration studies are actually screening studies, performed in order to select the strongest adsorbing agents for more detailed studies, e.g.:

- Diaz et al. have tested a series of bentonite clays and activated carbons and an esterified glucomannan (E-GM), all at 10 g/L: the %binding were in the 95%-99.9% range, corresponding to log $K_d$ values between 3.3 and 5 (Diaz et al., 2002);
- Mallmann has tested a series of 86 commercial clays, all at 50 g/L, in either hydro-alcoholic solution (pH 6, 1.2 µg/mL toxin) or artificial gastric juice (pH 2, 2 µg/mL toxin): the % binding ranged from 15% to 84% (log $K_d$ 0.6-2) in the first case, from 40% to 99.5% (log $K_d$ 1.1-3.6) in the second (Mallmann). The reason for this difference, as well as for the absence of high log $K_d$ values especially at pH 6, is unclear (note that Vekiru et al. have warned that at pH 2 some transformations of AFB1 into aflatoxin B2a may occur and be mistaken for adsorption) (Vekiru et al., 2007).

Gastro-intestinal models

Lemke et al. have used a gastro-intestinal experimental model which consisted essentially of 2 hours incubation at pH 1.3 with pepsin, followed by 2 hours at pH 7 with pancreatin and bile salts. The final percentages bound were >99%, 96% and 34% for charcoal, HSCAS and clinoptilolite respectively. The log $K_d$ values calculated from these %binding and the adsorbing agent concentration applied (3.1 g/L) are >4.5, 3.9 and 2.15 respectively, close to the values obtained in simpler set-ups (see above: 5->6, >4.5 and 2.5 respectively) (Lemke et al., 2001).

- Ergot toxins

Ergotamine (log Pow 2.5), ergocryptine and ergocristine are relatively large (MW c. 600) alkaloids. Only one study on the adsorption of these molecules has been found in the literature.

Single-concentration studies

The affinities of zeolite and organo-zeolites for ergotamine (log Pow 2.5) and other ergot alkaloids at pH 3 (Tomasevic-Canovic et al., 2002) were relatively high, with log $K_d$ ranging from 2.2 to 4 (BC50 7 to 0.1 g/L).

- Zearalenone

Zearalenone is a macrocyclic molecule, which is much more hydrophobic than aflatoxins (log Pow c. 3.6). The presence of a diphenolic group makes it a weak acid. Its pKa has been estimated at 7.62 by (Lemke et al., 1998). Therefore, at acidic pH, the dominant mechanism of binding will probably be hydrophobic interactions. At higher pH, the binding may be reduced by electrostatic repulsion if the adsorbing agent is anionic. Also if the adsorbing agent is neutral, binding may be reduced because anionic ZEA will either not bind at all or will bind to some extent but in doing so will build up a charge that will repel further anions. Inversely the presence of cationic groups on the adsorbing agent (e.g. anion-exchange resin) should enhance binding. However, due to the relatively high value of the pKa, these pH effects should be much weaker than for fumonisins, the carboxylic acid groups of which probably have a pKa around 5.

Isotherm studies

Avantaggiato et al. have carried out isotherm studies on several adsorbing agents and have estimated Csat between 34 and 112 mg/g. The initial slopes of the isotherms published are too steep to allow for an estimation of log $K_d$ (Avantaggiato et al., 2004; Avantaggiato et al., 2005).

Feng et al. have measured log $K_F$ of 2.3 and 3.7 respectively for montmorillonite clay and a hydrophobic organic cation -modified montmorillonite, confirming the importance of hydrophobic interactions (Feng et al., 2008).

Likewise, Lemke et al. have shown that exchanging the cations of a clay for organic cations increased the log $K_F$ values from -0.5 to values as much as 4.9. They also studied the effect of pH on a clay modified by the organic cation cetylpyridinium in excess (150%) of the CEC, and found as expected that the log $K_d$ was much lower at pH 10 (1.5) than at pH 2 or 6.5 (3.3-3.5) (Lemke et al., 1998).

(Ramos and Hernandez, 1996) have measured log $K_F$ between 1.4 for magnesium trisilicate to 2.5 for cholestyramine and >2.5 for crospovidone.

Yiannikouris et al. have obtained with purified yeasts cell walls an isotherm with a quite unusual sigmoid shape, which cannot be adequately fitted with either the Freundlich or the Langmuir equations. They have concluded that a specific cooperative mechanism was responsible for the increase of affinity with ZEA concentration, and used the Hill model (for cooperativity of binding of oxygen by hemoglobin) to interpret their data. The parameters obtained in this way are of a very different nature to the usual log $K_d$ and do not allow for any comparison. Therefore the log $K_d$ values have been estimated separately for each point of the isotherm curve: they increase from c. 3 for the first two points to c. 3.3 for the fifth point (Yiannikouris et al., 2003).

Single-concentration studies

The screening single-concentration studies of Avantaggiato et al. on a series of 21 adsorbing agents of all types provide some examples of the effects of pH on adsorption hypothesized hereabove (Avantaggiato et al., 2005). Increasing the pH from 3 to 8 has the following effects on the log $K_d$ estimated from the %binding:

- anion exchange resin (Dowex 1-X8) : increase, from 3.5 to 4.3
- non-ionic (hydrophobic) resin (Amberlite XAD-2) : decrease, from 3.7 to 3.2
- zeolite : decrease, from 2.9 to 2.6.

However in most cases, including another anion-exchange resin, the effect of pH was small. Most log $K_d$ were lower than 3 (corresponding to 50% binding at the 1 g/L binder concentration applied in this experiment). Besides the two resins discussed above, the adsorbing agents scoring higher than this were:

- activated carbon and standard-Q/FIS : log $K_d > 5$ ("100%" binding)
- cholestyramine : log $K_d$ >3.8 (90%-100% binding)
- HSCAS-based product : log $K_d$ = c. 4 (90%-96% binding)
- Combination of *Eubacterium* BBSH 797 with dried yeasts and clays: log $K_d$ = c. 3.1 (56%-57% binding)

Gastro-intestinal models

Avantaggiato et al. have applied the elaborate experimental gastro-intestinal tract model from TNO, in which the tract is actually mimicked by different physical compartments, with the test mixture travelling slowly from one compartment to the next. The end-point measured was the cumulated absorption in "jejunal" and "ileal" dialysate fluids. A dose-response study was carried out. The "$EC_{50}$", i.e. the doses reducing absorption to 50% of control, were c. 3 g/L for activated carbon and c. 20 g/L for cholestyramine (Avantaggiato et al., 2003).

- Ochratoxin A

Ochratoxin A is a polyaromatic molecule, more hydrophobic than ZEA when unionized (log Pow = c. 4.4) but with two weak acid groups, a carboxyle and a phenol (pKa 4.4 and 7.3 respectively).

Isotherm studies

In their isotherm studies on organic-cation modified zeolites, Dakovic et al. and Tomasevic-Canovic et al. have observed that binding increased with an increasing amount of organic cation (estimated log $K_d$ : from c.3 at 20% ECEC to c. 4 at 100% ECEC), but there seemed to be no pH effect (no difference at pH 3-7-9). The apparent capacities were rather low (c. 1.5-3.5 mg/g) (Dakovic et al., 2003; Tomasevic-Canovic et al., 2002).

The log KF estimated by Ringot et al. for different yeast-based products were between 1.1 and 2.3 (Ringot et al., 2007).

Single-concentration studies

In single-concentration studies, Galvano et al. have observed strong variations between different commercial and experimental activated carbons. Whereas three commercial carbons at 0.4 g/L bound >99% of the toxin (log $K_d > 5.4$, $BC_{50} < 0.001$ g/L), a fourth commercial carbon bound only c. 2% (log $K_d$ 1.7, $BC_{50}$ 20 g/L). The 15 different experimental carbons tested ranged from 0.8% to 100% binding. Sepiolite and HSCAS were in the low range (c. 11%-13% binding, giving log $K_d$ c. 2.5 and $BC_{50}$ c. 3 g/L) (Galvano et al., 1998).

Rotter et al. have tested another charcoal, in the absence and presence of a food matrix (chick diet, 200 g/L). The presence of the food matrix reduced the log $K_d$ from 3.25 to 2.6 and increased the $BC_{50}$ from 0.6 to 3 g/L, which, expressed relative to food matrix, corresponds to 15 g/kg (Rotter et al., 1989).

Var et al. tested sodium bentonite and activated carbon, in buffer and in white wine. Sodium bentonite at 0.2-0.4-1 g/L bound less than 12% of the toxin in buffer, and less than 24% in wine, corresponding to $BC_{50}$ above 1 g/L. Activated carbon bound up to 100% in buffer and 98% in wine, in a dose-dependent manner. The log $K_d$ estimated from these %binding range from 3.6 to >5 for buffer ($BC_{50}$ < 0.25 g/L) and from 2.9 to 4.8 in wine ($BC_{50}$ between 0.02 and 0.8 g/L) (Var et al., 2008).

- Fumonisin B1

Fumonisin B1 is an aliphatic long-chain molecule, with four carboxylic groups, the pKa of which are probably around 5. Strong pH effects are therefore expected.

Single-concentration studies

Avantaggiato et al. have performed single-concentrations screening tests on 21 different adsorbing agents and isotherm studies on four of these (Avantaggiato et al., 2005). The isotherms (in double-reciprocal form) were used to evaluate the capacities, which ranged from 45 mg/g (HSCAS-based product) to 390 mg/g (Standard Q/FIS). The initial slopes of the plots presented are too steep to allow for estimation of the log $K_d$. The single-concentration studies confirm the existence of strong pH effects:

- Several adsorbing agents bound less toxin at pH 7-8 than at pH 3 : Amberlite XAD-2 (non-ionic resin), glucomannans, Mycofix® Plus, Mycosorb®, Tixolex 28, zeolite
- Inversely cholestyramine and the anion-exchange resin Dowex 1-X8 adsorbed more at pH 7-8 than at pH 3

No pH effect was observed with activated carbon or Standard Q/FIS: both bound 100% of the toxin irrespective of pH. The log $K_d$ and $BC_{50}$ estimated from the %binding may be divided into three groups:

- very high values (log $K_d$ >5, $BC_{50}$ < 0.01 g/L) for activated carbon and Standard Q/FIS
- high values (log $K_d$ >3.8, $BC_{50}$<0.2 g/L) for cholestyramine and, at pH 7-8, for Dowex 1-X8
- low to moderate values (log $K_d$ <3.3, $BC_{50}$>0.5 g/L) for the other adsorbing agents tested.

- Deoxynivalenol (vomitoxin) and other trichothecenes

Trichothecenes range from rather hydrophilic (NIV and DON) to moderately hydrophobic (HT-2 and T-2). They are non-ionisable molecules with a bulky epoxy group, which does not favour adsorption to plane surfaces. As a consequence, they adsorb on very few adsorbing agents.
In Galvano's tests with 2 g/L adsorbing agent and 4 µg/mL DON, three commercial activated carbons bound 89% to 98% of the toxin (log $K_d$ 3.6-4.4, $BC_{50}$ 0.04-0.24 g/L) but a fourth one bound only 14% (log $K_d$ 1.9, $BC_{50}$ 12 g/L). HSCAS and sepiolite bound less than 5%. Tomasevic-Canovic et al. obtained less than 5% binding with organo-zeolites at 10 g/L (Tomasevic-Canovic et al., 2002). In the screening single-concentration tests of Avantaggiato et al., activated carbon (1 g/L) bound 84%-95% of the toxin at 2 µg/mL (log $K_d$ c.4, $BC_{50}$ c. 0.1 g/L), but only 52%-59% at 10 µg/mL, indicating saturation (Avantaggiato et al., 2005). Similarly, standard Q/FIS bound 50%-53% at 2 ug/mL, but only 13%-18% at 10 µg/mL. All of the other 19 adsorbing agents tested adsorbed less than 10% of the toxin (log $K_d$ < 2, $BC_{50}$ > 10 g/L).

NIV is even more hydrophilic than DON, and adsorbs even less, as shown again by Avantaggiato et al. (Avantaggiato et al., 2005).

Avantaggiato et al. have also applied the TNO experimental GI tract model to NIV and DON on activated carbon, in a dose-response study (5-10-20 g/L). Even the highest dose tested (20 g/L)

decreased the cumulated jujenal/ileal absorption by only c. 40%, showing that the $EC_{50}$ is higher than 20 g/L (Avantaggiato et al., 2004).

### 3.1.5. Link between BC50 from *in vitro* studies and EC50 from *in vivo* studies

A few examples will show how results from *in vitro* and *in vivo* studies may be compared quantitatively, using the $BC_{50}$ and $EC_{50}$ respectively.

Shi et al. have shown that 0.1 mg/kg aflatoxins in the diet significantly reduced the body weight gain of broiler chicks (-6.07% with $P<0.05$) and that a montmorillonite-based adsorbing agent added at 3 g/kg suppressed this effect: in this case the EC50 was therefore lower than 3 g/kg (Shi et al., 2006).

Santin et al. have seen no improvement in broiler chicks' weight gain and other parameters, negatively affected by 2 mg/kg OTA, when 2.5 g/kg HSCAS was added to the diet: this time the EC50 was higher than 2.5 g/kg (Santin et al., 2002).

Ortatatli and Oguz have tested clinoptilolite against aflatoxicosis, at two doses, 15 and 25 g/L. Both decreased the number of affected broilers and/or the severity of lesions moderately to significantly. For example afalatoxin increased the liver weight by 16%. This increase was reduced by clinoptilolite, to 12% and 9% at 15 and 25 g/L respectively. This suggests that the EC50 in this case was around 25-30 g/kg (Ortatatli and Oguz, 2001).

### 3.1.6. Summary and conclusions

The distribution coefficients $K_d$:

- are free of any model or underlying assumption
- provide a universal basis for comparing experimental results despite the differences in adsorbing agent and toxin concentrations
- can readily be recalculated or estimated from most types of data usually reported (% binding, isotherm plots, etc).

The interpretation of these $K_d$ as $1/BC_{50}$ does imply the following:

- the adsorbing agent largely exceeds the toxin, so that saturation is not relevant: this condition will most often be met in the case of interest, i.e. detoxication of mildly contamination feedstuffs using additives in the g/kg range
- the behaviour of the adsorbing agent is ideal in the sense that it would behave the same way at $BC_{50}$ concentration as at the concentration chosen for the experiment: deviations from an ideal result in this sense are of course expected, but will in most cases only be a second-order correction to ideal behaviour.

In other words the $BC_{50}$ should be regarded as a first approximation, and furthermore valid only at a sufficiently high adsorbing agent/toxin ratio. Keeping this precaution in mind, it is a very good and pragmatically meaningful indicator of an adsorbing agent's potential, allowing comparisons over the whole range of adsorbing agent-toxin affinities.

In simple experiments and in the absence of a food matrix, the following trends are observed concerning affinities:

- activated carbons generally have higher affinities for all toxins than any other adsorbing agent, and indeed are the only ones able to bind the hydrophilic trichothecenes DON and NIV
- activated carbons set aside, the highest affinities are observed for aflatoxin on clays with planar surfaces ($\log K_d > 5$, i.e. $BC_{50} < 0.01$ g/L) which may be ascribed to shape-enhanced

binding through several types of interactions, including coordination bonds between the metallic cations of the clay and the di-carbonyl system of aflatoxins

- ZEA, OTA and FB1:
    - in acidic conditions, may all be bound with moderate to high affinities (log $K_d$ typically between 2 and 4, i.e. $BC_{50}$ between 0.1 and 10 g/L) by various adsorbing agents, mostly through hydrophobic interactions
    - are ionized at higher pH (pH > 7.6, 4.4 and c. 5 respectively). The expected consequences are reduced adsorption on neutral or anionic adsorbing agents and inversely enhanced adsorption on cationic adsorbing agents such as anion-exchange resins. Such pH effects are indeed observed in some cases, but in other cases they are apparently negligible.
- DON and NIV adsorb only on activated carbons.

The capacities are typically in the 50-200 mg/g range. A capacity as high as 390 mg/g has been reported for FB1 on a carbon-based adsorbing agent (Avantaggiato et al., 2005). Values far below 50 mg/g, such as the 1.6 and 0.6 mg/g for DON and NIV reported for the same adsorbing agent, could reflect low affinities rather than low capacities. In actual practice, given the presumably low toxin concentrations (mg/kg range), relative to adsorbing agent dosages (g/kg range), capacities are less likely to be relevant than affinities or selectivities.

**Table 4: Results from single-concentration studies**

| Single-concentration studies : aflatoxin, zearalenone | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ref | toxin | binder | C(binder) | C(toxin) | solvent | pH | T | %binding | Kd | log(Kd) | BC50 |
| | | | | | | | ℃ | | mL/g | | g/mL |
| Di Natale et al., 2009 | aflatoxin M1 | Zeolite 13X | 50 | 0.0005 | milk | 6.7-7.7 | 4 | 2.0% | 0 | -0.39 | 2450 |
| Di Natale et al., 2009 | aflatoxin M1 | Zeolite 3A | 50 | 0.0005 | milk | 6.7-7.7 | 4 | 2.2% | 0 | -0.35 | 2223 |
| Di Natale et al., 2009 | aflatoxin M1 | Zeolite 4A | 50 | 0.0005 | milk | 6.7-7.7 | 4 | 3.0% | 1 | -0.21 | 1617 |
| Di Natale et al., 2009 | aflatoxin M1 | Char Carbon | 50 | 0.0005 | milk | 6.7-7.7 | 4 | 10.2% | 2 | 0.36 | 440 |
| Di Natale et al., 2009 | aflatoxin M1 | Clinoptilolite | 50 | 0.0005 | milk | 6.7-7.7 | 4 | 21.0% | 5 | 0.73 | 188 |
| Di Natale et al., 2009 | aflatoxin M1 | Bentonite | 50 | 0.0005 | milk | 6.7-7.7 | 4 | 85.0% | 113 | 2.05 | 8.82 |
| Di Natale et al., 2009 | aflatoxin M1 | Acquacarb 207 EA Carbon | 50 | 0.0005 | milk | 6.7-7.7 | 4 | 90.0% | 180 | 2.26 | 5.56 |
| Di Natale et al., 2009 | aflatoxin M1 | Filtrasorb 400 Carbon | 50 | 0.0005 | milk | 6.7-7.7 | 4 | 95.0% | 380 | 2.58 | 2.63 |
| Di Natale et al., 2009 | aflatoxin M1 | Norit GCN 1240 Carbon | 50 | 0.0005 | milk | 6.7-7.7 | 4 | 95.5% | 424 | 2.63 | 2.36 |
| Diaz et al., 2003 | aflatoxin B1 | sodium bentonite FG | 10 | 5 | 10% MeOH | 3-7-10-unadj. | room | 95.1% | 1941 | 3.3 | 0.52 |
| Diaz et al., 2003 | aflatoxin B1 | esterified glucomannan MTB-100 | 10 | 5 | 10% MeOH | 3-7-10-unadj. | room | 96.6% | 2841 | 3.5 | 0.35 |
| Diaz et al., 2003 | aflatoxin B1 | sodium bentonite AB20 | 10 | 5 | 10% MeOH | 3-7-10-unadj. | room | 98.0% | 4900 | 3.7 | 0.20 |
| Diaz et al., 2003 | aflatoxin B1 | sodium bentonite MS | 10 | 5 | 10% MeOH | 3-7-10-unadj. | room | 98.4% | 6150 | 3.8 | 0.16 |
| Diaz et al., 2003 | aflatoxin B1 | caalcium bentonite RC | 10 | 5 | 10% MeOH | 3-7-10-unadj. | room | 98.5% | 6567 | 3.8 | 0.15 |
| Diaz et al., 2003 | aflatoxin B1 | activated carbon A | 10 | 5 | 10% MeOH | 3-7-10-unadj. | room | 99.5% | 19900 | 4.3 | 0.05 |
| Diaz et al., 2003 | aflatoxin B1 | activated carbon D | 10 | 5 | 10% MeOH | 3-7-10-unadj. | room | 99.6% | 24900 | 4.4 | 0.040 |
| Diaz et al., 2003 | aflatoxin B1 | activated carbon B | 10 | 5 | 10% MeOH | 3-7-10-unadj. | room | 99.9% | 99900 | 5.0 | 0.010 |
| Diaz et al., 2003 | aflatoxin B1 | activated carbon C | 10 | 5 | 10% MeOH | 3-7-10-unadj. | room | 99.9% | 99900 | 5.0 | 0.010 |
| Lemke et al., 2001 | aflatoxin B1 | Clinoptilolite | 0.2 | 7.86 | water | | | 5.6% | 294 | 2.47 | 3.40 |
| Lemke et al., 2001 | aflatoxin B1 | HSCAS | 0.2 | 7.86 | water | | | 91.3% | 52273 | 4.72 | 0.02 |
| Lemke et al., 2001 | aflatoxin B1 | charcoal | 0.2 | 7.86 | water | | | 92.9% | 65000 | 4.81 | 0.02 |
| Mallmann et al., | aflatoxin B1 | clays (86 commercial samples) | 50 | 1.2 | hydro-alcoholic solution | 6 | 25 | 15-84% | 4-100 | 0.6- 2.0 | 10-250 |
| Mallmann et al., | aflatoxin B1 | clays (86 commercial samples) | 50 | 2 | artificial gastric juice | 2 | 25 | 40-99.5% | 13-4000 | 1.1-3.6 | 0.25-80 |
| Tomasevic et al., 2003 | aflatoxin B1 | organo-zeolites ((O)DMBA at 20-50% of external CEC) | 10 | 2 | buffer | 3 | room | 88-100% | > 730 | >2.9 | <1.4 |
| Tomasevic et al., 2003 | aflatoxin B1 | zeolite | 10 | 2 | buffer | 3 | room | 99% | 9900 | 4.00 | 0.10 |
| Vekiru et al., 2007 | aflatoxin B1 | activated charcoal | 0.2 | 4 | buffer | 5 | 37 | 88% | 37000 | 4.60 | 0.03 |
| Vekiru et al., 2007 | aflatoxin B1 | activated charcoal | 0.2 | 4 | swine gastric juice | 5 | 37 | 35% | 2700 | 3.40 | 0.4 |
| Vekiru et al., 2007 | aflatoxin B1 | HSCAS | 0.2 | 4 | buffer | 5 | 37 | 98% | 250000 | 5.40 | 0.004 |
| Vekiru et al., 2007 | aflatoxin B1 | HSCAS | 0.2 | 4 | swine gastric juice | 5 | 37 | 72% | 13000 | 4.10 | 0.08 |
| Vekiru et al., 2007 | aflatoxin B1 | HSCAS | 0.2 | 4 | buffer | 7 | 37 | 94% | 80000 | 4.90 | 0.01 |
| Vekiru et al., 2007 | aflatoxin B1 | 24 bentonite clays | 0.2 | 4 | buffer | 5 | 37 | 50%-95% | 5000-95000 | 3.7-5 | 0.01-0.2 |
| Vekiru et al., 2007 | aflatoxin B1 | 40 bentonite clays | 0.2 | 4 | swine gastric juice | 5 | 37 | 33%-86% | 2500-31000 | 3.4-4.5 | 0.03-0.4 |
| Vekiru et al., 2007 | aflatoxin B1 | 24 bentonite clays | 0.2 | 4 | buffer | 7 | 37 | 70%-98% | 12000-250000 | 4.1-5.4 | 0.004-0.09 |
| Shi et al., 2006 | aflatoxin B1 | | 5 | 100 of each afl. | water | 7 | 37 | 90% | 1840 | 3.30 | 0.5 |
| Shi et al., 2006 | aflatoxin B2 | | 5 | 100 of each afl. | water | 7 | 37 | 83% | 950 | 3.00 | 1 |
| Shi et al., 2006 | aflatoxin G1 | | 5 | 100 of each afl. | water | 7 | 37 | 82% | 900 | 2.95 | 1.1 |
| Shi et al., 2006 | aflatoxin G2 | | 5 | 100 of each afl. | water | 7 | 37 | 73% | 530 | 2.70 | 2 |
| | | | | | | | | | | | |
| Avantaggiato et al, 2005 | zearalenone | Glucomannan | 1 | 20 | phosphate buffers | 3-8 | room | 0%-11% | c. 50 | c. 1.7 | c. 20 |
| Avantaggiato et al, 2005 | zearalenone | Tixolex 28 | 1 | 2-20 | phosphate buffers | 8 | room | 3%-13% | c. 100 | c. 2 | c. 10 |
| Avantaggiato et al, 2005 | zearalenone | Glucomannan | 1 | 2 | phosphate buffers | 3-8 | room | 9%-21% | c. 180 | c. 2.25 | c. 6 |
| Avantaggiato et al, 2005 | zearalenone | Mycosorb | 1 | 20 | phosphate buffers | 3-8 | room | 18%-23% | c. 250 | c. 2.4 | c. 4 |
| Avantaggiato et al, 2005 | zearalenone | Mycofix Plus | 1 | 20 | phosphate buffers | 3-8 | room | 16%-32% | c. 300 | c. 2.6 | c. 3 |
| Avantaggiato et al, 2005 | zearalenone | Zeolite | 1 | 2-20 | phosphate buffers | 8 | room | 17%-36% | c. 300 | c. 2.6 | c. 3 |
| Avantaggiato et al, 2005 | zearalenone | Tixolex 28 | 1 | 2-20 | phosphate buffers | 3 | room | 30%-34% | c. 500 | c. 2.7 | c. 2 |
| Avantaggiato et al, 2005 | zearalenone | Dowex Marathon MSA (anion exch.resin) | 1 | 2-20 | phosphate buffers | 3-8 | room | 32%-51% | c. 700 | c. 2.8 | c. 1.5 |
| Avantaggiato et al, 2005 | zearalenone | Mycosorb | 1 | 2 | phosphate buffers | 3-8 | room | 38%-42% | c. 670 | c. 2.8 | c. 1.5 |
| Avantaggiato et al, 2005 | zearalenone | Zeolite | 1 | 2-20 | phosphate buffers | 3 | room | 33%-54% | c. 800 | c. 2.9 | c. 1.2 |
| Avantaggiato et al, 2005 | zearalenone | Mycofix Plus | 1 | 2 | phosphate buffers | 3-8 | room | 56%-57% | c. 1200 | c. 3.1 | c. 0.8 |
| Avantaggiato et al, 2005 | zearalenone | Amberlite XAD-2 (non-ionic, hydrophobic) | 1 | | phosphate buffers | 8 | room | 56%-66% | c. 1600 | c. 3.2 | c. 0.6 |
| Avantaggiato et al, 2005 | zearalenone | Dowex 1-X8 (anion exch.resin) | 1 | 2-20 | phosphate buffers | 3 | room | 69%-85% | 3000 | 3.5 | 0.3 |
| Avantaggiato et al, 2005 | zearalenone | Amberlite XAD-2 (non-ionic, hydrophobic) | 1 | | phosphate buffers | 3 | room | 80%-88% | c. 5000 | c. 3.7 | c. 0.2 |
| Avantaggiato et al, 2005 | zearalenone | Cholestyramine | 1 | 2-20 | phosphate buffers | 3 | room | 85%-100% | > 6000 | > 3.8 | < 0.2 |
| Avantaggiato et al, 2005 | zearalenone | Cholestyramine | 1 | 2-20 | phosphate buffers | 7-8 | room | 90%-100% | > 9000 | > 3.9 | < 0.1 |
| Avantaggiato et al, 2005 | zearalenone | Myco AD A-Z (purified clay) | 1 | 2-20 | phosphate buffers | 3-7-8 | room | 90%-96% | c. 10000 | c. 4 | c. 0.1 |
| Avantaggiato et al, 2005 | zearalenone | Dowex 1-X8 (anion exch.resin) | 1 | 2-20 | phosphate buffers | 8 | room | 89%-100% | 20000 | 4.3 | 0.05 |
| Avantaggiato et al, 2005 | zearalenone | Activated carbon | 1 | 2-20 | phosphate buffers | 3-7-8 | room | 100% | >99000 | >5 | <0.01 |
| Avantaggiato et al, 2005 | zearalenone | Standard Q/FIS (carbon/aluminosilicate-based product) | 1 | 2-20 | phosphate buffers | 3-7-8 | room | 100% | >99000 | >5 | <0.01 |
| Tomasevic et al., 2003 | zearalenone | zeolite | 10 | 2 | buffer | 3 | room | 5% | 5 | 0.72 | 190.00 |
| Tomasevic et al., 2003 | zearalenone | organo-zeolites ((O)DMBA at 20-50% of external CEC) | 10 | 2 | buffer | 3 | room | 90-99% | 900-9000 | 2.95-4 | 0.1-1.1 |
| | | | | | | | | | | | |

| Single-concentration studies : ochratoxine, fumonisine | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **ref** | **toxin** | **binder** | **C(binder)** | **C(toxin)** | **solvent** | **pH** | **T** | **%binding** | **Kd** | **log(Kd)** | **BC50** |
| | | | | | | | **°C** | | **mL/g** | | **g/mL** |
| Galvano et al., 1998 | ochratoxine-A | commercial activated carbon ("CAC3") | 0.4 | 4 | water | | room | c. 2% | 50 | 1.7 | 20 |
| Galvano et al., 1998 | ochratoxine-A | sepiolite | 0.4 | 4 | water | | room | c. 11% | 310 | 2.5 | 3 |
| Galvano et al., 1998 | ochratoxine-A | HSCAS | 0.4 | 4 | water | | room | c. 13% | 370 | 2.6 | 3 |
| Galvano et al., 1998 | ochratoxine-A | 3 commercial activated carbons ("CAC1", "CAC2", CAC4") | 0.4 | 4-10 | water | | room | >99% | >250000 | >5.4 | <0.001 |
| Galvano et al., 1998 | ochratoxine-A | 15 experimental acivated charcoals | 0.4 | 4 | water | | room | 0.8%-100% | 4->250000 | 1.3->5.4 | 50-<0.01 |
| Rotter & Frohlich, 1989 | ochratoxine-A | activated charcoal | 5 | 15 | citrate-phosphate buffer + chick diet (200 g/L) | 7 | room | 65% | 370 | 2.6 | 3 |
| Rotter & Frohlich, 1989 | ochratoxine-A | activated charcoal | 5 | 15 | citrate-phosphate buffer | 7 | room | 90% | 1800 | 3.25 | 0.6 |
| Tomasevic et al., 2003 | ochratoxine-A | zeolite | 10 | 4 | buffer | 3 | room | 40% | 67 | 1.82 | 15.00 |
| Tomasevic et al., 2003 | ochratoxine-A | organo-zeolites ((O)DMBA at 20-50% of external CEC) | 10 | 4 | buffer | 3 | room | 90-99% | 900-9000 | 2.95-4 | 0.1-1.1 |
| Var et al., 2008 | ochratoxine-A | sodium bentonite | 1 | 0.005-0.01-0.02 | PBS | 7 | 25 | 0-4.7% | … - 50 | … - 1.7 | >20 |
| Var et al., 2008 | ochratoxine-A | sodium bentonite | 0.4 | 0.005-0.01-0.02 | PBS | 7 | 25 | 0-5% | … - 130 | … - 2.1 | >7.6 |
| Var et al., 2008 | ochratoxine-A | sodium bentonite | 0.2 | 0.005-0.01-0.02 | PBS | 7 | 25 | 0-11.1% | … - 620 | … - 2.8 | >1.6 |
| Var et al., 2008 | ochratoxine-A | sodium bentonite | 1 | 0.005-0.01-0.02 | white wine | 3.2 | 25 | 0-23.7% | .... -310 | .... -2.5 | >3.2 |
| Var et al., 2008 | ochratoxine-A | sodium bentonite | 0.4 | 0.005-0.01-0.02 | white wine | 3.2 | 25 | 0-7.5% | .... -200 | .... -2.3 | >5 |
| Var et al., 2008 | ochratoxine-A | sodium bentonite | 0.2 | 0.005-0.01-0.02 | white wine | 3.2 | 25 | 0-19.2% | .... -1200 | .... -3.1 | >0.8 |
| Var et al., 2008 | ochratoxine-A | acivated carbon | 1 | 0.005-0.01-0.02 | PBS | 7 | 25 | 85.4-100% | 5850-…. | 3.8-…. | …-0.17 |
| Var et al., 2008 | ochratoxine-A | acivated carbon | 0.4 | 0.005-0.01-0.02 | PBS | 7 | 25 | 72-98.6% | 6400-180000 | 3.8-5.2 | 0.006-0.16 |
| Var et al., 2008 | ochratoxine-A | acivated carbon | 0.2 | 0.005-0.01-0.02 | PBS | 7 | 25 | 44-90% | 3900-45000 | 3.6-4.6 | 0.02-0.25 |
| Var et al., 2008 | ochratoxine-A | acivated carbon | 1 | 0.005-0.01-0.02 | white wine | 3.2 | 25 | 62.9-98.3% | 1700-58000 | 3.2-4.8 | 0.02-0.60 |
| Var et al., 2008 | ochratoxine-A | acivated carbon | 0.4 | 0.005-0.01-0.02 | white wine | 3.2 | 25 | 32.4-83.3% | 1200-12500 | 3.1-4.1 | 0.08-0.83 |
| Var et al., 2008 | ochratoxine-A | acivated carbon | 0.2 | 0.005-0.01-0.02 | white wine | 3.2 | 25 | 13.4-73.2% | 770-13700 | 2.9-4.1 | 0.07-1.3 |
| Tangni, 2003 | ochratoxine-A | micronised weath fibres | 4 | 0.057 | synthetic medium | 6.2 | 25 | 25%-43% | 90-190 | 1.9-2.3 | 5-12 |
| Tangni, 2003 | ochratoxine-A | micronised weath fibres | 10 | 0.057 | synthetic medium | 6.2 | 25 | 49%-53% | 95-115 | 2-2.1 | 9-11 |
| Tangni, 2003 | ochratoxine-A | micronised weath fibres | 20 | 0.057 | synthetic medium | 6.2 | 25 | 69%-72% | 70-95 | 2.05-2.1 | 8-9 |
| Tangni, 2003 | ochratoxine-A | micronised weath fibres | 30 | 0.057 | synthetic medium | 6.2 | 25 | 68%-74% | 70-95 | 1.85-1.95 | 410-15 |
| Tangni, 2003 | ochratoxine-A | acticated charcoal | 20 | 0.05 | synthetic medium | | 25 | 98% | 2500 | 3.4 | 0.4 |
| Tangni, 2003 | ochratoxine-A | Ceramil (yeast cell walls) | 20 | 0.05 | synthetic medium | | 25 | 53% | 56 | 1.75 | 18 |
| Tangni, 2003 | ochratoxine-A | zeolite Toxy-Nil Plus | 20 | 0.05 | synthetic medium | | 25 | 59% | 72 | 1.9 | 14 |
| Tangni, 2003 | ochratoxine-A | zeolite | 20 | 0.05 | synthetic medium | | 25 | 27% | 18 | 1.3 | 54 |
| Tangni, 2003 | ochratoxine-A | micronised weath fibres | 40 | 2 | wort | | 25 | 40% | 17 | 1.2 | 60 |
| Tangni, 2003 | ochratoxine-A | micronised weath fibres | 150 | 2 | wort | | 25 | 76% | 21 | 1.3 | 47 |
| Tangni, 2003 | ochratoxine-A | micronised weath fibres | 40 | 2 | wort | | 72 | 30% | 9 | 0.9 | 117 |
| Tangni, 2003 | ochratoxine-A | micronised weath fibres | 150 | 2 | wort | | 72 | 60% | 10 | 1 | 100 |
| | | | | | | | | | | | |
| Avantaggiato et al, 2005 | fumonisine B1 | Activated carbon | 1 | 2-20 | phosphate buffers | 3-7-8 | room | 100% | >99000 | >5 | <0.01 |
| Avantaggiato et al, 2005 | fumonisine B1 | Amberlite XAD-2 (non-ionic, hydrophobic) | 1 | 2 | phosphate buffers | 3 | room | 67% | 2000 | 3.3 | 0.5 |
| Avantaggiato et al, 2005 | fumonisine B1 | Amberlite XAD-2 (non-ionic, hydrophobic) | 1 | 20 | phosphate buffers | 3 | room | 25% | 330 | 2.5 | 3 |
| Avantaggiato et al, 2005 | fumonisine B1 | Amberlite XAD-2 (non-ionic, hydrophobic) | 1 | 2-20 | phosphate buffers | 7-8 | room | 1%-20% | <250 | <2.4 | >4 |
| Avantaggiato et al, 2005 | fumonisine B1 | Cholestyramine | 1 | 2-20 | phosphate buffers | 3 | room | 81%-89% | c. 6000 | c. 3.8 | c. 0.17 |
| Avantaggiato et al, 2005 | fumonisine B1 | Cholestyramine | 1 | 2-20 | phosphate buffers | 7-8 | room | 91%-100% | > 9000 | > 3.9 | < 0.1 |
| Avantaggiato et al, 2005 | fumonisine B1 | Dowex 1-X8 (anion exch.resin) | 1 | 20 | | 3 | | 23% | 300 | 2.5 | 3.3 |
| Avantaggiato et al, 2005 | fumonisine B1 | Dowex 1-X8 (anion exch.resin) | 1 | 2 | | 3 | | 55% | 1200 | 3.1 | 0.8 |
| Avantaggiato et al, 2005 | fumonisine B1 | Dowex 1-X8 (anion exch.resin) | 1 | 2-20 | | 7-8 | | 81%-100% | 9000 | 3.95 | 0.1 |
| Avantaggiato et al, 2005 | fumonisine B1 | Dowex Marathon MSA (anion exch.resin) | 1 | 20 | phosphate buffers | 3-7 | room | 22%-33% | c. 400 | c. 2.6 | c. 2.5 |
| Avantaggiato et al, 2005 | fumonisine B1 | Dowex Marathon MSA (anion exch.resin) | 1 | 2 | phosphate buffers | 3-7-8 | room | 42%-51% | c. 1000 | c. 3 | c. 1 |
| Avantaggiato et al, 2005 | fumonisine B1 | Glucomannan | 1 | 2-20 | phosphate buffers | 3 | room | 49%-50% | c. 1000 | c. 3 | c. 1 |
| Avantaggiato et al, 2005 | fumonisine B1 | Glucomannan | 1 | 2-20 | phosphate buffers | 7-8 | room | 2%-18% | c. 40 | c. 1.6 | c. 25 |
| Avantaggiato et al, 2005 | fumonisine B1 | Myco AD A-Z (purified clay) | 1 | 2-20 | phosphate buffers | 3-7-8 | room | 89%-95% | c. 10000 | c. 4 | c. 0.1 |
| Avantaggiato et al, 2005 | fumonisine B1 | Mycofix Plus | 1 | 2-20 | phosphate buffers | 3 | room | 77%-100% | c. 10000 | c. 4 | c. 0.1 |
| Avantaggiato et al, 2005 | fumonisine B1 | Mycofix Plus | 1 | 2-20 | phosphate buffers | 7-8 | room | 1%-18% | c. 100 | c. 2 | c. 10 |
| Avantaggiato et al, 2005 | fumonisine B1 | Mycosorb | 1 | 2-20 | phosphate buffers | 3 | room | 19%-25% | c. 300 | c. 2.6 | c. 3 |
| Avantaggiato et al, 2005 | fumonisine B1 | Mycosorb | 1 | 2-20 | phosphate buffers | 7-8 | room | 1%-6% | c. 40 | c. 1.6 | c. 25 |
| Avantaggiato et al, 2005 | fumonisine B1 | Standard Q/FIS (carbon/aluminosilicate-based product) | 1 | 2-20 | phosphate buffers | 3-7-8 | room | 100% | >99000 | >5 | <0.01 |
| Avantaggiato et al, 2005 | fumonisine B1 | Tixolex 28 | 1 | 2-20 | phosphate buffers | 3 | room | 31%-48% | c. 670 | c. 2.8 | c. 1.5 |
| Avantaggiato et al, 2005 | fumonisine B1 | Tixolex 28 | 1 | 2-20 | phosphate buffers | 7-8 | room | 4%-13% | c. 100 | c. 2 | c. 10 |
| Avantaggiato et al, 2005 | fumonisine B1 | Zeolite | 1 | 2-20 | phosphate buffers | 3 | room | 44%-59% | c. 1000 | c. 3 | c. 1 |
| Avantaggiato et al, 2005 | fumonisine B1 | Zeolite | 1 | 2-20 | phosphate buffers | 7-8 | room | 0%-9% | c. 50 | c. 1.7 | c. 20 |
| | | | | | | | | | | | |

| Single-concentration studies : deoxynivalenol, nivalenol, ergot alkaloids | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **ref** | **toxin** | **binder** | **C(binder)** | **C(toxin)** | **solvent** | **pH** | **T** | **%binding** | **Kd** | **log(Kd)** | **BC50** |
| | | | | | | | **°C** | | **mL/g** | | **g/mL** |
| Galvano et al., 1998 | deoxynivalenol | 3 commercial activated carbons ("CAC1", "CAC2", CAC4") | 2 | 4 | water | | room | 89%-98% | 4000-25000 | 3.6-4.4 | 0.04-0.24 |
| Galvano et al., 1998 | deoxynivalenol | commercial activated carbon ("CAC3") | 2 | 4 | water | | room | c. 14% | 80 | 1.9 | 12 |
| Galvano et al., 1998 | deoxynivalenol | 15 experimental acivated charcoals | 2 | 4 | water | | room | 1.8%-99% | 9->50000 | 0.95-4.7 | 100-0.02 |
| Galvano et al., 1998 | deoxynivalenol | HSCAS | 2 | 4 | water | | room | 3.9% | 21 | 1.3 | 50 |
| Galvano et al., 1998 | deoxynivalenol | sepiolite | 2 | 4 | water | | room | 4.5% | 26 | 1.4 | 40 |
| Tomasevic et al., 2003 | deoxynivalenol | organo-zeolites ((O)DMBA at 20-50% of external CEC) | 10 | 2 | buffer | 3 | room | <1%-9% | <1-10 | <1 | >100 |
| Tomasevic et al., 2003 | deoxynivalenol | zeolite | 10 | 2 | buffer | 3 | room | 1% | 1 | 0.00 | 990.00 |
| Avantaggiato et al, 2005 | deoxynivalenol | Activated carbon | 1 | 2 | phosphate buffers | 3-7-8 | room | 84%-95% | c. 10000 | c. 4 | c. 0.1 |
| Avantaggiato et al, 2005 | deoxynivalenol | Activated carbon | 1 | 10 | phosphate buffers | 3-7-8 | room | 52%-59% | c. 1200 | c. 3.1 | c. 0.8 |
| Avantaggiato et al, 2005 | deoxynivalenol | Standard Q/FIS (carbon/aluminosilicate-based product) | 1 | 2 | phosphate buffers | 3-7-8 | room | 50%-53% | c. 1000 | c. 3 | c. 1 |
| Avantaggiato et al, 2005 | deoxynivalenol | Standard Q/FIS (carbon/aluminosilicate-based product) | 1 | 10 | phosphate buffers | 3-7-8 | room | 13%-18% | c. 180 | c. 2.3 | c. 5.5 |
| Avantaggiato et al, 2005 | deoxynivalenol | Amberlite XAD-2 (non-ionic, hydrophobic) | 1 | 2-10 | phosphate buffers | 3-8 | room | 7% | 75 | 1.88 | 13.29 |
| Avantaggiato et al, 2005 | deoxynivalenol | Bentonite | 1 | 2-10 | phosphate buffers | 3-8 | room | 7% | 75 | 1.88 | 13.29 |
| Avantaggiato et al, 2005 | deoxynivalenol | Celite | 1 | 2-10 | phosphate buffers | 3-8 | room | 5% | 53 | 1.72 | 19.00 |
| Avantaggiato et al, 2005 | deoxynivalenol | Cholestyramine | 1 | 2-10 | phosphate buffers | 3-8 | room | 6% | 64 | 1.81 | 15.67 |
| Avantaggiato et al, 2005 | deoxynivalenol | Dowex 1-X8 (anion exch.resin) | 1 | 2-10 | phosphate buffers | 3-8 | room | 3% | 31 | 1.49 | 32.33 |
| Avantaggiato et al, 2005 | deoxynivalenol | Dowex Marathon MSA (anion exch.resin) | 1 | 2-10 | phosphate buffers | 3-8 | room | 7% | 75 | 1.88 | 13.29 |
| Avantaggiato et al, 2005 | deoxynivalenol | Flo Bond | 1 | 2-10 | phosphate buffers | 3-8 | room | 8% | 87 | 1.94 | 11.50 |
| Avantaggiato et al, 2005 | deoxynivalenol | Florisil | 1 | 2-10 | phosphate buffers | 3-8 | room | 9% | 99 | 2.00 | 10.11 |
| Avantaggiato et al, 2005 | deoxynivalenol | Glucomannan | 1 | 2-10 | phosphate buffers | 3-8 | room | 4% | 42 | 1.62 | 24.00 |
| Avantaggiato et al, 2005 | deoxynivalenol | Microton | 1 | 2-10 | phosphate buffers | 3-8 | room | 6% | 64 | 1.81 | 15.67 |
| Avantaggiato et al, 2005 | deoxynivalenol | Myco AD A-Z (purified clay) | 1 | 2-10 | phosphate buffers | 3-8 | room | 6% | 64 | 1.81 | 15.67 |
| Avantaggiato et al, 2005 | deoxynivalenol | Mycofix Plus | 1 | 2-10 | phosphate buffers | 3-8 | room | 8% | 87 | 1.94 | 11.50 |
| Avantaggiato et al, 2005 | deoxynivalenol | Mycosorb | 1 | 2-10 | phosphate buffers | 3-8 | room | 8% | 87 | 1.94 | 11.50 |
| Avantaggiato et al, 2005 | deoxynivalenol | Ryfix-Toxal | 1 | 2-10 | phosphate buffers | 3-8 | room | 8% | 87 | 1.94 | 11.50 |
| Avantaggiato et al, 2005 | deoxynivalenol | Tixolex 28 | 1 | 2-10 | phosphate buffers | 3-8 | room | 9% | 99 | 2.00 | 10.11 |
| Avantaggiato et al, 2005 | deoxynivalenol | Zeolite | 1 | 2-10 | phosphate buffers | 3-8 | room | 3% | 31 | 1.49 | 32.33 |
| Tangni, 2003 | deoxynivalenol | various micronised fibres (cereals, apple) | 20 | 0.05 | synthetic medium | | 25 | <13% | <7 | <0.9 | >130 |
| Tangni, 2003 | deoxynivalenol | acticated charcoal | 20 | 0.05 | synthetic medium | | 25 | >98% | >2500 | >3.4 | <0.4 |
| Tangni, 2003 | deoxynivalenol | Ceramil (yeast cell walls) | 20 | 0.05 | synthetic medium | | 25 | 15% | 9 | 0.95 | 110 |
| Tangni, 2003 | deoxynivalenol | zeolite Toxy-Nil Plus | 20 | 0.05 | synthetic medium | | 25 | 98% | 2450 | 3.4 | 0.4 |
| | | | | | | | | | | | |
| Avantaggiato et al, 2005 | nivalenol | Activated carbon | 1 | 10 | phosphate buffers | 3-7-8 | room | 23%-33% | c. 400 | c. 2.6 | c. 2.5 |
| Avantaggiato et al, 2005 | nivalenol | Activated carbon | 1 | 2 | phosphate buffers | 3-7-8 | room | 59%-63% | c. 1600 | c. 3.2 | c. 0.6 |
| Avantaggiato et al, 2005 | nivalenol | Standard Q/FIS (carbon/aluminosilicate-based product) | 1 | 2 | phosphate buffers | 3-7-8 | room | 20%-27% | c. 300 | c. 2.5 | c. 3 |
| Avantaggiato et al, 2005 | nivalenol | Standard Q/FIS (carbon/aluminosilicate-based product) | 1 | 10 | phosphate buffers | 3-7-8 | room | 4%-8% | c. 60 | c. 1.8 | c. 17 |
| Avantaggiato et al, 2005 | nivalenol | Amberlite XAD-2 (non-ionic, hydrophobic) | 1 | 2-10 | phosphate buffers | 3-8 | room | 7% | 75 | 1.88 | 13.29 |
| Avantaggiato et al, 2004 | nivalenol | Bentonite | 1 | 2-10 | phosphate buffers | 3-8 | room | 7% | 75 | 1.88 | 13.29 |
| Avantaggiato et al, 2004 | nivalenol | Celite | 1 | 2-10 | phosphate buffers | 3-8 | room | 5% | 53 | 1.72 | 19.00 |
| Avantaggiato et al, 2005 | nivalenol | Cholestyramine | 1 | 2-10 | phosphate buffers | 3-8 | room | 7% | 75 | 1.88 | 13.29 |
| Avantaggiato et al, 2005 | nivalenol | Dowex 1-X8 (anion exch.resin) | 1 | 2-10 | phosphate buffers | 3-8 | room | 4% | 42 | 1.62 | 24.00 |
| Avantaggiato et al, 2005 | nivalenol | Dowex Marathon MSA (anion exch.resin) | 1 | 2-10 | phosphate buffers | 3-8 | room | 8% | 87 | 1.94 | 11.50 |
| Avantaggiato et al, 2004 | nivalenol | Flo Bond | 1 | 2-10 | phosphate buffers | 3-8 | room | 9% | 99 | 2.00 | 10.11 |
| Avantaggiato et al, 2004 | nivalenol | Florisil | 1 | 2-10 | phosphate buffers | 3-8 | room | 9% | 99 | 2.00 | 10.11 |
| Avantaggiato et al, 2005 | nivalenol | Glucomannan | 1 | 2-10 | phosphate buffers | 3-8 | room | 5% | 53 | 1.72 | 19.00 |
| Avantaggiato et al, 2004 | nivalenol | Microton | 1 | 2-10 | phosphate buffers | 3-8 | room | 8% | 87 | 1.94 | 11.50 |
| Avantaggiato et al, 2005 | nivalenol | Myco AD A-Z (purified clay) | 1 | 2-10 | phosphate buffers | 3-8 | room | 7% | 75 | 1.88 | 13.29 |
| Avantaggiato et al, 2005 | nivalenol | Mycofix Plus | 1 | 2-10 | phosphate buffers | 3-8 | room | 10% | 111 | 2.05 | 9.00 |
| Avantaggiato et al, 2005 | nivalenol | Mycosorb | 1 | 2-10 | phosphate buffers | 3-8 | room | 6% | 64 | 1.81 | 15.67 |
| Avantaggiato et al, 2004 | nivalenol | Ryfix-Toxal | 1 | 2-10 | phosphate buffers | 3-8 | room | 6% | 64 | 1.81 | 15.67 |
| Avantaggiato et al, 2005 | nivalenol | Tixolex 28 | 1 | 2-10 | phosphate buffers | 3-8 | room | 9% | 99 | 2.00 | 10.11 |
| Avantaggiato et al, 2005 | nivalenol | Zeolite | 1 | 2-10 | phosphate buffers | 3-8 | room | 2% | 20 | 1.31 | 49.00 |
| | | | | | | | | | | | |
| Tomasevic et al., 2003 | ergocornine | organo-zeolites ((O)DMBA at 20-50% of external CEC) | 10 | 0.5 | buffer | 3 | room | 60-94% | 150-1600 | 2.2-3.2 | 0.62-6.7 |
| Tomasevic et al., 2003 | ergocryptine | organo-zeolites ((O)DMBA at 20-50% of external CEC) | 10 | 0.5 | buffer | 3 | room | 78-98% | 350-5000 | 2.55-3.7 | 0.2-2.9 |
| Tomasevic et al., 2003 | ergosine | organo-zeolites ((O)DMBA at 20-50% of external CEC) | 10 | 0.5 | buffer | 3 | room | 69-96% | 220-5000 | 2.35-3.7 | 0.2-4.5 |
| Tomasevic et al., 2003 | ergocristine | organo-zeolites ((O)DMBA at 20-50% of external CEC) | 10 | 0.5 | buffer | 3 | room | 87-99% | 700-9000 | 2.85-4 | 0.1-1.4 |
| Tomasevic et al., 2003 | ergotamine | organo-zeolites ((O)DMBA at 20-50% of external CEC) | 10 | 0.5 | buffer | 3 | room | 83-99% | 500-9000 | 2.7-4 | 0.1-2 |
| Tomasevic et al., 2003 | ergocornine | zeolite | 10 | 0.5 | buffer | 3 | room | 82% | 456 | 2.66 | 2.20 |
| Tomasevic et al., 2003 | ergocryptine | zeolite | 10 | 0.5 | buffer | 3 | room | 87% | 669 | 2.83 | 1.49 |
| Tomasevic et al., 2003 | ergosine | zeolite | 10 | 0.5 | buffer | 3 | room | 92% | 1150 | 3.06 | 0.87 |
| Tomasevic et al., 2003 | ergocristine | zeolite | 10 | 0.5 | buffer | 3 | room | 94% | 1567 | 3.19 | 0.64 |
| Tomasevic et al., 2003 | ergotamine | zeolite | 10 | 0.5 | buffer | 3 | room | 94% | 1567 | 3.19 | 0.64 |

## Table 5: Results from isotherm studies

| Isotherm studies :aflatoxin, zearalenone | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ref | toxin | binder | C(binder) | C(toxin) | solvent | pH | T | | KF | log(KF) | 1/n | K | Csat | Kd | log(Kd) | BC50 |
| | | | g/L | µg/mL | | | °C | | mL/g | | (Fr.exp.) | Langmuir | mg/g | mL/g | | g/L |
| Grant & Phillips, 1998 | aflatoxin B1 | HSCAS (848 m2/g) | 0.01 | 0.4-8 | water | | 12-25-37 | | 63000 | 4.80 | 0.76 | | 160 | 38000 | 4.58 | 0.026 |
| Grant & Phillips, 1998 | aflatoxin B1 | Col-HSCAS (77 m2/g) | 0.01 | 0.4-8 | water | | 12-25-37 | | | | 0.69 | | 18 | 4300 | 3.63 | 0.233 |
| Lemke et al., 2001 | aflatoxin B1 | charcoal | 0.02 | 0.5-10 | water | | | | | | | 6.23 | | 900000 | 5.95 | 0.00 |
| Lemke et al., 2001 | aflatoxin B1 | charcoal | 0.02 | 0.5-10 | maize extract (4 g/L) | | | | | | | 5.04 | | 70000 | 4.85 | 0.01 |
| Lemke et al., 2001 | aflatoxin B1 | HSCAS | 0.02 | 0.5-10 | water | | | | | | | 5.34 | | 47000 | 4.67 | 0.02 |
| Lemke et al., 2001 | aflatoxin B1 | Clinoptilolite | 0.02 | 0.5-10 | water | | | | | | | 4.9 | | <1000 | <3 | >1 |
| Jaynes et al., 2007 | aflatoxin B1 | activated carbon | 0.002-0.036 | 1 | water | | room | | | | | | 150-200 | >1000000 | >6 | <0.001 |
| Jaynes et al., 2007 | aflatoxin B1 | activated carbon | 0.002-0.036 | 1 | corn meal disp. (0.5 g/mL) | | room | | | | | | | 220 | 2.34 | 4.545 |
| Jaynes et al., 2007 | aflatoxin B1 | NOVASIL clay | 0.002-0.036 | 1 | water | | room | | | | | | c. 200 | 315000 | 5.50 | 0.003 |
| Jaynes et al., 2007 | aflatoxin B1 | NOVASIL clay | 0.002-0.036 | 1 | corn meal disp. (0.5 g/mL) | | room | | | | | | | 1300 | 3.11 | 0.769 |
| Jaynes et al., 2007 | aflatoxin B1 | Saz-1 clay | 0.002-0.036 | 1 | water | | room | | | | | | c. 200 | >1000000 | >6 | <0.001 |
| Jaynes et al., 2007 | aflatoxin B1 | Saz-1 clay | 0.002-0.036 | 1 | corn meal disp. (0.5 g/mL) | | room | | | | | | | 200 | 2.30 | 5.000 |
| Jaynes et al., 2007 | aflatoxin B1 | sepiolite | 0.002-0.036 | 1 | water | | room | | | | | | >= 60 | 88000 | 4.94 | 0.011 |
| Jaynes et al., 2007 | aflatoxin B1 | sepiolite | 0.002-0.036 | 1 | corn meal disp. (0.5 g/mL) | | room | | | | | | | 11400 | 4.06 | 0.088 |
| Jaynes et al., 2007 | aflatoxin B1 | Swy-2 clay | 0.002-0.036 | 1 | water | | room | | | | | | 200-300 | >1000000 | >6 | <0.001 |
| Jaynes et al., 2007 | aflatoxin B1 | Swy-2 clay | 0.002-0.036 | 1 | corn meal disp. (0.5 g/mL) | | room | | | | | | | 960 | 2.98 | 1.042 |
| Di Natale et al., 2009 | aflatoxin M1 | Bentonite | 5-50 | 0.0005 | milk | 6.7-7.7 | 4 | | | | | | | 100 | 2.00 | 10 |
| Di Natale et al., 2009 | aflatoxin M1 | Filtrasorb 400 Carbon | 5-50 | 0.0005 | milk | 6.7-7.7 | 4 | | | | | | | 67 | 1.82 | 15 |
| Di Natale et al., 2009 | aflatoxin M1 | Norit GCN 1240 Carbon | 5-50 | 0.0005 | milk | 6.7-7.7 | 4 | | | | | | | 67 | 1.82 | 15 |
| Di Natale et al., 2009 | aflatoxin M1 | Acquacarb 207 EA Carbon | 5-50 | 0.0005 | milk | 6.7-7.7 | 4 | | | | | | | 50 | 1.70 | 20 |
| Ramos & Hernandez, 1996 | aflatoxin B1 | montmorillonite | 10 | 0.25-8 | water | 7 | 25-37 | | 1057-952 | 3.02-2.98 | 0.89-0.81 | | | | | 9.5-10.5 |
| Ramos & Hernandez, 1996 | aflatoxin G1 | montmorillonite | 10 | 0.25-8 | water | 7 | 25-37 | | 454-426 | 2.66-2.63 | 0.86-0.84 | | | | | 2.2-2.3 |
| Ramos & Hernandez, 1996 | aflatoxin G2 | montmorillonite | 10 | 0.25-8 | water | 7 | 25-37 | | 236-233 | 2.37-2.37 | 0.90-0.96 | | | | | 4.3-4.3 |
| Ramos & Hernandez, 1996 | aflatoxin B2 | montmorillonite | 10 | 0.25-8 | water | 7 | 25-37 | | 203-203 | 2.31 | 0.84-0.86 | | | | | 5 |
| Desheng et al., 2005 | aflatoxin B1 | montmorillonite | 5 | 0.01-2 | water | 2 | 37 | | | | | | c. 1 | c. 4000 | c. 3.6 | c. 0.25 |
| Desheng et al., 2005 | aflatoxin B1 | montmorillonite | 5 | 0.01-2 | water | 8 | 37 | | | | | | c. 1 | c. 2000 | c. 3.3 | c. 0.5 |
| Tomasevic et al., 2003 | aflatoxin B1 | organo-zeolites ((O)DMBA at 20-50% of external CEC) | 2 | 1-4 | buffer | 7 | room | | | | | | | 3200-3800 | 3.5-3.6 | 0.26-0.31 |
| Vekiru et al., 2007 | aflatoxin B1 | HSCAS | 0.02 | 0.4-8 | phosphate buffer | 7 | 37 | 732000 | 89000 | 4.95 | | | 122 | | | |
| Vekiru et al., 2007 | aflatoxin B1 | 9 bentonite clays | 0.02 | 0.4-8 | phosphate buffer | 7 | 37 | 442000-1530000 | 33000-167000 | 4.5-5.2 | | | 66-131 | | | |
| Shi et al., 2006 | aflatoxin B1+B2+G1+G2 | montmorillonite nanocomposite | 5 | 20-100 of each | water | 7 | 37 | | | | | | 66.7 | 850 | 2.9 | 1.20 |
| | | | | | | | | | | | | | | | | |
| Avantaggiato et al, 2004 | zearalenone | activated carbon | 1 | 2-20 (?) | phosphate buffers | 7 | room | | | | | | 105 | | | |
| Avantaggiato et al, 2005 | zearalenone | activated carbon | 1 | 1-50 (?) | phosphate buffers | 7 | room | | | | | | 112 | | | |
| Avantaggiato et al, 2005 | zearalenone | cholestyramine | 1 | 1-100 (?) | phosphate buffers | 7 | room | | | | | | 34 | | | |
| Avantaggiato et al, 2005 | zearalenone | Myco AD A-Z | 1 | 1-400 (?) | phosphate buffers | 7 | room | | | | | | 57 | | | |
| Avantaggiato et al, 2005 | zearalenone | Standard Q/FIS (carbon/aluminosilicate-based product) | 1 | 1-50 (?) | phosphate buffers | 7 | room | | | | | | 66 | | | |
| Feng et al., 2008 | zearalenone | Ca-montmorilllonite | 1 | 1-10 | phosphate buffers | 7 | 37 | | 180 | 2.276 | 0.4616 | | 0.598 | | | 5.56 |
| Feng et al., 2008 | zearalenone | CTAB-montmorilllonite | 1 | 1-10 | phosphate buffers | 7 | 37 | | 5090 | 3.705 | 0.4879 | | 8.83 | | | 0.20 |
| Lemke et al., 1998 | zearalenone | Antitox Plus | 1 | 0.5-4 | water | 6.5 | 37 | | 8.46 | 0.927 | 0.458 | | | | | |
| Lemke et al., 1998 | zearalenone | non modified clay | 1 | 0.5-4 | water | 6.5 | 37 | | 0.283 | -0.548 | 0.207 | | | | | |
| Lemke et al., 1998 | zearalenone | organic(BTEA)-modified clay (100% CEC) | 1 | 0.5-4 | water | 6.5 | 37 | | 0.0234 | -1.631 | 0.047 | | | | | |
| Lemke et al., 1998 | zearalenone | organic(CP)-modified clay (150% CEC) | 1 | 0.5-4 | water | 6.5 | 37 | | 3260 | 3.513 | 0.959 | | | | | |
| Lemke et al., 1998 | zearalenone | organic(CP)-modified clay (150% CEC) | 1 | 0.5-4 | buffer (?) | 2 | 37 | | 2150 | 3.332 | 0.797 | | | | | |
| Lemke et al., 1998 | zearalenone | organic(CP)-modified clay (150% CEC) | 1 | 0.5-4 | buffer (?) | 10 | 37 | | 33.5 | 1.525 | 0.46 | | | | | |
| Lemke et al., 1998 | zearalenone | organic(CP)-modified clay (25% CEC) | 1 | 0.5-4 | water | 6.5 | 37 | | 0.0493 | -1.307 | 0.046 | | | | | |
| Lemke et al., 1998 | zearalenone | organic(CP)-modified clay (50% CEC) | 1 | 0.5-4 | water | 6.5 | 37 | | 10.5 | 1.021 | 0.427 | | | | | |
| Lemke et al., 1998 | zearalenone | organic(CP)-modified clay (75% CEC) | 1 | 0.5-4 | water | 6.5 | 37 | | 732 | 2.865 | 0.733 | | | | | |
| Lemke et al., 1998 | zearalenone | organic(CP)-modified clay (100% CEC) | 1 | 0.5-4 | water | 6.5 | 37 | | 2630 | 3.420 | 0.788 | | | | | |
| Lemke et al., 1998 | zearalenone | organic(DDPP)-modified clay (100% CEC) | 1 | 0.5-4 | water | 6.5 | 37 | | 817 | 2.912 | 0.745 | | | | | |
| Lemke et al., 1998 | zearalenone | organic(DDTMA)-modified clay (100% CEC) | 1 | 0.5-4 | water | 6.5 | 37 | | 75600 | 4.879 | 1.131 | | | | | |
| Lemke et al., 1998 | zearalenone | organic(HDTMA)-modified clay (100% CEC) | 1 | 0.5-4 | water | 6.5 | 37 | | 63900 | 4.806 | 1.05 | | | | | |
| Lemke et al., 1998 | zearalenone | organic(TDTMA)-modified clay (100% CEC) | 1 | 0.5-4 | water | 6.5 | 37 | | 3450 | 3.538 | 0.835 | | | | | |
| Lemke et al., 1998 | zearalenone | organic(TMA)-modified clay | 1 | 0.5-4 | water | 6.5 | 37 | | 8.91 | 0.950 | 0.546 | | | | | |
| Lemke et al., 1998 | zearalenone | PVP | 1 | 0.5-4 | water | 6.5 | 37 | | 663 | 2.822 | 0.914 | | | | | |
| Lemke et al., 1998 | zearalenone | sepiolite | 1 | 0.5-4 | water | 6.5 | 37 | | 0.125 | -0.903 | 0.178 | | | | | |
| Ramos et al, 1996 | zearalenone | bentonite | 50 | 1-10 | simulated intestinal fluid | 7.5 | 37 | | 112 | 2.05 | 1.08 | | | | | 8.9 |
| Ramos et al, 1996 | zearalenone | cholestyramine | 50 | 1-10 | simulated intestinal fluid | 7.5 | 37 | | n.d. (>) | | | | | | | |
| Ramos et al, 1996 | zearalenone | crospovidone | 50 | 1-10 | simulated intestinal fluid | 7.5 | 37 | | 314 | 2.50 | 0.98 | | | | | 3.2 |
| Ramos et al, 1996 | zearalenone | magnesium trisilicate | 50 | 1-10 | simulated intestinal fluid | 7.5 | 37 | | 23 | 1.36 | 0.75 | | | | | 43 |
| Ramos et al, 1996 | zearalenone | montmorillonite | 50 | 1-10 | simulated intestinal fluid | 7.5 | 37 | | 192 | 2.28 | 0.59 | | | | | 5.2 |
| Ramos et al, 1996 | zearalenone | sepiolite | 50 | 1-10 | simulated intestinal fluid | 7.5 | 37 | | 74 | 1.87 | 1.03 | | | | | 14 |
| Tomasevic et al., 2003 | zearalenone | organo-zeolites ((O)DMBA at 20-50% of external CEC) | 2 | 1-4 | buffer | 7 | room | | | | | | | 3700-3900 | 3.6 | 0.26-0.27 |
| Yiannikouris et al, 2003 | zearalenone | yeast cell walls | 0.1 | 2-20 | water | | 37 | | | | | | | 1000-2000 | 3-3.3 | |
| Debongnie et al., in prep. | zearalenone | micronized vegetal fibers | 10 | 0.05-5 | phosphate buffer | 3-6.5 | 37 | | 75-120 | 1.9-2.1 | 0.87-0.97 | | | | | 8-13 |
| Debongnie et al., in prep. | zearalenone | bentonite | 10 | 0.05-5 | phosphate buffer | 3-6.5 | 37 | | 100-600 | 2-2.8 | 0.97-1 | | | | | 1.7-10 |
| Debongnie et al., in prep. | zearalenone | clinoptilolite | 10 | 0.05-5 | phosphate buffer | 3-6.5 | 37 | | 40-55 | 1.6-1.7 | 0.76-0.82 | | | | | 18-24 |
| | | | | | | | | | | | | | | | | |

| Isotherm studies :ochratoxine, fumonisine, deoxynivalenol, nivalenol | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ref | toxin | binder | C(binder) | C(toxin) | solvent | pH | T | | KF | log(KF) | 1/n | K | Csat | Kd | log(Kd) | BC50 |
| | | | g/L | µg/mL | | | °C | | mL/g | | (Fr.exp.) | Langmuir | mg/g | mL/g | | g/L |
| Dakovic et al., 2003 | ochratoxine-A | organo-zeolite (ODMBA at 100% of external CEC) | 0.8 | 0.5-3 | phosphate buffers | 3-7-9 | room | | | | | | c. 3.5 | c. 10000 | c. 4 | c. 0.1 |
| Dakovic et al., 2003 | ochratoxine-A | organo-zeolite (ODMBA at 50% of external CEC) | 0.8 | 0.5-3 | phosphate buffers | 3-7-9 | room | | | | | | c. 2.5 | c. 5000 | c. 3.7 | c. 0.2 |
| Dakovic et al., 2003 | ochratoxine-A | organo-zeolite (ODMBA at 20% of external CEC) | 0.8 | 0.5-3 | phosphate buffers | 3-7-9 | room | | | | | | c. 1.5 | c. 1000 | c. 3 | c. 1 |
| Tomasevic et al., 2003 | ochratoxine-A | organo-zeolites ((O)DMBA at 20-50% of external CEC) | 2 | 1-4 | buffer | 7 | room | | | | | | | 1900-3600 | 3.3-3.6 | 0.28-0.53 |
| Ringot et al., 2007 | ochratoxine-A | vinasse "EX16" (16% yeast cell walls) | 50 | 500-10000 | water | | 25 | | 202 | 2.305 | 2.048 | | | | | 4.95 |
| Ringot et al., 2007 | ochratoxine-A | dried purified beta-glucan from yeast cell wall | 50 | 500-10000 | water | | 25 | | 16 | 1.204 | 1.03 | | | | | 62.50 |
| Ringot et al., 2007 | ochratoxine-A | "LEC" dry yeast cell wall fraction (industry by-product) | 50 | 500-10000 | water | | 25 | | 13 | 1.114 | 1.039 | | | | | 76.92 |
| | | | | | | | | | | | | | | | | |
| Avantaggiato et al, 2005 | fumonisine B1 | activated carbon | 1 | 1-100 (?) | phosphate buffers | 7 | room | | | | | | 124 | | | |
| Avantaggiato et al, 2005 | fumonisine B1 | Standard Q/FIS (carbon/aluminosilicate-based product) | 1 | 1-500 (?) | phosphate buffers | 7 | room | | | | | | 390 | | | |
| Avantaggiato et al, 2005 | fumonisine B1 | Myco AD A-Z | 1 | 1-100 (?) | phosphate buffers | 7 | room | | | | | | 45 | | | |
| Avantaggiato et al, 2005 | fumonisine B1 | cholestyramine | 1 | 1-100 (?) | phosphate buffers | 7 | room | | | | | | 190 | | | |
| | | | | | | | | | | | | | | | | |
| Avantaggiato et al, 2004 | deoxynivalenol | activated carbon | 1 | 0.5-20 | phosphate buffers | 7 | room | | 2200 | 3.34 | | | 10.4 | | | 0.45 |
| Avantaggiato et al, 2005 | deoxynivalenol | Standard Q/FIS (carbon/aluminosilicate-based product) | 1 | 1-25 (?) | phosphate buffers | 7 | room | | | | | | 1.6 | | | |
| | | | | | | | | | | | | | | | | |
| Avantaggiato et al, 2004 | nivalenol | activated carbon | 1 | 0.5-20 | phosphate buffers | 7 | room | | 980 | 2.99 | | | 2.75 | | | 1.02 |
| Avantaggiato et al, 2005 | nivalenol | Standard Q/FIS (carbon/aluminosilicate-based product) | 1 | 1-25 (?) | phosphate buffers | 7 | room | | | | | | 0.6 | 250 | 2.398 | 4 |

## Table 6: Results from gastro-intestinal tract model experiments

| Experimental gastro-intestinal tract model studies | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ref | toxin | binder | model, pH | effect measured | C(binder) | C(toxin) | solvent | C(toxin) | matrix | T | %binding | Kd | log(Kd) | EC50 |
| | | | | | g/L | µg/mL | | mg/kg | | °C | | mL/g | | g/L |
| Lemke et al., 2001 | aflatoxin B1 | charcoal | 2h pH 1.3 with pepsin, 2h pH 7 with pancreatin and bile salts | free toxin | 3.1 | 10 | G-I solutions | | | 37 | >99% | >30000 | >4.5 | <0.01 |
| Lemke et al., 2001 | aflatoxin B1 | HSCAS | 2h pH 1.3 with pepsin, 2h pH 7 with pancreatin and bile salts | free toxin | 3.1 | 10 | G-I solutions | | | 37 | c.96% | c. 8000 | c. 3.9 | c. 0.1 |
| Lemke et al., 2001 | aflatoxin B1 | clinoptilolite | 2h pH 1.3 with pepsin, 2h pH 7 with pancreatin and bile salts | free toxin | 3.1 | 10 | G-I solutions | | | 37 | c.34% | c. 150 | c. 2.15 | c. 7 |
| | | | | | | | | | | | | | | |
| Avantaggiato et al., 2003 | zearalenone | cholestyramine | TNO model / dose-resp. exp. / pH : 7 decr. to 2 (gastr.), 6.5-7.2 (intest.) | absorption in jejunal+ileal dialysate fluids | 2.5-5-10-20 | | G-I solutions | 4.1 | wheat | 37 | | | | c. 20 |
| Avantaggiato et al., 2003 | zearalenone | acivated carbon (Sigma) | TNO model / dose-resp. exp. / pH : 7 decr. to 2 (gastr.), 6.5-7.2 (intest.) | absorption in jejunal+ileal dialysate fluids | 2.5-5-10-20 | | G-I solutions | 4.1 | wheat | 37 | | | | c. 3 |
| Döll et al., 2004 | zearalenone | activated carbon | 2h pH 5, 2h pH 3, 2h pH 6, 3h pH 7 | free toxin | 0.82 | 0.334 | phosphate-citrate buffer | | | 37 | 100% | >100000 | >5 | <0.01 |
| Döll et al., 2004 | zearalenone | cholestyramine | 2h pH 5, 2h pH 3, 2h pH 6, 3h pH 7 | free toxin | 0.82 | 0.334 | phosphate-citrate buffer | | | 37 | 94% | 19106 | 4.28 | 0.052 |
| Döll et al., 2004 | zearalenone | modified aluminosilicate | 2h pH 5, 2h pH 3, 2h pH 6, 3h pH 7 | free toxin | 0.82 | 0.334 | phosphate-citrate buffer | | | 37 | 81% | 5199 | 3.72 | 0.192 |
| Döll et al., 2004 | zearalenone | Toxisorb | 2h pH 5, 2h pH 3, 2h pH 6, 3h pH 7 | free toxin | 0.82 | 0.334 | phosphate-citrate buffer | | | 37 | 55% | 1491 | 3.17 | 0.671 |
| Döll et al., 2004 | zearalenone | Mykosorb Extra | 2h pH 5, 2h pH 3, 2h pH 6, 3h pH 7 | free toxin | 0.82 | 0.334 | phosphate-citrate buffer | | | 37 | 24% | 385 | 2.59 | 2.597 |
| Döll et al., 2004 | zearalenone | Klinosan | 2h pH 5, 2h pH 3, 2h pH 6, 3h pH 7 | free toxin | 0.82 | 0.334 | phosphate-citrate buffer | | | 37 | 20% | 305 | 2.48 | 3.280 |
| Döll et al., 2004 | zearalenone | Mycofix Plus | 2h pH 5, 2h pH 3, 2h pH 6, 3h pH 7 | free toxin | 0.82 | 0.334 | phosphate-citrate buffer | | | 37 | 17% | 250 | 2.40 | 4.004 |
| Döll et al., 2004 | zearalenone | Bentonite | 2h pH 5, 2h pH 3, 2h pH 6, 3h pH 7 | free toxin | 0.82 | 0.334 | phosphate-citrate buffer | | | 37 | 13% | 182 | 2.26 | 5.488 |
| Döll et al., 2004 | zearalenone | Fix A Tox | 2h pH 5, 2h pH 3, 2h pH 6, 3h pH 7 | free toxin | 0.82 | 0.334 | phosphate-citrate buffer | | | 37 | 5% | 64 | 1.81 | 15.580 |
| Döll et al., 2004 | zearalenone | Likratox | 2h pH 5, 2h pH 3, 2h pH 6, 3h pH 7 | free toxin | 0.82 | 0.334 | phosphate-citrate buffer | | | 37 | 5% | 64 | 1.81 | 15.580 |
| Döll et al., 2004 | zearalenone | cholestyramine | 2h pH 5, 2h pH 3, 2h pH 6, 3h pH 7 / dose-resp. exp. | free toxin | | | | | | | | | | 0.0887 |
| Döll et al., 2004 | zearalenone | aluminosilicate | 2h pH 5, 2h pH 3, 2h pH 6, 3h pH 7 / dose-resp. exp. | free toxin | | | | | | | | | | 0.3548 |
| Döll et al., 2004 | zearalenone | Toxisorb | 2h pH 5, 2h pH 3, 2h pH 6, 3h pH 7 / dose-resp. exp. | free toxin | | | | | | | | | | 1.0644 |
| Döll et al., 2004 | zearalenone | Mykosorb Extra | 2h pH 5, 2h pH 3, 2h pH 6, 3h pH 7 / dose-resp. exp. | free toxin | | | | | | | | | | 6.7412 |
| Döll et al., 2004 | zearalenone | Bentonite | 2h pH 5, 2h pH 3, 2h pH 6, 3h pH 7 / dose-resp. exp. | free toxin | | | | | | | | | | 52.2443 |
| | | | | | | | | | | | | | | |
| Avantaggiato et al, 2004 | deoxynivalenol | activated carbon | TNO model / dose-resp. exp. / pH : 7 decr. to 2 (gastr.), 6.5-7.2 (intest.) | absorption in jejunal+ileal dialysate fluids | 5-10-20 | | G-I solutions | 2.8 | wheat | 37 | | | | >= 20 |
| Döll et al., 2004 | deoxynivalenol | activated carbon | 2h pH 5, 2h pH 3, 2h pH 6, 3h pH 7 | free toxin | 0.82 | 3.34 | phosphate-citrate buffer | | | 37 | 67% | 0 | -1.34 | 22000 |
| Döll et al., 2004 | deoxynivalenol | cholestyramine | 2h pH 5, 2h pH 3, 2h pH 6, 3h pH 7 | free toxin | 0.82 | 3.34 | phosphate-citrate buffer | | | 37 | 10% | 136 | 2.13 | 7.380 |
| Döll et al., 2004 | deoxynivalenol | modified aluminosilicate | 2h pH 5, 2h pH 3, 2h pH 6, 3h pH 7 | free toxin | 0.82 | 3.34 | phosphate-citrate buffer | | | 37 | 17% | 250 | 2.40 | 4.004 |
| Döll et al., 2004 | deoxynivalenol | Toxisorb | 2h pH 5, 2h pH 3, 2h pH 6, 3h pH 7 | free toxin | 0.82 | 3.34 | phosphate-citrate buffer | | | 37 | 1% | 12 | 1.09 | 81.180 |
| Döll et al., 2004 | deoxynivalenol | Mykosorb Extra | 2h pH 5, 2h pH 3, 2h pH 6, 3h pH 7 | free toxin | 0.82 | 3.34 | phosphate-citrate buffer | | | 37 | 24% | 385 | 2.59 | 2.597 |
| Döll et al., 2004 | deoxynivalenol | Klinosan | 2h pH 5, 2h pH 3, 2h pH 6, 3h pH 7 | free toxin | 0.82 | 3.34 | phosphate-citrate buffer | | | 37 | 0% | 0 | <1 | >80 |
| Döll et al., 2004 | deoxynivalenol | Mycofix Plus | 2h pH 5, 2h pH 3, 2h pH 6, 3h pH 7 | free toxin | 0.82 | 3.34 | phosphate-citrate buffer | | | 37 | 1% | 12 | 1.09 | 81.180 |
| Döll et al., 2004 | deoxynivalenol | Bentonite | 2h pH 5, 2h pH 3, 2h pH 6, 3h pH 7 | free toxin | 0.82 | 3.34 | phosphate-citrate buffer | | | 37 | 1% | 12 | 1.09 | 81.180 |
| Döll et al., 2004 | deoxynivalenol | Fix A Tox | 2h pH 5, 2h pH 3, 2h pH 6, 3h pH 7 | free toxin | 0.82 | 3.34 | phosphate-citrate buffer | | | 37 | 21% | 324 | 2.51 | 3.085 |
| Döll et al., 2004 | deoxynivalenol | Likratox | 2h pH 5, 2h pH 3, 2h pH 6, 3h pH 7 | free toxin | 0.82 | 3.34 | phosphate-citrate buffer | | | 37 | 2% | 25 | 1.40 | 40.180 |
| | | | | | | | | | | | | | | |
| Avantaggiato et al, 2004 | nivalenol | activated carbon | TNO model / dose-resp. exp. / pH : 7 decr. to 2 (gastr.), 6.5-7.2 (intest.) | absorption in jejunal+ileal dialysate fluids | 5-10-20 | | G-I solutions | 3.8 | wheat | 37 | | | | >= 20 |

## 3.2. *In vitro* mechanisms of action of biotransforming agents

When mycotoxin formation cannot be avoided, some remediation strategies can also be used to reduce its levels on products, its bioavailability or its toxic effects. Among these remediation strategies, biological methods are at the forefront, being widely studied over the last few years. As a result, a great number of microorganisms that can degrade or detoxify several mycotoxins have been reported (Abrunhosa et al., 2009).

Biological detoxification is regarded as the biotransformation or degradation of the toxin by microorganisms/enzymes to produce metabolites that are either non-toxic when ingested by animals or less toxic than the parent toxin molecule.

### 3.2.1. Bacteria

Bacteria were screened for their ability to modify or inactivate the different types of mycotoxins.

#### 3.2.1.1. Aflatoxin

Many bacteria in soil are able to degrade aflatoxins (Wu et al., 2009).
Ciegler et al. reported that *Flavobacterium aurantiacum* (NRRL B-184), a kind of bacteria from soils and water, was able to remove aflatoxin from a liquid medium significantly without producing toxic by-products and showed a very high capability for detoxifying aflatoxins. The aflatoxin-contaminated substance and *F. aurantiacum* NRRL B-184 were mixed together and incubated at 28°C for 12 hours. All of the aflatoxin G was removed, as well as a part of aflatoxin B, which was diminished (Ciegler et al., 1966).
Later, it was observed that the radioactively labeled 14C-AFB1 was partially metabolized and partially adsorbed to *F. aurantiacum* cells (Line and Brackett, 1995).

Other microorganisms were also tested for their possible ability to degrade aflatoxins. The strain *Nocardia asteroids* reduced AFB1 by biotransformation to another fluorescent product, and *Corynebacterium rubrum* was able to detoxify aflatoxin as well.

In another work, *Mycobacterium fluoranthenivorans* sp. nov. DSM44556T isolated from soils of a former coal gas plant, which was polluted with polycyclic aromatic hydrocarbons, was found to be able to degrade AFB1 as a single carbon source. The AFB1 concentration was reduced to 70–80% of the initial concentration within 36 hours, and no AFB1 was detectable after 72 hours. In addition, the cell-free extracts of *M. fluoranthenivorans* sp. nov. DSM44556T degraded AFB1 more efficiently. More than 90% of the initial amount of AFB1 was degraded at 30°C within 4 hours, and no AFB1 was detected after 8 hours (Wu et al., 2009).

*Rhodococcus erythropolis* isolated from polycyclic aromatic hydrocarbon (PAH) soils was investigated for AFB1 degradation activity. Dramatic reduction of AFB1 was observed during incubation in the presence of *R. erythropolis* cells. Then, 17% residual AFB1 was left after 48 hours and only 3–6% was detectable after 72 hours. In addition, this research team also found that AFB1 was effectively degraded by extracellular extracts from *R. erythropolis* (only 32% residual AFB1 was detectable after 72 hours) (Wu et al., 2009).

The enzyme peroxidase seems to be participating in this mode of aflatoxin degradation.

### 3.2.1.2. T-2 toxin

Several experiments were performed to study the effects of bacterial communities on T-2 toxin and other trichothecenes toxin.

Beeton and Bull have investigated the role of natural bacterial communities and monocultures as agents for the detoxification and biodegradation of T-2 toxin and related trichothecenes. Bacterial communities capable of detoxification and biodegradation were enriched from soil and freshwater samples, collected from different sites such as leaf litter and soils, rivers, effluent water from screen paper manufacture, and soils. The experiments showed that 85% of the soil and water isolates screened were able to use T-2 toxin as a sole source of carbon and energy and that this activity was associated with the removal of T-2 toxicity. Two communities, TS4 and KS10, degraded the trichothecene nucleus within 24 to 48 h (Beeton and Bull, 1989).

The major degradation pathway of T-2 toxin in most of isolates involved side chain cleavage of acetyl moieties to produce HT-2 toxin and T-2 triol. A minor degradation pathway of T-2 toxin that involved conversion to neosolaniol and thence to 4-deacetyl neosolaniol was also detected. Co-metabolic interaction between species is suggested as a significant factor in T-2 toxin degradation.

Ueno et al. used *Curtobacterium* sp. strain 114-2, isolated as a T-2 toxin-assimilating bacterium, in this experiment. During incubation with *Curtobacterium* sp. strain 114-2 in basal medium T-2 toxin was converted into T-2 triol via HT-2 toxin and the resulting T-2 triol was further assimilated by the bacterium without formation of neosolaniol and T-2 tetraol. This study indicated that *Curtobacterium* sp. and other bacteria found in soil were capable of degrading toxic trichothecenes (Ueno et al., 1983).

Furthermore, Fuchs et al. investigated whether the bacterial strain BBSH 797, a microbial feed additive, could degrade the following trichothecene mycotoxins: T-2 toxin, HT-2 toxin, T-2 triol, T-2 tetraol, scirpentriol and diacetoxyscirpenol (Fuchs et al., 2002).
Products which would appear after degradation were also observed. The active biotransforming bacterial strain BBSH 797 was isolated from rumen fluid. It is a Gram-positive, non-spore-forming irregular rod which is a strictly anaerobic organism. It is 0.2-0.4 x 1-1.5 µm and occurs singly and in long chains up to 100 µm.
In the case of treatment of T-2 toxin with BBSH 797, T-2 toxin was partially hydrolyzed into HT-2 toxin. The metabolism of this transformation could be a deacetylation.
Treating HT-2 toxin with BBSH 797 caused almost complete transformation into its deepoxy form.
The result of the microbial transformation of T-2 tetraol was a complete degradation into deepoxy T-2 tetraol.
The results of the characterization of the metabolites of T-2 triol showed clearly that the microbial transformation resulted in a detoxification into deepoxy T-2 triol.
The results indicated that scirpentriol transformed into non-toxic deepoxyscirpentriol metabolite.
Observing the mechanism of microbial detoxification of type A trichothecenes by BBSH 797, two important steps can be highlighted. In most cases, the first step is a transformation of an acetyl group into a hydroxyl group or the hydrolysis of other ester groups. The second step is important for detoxification, namely the transformation of the epoxide into a double bond.

### 3.2.1.3. Deoxynivalenol

Deoxynivalenol is enzymatically reduced by an epoxidase of *Eubacterium* BBSH 797 to the metabolite deepoxy-deoxynivalenol (DOM-1) which is known to be non-toxic. Indeed, the metabolite

DOM-1 is 500 times less toxic than DON. This strain was isolated out of bovine rumen fluid. The mode of action was proven *in vitro* (Fuchs et al., 2002).
In the case of trichothecenes it is known, that the 12,13-epoxide ring is responsible for their toxic effects and removal of this ring results in a significant loss of toxicity (Schatzmayr et al., 2006).

Young et al. studied the biodegradation of DON and other trichothecenes by chicken intestinal microorganisms. The sub-cultures of the mixed microbes or isolates LS100 and SS3 were used for the biodegradation assays and studies showed that all twelve trichothecenes were metabolized. Deepoxidation seemed to be the predominant biochemical metabolic reaction (Young et al., 2007).

Microbial treatment of the acylated trichothecenes turned out differently. In the presence of an acetyl group (e.g. 3ADON, 15ADON, and FUS), deacetylation was greatly favored over deepoxidation.Pure cultures of microbial isolates from single colonies are therefore able to degrade a variety of trichothecene mycotoxins through deepoxidation and deacetylation functions and the route depends upon the presence and position of acyl functionalities.

#### 3.2.1.4. Zearalenone

Megharaj et al. have investigated the possibility of eliminating ZEA by a mixed culture of bacteria from soil. When ZEA was supplied in combination with phenanthrene, an easily usable carbon source, the ZEA was almost completely degraded (only 0.4% recovered). The increased degradation of ZEA in the presence of phenanthrene was associated with increased bacterial growth (40% greater bacterial protein) suggesting phenanthrene might be acting as a co-substrate in metabolism of ZEA (Megharaj et al., 1997).
These findings demonstrated biodegradation of ZEA by a mixed culture (*Alcaligenes*, *Bacillus*, *Achromobacter*, *Flavobacterium*, and *Pseudomonas)* and showed its ability to use ZEA as a carbon and energy source.

Furthermore, total biodegradation of ZEA by a soil bacterial mixture was reported recently as the first report of complete ZEA degradation by bacteria. HPLC and ELISA analyses of the culture extracts revealed no ZEA or ZEA-like products and means that transformation of ZEA to the more estrogenic zearalenol was not observed (Styriak and Conkova, 2002).

#### 3.2.1.5. Ochratoxin A

The degradation of OTA was described by Hult et al., and it was concluded that OTA was cleaved into the non-toxic ochratoxin α and phenylalanine by rumen content (Hult et al., 1976).
The disappearance of OTA from the free solution was at first very rapid. Indeed, 50% of added OTA disappeared in less than 15 min. A slower phase followed, and after 4 h, less than 5% of added OTA remained in the solution. It was possible to detect the formation of ochratoxin α in the solution after 1 to 4 h. 40% of added OTA was found as ochratoxin α.

#### 3.2.1.6. Fumonisin B1

There is a lack of information on the effect of swine caecal microbiota on fumonisin metabolism. In an *in vitro* study, the biotransformation of FB1 by the gut microbiota of pigs was examined. Suspensions of caecal contents were incubated anaerobically with pure FB1 for 0, 12, 24, 48 and 72 h. The caecal chyme contained 1.6 ± 0.23 x $10^6$ and 5.8± 0.4 x $10^8$ *Escherichia coli* and *Bacteroides* spp., respectively. After 48 h, the conversion of FB1 to partially hydrolysed FB1 (46%) was nearly equal to the percentage ratio of FB1, while by 72 h it was 49%. *In vitro*, the conversion of FB1 to

aminopentol was less than 1%. The results show that the caecal microbiota are capable of transforming FB1 to the above metabolites (Fodor et al., 2007).

#### 3.2.1.7. All toxins

Kiessling et al. reported the ability of rumen microorganisms from sheep to degrade important mycotoxins *in vitro*. They showed that DAS, T-2 toxin, OTA, and ZEA were degraded by rumen microorganisms, but that the protozoa are more active than the bacteria (Kiessling et al., 1984).
In the presence of OTA and ZEA, the bacterial fraction is almost inactive. AFB1 and DON were not degraded by rumen microorganisms. OTA was cleaved primarily into ochratoxin α and phenylalanine. This is a detoxification process because ochratoxin α is nontoxic as regards renal effects in pigs. More than 90% of the ZEA was degraded to zearalenol, and about twice as much α-zearalenol as β-zearalenol was formed. Zearalenol has three to four times as much estrogenic activity as does the parent compound.
DAS was rapidly and completely deacetylated to monoacetoxyscirpenol. T-2 toxin was similarly deacetylated to HT-2 toxin.
Monoacetoxyscirpenol and HT-2 toxin are less active inhibitors of protein synthesis than their mother compounds.

### 3.2.2. Fungi

A number of fungal species have been shown to be capable of degrading mycotoxins.

#### 3.2.2.1. Aflatoxin

Nakazato et al. screened four fungal strains and reported that *Aspergillus niger*, *Eurotium herbariorum*, a *Rhizopus sp*., and nonaflatoxin-producing *A. flavus*, were able to convert $AFB_1$ to aflatoxicol (AFL) by reducing the cyclopentenone carbonyl of AFB1. AFB1 was converted into aflatoxicol-A (AFL-A), which was then converted into aflatoxicol-B (AFL-B) by the actions of medium components or organic acids produced from the fungi (Nakazato et al., 1990).

Fungi *A. niger* is able to convert AFL into AFB1, which can be converted further to AFB2a. However, the sum of AFL and AFB1 was found to decrease with time, which suggested that both AFB1 and AFL were further metabolized to unknown substances by the fungi (see Figure below).

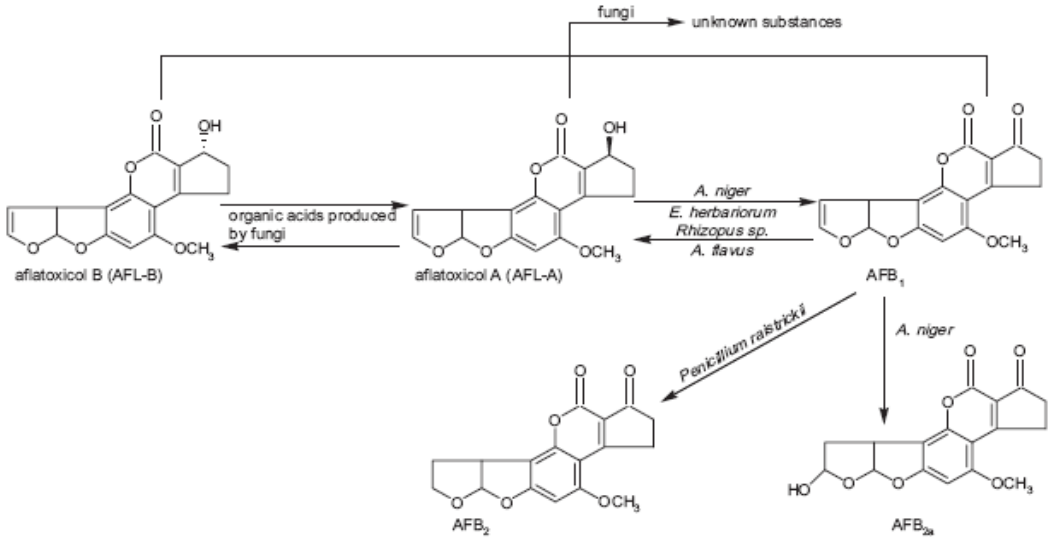


**Figure 4: Metabolic pathways of aflatoxin B1 by fungi**

*Rhizopus sp.* and *Aspergillus flavus* were both capable of degrading aflatoxins and reducing their inherent toxicity and potential mutagenicity. The interconversion (from AFL to AFB1) occurred due to intracellular enzyme (cytochrome P-450 mono-oxygenases) of *A. flavus* and *Rhizopus sp.*

Wu et al. mentioned that other fungi were able to degrade AFB1. Indeed, *A. parasiticus* NRRL 2999 and NRRL 3000 actively degraded aflatoxins. In the presence of asparagines, *Aspergillus candidus* converts AFB1 to aflatoxin D. Fungi *Penicillium raistrickii NRRL* 2038 was able to transform AFB1 to a new compound that is similar to AFB2 (Wu et al., 2009).

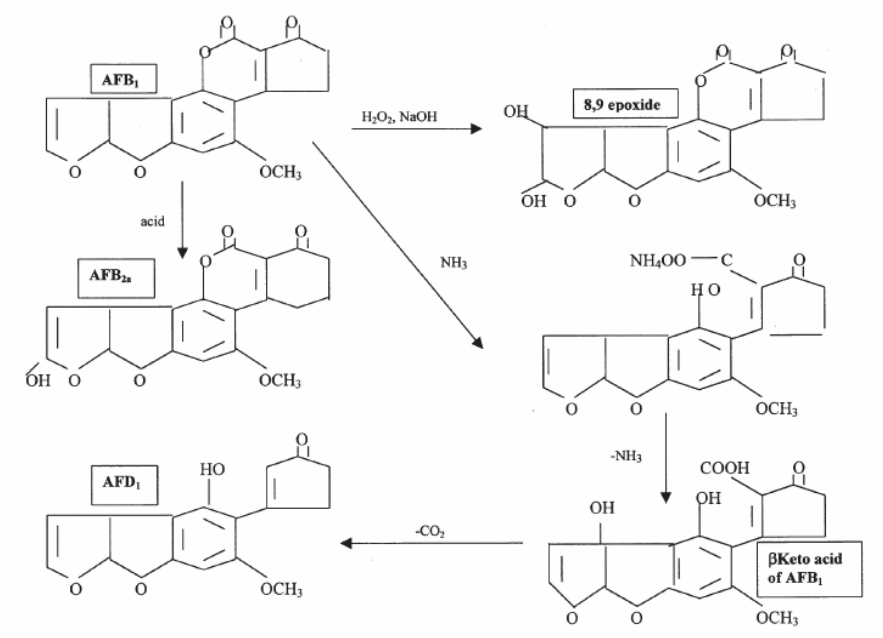


**Figure 5: Probable mechanism of degradation of aflatoxin B1**

### 3.2.2.2. T-2 toxin

Beeton and Bull mentioned that biotransformation of T-2 toxin to HT-2 toxin has been reported for fungal systems (*Fusarium* spp.) (Beeton and Bull, 1989).

### 3.2.2.3. Zearalenone

Recently, ZEA was found to be completely degraded by several Rhizopus isolates including *R. stolonifer*, *R. oryzae* and *R. microsporus* strains (Varga and Toth, 2005), but further studies are needed for the identification of ZEA-degrading enzymes in Rhizopus isolates.

A preliminary study was performed to screen twelve black *Aspergillus* strains for their ZEA transformation activity by being incubated in contaminated culture medium. HPLC analyses showed that ZEA was removed but not adsorbed by two *A. niger* strains. ZEA was completely metabolized in 48h by the mycelium even with high concentrations of ZEA in culture medium but, up to now, no degradation product has been observed by HPLC (Jard et al., 2009).

### 3.2.2.4. Ochratoxin A

Varga et al. showed that *Aspergillus niger* was also able to degrade OTA to the less toxic compound ochratoxin α, and then OTα into an unknown compound. The pathway leading to the opening of the isocoumarin ring is unknown (Varga et al., 2000).

### 3.2.2.5. Fumonisin

Two species of black yeast fungus (*Exophalia spinifera*, *Rhinocladiella atrovirens*) have been found to extensively metabolize fumonisins to $CO_2$ (Blackwell et al., 1999). These microorganisms produce fumonisin catabolising enzymes, such as esterase which lead to the formation of hydrolyzed FB1 (aminopentol 1 or AP1) and tricarbalylic acid. Aminopentol is 10 times more toxic than FB1 (Varga and Toth, 2005).

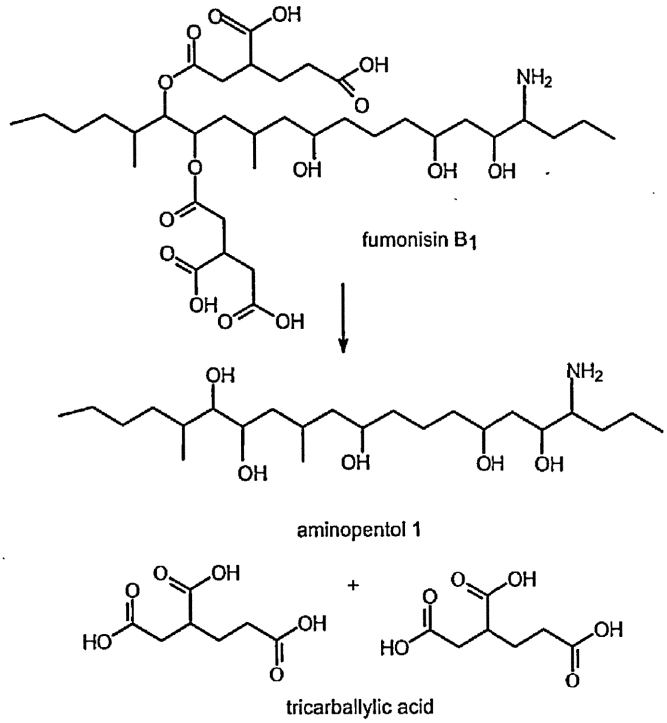


**Figure 6: Degradation of fumonisin B1 by fumonisin esterase of Exophiala spinifera**

## 3.2.3. Protozoa

### 3.2.3.1. Aflatoxin B1

Wu et al. reported that cells of the protozoon *Tetrahymena pyriformis* had the ability to degrade pure AFB1 to another bright-blue fluorescent product and decrease the AFB1 concentration to 25% in 30 hours. *Tetrahymena pyriformis* reduced the carbonyl in the cyclopentane ring of AFB1 to a hydroxyl group (**Figure 7**) (Wu et al., 2009). The biologically reduced aflatoxin appears to be aflatoxicol, which is 18 times less toxic (Petchkongkaew, 2008).

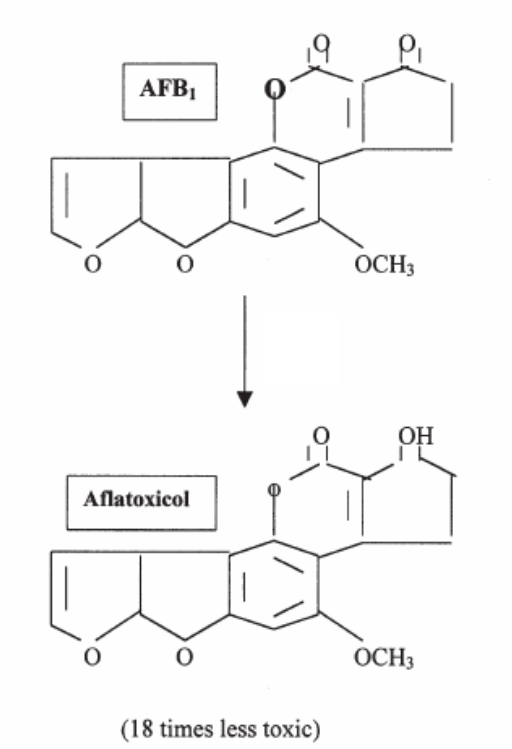


**Figure 7: Aflatoxin $B_1$ biologically reduced by T. pyriformis, Rhizopus spp**

#### 3.2.3.2. T-2 toxin

Biotransformation of T-2 toxin to HT-2 toxin has been reported for ruminal protozoa (Beeton and Bull, 1989).

#### 3.2.3.3. Zearalenone

Evidence of ruminal microbial degradation of ZEA has been demonstrated in isolates cultures of rumen contents. Kiessling et al. reported that rumen protozoa were more active than bacteria in ZEA degradation and demonstrated that 90–100% of ZEA concentration was transformed to α-ZEA and to a lesser degree to β-ZEA. They concluded that protozoa were considered to be the most important ruminal microbial population in ZEA biodegradation. However this degradation should not be regarded as a detoxification since α-ZEA (more oestrogenic than ZEA) was the major metabolite obtained (Kiessling et al., 1984).

### 3.2.4. Yeast

#### 3.2.4.1. Ochratoxin A

Peteri et al. examined astaxanthin-producing yeast isolates (*Phaffia rhodozyma* and *Xanthophyllomyces dendrorhous* isolates) for their ability to degrade and/or adsorb OTA in a liquid medium (Peteri et al., 2007).

The results indicated that besides producing astaxanthin, *P. rhodozyma* was also able both to detoxify and adsorb OTA at temperatures well above the temperature optimum for growth of Phaffia cells.

Ochratoxin α was detected in the ferment broths derived from kinetic studies, indicating that a carboxypeptidase enzyme could be responsible for OTA degradation.

To prove this hypothesis, the effect of various carboxypeptidase inhibitors was tested on OTA degradation activities of *P. rhodozyma* cells. Two of these inhibitors, the chelating agents EDTA and 1,10-phenanthroline significantly inhibited OTA degrading activities of the *P. rhodozyma* cells, indicating that the enzyme responsible for OTA degradation is a metalloprotease. The other inhibitors tested had no significant effect on OTA degradation.

#### 3.2.4.2. Zearalenone

Recently, Molnar et al. isolated and characterized a new yeast strain, *Trichosporon mycotoxinivorans*, which has the ability to degrade OTA and ZEA (Molnar et al., 2004).
The yeast can detoxify OTA by cleavage of the phenylalanine moiety from the isocumarin derivate ochratoxin α. This metabolite has been described to be non-toxic or at least 500 times less toxic than the parent compound.
*T. mycotoxinivorans* was able to degrade ZEA to carbon oxide and other non-toxic metabolites. ZEA has no acute toxicity, but it mimics the reproduction hormone estrogen, and therefore causes substantial fertility problems. The metabolization of ZEA by *T. mycotoxinivorans* leads to a compound that is no longer estrogenic (Schatzmayr et al., 2006).

The biotransformation of ZEA has also been reported in other yeast cultures, where *Candida tropicalis*, *Torulaspora delbruckii*, *Zygosaccharomyces rouxii* and several *Saccharomyces* strains reduced ZEA to α and β-ZEA (Boswald et al., 1995).

#### 3.2.4.3. Fumonisins

It is difficult to find strains capable of fumonisin biodegradation. More than 30 yeast strains were tested for this ability, but only 2 were found to degrade fumonisins, 1 to75% and another to 50% of original concentration (Styriak and Conkova, 2002).

#### 3.2.4.4. Patulin

In the study performed by Coehlo et al., the effectiveness of two yeasts, *Pichia ohmeri* and *Saccharomyces cerevisiae,* in the biodegradation of patulin was evaluated *in vitro* (Coelho et al., 2008).
Patulin levels were reduced by over 90% in the presence of *Saccharomyces cerevisiae* and completely destroyed by *Pichia ohmeri*, after incubation at 25 ºC under static conditions. Two hypotheses concerning the mechanism by which patulin biodegradation by yeasts could have occurred have been formulated.
The first one is that patulin degradation occurs by enzymatic action during the fermentative condition associated with an inducible process.
The second hypothesis is that the decrease in toxin levels could be associated with the adsorption capacity of yeast cell wall components, such as glucan/mannan.
The effective biodegradation of patulin using *P. ohmeri* 158 and *S. cerevisiae* indicated the promising application of innocuous yeasts isolated from natural ecosystems. The combined biodegradation / adsorption / anti-fungal properties of such yeasts offer an effective and profitable choice of biological control.

### 3.2.5. Enzymes

Several microbes and their enzymes have been identified and considered as able to detoxify mycotoxins, including several bacteria, yeasts, filamentous ascomycetes and basidiomycetes.

Specific enzymes that are capable of degrading aflatoxins have been purified from microbial systems.

### 3.2.5.1. Aflatoxin B1

A new aflatoxin degradation enzyme isolated and purified from *Pleurotus ostreatus* showed a great aflatoxin-degradation activity at 25°C with a pH of 4.0–5.0. Fluorescence measurements suggested that this specific enzyme cleaved the lactone ring of aflatoxin.
Liu et al. isolated and purified from *Armillariella tabescens* (E-20) an enzyme named aflatoxin-detoxifizyme (ADTZ), which exhibited detoxification activity on AFB1. AFB1 seemed to be degraded into difuran ring-opening AFB1, which was less toxic than AFB1, and the optimum activity for the enzyme was at 35°C with a pH of 6.8 (Liu et al., 2001). A multienzyme had previously been isolated and was able to degrade AFB1 from *A. tabescens*. The proposed pathway for the degradation of AFB1 by this multienzyme is as follows: AFB1 was first transformed to AFB1-epoxide, followed by hydrolysis of the epoxide to give the dihydrodiol. Then, the difuran ring is assumed to open in the subsequent hydrolysis step (**Figure 8**).

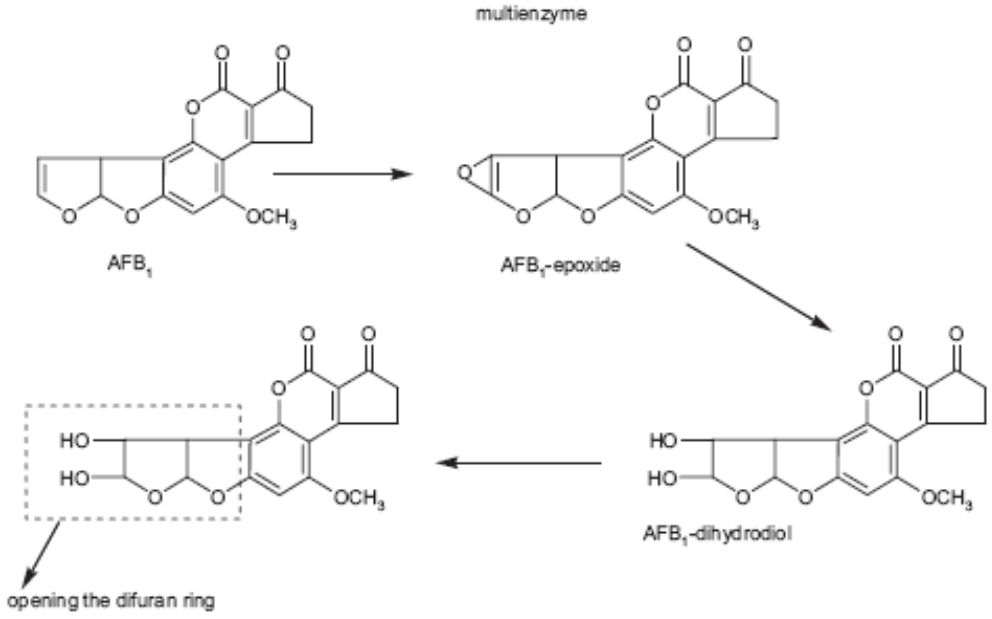


**Figure 8: Proposed pathway of degradation of $AFB_1$ by Armillariella tabescens**

### 3.2.5.2. Zearalenone

Several studies have dealt with the degradation of ZEA by enzymes (Zinedine et al., 2007).

Malekinejad et al. have shown that following adsorption, ZEA is biotransformed in the liver to α-zearalenol and β-zearalenol implying reduction of the keto group at the 6'-position.The conversion of zea into its hydroxylated metabolites is catalyzed by 3α-HSD and probably also by 3β-HSD. These enzymes are involved in the synthesis and inactivation of many steroids and are expressed not only in the liver but also in steroidogenic target tissues (Malekinejad et al., 2006).

Takahashi-Ando et al. also reported that ZEA was converted into a less oestrogenic product, 1-(3,5-dihydroxyphenyl)-10«-hydroxy-1«-undecen-6«-one (**Figure 9, compound 2**) by incubation with the fungus *Clonostachys rosea* IFO 7063 (Takahashi-Ando et al., 2002).
The lactonohydrolase responsible for the detoxification was purified to homogeneity from the fungus and the encoding gene zhd101 was cloned (El-Sharkawy 1988).

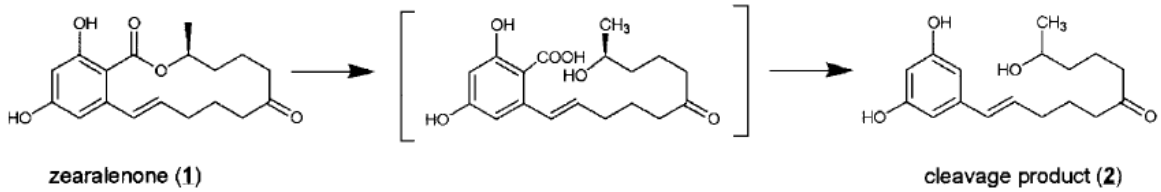


**Figure 9: Detoxification of Zearalenone**

A hypothetical pathway for the detoxification of ZEA is presented. Structures of ZEA (1) and 1-(3,5-dihydroxyphenyl)-10«-hydroxy-1«-undecen-6«-one (compound 2) are indicated. A putative unstable intermediate is shown in square brackets.

### 3.2.5.3. Ochratoxin

Pitout presented the *in vitro* hydrolysis of OTA by carboxypeptidase A and, in lower amounts, by α-chymotrypsin (Pitout, 1969).

Abrunhosa et al. reported the ability of several commercial proteases to hydrolyze OTA into ochratoxin α in different amounts (Abrunhosa et al., 2006). After an incubation period of 25 h, a significant hydrolytic activity was detected at pH 7.5 for Protease A (87.3%), and for Pancreatin (43.4%). Also, the isolation of an enzyme extract from an *Aspergillus niger* strain with very strong OTA hydrolytic activity at pH 7.5 (99.8%) was reported. This activity is similar to the activity detected in Protease A. The authors also showed that EDTA (ethylene diamine tetra acetic acid) significantly inhibits protease A, pancreatin and carboxypeptidase, so these enzymes could be metalloproteases (since EDTA is a specific inhibitor of metalloproteases). Prolyve is not inhibited by EDTA but by PMSF (specific inhibitor of serine type-proteases).

## CONCLUDING REMARKS

### ADSORBING AGENTS

- Mycotoxins may bind to adsorbing agents through different types of interactions, often simultaneously. Some of the most important types of interactions are hydrophobic binding, hydrogen bridges, electrostatic attraction or repulsion, and coordination bonds.

- There are several different ways to define and express affinity, depending on the model and equations used. The "distribution coefficient" $K_d$ defined as the ratio of bound toxin to free toxin does not depend on any assumption or model and provides a universal basis for quantitative comparison of affinity. It can be interpreted as equal to the inverse of the "BC50, i.e. the adsorbing agent concentration that will bind 50% of the toxin present. This "BC50" is also readily comparable with the various "EC50" endpoints that may be derived from *in vivo* studies.

- Several types of experimental studies can be found in the literature: "single-concentration studies", "isotherm studies", "modified isotherms" and "gastro-intestinal tract models" (static or dynamic).

- Activated carbons have high affinities for all toxins, but they also have low selectivities and therefore are readily saturated by food matrix; planar adsorbing agents such as montmorillonite clays are very good adsorbing agents for aflatoxins, because they combine very high affinity and high selectivity; in acidic conditions, ZEA, OTA and fumonisins bind several types of adsorbing agents mainly through hydrophobic interactions, with moderate to high affinities; DON and other trichothecenes do not adsorb readily on any type of adsorbing agent except activated carbons.

- Generally the experiments conducted in water or buffers are 'best-cases' and are only the first step in the assessment. It is strongly recommended to study the influence of digestive fluids and especially of food matrix, and if possible to apply a gastro-intestinal tract model, before concluding on the *in vitro* properties of the adsorbing agent and considering further tests *in vivo*.

### BIOTRANSFORMING AGENTS

- Biological detoxification involves enzymatic degradation or modification of mycotoxins, resulting in a decrease in potential toxicity. It is one of the well-known strategies for the management of the mycotoxins in the feed chain.

- Many different types of biological agents, such as bacteria, yeast, fungi, protozoa and enzymes, have been reported to possess the ability to degrade mycotoxins. It seems that, according to results of *in vitro* experiments realized till present time, bacteria and yeast are the main living organisms applicable for mycotoxin biodegradation.

- Despite the many publications on biological transformation of mycotoxins by microorganisms, their application in practice in detoxification of animal feeds has been limited. This may be due to lack of information about mechanisms of transformation, toxicity of transformation products, effects of the transformation reactions on nutritional values of the feeds, and safety towards animals.

# 4. EFFICACY AND BIOLOGICAL EFFECTS OF DETOXIFYING AGENTS IN ANIMALS

**Objectives:**

- Describe the uses and the efficacy of mycotoxin detoxifying agents in animal nutrition.
- Identify relevant end-points to be studied when testing the efficacy of mycotoxin detoxifying agents in *in vivo* trials.

As reviewed in **chapter 1**, mycotoxins are highly toxic secondary products of the metabolism of some fungi, mainly belonging to *Aspergillus, Penicillium and Fusarium spp*. that affect the health of animals and their performance to a different extent. There is a great diversity among animal species as regards susceptibility to a particular mycotoxin. In addition, within an animal species, susceptibility is influenced by factors such as age, sex, breed, general health and nutritional status.

As reported in **chapter 2**, the use of additives with protective properties against mycotoxins and addition of non-nutritive adsorbing agents, microorganisms or biomolecules, capable of reducing the bioavailability of mycotoxins (mycotoxin-detoxifying agents) is increasing.
Detoxifying agents are supposed to detoxify the contaminated feedstuffs during passage through the digestive tract by adsorbing and/or degrading the mycotoxins under the pH-, temperature- and moisture- conditions of the digestive tract (Döll and Dänicke, 2004).

Most of the studies on the efficacy of detoxifying agents do not include pharmacokinetic analysis and assess the effects of the products on recording of performance parameters of the animals. Other parameters usually assessed to study the effectiveness of detoxifying agents are the relative weight of target organs such as liver, kidneys, heart, spleen, etc. Some haematological and biochemical serum parameters, such as total proteins, albumins, globulins, enzymatic activities as γ-glutamyl-transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT) are also indicators of the effects of mycotoxins and the preventive action of detoxifying agents.

The first part of this chapter focuses on the evaluation of the efficacy of mycotoxin-detoxifying agents in animal nutrition while the second part aims to identify the relevant end-points to be studied when testing the efficacy of mycotoxin-detoxifying agents in *in vivo* trials.

## 4.1. Description of the uses and efficacy of mycotoxin detoxifying agents in animal nutrition

The efficacy depends on the chemical structure of both the detoxifying agent and the mycotoxin (Avantaggiato et al., 2005). Moreover, the effects of specific mycotoxins and detoxifying agents differ for each animal species. Consequently, the evaluation of the efficacy of detoxifying agents against the different mycotoxins presents in feeds is undertaken separately for poultry, swine, ruminants, rodents and other species.

### 4.1.1. Poultry

The *in vivo* studies on the efficacy of detoxifying agents in ameliorating the performance depressive effects of mycotoxins in poultry are summarized in **Annex 12**. Other parameters usually assessed to study the efficacy of detoxifying agents are the relative weight of target organs such as liver, kidney, heart, spleen, bursa Fabricius, etc. It is known that mycotoxins in birds produce degenerative

processes of these organs which consequently increase their relative weight, and the action of detoxifying agents could be assessed by the reduction of these weights.

Effects of mycotoxin-detoxifying agents in poultry are summarised in **Table 7**.

#### 4.1.1.1. Aflatoxin

As shown in **Table 1 of Annex 12**, most of the detoxifying agents referenced in **chapter 2** have been tested to alleviate the negative effects of aflatoxins in feeds. The toxic effects of aflatoxins on broilers depend on the doses and the time of exposure, therefore acute and chronic aflatoxicosis can be distinguished. Patterson considers that chickens are somewhat resistant to aflatoxins intoxication and reports a LD50 for chickens from 6.5 to 16.5 mg/kg body weight (BW) (Patterson, 1973).

Most of the aluminosilicates are effective as aflatoxin-detoxifying agents.
HSCAS (hydrated sodium calcium aluminosilicate) has demonstrated an ability to sorb mycotoxins with a high affinity and has shown a protective effect against the development of aflatoxicosis in farm animals (Ramos and Hernandez, 1997). Phillips et al. showed that 0.5% HSCAS fed to broiler and Leghorn chicks significantly decreased the growth inhibitory effect of feeding 7.5 mg AFB1/kg feed (Phillips et al., 1988). Kubena et al. confirmed that the relative weights of liver, kidney, proventriculus and gizzard were not affected by 5 mg of aflatoxin in the diet when 0.5% HSCAS was added to the feedstuff (Kubena et al., 1990).

Sodium bentonite has the ability to adsorb aflatoxin molecules. The bound aflatoxins are then excreted in faeces. Pasha et al. reported that chicks receiving an aflatoxin-contaminated diet had suppressed body weight, feed consumption and feed conversion ratio (FCR) value, which was significantly improved with the addition of 0.5% simple sodium bentonite. The relative weight of liver (5.34%), heart (0.72%), gizzard (2.05%) and mortality (40%) increased significantly with the addition of 100 mcg/kg aflatoxin and were restored with the dietary inclusion of 0.5% simple sodium bentonite (Pasha et al., 2007). Other researchers have reported similar results (Eraslan et al., 2004; Rosa et al., 2001). Rosa et al. selected by *in vitro* studies a sodium bentonite from South Argentina showing a high ability in sequestering AFB1 from aqueous solution (Rosa et al., 2001). In an *in vivo* assay, the addition of 0.3% of this bentonite to aflatoxin contaminated diet (5 mg/kg feed) had a moderate protective effect against the development of aflatoxicosis in broiler chickens. Body weight gain and feed:gain data agreed with the results of the *in vitro* studies, as bentonite fed chickens had values similar to those of control chickens. However, the biochemical indicators and histopathological findings showed that the amelioration of aflatoxin-toxic effects was not as great as might have been predicted.

Desheng et al. showed that montmorillonite, when added to the diet of broiler chicken at 0.5%, significantly reduced the adverse effects of feeding 200 $\mu$g of AFB1 /kg of feed (Desheng et al., 2005).

Shi et al. studied the effects of adding 3 g modified montmorillonite nanocomposite (MMN)/kg diets containing 0.1 mg aflatoxin /kg were determined in broiler chicks from 0 to 42 days of age. Compared to the control, aflatoxin alone significantly decreased BW gain and feed efficiency. However, no differences in BW gain and feed/gain ratio were found between the chicks fed 3 g MMN or 3 g MMN + aflatoxin/kg treatment groups and the control group, indicating apparent protection against the deleterious effects caused by aflatoxin. Furthermore, the addition of 3 g MMN/kg aflatoxins-contaminated diet diminished the adverse effects of aflatoxin on most relative organ weights, hematological values, serum and liver biochemical values and enzymatic activities associated with

aflatoxicosis. These findings suggested that MMN can effectively reduce the toxicity of aflatoxin in broiler chicks and MMN can be a potential ameliorator of aflatoxicosis in broiler chicks (Shi et al., 2006).

Because of its high affinity and stable association with AFB1, synthetic Zeolite NaA (ZN) was incorporated into diets (1%) containing 2.5 mg/kg AFB1 and tested in broiler chicks from 21 to 42 d of age. When compared with controls, BW gains were lower for broilers that were fed AF in their diets. No differences were found between the BW gains of chicks fed diets without AF and those of chicks fed AF and ZN, indicating almost total protection against the effects caused by AF. These findings suggest that ZN can counteract some of the toxic effects of AF in growing broiler chicks (Miazzo et al., 2000).

Oguz et al. observed that the use of clinoptilolite at 1.5% and 2.5% concentration to broiler chickens diet containing 2.5 mg/kg aflatoxin was effective to avoid aflatoxicosis (Oguz et al., 2000).
Similarly, Parlat et al. showed that the addition of clinoptilolite significantly reduced the negative effects of aflatoxin on feed consumption and body weight gain in Japanese quail (Parlat et al., 1999).

Edrington performed a study to evaluate the effectiveness of a superactivated charcoal (SAC) in alleviating aflatoxicosis. Broiler chicks were fed diets containing 4 mg aflatoxin/kg, with or without 0.5% SAC, from 1 to 21 d of age. Feeding aflatoxin significantly decreased BW gain over the experimental period while inclusion of SAC in the diet containing aflatoxin resulted in moderate BW gains. The addition of dietary SAC is marginally effective in alleviating some of the toxic effects associated with aflatoxins (Edrington, 1997).

Several products derived from yeast cell wall (YCW) and glucomannans have been evaluated as aflatoxins-detoxifying agents in broilers (Karaman et al., 2005; Santin et al., 2003; Stanley et al., 2004) and breeders (Stanley et al., 2004). Yeast glucomannan, incorporated into the diet up to 1 g/kg, reduced the detrimental effects of 2 mg aflatoxin/kg diet on growing broiler chicks from 1 to 21 d of age and decreased the severity of pathological changes caused by aflatoxin. In breeders 35-weeks old, 1mg/ kg aflatoxin did not negatively affect fertility. However, hen-day egg production (57.6%), percentage of hatchability (67.6%), embryonic mortality (24%), serum total protein, globulin, and albumin were significantly affected by aflatoxin. The inclusion of 0.1% YCW in the aflatoxin-treated diet raised the level of hatchability (74.9 vs. 67.6%), egg production (65.83 vs. 57.26%), and lowered embryonic mortality (16.8 vs. 24%). Serum globulin and albumin were lowered in the aflatoxin-fed hens but were partially restored with the addition of YCW. The data demonstrated that YCW may enhance the performance of broiler breeder hens that are provided feed contaminated with aflatoxin (Karaman et al., 2005).

*Nocardia corynebacteroides* bacteria has proven effects on broilers and is useful for partly detoxifying aflatoxin-contaminated feed, that reduces the amount of AFB1 by forming other compounds having a lower toxicity (Tejada-Castaneda et al., 2008).

Polyvinylpolypyrrolidone is an other compound that was shown to be effective as an aflatoxin-detoxyfying agent (Celik et al., 2000).

The alleviation of aflatoxicosis produced by 2.5 mg total aflatoxin/kg in Japanese quails was examined by the dietary addition of 1 g/kg live yeast (*Saccharomyces cerevisiae)*. The aflatoxin treatment significantly and dramatically decreased food consumption and BW gain from the first week onwards. The addition of *Saccharomyces cerevisiae* to the aflatoxin-containing diet significantly reduced these deleterious effects of aflatoxin on food consumption, BW gain and FCR (Oguz et al., 2001).

#### 4.1.1.2. Ochratoxin A

In the studies mentioned below, the presence of OTA in feeds impaired the productive parameters of birds, reduced BW of broilers and impaired feed to gain ratio. Moreover, increased relative weights of liver and gizzard were observed in chicks fed contaminated diets.

Not all the mycotoxin-detoxifying agents were equally effective in reducing the negative impact of OTA.

The esterified glucomannan (E-GM) effectively alleviated the growth depression caused by a naturally OTA contaminated diet (Aravind et al., 2003). In a study conducted to evaluate the individual and combined effects of AFB1, OTA and T-2 toxin on performance, E-GM increased body weight (2·26%) and food intake (1.6%), and reduced the relative weight of liver and kidney, indicating its possible beneficial effect on mycotoxicosis in broiler chickens (Raju and Devegowda, 2000).

Likewise, FCR in groups fed with a combination of clay minerals with *Eubacterium* and *T. mycotoxinivorans* were improved compared with groups receiving only OTA (Hanif et al., 2008). The yeast strain *T. mycotoxinivorans* was shown to cleave OTA into phenylalanine and the non-toxic OTα. OTA produced an increase in the relative weights of liver and kidney likely due to the enlargement of the epithelium and to hyperanaemia in these organs, and the yeast tested was effective in counteract these effects.

However, HSCAS was not effective in improving performance of broilers fed OTA contaminated diet (Santin et al., 2002) and there was no interaction between HSCAS and dietary ochratoxin.

Rotter et al. described that the inclusion of 1% activated charcoal reduced feed consumption by 10% and body weight gain by 20% compared to those whose feed did not include activated charcoal, and was not effective against mycotoxicosis produced by OTA. An additional management problem was associated with the propensity of charcoal to blacken the feed, the birds and their environment (Rotter et al., 1989).

Ocratox, an activated diatomaceous earth, has been tested as a detoxifying agent of OTA in layer hens (Denli et al., 2008). Addition of OcraTox to the contaminated diet alleviated the negative effects resulting from OTA, reaching values not significantly different from the control diet for most of the parameters except the relative weight of the liver, showing that OcraTox counteracted the deleterious effects caused by OTA.

#### 4.1.1.3. Trichothecenes

Different detoxifying agents have been tested to counteract the effects of these toxins in poultry. Aluminosilicates can effectively adsorb aflatoxins but are not effective against trichothecene mycotoxins, such as DAS or T-2 toxin (Kubena et al., 1993; Phillips, 1999; Stanley et al., 1993). Aravind et al. suggested that E-GM is effective in counteracting the toxic effects of mycotoxins, including T-2 toxin (Aravind et al., 2003). A more specific dietary treatment has been recently developed using microorganisms capable of inactivating the trichothecenes through enzymatic modification of the basic trichothecene structure. The inhibitory activity of trichothecenes requires the presence of the C-12,13 epoxide, and opening of the 12,13-epoxide ring results in loss of any apparent toxicity (Diaz et al., 2005).

T2 toxin

Diaz et al. investigated the possible effectiveness of 4 commercially available feed additives against the adverse effects of 2 ppm dietary T-2 toxin in broiler chickens 28 days old (Diaz et al., 2005). Two of the selected products were aluminosilicates (at 2.5 g/kg and 3g/kg), one was based on E-GM (2 g/kg), and the other one was based on the enzymatic inactivation of the 12,13-epoxy trichothecene ring (2 g/kg). The trial confirmed that only the product responsible for this inactivation and which is a combination of *Eubacterium* BBSH 797 with dried yeasts and clays alleviates the adverse effects caused by T2-toxin: reduced animal performance, lesion in liver, heart, spleen and guizzard, altered levels of AST and lactate deshydrogenase (LDH) in serum. Aluminosilicates and E-GM were not effective as detoxifying agents; even, chickens receiving these products had significantly greater relative gizzard weights than the control group and those receiving the combined product.

Curtui also found that the inclusion of 0.5% zeolite to diets contaminated with T2 toxin (25 mg/kg) did not exert any positive effect on performance and relative weight of organs of 28 days old-broilers (Curtui, 2000).

However, Aravind et al. reported that the addition of 0.05% of E-GM to a naturally multicontaminated diet effectively alleviates the growth depression caused by the contaminated diet (see chapter 4.1.1.6. on co-contamination) (Aravind et al., 2003).

Activated charcoal was not effective in alleviating the toxic effects associated to T2 toxin in chicks: decreased body weight gain of 21-days old birds, increased relative weight of liver, spleen and kidney, and decreased serum cholesterol, phosphorus and total protein contents. However, birds fed T-2 contaminated feed plus 0.5% activated charcoal had a significantly lower oral lesions scores than those fed T2-toxin alone (Edrington, 1997).

DON and NIV

Several researchers reported that broiler chicks could tolerate up to 15 mg/kg dietary DON from naturally contaminated wheat and oats without any adverse effects (Kubena et al., 1997). Other researchers, however, observed reduced performance and immune function and changes in haematology and serum chemistry in broiler chicks fed diets containing 16 to 18 mg DON/kg from naturally contaminated wheat (Kubena et al., 1990; Yegani et al., 2006).

Swamy et al. studied the effects of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins on production parameters, clinical chemistry, and muscle coloration in broilers and the possibility of E-GM to counteract these adverse effects (Swamy et al., 2002). Probably due to the level of DON found in diets, feeding of DON-contaminated grains did not affect feed consumption, weight gain, or feed efficiency in the starter and grower periods. E-GM prevented the *Fusarium* mycotoxin-induced alterations in haematology, serum chemistry, and biliary IgA concentrations but these alterations were not reflected in growth (Swamy et al., 2004).

Danicke et al. performed trials with broilers and turkeys to test the hypothesis that *Fusarium* infection-related increases in nonstarch polysaccharide (NSP)-hydrolyzing enzyme activities could compensate for the deleterious effects of mycotoxins such as DON. Weight gain of birds decreased slightly with increasing proportions of *Fusarium* contaminated wheat, whereas other performance parameters remained unaffected. NSP enzyme supplements to the diets had no influence (Dänicke et al., 2007).

The use of zeolite as detoxifying agent of *Fusarium* toxins (T-2 toxin, NIV and DAS) has been reported by Curtui, who stated that the addition of 0.5% zeolite did not diminished the adverse effects

produced by these toxins on the performance of broilers, on the relative weights of organs and on serum biochemical values (Curtui, 2000). In the same way polyvinylpyrrolidone was not effective to counteract the deterious effects of *Fusarium* toxins in broilers (Dänicke et al., 2003).

DAS

An *Eubacterium*-based product (Diaz, 2002) and zeolite (Curtui, 2000) have been tested to determine their possible effectiveness against the adverse effects of dietary DAS in growing broiler chickens. Zeolite was not effective in counteract the deleterious effects of DAS on broilers. The adverse effects of 1 ppm dietary DAS on feed intake and BW were counteracted by dietary supplementation of 0.75 or 1.5 g/kg of the *Eubacterium*-based product. However, when 2 ppm DAS is present in the diet, only partial protection is afforded by up to 1.5 g/kg *Eubacterium*-based product supplementation. More studies are needed to determine if higher doses of *Eubacterium*-based product can completely overcome the adverse effects of higher contents of DAS.

#### 4.1.1.4. Zearalenone

The detoxification of ZEA-contaminated feeds using E-GM or *T. mycotoxinivorans* has been evaluated in broilers (Aravind et al., 2003; Dänicke et al., 2001; Swamy et al., 2002). E-GM at 0.05% added to a naturally contaminated diet containing 54 ppb of ZEA increased performance of broilers and decreased the serum activity of GGT suggesting that the level of 0.05% might be sufficient to counteract the adverse effects of mycotoxins. E-GM at 0.2% was also effective in counteract the negative effects of performance of broilers fed a naturally *Fusarium* contaminated diets, containing up to 0.8 mg/kg ZEA.

#### 4.1.1.5. Fumonisins

Sodium bentonite (0.3%) was evaluated for its ability to reduce the deleterious effects of 200 mg/kg FB1 in broiler diets. FB1 or the combination of FB1 and sodium bentonite had no effect on BW gain. Feeding FB1 alone did not alter relative weights of any organs (Miazzo et al., 2005).

#### 4.1.1.6. Co-contamination

Co-contamination is more likely to occur in the field than monocontamination of cereals by mycotoxins. At the experimental level, when researchers use naturally contaminated grains, multimycotoxicoses are also observed. If these multicontaminations prevent from evaluating the effect of one detoxifying agent against one mycotoxin, they would allow for studying whether some agents are able to provoke a simultaneous deactivation of several mycotoxins in far animals.

Broilers are fed with compounded diets composed of several feed ingredients grown in different agroclimatic conditions. Thus, the use of multiple feed ingredients, contaminated with individual mycotoxins, when combined, may lead to co-occurrence of all the mycotoxins present in the individual ingredients (Aravind et al., 2003).

The toxicity and clinical signs observed in animals when feed is multi-contaminated by mycotoxins are complex and diverse. Co-contamination of animal feedstuffs by aflatoxin and OTA (Huff and Doerr, 1981), aflatoxin and ZEA (Ravindran et al., 1996), T-2 with OTA (Chandrasekaran, 1996), and T-2 toxin with other *Fusarium* metabolites (Bata et al., 1983) has been reported in field conditions.

Most of the studies regarding the efficacy of the mycotoxin-detoxifying agents in poultry have been performed assessing their effects against a specific mycotoxin: AFB1, OTA, etc.

Many inorganic adsorbing agents have shown considerable promise in countering aflatoxins. However, many of these agents lack a similar effect against other mycotoxins (Edrington, 1997; Rotter et al., 1989).

Miazzo et al. used a sodium bentonite (0.3%) to detoxify diets containing AFB1 (2.5 mg/kg), FB1 (200 mg/kg) or a combination (2.5 mg AFB1/kg + 200 mg FB1/kg) in chicks. Protective effects of sodium bentonite on gross hepatic changes produced by AFB1 or by a combination of AFB1 and FB1 were observed. Changes in concentrations of serum total protein, albumin, and globulin were also completely reversed. These authors concluded that sodium bentonite was effective to counteract some of the aflatoxin promoted effects. In fact, addition of sodium bentonite was not able to return body weight gain to equal that of the control. They attributed this result to a competition between FB1 and AFB1 for the active surface sites of the sodium bentonite, rendering a greater bioavailability of AFB1 in the presence of high dose of FB1. The data indicated toxicity for chicks of AFB1 alone or in combination with FB1. Neither additive nor synergistic toxic interaction between AFB1 and FB1 seems to be operating as shown with most of the parameters (Miazzo et al., 2005). **Table 7** confirms that sodium bentonite is effective to detoxify AFB1, but no further data is available concerning its potential effect on FB1.

Esterified glucomannans have shown considerable binding ability with several commonly occurring mycotoxins. Aravind et al. assessed the ability of E-GM to alleviate the adverse effects of several combinations of mycotoxins (168 ppb AFB1 + 8.4 ppb OTA + 53 ppb ZEA + 32 ppb T-2 toxin) naturally found in feed on productivity and serum biochemical and hematological parameters of broilers. Compared with the control, the naturally contaminated diet significantly decreased BW and feed consumption and resulted in poor feed efficiency and increased relative weights of liver and gizzard. E-GM (0.05%) effectively counteracted the toxic effects of contaminated feed with mycotoxins alleviating the growth depression and reducing the organ weights (Aravind et al., 2003).

These beneficial effects of E-GM in counteracting the deleterious effects of several mycotoxins have also been reported by Raju and Devegowda. In this study the individual and combined effects of 0.3 ppm AFB1, 2 ppm OTA and 3 ppm T-2 toxin on performance, organ morphology, serum biochemistry and haematology of broiler chickens and the efficacy of 0.1% E-GM in their counteraction were evaluated. BW and feed intake were depressed by all the mycotoxins, OTA being the most toxic during early life. Significant interactions were observed between any 2 toxins for their additive effects on productive parameteres and bone ash content. Simultaneous feeding of all 3 mycotoxins did not show increased toxicity above that seen with any 2. E-GM counteracted these negative effects, indicating its possible beneficial effect on mycotoxicosis in broiler chickens (Raju and Devegowda, 2000).

In addition, according to the compilation of data showed in **Table 7**, E-GMs are capable of detoxifying AFB1, DON, OTA, ZEA and fusaric acid when they are administraded separatly to poultry.

Usually ZEA has been detected when feeds are contaminated with trichothecenes. Several researchers studied the effects of E-GM as a detoxifying agent in feed multi-contaminated with DON and ZEA, in broilers (Swamy et al., 2002), broiler breeders (Yegani et al., 2006) and turkeys (Girish et al., 2008). Swamy et al. studied the effects of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins (9.7 mg/kg DON, 21.6 mg/kg fusaric acid, 0.8 mg/kg ZEA) on production parameters, clinical chemistry, and muscle coloration in broilers, and the possibility of 0.2% of E-GM (from *Saccharomyces cerevisiase*) in counteracting these adverse effects. At the levels assayed, productive parameters were not affected by the diets. The supplementation of E-GM to the diets high in

contaminated grains did not have any effect on feed consumption, weight gains, and feed efficiency but counteracted most of the blood parameter alterations caused by the *Fusarium* mycotoxin-contaminated grains and reduced breast muscle redness (Swamy et al., 2002). Similar results were reported by Girish et al., describing the efficacy of glucomannans as adsorbing-detoxifying agents included in naturally *Fusarium* contaminated feeds on turkey production (Girish et al., 2008).

The effects of feeding grains naturally contaminated with *Fusarium* mycotoxins (13.8 mg/kg DON, 0.5 mg/kg ZEA and 1 mg/kg 15-acetyl-DON) on the performance and metabolism of broiler breeders and the efficacy of 0.2% of glucomannans in preventing these effects have been reported by Yegani et al. Feed consumption, BW and egg production were not affected by the diet. Decreases in eggshell thickness and increases in early (1 to 7 d) embryonic mortality were observed at the end of week 4, and dietary supplementation with glucomannans prevented this effect (Yegani et al., 2006).

Furthermore, when young broilers are fed a feed containing a natural mixture (*Fusarium poae* extract) of trichothecenes (6.4 ppm NIV + 1.3 ppm toxin T-2 + 2.6 ppm DAS), a natural zeolite (0.5% in feed) is not able to reduce the deleterious effects of these mycotoxins on the animal performance, organ abnormalities and immune parameters (Curtui, 2000).

A NSP enzyme (250 U/kg) was studied in turkeys from day 21 to day 56 of age as mycotoxin-detoxifying agent of feed contaminated simultaneously with 5.42 mg DON/kg and 0,44 mg ZEA/kg ZEA. Weight gain decreased slightly when turkeys were fed contaminated diet compared to control group whereas other performance parameters remained unaffected. The NSP enzyme supplements to the diets had no influence (Dänicke et al., 2007).

**Table 7: Mycotoxin detoxifying agents tested *in vivo* in poultry**

| DETOXIFYING AGENTS | MYCOTOXIN | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Aflatoxin (AFB1) | Cyclopiazonic acid | DAS | DON | Fusaric acid | Nivalenol | OTA | T2 toxin | ZEA |
| HSCAS | + | - | | | | | - | - | |
| Clinoptilolite | +/- | - | | | | | | | |
| Modified nano montmorilonite | + | | | | | | | | |
| Mg K aluminosilicate | +/- | | | | | | | | |
| Sodium bentonite | + | | | | | | | | |
| Ca montmorilonite | + | | | | | | | | |
| Synthetic crystialline aluminosilicate | +/- | | | | | | | | |
| Acidic phyllosilicate | | - | | | | | | | |
| Zeolite | + | | - | | | - | | - | |
| Diatomaceaus earth | | | | | | | + | | |
| Charcoal | | | | | | | - | | |
| Superactivated charcoal | +/- | | | | | | | +/- | |
| BHT | + | | | | | | | | |
| Cell wall saccharomyces cerv. | + | | | | | | | | |
| Yeast glucomannans | + | | | | | | | | |
| Esterified glucomannans | + | | | +/- | + | | + | - | + |
| Xylanase | | | | - | | | | | |
| Live yeast culture residue | + | | | | | | | | |
| Nocardia corynebacteroides | +/- | | | | | | | | |
| *Eubacterium* | | | + | + | | | | + | |
| Yeast *Trichosporon mycotoxinivorans* | | | | | | | + | | - |
| *Saccharomyces cerevisiae* | + | | | | | | | | |
| Ammonia | + | | | | | | | | |
| Calcium propionatex | +/- | | | | | | | | |
| PVPP | +/- | | | - | | | | | |

Cells highlighted in green indicate that the product has shown positive effects in counteracting deleterious effects of mycotoxins. Cells marked in pink indicate that the product was not effective.

+ : positive effect of the mycotoxin-detoxifying agent

- : negative effect of the mycotoxin-detoxifying agent

+/- : positive effect of the mycotoxin-detoxifying agent on some parameters, no effect on other parameters

### 4.1.2. Swine

In pigs, most studies concerned the efficacy of detoxifying agents for contaminations with trichothecenes (mainly DON) and ZEA. A fewer number were related to aflatoxin, and studies concerning fumonisins and OTA were rare. In pigs, as in poultry, most studies on the detoxifying agents focus on the consequences of the intake of mycotoxins on the animal (zootechnical performance, immune status, organ abnormalities...) and not on the direct effect of these agents on the mycotoxins themselves.

The *in vivo* studies on the efficacy of detoxifying agents in ameliorating the performance depressive effects of mycotoxins in pigs are summarized in **Table 2 in Annex 12** (both specific and unspecific parameters).

Effects of mycotoxin-detoxifying agents in swine are summarised in **Table 8**.

#### 4.1.2.1. Aflatoxin

Pigs are highly susceptible to aflatoxin, especially in the weaning stage as it can cause a variety of chronic or acute syndromes depending on the level of consumption. Extreme effects can lead to death, but the greatest impact comes from weight loss and poor performance, reduced reproductive capability, changes in clinical biochemistry patterns, suppressed immune function, increased susceptibility to infectious diseases and increased mortality (Harvey et al., 1988; Marin et al., 2002; Miller et al., 1982; Phillips et al., 1988; Sharma, 1993).

The specific effects of a mineral adsorbing agent (HSCAS, 0.05% in feed) have been monitored in growing pigs fed an aflatoxin-contaminated diet (0.5-0.6 ppm) (Beaver et al., 1990). AFB1, AFB2 and AFM1 have been found in the liver, muscle, kidney and adipose tissue of the pigs, but the dietary incorporation of the aluminosilicate allowed reducing the level of AFM1 in the liver, muscle, kidneys and of AFB1 in muscle.

Studies of Harvey et al., Schell et al. and Thieu et al. confirmed the beneficial effect of various mineral adsorbing agents (sodium or calcium bentonites, from 0.25 to 5% in feed and hydrated sodium calcium aluminosilicates from 0.5 to 2%) on the zootechnical performance (weight gain, feed intake or feed efficiency) of pigs (piglets and adults) fed aflatoxin-contaminated diets (0.2-3 ppm). The intake of aflatoxin results also in deleterious effects on some serum enzymes activities (GGT, ALP, AST…), some blood metabolites (cholesterol, albumin, globulin, total proteins) and liver weight. The incorporation of aluminosilicates can reduce these deleterious effects (Harvey et al., 1988; Schell et al., 1993; Thieu et al., 2008), through obviously a reduction of the aflatoxin absorption.

Only Colvin et al. did not show any beneficial effect of HSCAS (0.5%) on the zootechnical performance of growing pigs. At the opposite, they related a deleterious effect (Colvin et al., 1989). In fact, surprisingly, the presence of aflatoxin can induce an increase of the absorption and retention of various elements (Ca, P, Na, Zn, Fe), while the aluminosilicate tested can reduce the digestibility of dry matter, and the absorption and retention of Mg, Na and Fe. The concomitant presence of aflatoxin and the aluminosilicate accentuates the reduction of the absorption and retention of elements (Ca, P, Na, Zn, Fe) (Schell et al., 1993).

Meissonnier et al. have tested the efficacy of an organic adsorbing agent (yeast-derived glucomannan, 0.2% in feed) to improve the vaccinal response in piglets fed an aflatoxin-contaminated diet (0.48-1.91 ppm). Results showed deleterious effects of aflatoxin on weigh gain of piglets, but also on liver (lesions, total cytochrome P450, EROD activity, 6β-testosterone hydroxylation activity), on serum

albumin concentration and on serum ALP activity. The vaccinal response was also reduced (antiovalbumin immune response). The incorporation of this organic adsorbing agent was only able to improve this vaccinal response (Meissonnier et al., 2009).

To conclude, as shown in **Table 8**, for all the adsorbing agents tested, including the yeast derived-glucomannan tested, a beneficial effect has been observed in pigs fed aflatoxin-contaminated diets. The best results have been obtained when using HSCAS.

#### 4.1.2.2. Ochratoxin A

Pigs are also susceptible to OTA. It can cause a variety of chronic or acute syndromes depending on the level of consumption. The most famous effect in pigs is the occurrence of kidney lesions, and this pathology is called MPN (mycotoxic porcine nephropathy). OTA residues have been found in the kidney of pigs. Zootechnical impacts have also been observed: poor weight gain, feed intake and feed efficiency. Low levels of OTA can induce several clinical symptoms, such as polydipsie and polyury, while higher levels can provoke vomiting, anorexia, serious diarrheas, and even death of pigs. The LD50 has been experimentally evaluated between 1 and 6 mg/kg body weight. In addition, OTA can also reduce reproductive capability in pig males, change clinical biochemistry patterns, and suppress immune function (Afssa, 2009).

Despite the well-known sensitivity of pigs to OTA, only the results from one study concerning the effect of a detoxifying agent on this mycotoxin in pigs are available (Hofstetter et al., 2006). But although beneficial effects have been related, this study does not allow for evaluating the effect of this product (combination of clay minerals, rumen bacterium (*Eubacterium* BBSH 797) and yeast (*T. mycotoxinivorans*) on OTA because a co-contamination was tested: 0.5 ppm OTA and 0.2 ppm ZEA.

#### 4.1.2.3. Trichothecenes

Among the farm animals, pigs react most sensitively to exposure to trichothecenes (Eriksen and Petterson, 2004). The majority of studies on the efficacy of detoxifying agents in pigs were carried out with the inclusion of *Fusarium* toxin-contaminated grains, with the most frequently naturally occuring trichothecenes, DON, as the main contaminant.
However, the presence of further *Fusarium* toxins, such as ZEA, or trichothecenes besides DON, was indicated in many cases, and the presence of *Fusarium* metabolites which were not analysed cannot be excluded in naturally or artificially *Fusarium* infected feed.

<u>DON and NIV</u>

The particularities of DON metabolism in swine explain why this species is more susceptible to DON than other domestic animals. Indeed, DON is rapidly and efficiently absorbed, extensively distributed in tissues and body fluids, and poorly metabolized in pigs (Prelusky et al., 1988).
Swine are more sensitive to trichothecenes than rats (Vesonder et al., 1979), poultry, and especially cattle which seem to be more tolerant (Eriksen and Petterson, 2004; Rotter et al., 1996; Trenholm et al., 1984). The most obvious effect of DON in pigs is the decrease of feed intake. This reduction is observed from DON levels of 1 ppm in feed. To explain this, a direct effect of DON on brain transmitters is considered. As a direct consequence of decreased feed intake, DON alters the growth rate of pigs (Foster et al., 1986; Friend et al., 1982). The effects of DON on the growth rate of pigs result then rather from a lower feed intake, and not from an alteration of their metabolism.
DON affects also the immunological status of pigs: one of the its most pronounced effects is an increase of total IgA in the serum (Oswald, 2007; Pestka, 2007). In pigs, *in vitro* and *ex vivo* data

suggest also that DON could decrease the barrier function of intestinal epithelial cells with a concomitant increase of bacterial translocation (Pinton et al., 2007).

When regarding the few studies where the specific effects of the detoxifying agents have been monitored in pigs, mineral adsorbing agent seems to be ineffective in reducing DON absorption. (Döll et al., 2005) showed that 0.4% of in-feed organophil modified montmorillionite was unable to reduce the concentration of DON in the serum of weaned piglets fed a DON contaminated diet (8.6 ppm). However, a detoxifying agent (content of the large intestine of hens mixed with contaminated corn prior the incorporation of the corn in the pig's feed, 5 ml/g corn) was effective to partially detoxify a corn contaminated with 450 ppm of DON (He et al., 1993). A reduction of 56% has been measured in the feed containing the detoxified corn compared to the non-detoxified feed. Studies have been performed also with a combination of an adsorbing agent and a detoxifying agent. (Dänicke et al., 2004) have shown that this product (0.25% in feed) led to the reduction of the DON concentration in the faeces of growing pigs fed a diet contaminated with 6.6 ppm DON. However no beneficial effect has been observed on the DON levels in urine, nor in serum, suggesting that the absorption of DON by pigs remained unchanged.

The unspecific effects of these three detoxifying agents confirm what was previously observed. Indeed, the modified aluminosilicate tested by Döll et al. had no beneficial effect on the few parameters affected by the DON intake: growth performance and vulva swelling (Döll et al., 2005). Similarly, a combination of *Eubacterium* BBSH 797 with dried yeasts and clays showed no beneficial effect on growth performance, which was the only parameter that was affected by DON intake. At the opposite, the single product that had shown a beneficial effect on a specific parameter (DON level in feed) showed also beneficial effects on the zootechnical performances of pigs (He et al., 1993). Even if the process used in this study is not suitable for a practical application, it raises the possibility of using a microbial system to reduce the toxicity of DON contaminated feed.

Other studies have tested the efficacy of detoxifying agents for DON taking into account only the unspecific effects.

Concerning mineral adsorbing agents and looking at the effects of the detoxifying agent in the DON-contaminated diet, it becomes clear that none of the classical growth studies (Friend, 1984; Patterson, 1973; Patterson and Young, 1993; Wetscherek et al., 1998; Williams et al., 1994) were able to demonstrate that the addition of a mineral adsorbing agent to a DON-contaminated diet could clearly ameliorate the depressive effect on the zootechnical performance of pigs.

Two commercialised adsorbing agents (polymeric glucomannans extracted from cell wall of yeasts) have been tested and the results are more variable. Indeed, Wetscherek et al. have tested a polymeric glucomannan (1% in feed) in growing pigs fed DON-contaminated diet (2-2.5 ppm), and no beneficial effect on the zootechnical performance has been observed, suggesting that this polymeric glucomannan is not able to sequester DON (Wetscherek et al., 1998). However, Diaz-Llano and Smith have demonstrated a positive effect of another polymeric glucomannan in sows fed DON-contaminated diets (5.5 ppm) during a whole reproductive cycle. The observed deleterious effects of DON (reduced performance and increased stillbirth piglets) were reduced (Diaz-Llano and Smith, 2006). However, this beneficial impact of this product on the sow's performance was not found again when the experiment was repeated (Diaz-Llano and Smith, 2006).

A most original organic adsorbing agent has been also tested: apple pommace incorporated at the level of 8% in DON-contaminated feed (3.1 ppm) of piglets. The deleterious effect of DON on weight

gain is reduced, and the results obtained with the "positive control" suggest that apple pommace is able to reduce DON absorption in pigs (Gutzwiller et al., 2007).

Compared to adsorbing agents, bacterial agents are much capable in detoxifying DON in pigs (EFSA, 2005; He et al., 1993). When DON-contaminated corn is mixed with the content of large intestine of hens (5 ml/g corn) previous the feed fabrication, the resulting DON level in feed (5 ppm) is decreased and the deleterious effect of DON on the zootechnical performance of the piglets are reduced (He et al., 1993). A commercial *Eubacterium*-based product seems also to be able to reduce the deleterious effect of DON (1.2-2.5 ppm) on the zootechnical performance of piglets from the dosage of $5x10^8$ cfu/kg feed in piglets, while no beneficial effect was observed in fattening pigs (EFSA, 2005). However, the designs of the studies related in (EFSA, 2005) were not appropriate to conclude for sure to a detoxifying action of this product. Indeed, only two treatments were used (myco +/detoxifying agent- and myco +/ detoxifying agent +).

A combination of an adsorbing agent and a detoxifying agent (0.25% in feed) can also reduce the deleterious effect of a DON contaminated diet (2.7-3 ppm) in growing pigs (Dänicke et al., 2004), even if the previous commercial product, which was an *Eubacterium*-based product (1% in feed) was not working (Wetscherek et al., 1998). The accurate experimental design of the study of (Dänicke et al., 2004) allows concluding to a direct detoxifying action of the combined product on DON.

To conclude, as shown in **Table 8**, none of the mineral adsorbing agents tested has a beneficial effect in pigs fed DON-contaminated diets. Polyvinylpyrrolidone, which is an organic adsorbing agent, has no beneficial effect either, while yeast derived glucomannan and apple pommace are both beneficial. Biotransforming agents, such as the content of the large intestine of hens or *Eubacterium* tested alone, are able to detoxify DON-contaminated diets. When combinated products are tested the results are more variable.

T2-toxin

Chronic exposure to T2-toxin reduces feed intake and BW gain in pigs and induces muco-cutaneous lesions in the gastro-intestinal tract. Similarly, as with other trichothecenes, T2-toxin is an inhibitor of protein synthesis. It is also hematotoxic and immunotoxic with deleterious effects on the cell-mediated and humoral acquired responses. Acute exposure to T2-toxin induces oxidative stress in the liver and lipid peroxydation and it has recently been demonstrated that sub-clinical doses of T2-toxin has deleterious effects on a number of liver metabolising enzymes. The sensibility of T2-toxin to detoxifying agents has been the subject of a single study (Meissonnier et al., 2009). The authors studied if a yeast-derived glucomannan (0.2% in feed) was able to improve the vaccinal response in piglets fed T2-toxin-contaminated diets (0.54-2.10 ppm). Indeed, glucomannan demonstrated *in vitro* binding properties on mycotoxin (Yiannikouris et al., 2006; Yiannikouris et al., 2004), but as a yeast extracted component, it could also display immuno-modulatory effects (Zekovic et al., 2005). Results showed a deleterious effect of T2-toxin on the liver EROD and benzphetamine N-demethylation activities, but the glucomannan tested was not able to reduce these deleterious effects.

#### 4.1.2.4. Zearalenone

Pigs are very sensitive to ZEA. Diarrhea, vomiting, feed refusal, BW loss and hemorrhages could be seen, but estrogenic syndrome is the dominant clinical sign. Symptoms of intoxication in pigs are hyperemia and oedema of vulva with lightly clouded mucilaginous vaginal discharge. In severe cases, vaginal and rectal prolapse can be observed.

When regarding the only study (Döll et al., 2005) where the specific effects of a detoxifying agent (organophil modified montmorillonite, 0.4% in feed) have been monitored in piglets fed a ZEA-contaminated diet (1.2 ppm), the mineral adsorbing agent seems to be ineffective in reducing ZEA absorption. Indeed, the concentration in bile fluid of ZEA, α-ZOL and β-ZOL remained unchanged despite the incorporation of the modified aluminosilicate into the contaminated feed.

However, Sinovec et al. have demonstrated a beneficial effect of a modified clinoptilotite (0.2% in feed) on the growth performance of piglets fed a ZEA-contaminated diet (0.84 ppm). But if the deleterious effects of ZEA on feed intake, weight gain and feed efficiency were reduced, the experimental design (lack of a "positive control") does not enable to conclude in a reduction of the toxicity of ZEA or in a direct effect of the mineral adsorbing agent on the feed use (Sinovec et al., 2006). Coenen and Boyens have nevertheless showed a reduction of the uterus weight gain ZEA-induced (0.18-0.36 ppm) in the piglets receiving a ZEA contaminated diet with zeolite (2% in feed) (Coenen and Boyens, 2001).

E-GM, (0.1% in feed) was also able to reduce the deleterious effect of a ZEA-contaminated diet (0.84 ppm) on growth performance of piglets. But the lack of positive control keeps us from concluding in a reduction of the absorption of ZEA (Sinovec et al., 2006).

Oldest studies (James and Smith, 1982; Smith, 1980) have also tested the effect of a vegetal (alfafa, 15 or 25%) in piglets fed ZEA-contaminated diets (10-50 ppm). The results of the first study (1980) were not very good, but in 1982, the deleterious effect of ZEA on the weight gain of piglets was reduced, mainly at the level of 25% of alfafa in the feed, and a reduction of the uterus weight gain ZEA-induced was also observed. However, the alfafa had also a direct effect on the piglets: a reduction of the weight gain and of the uterus weight.

To conclude, as shown in **Table 8**, montmorillonite is not efficient to reduce the absorption of ZEA, whereas zeolite, yeast derived glucomannan and alfafa have this ability.

#### 4.1.2.5. Fumonisins

Pigs are very susceptible to fumonisins (mainly FB1), and depending on its concentration, route of uptake and the duration of exposure, FB1 can cause rather diverse pathological lesions such as pulmonary edema, cardiovascular lesions, pancreatic necrosis (Harrison et al., 1990) and hepatic intoxication (Theumer et al., 2002). The mechanism of action of fumonisins involves inhibition of the enzyme ceramide synthase, a key enzyme in the biosynthesis of sphingolipids (Wang et al., 1991). Unfortunately, until now, the effects of fumonisin on the cellular immune response of the pig and its defense mechanisms are only partially understood.

Piva et al. have monitored the variations in the sphinganin and sphingosin levels in serum and urine of pigs fed FB1-contaminated diets (30 ppm) with or without a mineral adsorbing agent (activated carbon, Sorbopor MV 125, 1% in feed). The intake of FB1 induced an increase of the concentrations of free sphinganine and of free Sa/So in urine and serum, and of sphinganine 1-phosphate, sphingosine 1-phosphate and total sphingosine concentrations in serum. Activated carbon has reduced the impact of FB1 on the free sphinganine and on the free Sa/So concentrations in serum, suggesting a reduction of the FB1 absorption. However, the deleterious effects of FB1 on the weight gain, feed efficiency, serum glutamic oxaloacetic transaminase and GGT activities, and of serum cholesterol concentration were not reduced. In addition, the intake of activated carbon emphasized the abnormalities caused by FB1 in lungs, heart, liver, pancreas and intestine. It had a beneficial effect only on kidney, spleen and lymph nodes. Concerning the immune status of the piglets, FB1 provoked

a decrease of the blood CD14 (monocyte) levels, while FB1 + activated carbon provoked a reduction of the blood CD4 and CD8 (T lymphocytes) levels (Piva et al., 2005). At the opposite, Cabassi et al. related no beneficial effect of activated carbon on these immunological parameters (Cabassi et al., 2005).

#### 4.1.2.6. Co-contamination

Amoung the studies performed to evaluate the efficacy of detoxifying agents in pigs, the presence of trichothecenes (mainly DON) in feed is concomitant with ZEA (Danicke et al., 2004; Diaz-Llano and Smith, 2006; Döll and Dänicke, 2004; Döll et al., 2005; Grunert, -; Gutzwiller et al., 2007; Hoppenbrock, 2002; Jaunet et al., 2006; Kyriakys et al., 2002; Papaioannou et al., 2002; Pietri et al., 1999; Swamy et al., 2002; Swamy et al., 2003; Weiβ et al., 1999).

Concerning mineral adsorbing agents, when the levels of ZEA + DON are high in the feed (2.5-8.6 ppm DON + 0.35-1.2 ppm ZEA), no beneficial effect of montmorillonite (0.4% in feed) has been detected in piglets (Döll and Dänicke, 2004; Döll et al., 2005). However, when much lower dosages of ZEA+DON are tested (0.14-0.31 ppm trichothecenes + 0.16-1.55 ppm ZEA) in sows, a beneficial effect of zeolite has been observed on the reproductive performance of the animals, although the experimental design of these experiments does not allow for concluding about an impact of zeolite on the ZEA+DON or ZEA or DON absorption in swine (Kyriakys et al., 2002; Papaioannou et al., 2002). These observations are in good agreement with what is observed for both mycotoxins when studied alone (see Table 8). Indeed, montmorillonite shows no beneficial effect in animal fed DON or ZEA contaminated diet, while zeolite can reduce the deleterious effect of ZEA.
A combination of *Eubacterium* BBSH 797 with dried yeasts and clays (0.15% in feed) is also efficient to improve the reduced reproductive performance of sows fed DON+ZEA low contaminated diets (0.3-0.5 ppm DON + 0.02-0.09 ppm ZEA), although the experimental design do not allow clear conclusions.

When fumonisin is largely predominant in a diet (23-30 ppm) compared to DON (4.6-6 ppm), a yeast derived glucomannan (0.05 to 0.2% in feed) demonstrated no capacity for reducing the deleterious effect of these mycotoxins on the performance of piglets, but some beneficial effects were observed on some physiological parameters which were affected by mycotoxins (Swamy et al., 2002).

Finally, a detoxifying agent composed of a blend of clay minerals, *Eubacterium* BBSH 797 and *T. mycotoxinivorans* is capable of counteracting the deleterious effect of a diet co-contaminated diet with OTA (0.5 ppm) and ZEA (0.2 ppm). A beneficial effect has been observed on the daily weight gain, but also on clinical signs such as swollen vulva and prepuce, rectum prolapse, vomiting and diarrhea, frequent urination and kidney damages (Hofstetter et al., 2006).

**Table 8: Mycotoxin detoxifying agents tested *in vivo* in pigs**

| DETOXIFYING AGENTS | MYCOTOXINS | | | | | |
|---|---|---|---|---|---|---|
| | Aflatoxins (AFB1) | DON-NIV | Fumonisin | OTA | T2 toxin | Zearalenone |
| HSCAS | + | - | | | | |
| Montmorilonite | | - | | | | - |
| Sodium bentonite | + | - | | | | |
| Calcium bentonite | + | | | | | |
| Zeolite | + | | | | | + |
| Sepiolite | + | | | | | |
| palygorskite | + | | | | | |
| Ammonium carbonate | | - | | | | |
| Charcoal | | | +/- | | | |
| Polyvinylpyrrolidone | | - | | | | |
| Yeast glucomannans | + | +/- | | | - | + |
| Apple pommace | | + | | | | |
| Alfafa | | | | | | + |
| Content of large intestine of hens | | + | | | | |
| *Eubacterium* | | + | | | | |
| Combination of *Eubacterium* BBSH 797 with dried yeasts and clays | | - | | | | |

Cells highlighted in green indicate that the product has shown positive effects in counteracting deleterious effects of mycotoxins. Cells marked in pink indicate that the product was not effective.
+ : positive effect of the mycotoxin-detoxifying agent
- : negative effect of the mycotoxin-detoxifying agent
+/- : positive effect of the mycotoxin-detoxifying agent on some parameters, no effect on other parameters

### 4.1.3. Ruminants

Ruminants are more resistant to mycotoxins than other species such as poultry and swine, and negative effects of mycotoxins are attenuate. However, a special attention should be paid to the possible transfer of mycotoxins, in particular aflatoxins, in milk, which remains the main concern for this species. This is the reason why this paragraph is not organized as the same way as paragraphs on poultry and swine and is focused on the fate of mycotoxins in ruminants and their transfer into milk.
The use of adsorbing agents capable of binding mycotoxin molecules can reduce toxin absorption from the gastro-intestinal tract. *In vivo* efficacy of detoxifying agents is assessed in two main ways: a) by monitoring the concentration of mycotoxins in body tissues and excreta, b) by assessing the toxicity and effect on animal performances in the presence and absence of the additive (**see Annex 12 table 3**).

#### 4.1.3.1. Fate of mycotoxins in ruminants

This is the most precise way as it considers the decrease in concentration of mycotoxin and/or its metabolites in different biological fluids and by comparing the balance excretion (faecal vs urine) or

the transfer into milk. The latter is the most commonly reported in ruminants where most of the studies have been performed on aflatoxin B and its metabolite (aflatoxins M) that is readily found in milk. A decrease in milk excretion (or in urine) associated with an increase in faecal excretion implies that mycotoxin absorption is impaired and has the potential to protect the animal as well as consumers against exposure. Both toxicokinetic and milk carry over studies provide reliable and specific results that can be satisfactory interpreted.

#### 4.1.3.2. Transfer into milk

As mentioned in **chapter 1**, the presence of mycotoxins in feeds could pose a risk to consumers if these toxins and/or their metabolites are excreted and accumulated in animal products such as milk and meat. The presence of mycotoxins in milk is a public health concern and has to be regularly monitored. A great majority of mycotoxins found in feeds do not cause problems either because they are not excreted into milk such as the majority of *Fusarium* toxins or they are excreted as a less toxic metabolite like ochratoxinα. This is why the majority of studies have been done on AFM1. When contaminated AFB1-feeds are ingested by ruminants, AFB1 is mainly transformed into AFM1 which is then excreted into milk. The amount of AFM1 found in milk represents normally 1 to 2 % of the ingested AFB1. However, it can reach 6% in high producing cows (Veldman and Meijs, 1992). AFM1 is toxic and carcinogenic; it is classified by the International Agency of Research on Cancer as class 2B, possible human carcinogens. Because of this concern, the AFM1 level in milk is regulated in several countries; the European Union limit of 0.05 µg/kg being one of the lowest in the world (EFSA, 2004).

#### 4.1.3.3. Available information on the use of mycotoxin-detoxifying agents to reduce AFM1 in milk

The detoxifying agents used in ruminants are mainly adsorbing agents and most of them are inorganic. They include some commercial clay products, such as HSCAS, calcium and sodium-bentonite and activated carbon.

- Inorganic adsorbing agents

Several *in vivo* experiments reported that inorganic adsorbing agents were able to reduce the concentration of AFM1 in milk without any modification of the milk yield.

Kutz et al. tested 2 different commercial HSCAS at 0.56% inclusion in dairy cow's diets, and reported similar reduction of AFM1 concentration (45 vs 48%, respectively) as compared to controls (Kutz et al., 2009). In another study, HSCAS was shown to be less effective (21.7%) even if it was used at 2% in diets (Galvano et al., 1996). In contrast, Smith and Phillips showed an increased efficacy of HSCAS in reducing milk AFM1 concentration with an increasing level of inclusion. A reduction of 51.9% was obtained with 1%, and it was increased up to 82.2 and 86.9% with 2 and 4% of adsorbing agent in the diet (Smith and Phillips, 1994).

A similar efficacy was obtained with two sodium-bentonites (58% for the first sodium bentonite and 65% the second one) when added at 1.2% to dairy cow diet (Diaz et al., 2004). In a second experiment, by the same authors, the result obtained with the first sodium bentonite (61%) was confirmed, but a lower efficacy (31.4%) was obtained with calcium-bentonite tested in the same experiment at a same level (1.2%) in the diet. In a study carried out with dairy goats (Nageswara and Chopra, 2001), the effect of sodium bentonite was similar to that reported for cows (Diaz et al., 2004).

Activated carbon was also tested in ruminants. The addition of activated carbon in the feeds contaminated with AFB1 also showed a significant reduction of milk AFM1 concentration. Galvana et al. investigated 2 different commercial activated carbon (AC1 vs AC2) included at 2% of the diet, and observed a slight reduction in AFM1 concentration in cow's milk (45.3 vs 32.5 as compared to controls) (Galvano et al., 1996). In a dairy goat experiment (Nageswara and Chopra, 2001), using a 1% inclusion in the diet reported a higher reduction (81.7%). In contrast, at lower level of inclusion (0.25%), activated carbon was not effective in dairy cows (Diaz et al., 2004).

- Organic adsorbing agents

For organic adsorbing agents, few data are available on AFM1 excretion in milk. There are two published experiments carried out on dairy cows fed different levels of AFB1 from contaminated feeds, and with different inclusion levels of yeast-derived glucomannan. In the first experiment, this product added at 0.05% reduced AFM1 concentration by 58.5% (Diaz et al., 2004); while in the second experiment a non-significant reduction (only 4%) was obtained with a higher inclusion rate (0.56%) (Kutz et al., 2009).

#### 4.1.3.4. Zootechnical performances and toxicity

This way to evaluate the efficacy of detoxifying agents is indirect and consequently less precise; it is based on measuring animal toxicity and production performances with and without the feed additive. However, these criteria are unspecific and differences obtained between treated and not treated animals cannot be solely attributed to the detoxifying effect of the additive. Indeed some negative and/or positive side effects can be confounding, e.g. immunomodulating activity of β-glucans. Most *in vivo* studies have evaluated the efficacy of detoxifying agents using this indirect methodology. In general, concentrations of mycotoxins used in the ruminant experiments, even if they were higher than the average level found in naturally contaminated feeds, do not usually cause reductions in feed intake and milk production (Kutz et al., 2009).

#### 4.1.3.5. Biotransforming agents

The most recent approach tested is the use of the capacity of bacteria to biotransform some mycotoxins. To the best of our knowledge there is not published information on the use of biotransforming agents in ruminants.

### 4.1.4. Rodents

In contrast with other animal species where the *in vivo* efficacy of mycotoxin-detoxifying agents has been assessed evaluating their effects on performance parameters, the trials with rodents (rats and mice) have been more focused on the changes in the metabolism of mycotoxins.

The action of several mycotoxin-detoxyfing agents has been evaluated in rats and/or mice to reduce the toxiticy of aflatoxins, such as HSCAS and clinoptilolite (Mayura et al., 1998) and also some carotenoids (Gradelet et al., 1998).

Mayura et al. feeding pregnant rats to compare the potential of HSCAS and clinoptilolite to prevent the developmental toxicity of AFB1 found that both adsorbing agents alone were not toxic (Mayura et al., 1998). Pregnant rats were used as a sensitive model, because the developing rat embryo is susceptible to nutritional deficit and vulnerable to toxic insult from aflatoxin. HSCAS or clinoptilolite

were added to the diet at a level of 0.5%. Evaluations of toxicity included: maternal (mortality, BW, feed intake, and litter weights), developmental (embryonic resorptions and fetal BW), and histological (maternal livers and kidneys) parameters. AFB1 alone and with clinoptilolite resulted in maternal and developmental toxicity, and more important, feeding clinoptilolite+AFB1 produced severe maternal liver lesions (more than AFB1 alone), suggesting that this zeolite may interact with dietay components that modulate aflatoxicosis. Animals treated with HSCAS + AFB1, were comparable to non-contaminated controls. The metabolism study was performed by feeding male rats with diets containing 0.5% of HSCAS or clinoptilolite and, orally dosing 2.0 mg AFB1/kg BW. The concentration of AFM1 was decreased in presence of HSCAS, suggesting that the protection mechanism involves absorption and reduction of AFB1 bioavailability.

Gradelet et al. studied the effects of carotenoids on the initiation of liver carcinogenesis by AFB1, using male weaning rats which were fed β-carotene, β-apo-8'-carotenal, canthaxanthin, astaxanthin or lycopene (300 mg/kg diet), or an excess of vitamin A (21000 U/kg) before and during i.p. treatment with AFB1 (2 mg/kg body wt) (Gradelet et al., 1998). The *in vivo* effects of carotenoids on AFB1-induced liver DNA damage were evaluated measuring the *in vivo* binding of [3H]-AFB1 to liver DNA and plasma albumin. β-apo-8'-carotenal, astaxanthin and canthaxanthin, decreased these parameters, suggesting that these carotenoids exert their protective effect through the deviation of AFB1 metabolism towards detoxication pathways.

The effectiveness of some mycotoxin-detoxifying agents in decreasing the impact of OTA in feeds has also been evaluated in rats. Micronized wheat fibres (2% MWF) alone or in association with yeast cell walls (0.2% YCW) as active adsorbing agents of OTA (2 mg/kg) were reported by Aoudia et al. (Aoudia et al., 2008). The adsorbing agents did not significantly alleviate the growth depression caused by the contaminated diet. However, a significant protective effect of MWF was observed in terms of OTA concentration in plasma (40.5% decrease), kidney (28.1% decrease) and liver (38.8% decrease). Mixing this adsorginb agent with YCW did not significantly improve its protective activity against OTA. The faecal OTA concentrations were higher for MWF and MWF + YCW treated animals, as compared to the positive control. Cholestyramine (2%) tested *in vivo* in rats fed diets contaminated with OTA (1mg/kg) greatly increased the amount of OTA eliminated in faeces and reduced the amount in urine, and as a result, it decreased the amount found in the systemic circulation (Madhyastha et al., 1992).

Cholestyramine (2%) has also been effective in adsorbing the FB1, tested *in vivo* in rats fed a diet contaminated with toxigenic *Fusarium* verticillioides (20 mg/kg). The increase of sphinganine/sphingosine (SA/SO) ratio in urine and kidney of rats was used as a specific and sensitive biomarker of fumonisin exposure. The addition of cholestyramine to the FBs-contaminated diets consistely reduced both urinary and renal SA/SO ratios (Solfrizzo et al., 2000). On the contrary, the addition of activated carbon (2%) to fumonisin-contaminated diets (4 mg/kg) and tested *in vivo* with rats did not alter the change of SA/SO biomarker for fumonisin exposure, thus indicating that activated carbon is unlikely to be effective *in vivo* (Solfrizzo et al., 2001).

### 4.1.5. Fish

Fish are also susceptible to the deleterious effects of mycotoxins. For instance, trouts are the most sensitive to aflatoxin among domesticated animals (CAST, 1989). Tumors can take a year or more to develop, but 20 ppm is well above safe limits for trout, even for temporary feeding (Lee et al., 1971). Once ingested, AFB1 is metabolized into other forms. Carcinogenesis occurs when epoxide derivatives penetrate the nucleus of a cell and bind to the genetic material (Nakatsuru et al., 1990).

Smith reported unexpected improvements in growth and FCR in rainbow trouts, when clay minerals were added to the diet (Smith, 1980). He was unable to provide a suitable explanation until several years later, when researchers working with farm animals presented evidence of clay-aflatoxin interactions in fish intestinal tracts (Harvey et al., 1988; Lindemann, 1997). An examination of Smith's data supported this hypothesis and initiated a later study conducted by Ellis et al. in which the absorption, metabolism and elimination of dietary aflatoxin was traced in rainbow trout consuming AFB1 (20 ppb), with or without 2% dietary sodium bentonite added to the feed of growing fish (Ellis et al., 2000). Results demonstrated that the addition of 2% dietary bentonite blocked intestinal absorption of dietary aflatoxin, reducing liver and kidney aflatoxin loads by about 80% and increasing the amount of AFB1 found in the feces by about 470%, compared to control fish not fed bentonite. AFB1 or metabolite concentrations in urine increased for both groups of fish, but were always significantly lower in the bentonite-fed group. On average, insoluble aflatoxin metabolites accounted for 40-60% of the total aflatoxin load in tissues, indicating a high percentage conversion to an adduct that binds to protein (or other materials in the tissues) and is not extracted by solvents. All data indicate that 2% bentonite contained in trout diets contaminated with 20 ppm AFB1 significantly reduces the amount of AFB1 absorbed from the digestive system following ingestion of contaminated diets.

Another mineral adsorbing agent, an Egyptian montmorillonite, has been tested in Nile tilapia fish (*Oreachromis nilaticus*) (Abdel-Wahhab, 2005). Fish received an intragastric dose of montmorillonite in corn oil (0.5 µg/kg body weight) with or without sterigmatocystin (1.6 µg/kg body weight) twice a week. Sterigmatocystin (Stg), produced by *Aspergilllus versicolor* and *Aspergillus nidulans*, is closely related to aflatoxin and is a precursor in aflatoxin biosynthesis (Barnes et al., 1994). However, the acute and chronic toxicities of Stg are considerably lower (Scudamore and Livesey, 1997). The results of this study show that Stg was toxic and clastogenic to tilapia as indicated by the significant decrease of body weight and the increase in frequencies of micronucleated red blood cells (MN RBC) and chromosomal aberrations in the kidney. The intragastric administration of montmorillonite combined with Stg to fish resulted in a reduction of the number of MN RBCs and the frequency of chromosomal aberrations in the kidney compared with the group tested with Stg itself was found to be safe and successful in preventing Stg toxicity and clastogenicity.

### 4.1.6. Other species (Cats, Dogs and Horses)

Very little information has been found in the literature on the use of mycotoxin-detoxifying agents in *in vivo* trials with pets or other animal species such as horses.

Leung et al. reviewed the prevalence and preventive strategies for countering mycotoxins in pet foods. The use of some mycotoxin-sequestering agents, such as activated charcoal, silicate minerals, and cholestyramine, to prevent intestinal mycotoxin absorption in domestic animals, and also the application of microorganisms capable of detoxifying mycotoxins into non-toxic metabolites in animal feed were described (Leung et al., 2006).

The effectiveness of HSCAS as AFB1-detoxifying agent in dogs was evaluated by Bingham et al. The trial was undertaken to determine whether this clay (included at 0.5%) could protect dogs against aflatoxin absorption as indicated by a decrease in the amount of metabolites excreted in the urine. Dogs were fed a diet with low-level, sub-clinical dose of AFB1 (100 µg/kg BW). Urinary aflatoxin metabolites M1 and Q1 were identified and compared between samples from dogs fed the control diet

(no-clay). The HSCAS diet significantly reduced urinary AFM1 by 48.4% versus the control diet, protecting the dogs against aflatoxicosis (Bingham et al., 2004).

A polymeric glucomannan (GM) mycotoxin adsorbing agent was also tested for efficacy in preventing *Fusarium (*DON+DAS+fusaric acid+ZEA) mycotoxicoses in horses. A supplement of 0.2% GM polymer to the contaminated diet increased feed intake of horses compared with those fed the unsupplemented contaminated diet. The supplement of GM polymer prevented other mycotoxin-induced adverse effects, such as increases in serum GGT activity (Raymond et al., 2003).

Several studies have demonstrated the potential for hidden risks associated with the inclusion of non selective aflatoxins-adsorbing agents in feeds. Aflatoxin-adsorbing agents should be tested individually and thoroughly characterized *in vivo*, paying particular attention to their effectiveness and safety in target animals and their potential for harmful interactions (Mayura et al., 1998).

## 4.2. Identification of relevant end-points to be studied when testing the efficacy of mycotoxin detoxifying agents in *in vivo* trials

In order to clarify and verify the action of mycotoxin-detoxifying agents *in vivo* as adsorbing and/or biotransforming agents, several specific toxicokinetic parameters should be measured in the trials, based on the ADME (Absorption, Distribution in organs, Metabolism, Excretion) approach. This toxicokinetic model could be used to describe and predict the behaviour of a toxin in an animal body; for example, which parts (compartments) of the body a chemical may tend to enter (e.g. fat, liver, spleen, etc.), and whether or not the chemical is expected to be metabolized or excreted and at what rate.

According to the ADME approach, there are four potential steps: absorption, distribution, metabolism (biotransformation) and excretion. Absorption describes the entrance of the chemical into the body, and can occur through air, water, food, or soil. Once a chemical is inside a body, it can be distributed to other areas of the body through diffusion or other biological processes. At this point, the chemical may be biotransformed through metabolism into other chemicals (metabolites). These metabolites can be more or less toxic than the parent compound. After this potential biotransformation occurs, the metabolites may leave the body, be transformed into other compounds, or continue to be stored in the body compartments.

In the *in vivo* evaluation of mycotoxin-detoxifying agents as adsorbing agents or/and biotransforming agents of mycotoxins, the specific toxicokinetics parameters to be measured in the trials should be:

- Bio-availability of the toxin by analysis of the mycotoxin and/or its metabolites
- Excretion/absorption of the toxin
- Transfer of toxins to animals products.

Nevertheless in most trials, non-specific parameters are measured to estimate the negative impacts of the mycotoxins and the beneficial effects of the mycotoxin-detoxifying agents, such as:

- Productive parameters: body weight gain, feed intake, feed conversion ratio.
- Mortality/morbidity
- Weight (and relative weight to body weight) of target organs: liver, kidney, spleen, heart and guizzard (in poultry)
- Physiological samples, that need to be justified to the target-mycotoxin evaluated
- Blood serum parameters: total protein, albumins, globulins, key enzymatic activities
- Histologial evaluation of liver, or other target tissues.

When only these non-specific parameters are evaluated (biological effects), and in order to evaluate the effects of the detoxifying agents, even in the absence of mycotoxins, the design of the trial should include at least the following dietary treatments:

- Non contaminated diet (negative control)
- Mycotoxin-contaminated diet (positive control)
- Non contaminated diet with the mycotoxin-detoxifying agent
- Mycotoxin-contaminated diet with the mycotoxin-detoxifying agent.

Few *in vivo* trials using farm animals have been found in the literature with specific parameters determined, such as the analysis of toxins or metabolites in biological samples, the quantification of toxins found in urine and faeces or excreta, or the transfer of toxins to animal food products. These trials have been marked in **Annex 12**.

In the past, most *in vivo* trials performed to evaluate the efficacy of the detoxifying agents have focused on their effects on productive parameters.
Several papers reporting experimental *in vivo* and in farm studies are more and more considering and combining other parameters than purely zootechnical ones (BW and feed consumption) and/or the mycotoxin concentration in some excreta. These parameters are based not only on the toxicokinetic principle but also on effects such as:

- The weight of organs (liver, kidney, gizzard) and concentration of mycotoxin(s) in these organs;
- The concentration of mycotoxin adduct(s) and metabolite(s) in serum and bile;
- Serum chemistry, e.g. total protein, albumin, globulins, glucose, uric acid, cholesterol, bilirubin, inorganic phosphate, magnesium, glutamate dehydrogenase, γ-glutamyl transferase, aspartate aminotranferase, alanine aminotransferase, lactate dehydrogenase, creatine kinase, α-hydroxybutyrate dehydrogenase;
- Hematology measurements, e.g. red blood cell count, hematocrit and hemoglobin;
- Analysis of immunoglobulins in serum and bile: IgA, IgG, and IgM in serum and IgA in bile;
- Determination of antiboby titers for an infectious disease specific to a considered species;
- Ratio of sphinganine to sphingosine (SA/SO) which has been proposed specifically for fumonisins as a valid biomarker in rats (Solfrizzo et al., 1997; Solfrizzo et al., 1997; Solfrizzo et al., 2001);
- Brain transmitters concentrations (norepinephrine, 3,4-dihydroxyphenylacetic acid, dopamine, homovanillic, tryptophan, 5-hydroxytryptamine, 5-hydroxyindoleacetic acid) which are directly affected by DON and which affect in turn the feed intake.

Swamy et al. in an experimental study on the effects and efficacy of an esterified GM polymer derived from *Saccharomyces cerevisiae* diet in broilers chickens considered not only most of the above quantitative parameters on zootechnical performance, haematology and serum chemistry, analysis of immunoglobulins in serum and bile, determination of antiboby titer to infectious bronchitis vaccination, but also the qualitative paramater on the musculature look and color (Swamy et al., 2002).

Madhyastha et al. showed that dietary cholestyramine reduced the OTA concentration in blood and urine in rats, with an increased OTA excretion in feces. This finding was corroborated by (Kerkadi et al., 1998) with 5 % cholestyramine effective at 3 ppm OTA, in addition a decreased nephrotoxicity was demonstrated (Madhyastha et al., 1992). For sodium bentonite clay, a reduced aflatoxin concentration in liver and kidney and increased amount in feces has been shown for trout (Ellis et al.,

2000). On the other hand, activated carbon proved to be ineffective in reducing the SA/SO ratio in rats fed with a fumonisin-contaminated diet (Afssa, 2009; Solfrizzo et al., 2001).

More recently, glucomannan polymers, a combination of *Eubacterium* BBSH 797 with dried yeasts and clays, and micronized wheat fibers have been studied for their detoxifying capacity *in vivo*. E-GM has been shown to alleviate the growth depression, food conversion and some serological parameters in broilers (Aravind et al., 2003; Raju and Devegowda, 2000; Swamy et al., 2002). The effects of the combined product were mycotoxin-independent in chickens fed with naturally contaminated wheat or maize (Dänicke, 2002; Dänicke et al., 2003). However, a *T. mycotoxinivorans*-based product increased the FCR and attenuated the histological changes in the organs due to OTA contamination (Hanif et al., 2008). Aoudia et al. tested the effectiveness of mycotoxins sequestration activity of micronized wheat fibres on the biodistribution of OTA in rats and piglets (Aoudia et al., 2009; Aoudia et al., 2008). In these studies, micronised wheat fibres protected against weight increase of kidney and liver in piglets, although in rats the growth depression was not alleviated. A lower concentration of OTA was measured in plasma, kidney and liver in rats and piglets, with a higher fecal OTA in rats.

**CONCLUDING REMARKS**

- Most of the *in vivo* trials performed with farm animals have evaluated the efficacy of mycotoxin-detoxifying agents by studying the effects on animal performance, and other non specific parameters, such as weight of key organs, serum biochemistry or histological measurements.

- In some of these performance studies, the design of the trials did not allow for elucidation of the specific action of the mycotoxin-detoxifying agent, or for observation to determine whether the product itself presented other side effects.

- Very few trials have measured specific parameters (analysis of the mycotoxin and/or its metabolites, excretion/absorption of the toxin, or transfer of toxins to animal products) to define the specific action of the tested agents on the mycotoxins.

- Most of the *in vivo* trials evaluating the effects of detoxifying agents on the mycotoxin metabolism have been performed using rodents. This could be of concern for data extrapolation because of different sensitivity of the different animal species to each specific mycotoxin.

- Very little data is available on the effect of detoxifying agents tested on diets with mycotoxins found below the regulatory level.

- In poultry, many detoxifying agents have been tested in broilers, but there is very little information available on laying hens, breeders and turkeys. Adsorbing and biotransforming agents have been effective in detoxifying aflatoxins. In general, mineral adsorbing agents were not effective against thricothecenes. There is a lack of information on the effects of biotransforming agents on mycotoxins and on the metabolites produced by their action.

- In pigs, the efficacy of detoxifying agents has been tested mainly in weaned piglets, but also in sow and their litter during a reproductive cycle. Most studies concerned the efficacy of detoxifying agents for contaminations with trichothecenes (mainly DON) and ZEA. A fewer number were related to aflatoxin, and studies concerning fumonisins and OTA were rare. In pigs, the mineral adsorbing agents are effective in detoxifying aflatoxins, but seem to have no beneficial effect on trichothecenes. No data is available for OTA.

- In pigs, some prebiotics and probiotics are effective in detoxifying trichothecenes, and prebiotics also seem to be efficient for aflatoxin. But there is no available data concerning the other mycotoxins or metabolites potentially produced by biotransforming agents.

- Ruminants are more resistant to mycotoxins than other species, but a special attention should nevertheless be paid to the possible transfer of mycotoxins, in particular aflatoxins, in milk.

- *In vivo* efficacy of detoxifying agents is assessed by monitoring the concentration of mycotoxins in body tissues and excreta, and by assessing the toxicity and effect on animal performances in the presence and absence of the additive.

- The detoxifying agents used in ruminants are mainly adsorbing agents and most of them are inorganic.

- In *in vivo* trials, it is recommended to evaluate the efficacy of the detoxifying agents by measuring specific parameters (evolution of toxins); if this is not possible, factorial designs of the trials should include at least a non-contaminated diet and a contaminated diet, both with and without the mycotoxin-detoxifying agent.

# 5. RELATIONSHIP BETWEEN *IN VITRO* AND *IN VIVO* STUDIES

Mycotoxin-detoxifying agents have been assessed using both *in vitro* (see **chapter 3**) and *in vivo* models (see **chapter 4**). Most of these studies deal with feed additives acting as mycotoxin-adsorbing products. *In vitro* evaluations have been useful as a screening method for potential detoxifying agents. Unfortunately, *in vitro* methods are not standardized; therefore, results from different studies may not be directly compared or, as a first approximation, can be compared in terms of $K_d$ or BC50 (**see chapter 3**). Similarly, a standardized animal model for the assessment of efficacy of mycotoxin-detoxifying agents is not available (see **chapter 4**).

As underlined by Döll and Dänicke, efficacy of some detoxifying agents in preventing toxic effects in animals has not or inadequately been proven (Döll and Dänicke, 2003). Some of the *in vivo* studies used incomplete experimental designs or were limited to a recording of performance responses, which are rather non-specific. Any attempt to evaluate results across animal studies should consider that many factors/conditions can affect the outcomes of the studies. These factors can be related to the detoxifying agents (type and source of detoxifying agent, mode of action, inclusion rate and method of inclusion into feed…), the mycotoxins (single or multiple contaminations, natural or artificial contaminations…), the animals (species, ages, genders, health conditions, animal husbandry …) and even to the lots of product used for the study. Taking into account all these constraints, in order to make a comparison across *in vitro*/*in vivo* studies, those where detoxifiers are easily traceable and were the same from the beginning (*in vitro* testing) to the end (*in vivo* testing) of the study should be taken into consideration. This might allow reducing at least the variability due to the product(s) assayed, and check for a direct correlation between *in vitro*/*in vivo* models. Main results from studies reported in literature (some of them already cited in other chapters of the present document) are summarised below and arranged according to the mycotoxins and the adsorbing agents assayed. Studies were regarded in order to draw a possible, rough correlation between *in vitro* and *in vivo* models. Some results from all reviewed papers can be summarized as following.

## 5.1. Relationship between *in vitro* and *in vivo* studies for adsorbing agents

### 5.1.1. Aflatoxins-adsorbing agents

#### 5.1.1.1. Hydrated sodium calcium aluminosilicate (HSCAS)

Phillips et al. showed that HSCAS was able to form the most stable complex with AFB1 (with an adsorption of more than 80% of toxin present in the medium). The complex was demonstrated to be stable in water at pH 2, 7 and 10 and a temperature of 25 and 37°C (Phillips et al., 1988). Further studies with molecular modelling assessed that aflatoxin binding to HSCAS may be attributed to the chemisorption of aflatoxins at surfaces within the interlamellar region of HSCAS, whereas the exterior surfaces of the clay were responsible for only minor adsorption of toxins (Phillips, 1999; Phillips et al., 2008). Kubena et al. reported that the reaction between AFB1 and HSCAS was fast and reached the equilibrium after 30 min. Calculations based on the equilibrium binding experiments indicated that approximately 200-230 nmol of aflatoxin could be maximally bound per milligram of HSCAS (Kubena et al., 1990).

*In vitro* efficacy and specificity of HSCAS for aflatoxins was then confirmed by several *in vivo* studies as listed below (Phillips et al., 1995) and mentioned in **chapter 4**. In the first enterosorbent

study with aflatoxins, HSCAS (when incorporated into the diet of chicks at a level of 0.5% by weight) significantly reduced the effects of purified AFB1 (Phillips et al., 1988). Since these early studies, HSCAS has been reported to reduce the effects of aflatoxins in a variety of young animals including rodents, chicks, turkey poults, ducklings, lambs, pigs, mink and trout (Phillips, 1999). Levels of AFM1 in milk from lactating dairy cattle and goats were also diminished with the inclusion of HSCAS in the diet (Ellis et al., 2000; Harvey et al., 1991; Smith and Phillips, 1994). All these studies support the conclusion that HSCAS has a notable capacity for the aflatoxins at levels in the diet at, or below, 0.5% w/w (the level that is recommended for anticaking activity in animal feeds).

It can be concluded that there is a correlation between the *in vitro* and *in vivo* efficacy of HSCAS. Conclusions from these studies are detailed in **chapter 7**.

#### 5.1.1.2. Smectite clays (montmorillonites/bentonites)

Masimango et al. demonstrated early on that several bentonites had the ability to bind AFB1 in a buffer solution (Masimango et al., 1978). Dvorak showed that 2 samples of bentonite were effective in sequestering AFB1 in different liquid media such as water, saline solution, blood serum of pigs, stomach fluid of pigs, and bovine rumen fluid (Dvorak, 1989). The initial concentration of 3.6 mg or 18 mg AFB1 per litre was reduced to 0.3-27% after exposure to 50 g of adsorbing agent. Authors Winfree and Allred, and Ramos and Hernandez also demonstrated the high capacity of bentonites to sequester aflatoxins in several different solutions. These authors hypothesised that bentonites would sequester aflatoxins *in vivo*, thus reducing aflatoxicosis (Ramos and Hernandez, 1996; Winfree and Allred, 1992).

Over the past two decades the use of smectite clays to suppress aflatoxins has been demonstrated for many farm animals. In a series of experiments on growing swine, Lindemann et al. demonstrated that the addition of sodium bentonite (0.5%) to diets contaminated with 800 ppb AFB1 improved average daily feed intake and increased average daily gain. Bentonite supplementation significantly improved concentrations of blood urea, total protein, albumin and activities of AST, ALP and GGT, which were significantly altered by AFB1 (Lindemann et al., 1993).

Schell et al. also saw a positive effect of bentonite on growing pigs consuming aflatoxin contaminated diets without negatively affecting mineral metabolism (Schell et al., 1993; Schell et al., 1993).

Addition of a montmorillonite (5 g/kg) to aflatoxin-exposed rats prevented the degenerative changes in hepatic and renal tissue thus offering protection against serum biochemical parameters affected by the toxin (Abdel-Wahhab, 2005). These authors postulated that the bentonite formed a complex with the toxin thus preventing the absorption of aflatoxin across the intestinal epithelium.

Scheideler examined the effects of various commercial aluminosilicate products on aflatoxin toxicity to chicks and reported that these additives lessened the growth reduction caused by AFB1 (Scheideler, 1993).

Desheng et al. measured *in vitro* 0.6 g AFB1/kg adsorption to Ca2+-montmorillonite from water and observed that a 0.5% Ca2+-montmorillonite addition to feed contaminated with 200 μg AFB1/kg significantly reduced aflatoxicosis in chickens. In the mean time, they assessed that the concentrations of Ca, P, Cl, Fe, Zn in broiler bones were not affected by aflatoxin and montmorillonite, but the concentrations of Mn, Pb, and F were decreased by the clay (Desheng et al., 2005).

Recently, as mentioned in **chapter 4**, the efficacy of a modified montmorillonite nanocomposite to reduce the toxicity of aflatoxins in broiler chicks has been assessed (Shi et al., 2006). *In vitro* data

demonstrated that montmorillonite nanocomposite had a high ability to adsorb aflatoxins from aqueous solution and it was supposed to be a good candidate for *in vivo* testing. The results of *in vivo* study with broilers indicated aflatoxins can significantly affect overall animal health and performance, and MMN was effective in reducing the growth inhibitory effects and the increased relative organ weights produced by aflatoxins at the level used in the study. Moreover, there was apparent protection noted for some of the hematological, serum and liver biochemical and enzymatic changes associated with aflatoxin toxicity.

The work suggested that *in vitro* binding of aflatoxins to MMN was predictive of its efficacy *in vivo*.

Nageswara and Copra examined the effects of 1% sodium bentonite and activated charcoal additions to feed that contained 100 µg AFB1/kg on the AFM1 content in goat's milk. Concentrations of AFM1 excreted in the milk were reduced 65% by bentonite and 76% by activated charcoal (Nageswara and Chopra, 2001). Similarly, Diaz et al. found that 0.25% activated carbon had no effect in reducing AFM1 in cow's milk, but several commercial Na+-bentonite products effectively reduced AFM1 in milk (Diaz et al., 2004).

In conclusion, the results of the studies listed above indicate a correlation between the *in vitro* and *in vivo* efficacy of smectite clays in adsorbing aflatoxins. Conclusions from these studies are detailed in **chapter 7**.

#### 5.1.1.3. Tectosilicates (zeolites, clinoptilolites)

Since zeolites were identified as effective *in vitro* sequestrants of AFB1 (Dvorak, 1989), several studies have assessed their *in vivo* response in different animal models.

Scheideler fed a natural zeolite to broilers consuming 2.5 µg/kg AFB1. Zeolite added at 1% of the diet alleviated the growth depression and reduced the increase in liver lipid concentration caused by AFB1. Zeolite did however decrease serum phosphorus and chloride concentrations independently of aflatoxin exposure (Scheideler, 1993).

Sova et al. showed that zeolite inclusion at 5% of the diet significantly reduced heterophilia and lymphopenia in broilers consuming an aflatoxin-contaminated diet. The authors however noted that zeolite did not protect the animals against the acute alteration of liver parenchyma caused by AFB1 (Sova et al., 1991).

Harvey fed growing broiler chickens a diet contaminated with 3.5 ppm total aflatoxins with or without the inclusion of five different commercially available zeolites incorporated into the diets at 0.5%. Three of five tested zeolites were not effective in alleviating the aflatoxin-associated problems. One of the two effective zeolites (Zeomite mordenite) reduced the toxicity of aflatoxin by 41%, as indicated by weight gains, liver weight, and serum biochemical measurements. This result is compared positively with its *in vitro* capacity of binding aflatoxins (Harvey et al., 1993).

Zagnini et al. fed layers 2.5 ppm AFB1 with or without the inclusion of clinoptilolite. This at 3% of the diet was effective in reducing liver concentrations of AFB1 (Zagnini et al., 1998).

In another study, four synthetic zeolites were evaluated *in vitro* for their ability to adsorb AFB1 from an aqueous solution. One of them was selected to be tested *in vivo* in broiler chicks because of its high affinity and its stable association with AFB1. The efficacy of the synthetic zeolite (incorporated into diets (1%) containing 2.5 mg/kg AFB1) in binding *in vitro* AFB1 was confirmed *in vivo*, as it counteracted some of the toxic effects of AFB1 in animals (Miazzo et al., 2000).

Abdel-Wahhab and Nada found a significant reduction in AFM1 output in urine of rats exposed to aflatoxin, and concluded that zeolite reduced intestinal absorption of the toxin and hepatic metabolism into AFM1 and hence excretion in urine (Abdel-Wahhab and Nada, 1998).

Earlier work *in vitro* indicated that clinoptilolite can bind AFB1 (but less than 50% of that of HSCAS). *In vivo* adsorption of AFB1 by HSCAS or clinoptilolite in the gastro-intestinal tract of rats was confirmed in metabolism studies. The concentration of AFM1 was significantly reduced in animals treated with HSCAS or clinoptilolite + AFB1. Although reduction in AFM1 was significant in rats treated with both adsorbing agents, the greatest reduction in AFM1 concentrations was observed in animals treated with HSCAS. This finding suggests strong correlation between *in vitro* and *in vivo* results on binding of AFB1 by HSCAS or clinoptilolite. The strong AFB1 binding by HSCAS assessed *in vitro* resulted in significant reduction of bioavailability in the gastro-intestinal tract and in reduced animal toxicity. Clinoptilolite sequestered aflatoxin with low efficiency both *in vitro* and *in vivo* experiments, and resulted even toxic to animals. Importantly, the study demonstrates the potential for significant hidden risks associated with the inclusion of non-selective aflatoxin adsorbing agents.

In conclusion, zeolitic minerals showed much lower adsorption abilities towards aflatoxins than smectite clays. Results are given in **chapter 7**.

### 5.1.2. Mycotoxins other than aflatoxins adsorbing agents

#### 5.1.2.1. Silicate materials

HSCAS is characterized as “aflatoxin-selective clay,” and is not a good adsorbing agent of other mycotoxins (Phillips, 1999). Numerous *in vivo* studies have supported earlier *in vitro* findings that aluminosilicates have a notable preference for aflatoxins, i.e., they do not significantly prevent the toxicity of other chemically diverse mycotoxins such as cyclopiazonic acid, ergotamine, ZEA, DON, T-2 toxin, OTA, etc. and therefore, are not expected to be protective against feeds containing multiple mycotoxins. However, they can be protective for sterigmatocystin, a mycotoxin closely related to aflatoxin as a precursor in aflatoxin biosynthesis.

Indeed, good correlation was found between *in vitro* and *in vivo* results for sterigmatocystin binding onto montmorillonite (Abdel-Wahhab, 2005). The results of *in vitro* study showed that montmorillonite had a high capacity of adsorbing Stg, forming and adsorption complex that was stable under different pHs (from 2 to 10) at 37°C, and in different organic solvents. An *in vivo* experiment was conducted to evaluate the ability of montmorillonite (0.5 mg/kg bw) to prevent the toxicity and chromosomal aberrations induced by toxin (1.6 µg/kg bw) in the Nile tilapia fish. The intragastric administration of clay combined with Stg to fish resulted in a reduction of toxicity and frequency of chromosomal aberrations in the kidney compared with the group treated with toxin alone. The *in vivo* adsorption of Stg in the gastro-intestinal tract by the clay was confirmed by the reduction of toxin residues in fish tissues. It was concluded that montmorillonite itself was safe and successful in reducing toxin bioavailability, thus preventing its toxicity and clastogenicity.

Cyclopiazonic acid is not sequestered *in vivo* by HSCAS (Dwyer et al., 1997). Dwyer et al. tested three minerals (an acidic phyllosilicate, a neutral phyllosilicate and a zeolite), which had previously shown *in vitro* adsorption of cyclopiazonic acid. None of the three mineral clays were found to be effective in reducing the toxic effects of this toxin in broiler chicks when added to the diet at 1%. The results of the study suggested that *in vitro* binding of cyclopiazonic acid to clay did not forecast its

efficacy *in vivo*. The reasons for this discrepancy were not clear; the authors suggested that they may be related to differences in clay binding capacity and ligand selectivity for toxin *in vitro* vs *in vivo*.

No in *vitro/in vivo* correlation was found as regards the efficacy of HSCAS in adsorbing ergotamine. Huebner et al. and Chesnut et al. found that clays bind well ergotamine *in vitro* (Huebner et al., 1999). In particular, Chesnut et al. showed that HSCAS removed >90% of the ergotamine from aqueous solutions at pH 7.8 or lower, while no binding was recorded at pH 8. Supplementing ergotamine contaminated diets with HSCAS added at 2% did not reduce fescue toxicity both in rats and sheep. Moreover, addition of HSCAS to tall fescue hay diets did not affect apparent absorption by sheep of OM, N, Ca, P, Na, K, or Cu, but it reduced ($P < 0.05$) the apparent absorption of Mg, Mn, and Zn. Results suggest that HSCAS did not protect livestock from xenobiotic compounds responsible for fescue toxicosis and that HSCAS impaired the absorption of Mg, Mn, and Zn.

Results regarding the efficacy of silicate materials towards ZEA are somewhat contradictory. Bueno et al. indicated that bentonite could bind to ZEA *in vitro* and proposed this adsorbing agent as a good candidate for detoxification of ZEA present in feed (Bueno et al., 2005). Ovariectomized mink were fed diets containing ZEA at concentrations of 0, 10 or 20 ppm with or without 0.5% HSCAS for 24 days (Bursian et al., 1992). ZEA caused a significant increase in uterine weights, while 20 ppm ZEA resulted in significantly higher vulva swelling scores when compared to controls. HSCAS in the diet did not alter these hyperestrogenic effects of ZEA. However, HSCAS could alleviate some of the reproductive effects of ZEA which were not related to its estrogenic action.

In another study, HSCAS was added alone (at 400 mg/kg bw or 5 g/kg bw) or simultaneously with a toxic ZEA dose (40 or 500 mg/kg bw) to mice. The mixture of HSCAS with ZEA induced a reestablishment of haematological parameters, levels of serum biochemical enzyme activities and histological pictures of both liver and kidney (Abbes et al., 2006). It also prevented general toxicity of toxin. In general, data suggested that deleterious effects of ZEA could be overcome or, at least, significantly diminish by HSCAS, which did not exhibit any toxic effects by itself.

No effect in preventing toxic effects of ZEA by silicate materials were recorded *in vivo* studies by Williams et al. and Lemke et al. A pig assay assessed the efficacy of sweeteners (raw sugar or dehydrated molasses) or bentonite at dietary concentrations of either 20 or 50 g/kg in overcoming the adverse effects of a diet containing mouldy maize, naturally contaminated by ZEA and NIV (Williams et al., 1994). Pigs given diets containing the mouldy maize showed marked feed rejection typical of trichothecene toxicity and some scouring and vomiting were also observed. Females exhibited signs of precocious oestrus typical of ZEA toxicity. None of the additives was effective in overcoming either the oestrogenic or depressed performance effects of the mouldy maize (Lemke et al., 2001).

No correlation between *in vitro* and *in vivo* results was found by Lemke et al. in investigation of organophilic montmorillonite clay inclusion in ZEA contaminated diets of mice. Preliminary *in vitro* study showed that two organo clays were quite effective in adsorbing ZEA from aqueous solution. Besides, their *in vivo* effectiveness was tested using the mouse uterine weight bioassay. At a dietary inclusion level of 0.25%, the clays did not have a negative impact on overall animal health as measured by final body weight; however, they did not protect the animals from the estrogenic effects induced by 35 mg ZEA/kg in the feed (Lemke et al., 2001).

Silica materials did not reduce the toxicity of T-2 toxin (Dvorska and Surai, 2001; Kubena et al., 1997; Kubena et al., 1998; Kubena et al., 1990). In the study by Kubena et al. with young broiler chickens, an HSCAS was added at 0.80% to a diet containing 8 mg T-2 toxin/kg of diet (Kubena et al., 1998). The adsorbing agent did not reduce the toxic effects of T-2 toxin as evidenced by the lack

of significant differences between the T-2 toxin alone and the T-2 toxin plus adsorbing agent treatments for BW gains, feed consumption per bird, or efficiency of feed utilization. Similarly, inclusion of zeolite in a quail diet (containing 8.1 mg/kg T-2 toxin) at the level of 3% was ineffective in preventing antioxidant depletion in the liver by mycotoxicosis (Dvorska and Surai, 2001).

Silica materials did not prevent toxic effects of diacetoxyscirpenol (Kubena et al., 1993). HSCAS was incorporated into diets (0.5%) of broiler chicks containing 3.5 mg/kg aflatoxin and 5.0 mg/kg diacetoxyscirpenol singly and in combination. A significant interaction occurred between aflatoxin and diacetoxyscirpenol for some biochemical values and enzyme activities. Adding HSCAS resulted in almost total protection against the effects caused by aflatoxin alone, limited protection against the combination, but no protection against the diacetoxyscirpenol alone.

No protective effect by silica materials towards OTA has been recorded (Huff et al., 1992; Plank et al., 1990). Adsorption of the OTA by various bentonites (acid, alkaline, neutral), HSCAS and activated charcoal was tested *in vitro* as well as in feeding experiments with pigs (Plank et al., 1990). *In vitro* tests showed that the 1% addition of activated charcoal leads to complete adsorption of toxin from aqueous solutions, regardless pH (3-8). In contrast, adsorption by bentonite and HSCAS occurred primarily in the acid range (pH 3-4). Unlike charcoal (1%, 10% inclusion level) which caused a decrease of OTA in the blood; dietary addition of HSCAS (1%) and acid bentonite (1%, 10%) to OTA-contaminated feed (1.0 mg/kg) had no effect on the blood or tissue levels of the toxin in pigs (Plank et al., 1990).

Several *in vivo* studies confirmed the ineffectiveness of silica materials in protecting animals from the harmful effects due to the ingestion of multi-mycotoxins contaminated feeds. Garcia et al. did not find a relation between *in vitro* and *in vivo* trials with broilers, as respect the efficacy of two commercial adsorbing agents in sequestering OTA and T-2 toxin. An aluminosilicate-based product had the highest OTA and T-2 toxin *in vitro* binding ability (100% and 8.67%, respectively). Mycotoxins (0.6 ppm OTA and 0.9 ppm T-2 toxin) were fed alone or combined in treatments. After 21 days, blood chemistry, gross, and histological evaluations were performed. OTA toxic effects could not be counteracted by any adsorbing agent. T-2 toxicity could be partially counteracted by an adsorbing agent used in this experiment (Garcia et al., 2003).

Huff et al. also failed to see a benefit from adding HSCAS (0.5%) to diets of broilers containing OTA (2.0 ppm) and/or AFB1 (3.5 ppm). Addition of HSCAS alone did not alter any of the parameters evaluated (blood chemistry, gross). As expected HSCAS reduced the toxicity of aflatoxin, but had little effect on either toxicity of OTA alone or toxicity resulting from the combination of aflatoxin and OTA (Huff et al., 1992).

Same finding was obtained by Watts et al., which evaluated the effects of feeding a combination of mycotoxins at naturally occurring levels (1 mg DON, 5 mg moniliformin, 5 mg FB1, 100 µg AFB1, 1 mg ZEA and 0.5 mg OTA per kg of diet) in broiler chicks and turkey poults. The efficacy of HSCAS to ameliorate the effects of the combination of mycotoxins was also determined. Results of this study indicated that a combination of low levels of mycotoxins decreases chick performance and alters several haematological and serum biochemical values in poults. Addition of HSCAS at 1% to diets containing multiple mycotoxins did not prevent the negative effects (Watts et al., 2003).

#### 5.1.2.2. Charcoals

As reported in **chapter 3**, by *in vitro* studies, charcoals as well as activated charcoals have shown higher affinities for all toxins than any other adsorbing agents, and so far they are the best adsorbing

agents for the hydrophilic trichothecenes DON and NIV. However, charcoal based products are not selective in binding mycotoxins as clays may be; consequently, their efficacy in adsorbing *in vitro* mycotoxins can be dramatically reduced when more complex *in vitro* studies miming the digestion process of a contaminated feed are simulated (for more details see **chapter 3**). This can explain why the effectiveness of charcoal based products in sequestering *in vitro* mycotoxins has not been confirmed in most of animal studies, unless high dosages have been used.

Hatch et al. used activated charcoal as an antidote against a lethal dose of AFB1 in goats (3 mg AFB1/ kg BW). Animals consuming activated charcoal 8 hours after aflatoxin exposure had no visible liver lesions and had a lower percentage of hepatic damage (3%) compared to animals receiving no antidote (25%). In subsequent studies, the effects of activated charcoal have been variable (Hatch et al., 1982).

Studies by Dalvi et al. have shown protective effects by activated charcoals towards aflatoxicosis in chickens. When chickens were fed a mixture of 6 mg AFB1/kg BW and 200 mg of activated charcoal/ kg BW, functional alterations to the liver were reduced or prevented during a 2-month study (Dalvi and Ademoyero, 1984). (Dalvi, 1984) fed broiler chicks 10 ppm aflatoxin for 8 weeks. Improvement in feed consumption and weight gain was observed in the charcoal treated birds. The treatment was also able to reduce the effect of AFB1 on the microsomal cytochrome P450 and the activities of benzphetamine demethylase and SGOT.

Kubena et al. and Edrington et al. had very different results. Using broiler chicks, Kubena et al. found that activated charcoal included at 0.5% of the diet was not effective in reducing toxicity of purified AFB1 or of aflatoxins from naturally contaminated material and that activated charcoal may even enhance the toxic effect (Kubena et al., 1990). Urinary excretion of AFM1, an indicator of absorbed aflatoxin, was reduced by adding 0.5% activated charcoal (or 0.5% aluminosilicates) to feedstuffs consumed by turkey poults (Edrington et al., 1996). In this experiment however, the performance-associated effects produced by aflatoxin were not ameliorated by dietary charcoal inclusion. The authors suggested that aflatoxin adsorption by activated charcoal may occur via non-specific binding of AFB1 and may be altered in the presence of competing ligands. Similarly to this latter experiment with turkey poults (Edrington et al., 1996), responses to charcoal with broilers (Edrington, 1997), rats (Abdel-Wahhab, 1999) and mink (Bonna et al., 1991) also suggest that charcoal may not as effective in binding aflatoxin as are clay based adsorbing agents.

Galvano et al. showed reduced aflatoxin residues in milk of cows consuming different sources of charcoal, but responses to charcoal did not exceed that seen with HSCAS. Moreover, one of two activated charcoals tested in the study was ineffective in reducing transfer of AFB1 to AFM1 in milk (Galvano et al., 1996). Likewise, Diaz et al. showed that low levels (45 g/cow daily) of activated carbon did not significantly reduce milk aflatoxin residues, whereas clay –type adsorbing agents (225 g/cow daily) or an organic polymer of esterified glucan (10 g/cow daily) significantly reduced milk aflatoxins (Diaz et al., 2004).

Activated charcoal may be important in binding *in vitro* T-2 toxin (Bratich, 1985) and in preventing T-2 toxin toxicosis in animals (Buck and Bratich, 1986; Galey et al., 1987; Poppenga et al., 1987). Buck and Bratich observed a 70% survival rate in rats orally dosed with 6 times the $LD_{50}$ for T-2 toxin (25 ppm) when a commercially available superactivated charcoal was administered immediately after toxin exposure. However, two other activated charcoals assayed (with adsorptive capacity two or three times lower than superactivated charcoal) did not protect the rats exposed to the lethal dose of the toxin. Similarly, Galey et al. demonstrated that there is a potential benefit in giving a superactive charcoal as late as 3 and possibly even 5 h after rats have been given a lethal oral dose of T-2 toxin (8

mg/kg BW) (Galey et al., 1987). Poppenga et al. also demonstrated that the use of 2 g of activated charcoal/kg BW 30 min and 4 h after a 3.6 mg of T-2 toxin/kg BW as parenteral intoxication could have some beneficial effects (Poppenga et al., 1987).

Rotter et al. showed that although activated charcoal has a powerful activity in the adsorption of OTA *in vitro*, it has no effect on the OTA toxicity *in vivo*. In particular, they demonstrated that 50 mg of activated charcoal was able to adsorb 90% of OTA (150 µg) contained in 10 ml of a citrate-phosphate buffer (pH 7.0), but it was less effective in the presence of a complete chicken diet. The authors concluded that the addition of activated carbon was an impractical method of reducing OTA toxicity in poultry chronically exposed to this toxin (Rotter et al., 1989).

Activated charcoal treatment seems also to be ineffective at reducing the toxicity of fumonisins, despite the promising results obtained *in vitro* (Galvano et al., 1997). Solfrizzo et al. performed both *in vitro* and *in vivo* testing of activated charcoal for detoxifying fumonisin-contaminated feedstuffs (Solfrizzo et al., 2000). The activated charcoal showed a good capacity to adsorb FB1 from water solution and was proposed as a candidate to reduce fumonisin toxicity. However, it was not effective *in vivo* at least with respect to the alteration of sphingolipid metabolism. In particular, the biomarker of fumonisin exposure (kidney sphinganine/sphingosine ratio) in rats fed fumonisin-contaminated diet (4 ppm FB1+FB2) mixed with 2% activated charcoal was not significantly different from that of rats fed fumonisin-contaminated diet alone, suggesting that activated charcoal may not be effective at reducing the toxicity of fumonisins.

Activated charcoal may be important in binding *in vitro* ZEA and/or DON (Avantaggiato et al., 2005; Bueno et al., 2005; Döll et al., 2004); however, the relevant *in vivo* effectiveness needs to be assayed.

#### 5.1.2.3. Biological products

In the poultry industry, in the early 1990s, *Saccharomyces cerevisiae* has been used as general performance promoter in poultry feeds. It has been shown to bind AFB1 *in vitro* and have beneficial effects against aflatoxin exposure in animals (Celik et al., 2000; Celyk et al., 2003; Devegowda et al., 1996; Devegowda et al., 1998; Stanley et al., 1993). In an *in vitro* study with the cell wall material, there was a dose dependent aflatoxin binding of as much as 77% (w/w) and modified mannan-oligosaccharides derived from the *S. cerevisiae* cell resulted in 95% binding (Devegowda et al., 1996). Further *in vitro* studies have helped to characterise the interactions between a commercial E-GM and aflatoxin (Dawson, 2001). Serial elution of the aflatoxins adsorbed to E-GM clearly demonstrated that the adsorption of the mycotoxin is a concentration-dependent reversible process and that aflatoxins are not modified during adsorption.

In accordance with *in vitro* trials, *S. cerevisiae* 1026 live yeast, incorporated at 0.05% or 0.1% of feed into a standard broiler diet (containing 5 ppm aflatoxin), was shown to reduce the detrimental effects of aflatoxin on BW and weights of liver, heart and proventriculus in broilers (Stanley et al., 1993). The protective effect towards AFB1 (400 ppb in the diet) of live yeast (1%) was confirmed in rats, but thermolysed yeast (1%) was shown ineffective (Baptista et al., 2002). In another study, these authors assayed the capacity of the addition of 0.1 and 0.2% manno-oligosaccharides, 1% thermolysed yeast or 1% dehydrated active yeast to reduce the effect of aflatoxins (400 ppb in the diet) in rats (Baptista et al., 2004). No significant differences were observed for the weights of body organs from the animals fed with the different rations. However, the analysis of the liver tissue showed animals fed with aflatoxin, and those fed diets with aflatoxin amended with either manno-oligosaccharides or with thermolysed yeast had clear signs of toxicity and damage, while those fed with dehydrated active yeast showed less intense toxicity and less liver damage. Therefore, the thermolysed yeast and manno-

oligosaccharides did not suppress damage to liver tissue caused by aflatoxins, while active yeast reduced the aflatoxin symptoms in the hepatocytes (Baptista et al., 2004). On the other side, additions of E-GM at 0.5 or 1.0 g/kg to diets supplying 2 mg of total aflatoxin/kg diet resulted in dose dependent responses in broiler chicks (Basmacioglu et al., 2005).

Conflicting results have also been obtained with dairy animals consuming aflatoxin contaminated diets supplemented with yeast or E-GM products (Battacone et al., 2009; Diaz et al., 2004; Kutz et al., 2009; Stroud, 2006; Waltman et al., 2008). Diaz et al. reported that yeast-derived glucomannan bound 96.6% of AFB1 *in vitro* and reduced AFM1 concentrations by 59% *in vivo* when fed (0.05%) to lactating Holstein cows consuming diets contaminated with 55 µg AFB1/kg of diet (Diaz et al., 2004). Stroud also conducted *in vitro* and *in vivo* studies using the same product (Stroud, 2006). *In vitro* studied reported that yeast-derived glucomannan bound 96.2%, but the subsequent *in vivo* study reported that this product at 0.5% of a diet containing 170 µg of AFB1/kg of feed was not effective in reducing milk AFM1 concentrations (−8%), aflatoxin excretion (−7%), or aflatoxin transfer (−4%) from feed to milk. Similarly, Kutz et al., Waltman et al., Battacone et al. found that the addition of different kinds of non-digestible yeast oligosaccharides were not effective in reducing the AFM1 concentrations in milk (Battacone et al., 2009; Kutz et al., 2009; Waltman et al., 2008). In the study of Waltman et al., experimental sequestering agents (10g/cow/daily) consisting of yeast-derived glucomannan did not affect AFM1 concentrations when cows were fed diets containing 80 to 100 µg of AFB1/kg of diet. Kutz et al. found that yeast-derived glucomannan (0.56%) was not effective in reducing milk AFM1 concentrations (-4%), AFM1 excretion (-5%), or aflatoxin transfer from feed to milk (-2.52%) in cows consuming a total mixed ration containing 112 µg of AFB1/kg of diet. Battacone et al. showed that a dried yeast culture product (which is marketed as a probiotic feed supplement for high-producing dairy ruminants) fed at 12 g/day per ewe did not affect absorption of the aflatoxin in the gastro-intestinal tract of dairy ewes fed diets naturally contaminated with 1-5 µg of AFB1/kg of feed.

Fibrous material from the yeast cell wall was shown to have a potential to bind several mycotoxins (Devegowda et al., 1998). Raju and Devegowda have shown that mannans can also bind mycotoxins other than aflatoxins such as OTA and T-2 toxin (Raju and Devegowda, 2000). Yiannikouris et al. demonstrated the mechanism of binding of ZEA to β-d-glucans (Yiannikouris et al., 2004) and another study showed that modified yeast β-1,3-glucan have excellent binding with T-2 toxins in addition to ZEA mycotoxin (Freimund et al., 2003). The *in vitro* effectiveness of these glucan products in binding multiple mycotoxins was then confirmed *in vivo*. Raju and Devegowda demonstrated that the inclusion of a commercial E-GM improved BW gains and antibody titres suppressed by aflatoxin, OTA and T-2 toxin (Raju and Devegowda, 2000). The polymer also improved serum biochemical and haematological parameters. The glucan polymer product was protective against depression in antioxidant activities resulting from T2-toxin consumed by growing quail (Dvorska and Surai, 2001). Similarly, the glucan polymer product protected swine (Swamy et al., 2002), broilers (Swamy et al., 2004) and hens (Chowdhury and Smith, 2005) against some of the detrimental effects of multiple mycotoxins, but without restoring growth rate. A glucan polymer product was effective in preventing aurofusarin toxicity in quail (Dvorska, 2003). However, a glucan polymer product did not alleviate the toxic effects on mink consuming diets contaminated with fumonisin, OTA, moniliformin and ZEA (Bursian, 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., 2002; El-Nezami et al., 2000; El-Nezami et al., 2002; Haskard et al.,

2001; Oatley et al., 2000; Yoon and Baeck, 1999). The binding appears to be physical with DON, DAS, NIV, and other mycotoxins associated with hydrophobic pockets on the bacterial surface. Research reports on the subject are limited.

#### 5.1.2.4. Polymers

Polyvinylpyrrolidone is reported to bind with AFB1 and ZEA *in vitro* (Alegakis et al., 1999). A total of 0.5g/kg of polyvinylpyrrolidone can bind up to 50 µg/kg of AFB1 contained in feed (Thalib, 1995). Polyvinylpyrrolidone + bentonite partially ameliorated some haematological parameters altered by AFB1 administration to broiler chickens (Kececi et al., 1998). Polyvinylpyrrolidone did not alleviate the toxicity of DON seen in pigs (Friend, 1984). An experiment involving the addition of 5% of a divinylbenzene-styrene polymer (anion exchange resin) to the diets of rats supplemented with 10 mg of ZEA per 100 g of body weight resulted in reduced renal and hepatic residues of ZEA and its metabolites (Smith, 1982). The authors postulated that this may have been due to increased excretion of these compounds in bile. Carson and Smith demonstrated that when divinylbenzene-styrene polymers were added to diets of T-2 intoxicated rats, a beneficial effect was only achieved when anion-exchange and not cation-exchange resins were used (Carson and Smith, 1983). The use of a 5% anion-exchange resin minimized the growth-depressing effects or the reduction in feed consumption caused by T-2 toxin.

Cholestyramine was shown to adsorb ZEA (Avantaggiato et al., 2003; Avantaggiato et al., 2005; Döll et al., 2004; Ramos et al., 1996) and fumonisins (Solfrizzo et al., 2001). The *in vitro* efficacy of cholestyramine in binding fumonisins was confirmed in *in vivo* tests with rats, using the increase of the sphinganine/sphingosine ratio in urine and tissues as a biomarker of fumonisin exposure. When 2% cholestyramine was added to a diet containing 20 mg/kg fumonisins, the mean sphinganine/sphingosine ratios decreased significantly both in kidney and urine (Solfrizzo et al., 2001). Cholestyramine was able in reducing intestinal absorption of ZEA in a gastro-intestinal model simulating the gastro-intestinal tract of healthy pigs (Avantaggiato et al., 2003). In rats consuming OTA, cholestyramine reduced plasma OTA and increased fecal OTA excretion (Kerkadi et al., 1998). In another *in vivo* study, cholestyramine did not bind OTA (Bauer, 1994). Because of cost, cholestyramine use is questionable.

#### 5.1.2.5. Fibers

Indigestible dietary fibers have adsorbance potential for mycotoxins. Alfalfa fiber has reduced the effects of ZEA (James and Smith, 1982; Stangroom and Smith, 1984) in rats and swine and T2-toxin in rats (Carson and Smith, 1983). Interestingly, Tangni found that micronized plant-derived fibres efficiently adsorb OTA from a liquid medium (Tangni, 2003). Further, the effect of one micronized wheat fibres on OTA *in vivo* detoxification was evaluated through its incorporation (1-2% in the diet) into the daily feed of rats (Aoudia et al., 2008) or pigs. In both studies, a significant protective effect of MWF was observed in terms of reduction of OTA concentration in plasma, kidney and liver. These results suggest that the addition of micronized fibres is effective in decreasing the bioavailability of OTA from contaminated diets in piglets and rats.

#### 5.1.2.6. Humic acids

Humic acids are ubiquitous and are found wherever matter is being decomposed or has been transposed, as in the case of sediments. Van Rensburg et al. assessed *in vitro* and *in vivo* efficacy of oxihumate as an aflatoxin-adsorbing agent (Van Rensburg et al., 2006). Oxihumate showed a high *in vitro* affinity for AFB1, and bound 10, 7, and 12 mg of AFB1/g of oxihumate at pH 3, 5, and 7,

respectively. The *in vivo* efficacy of oxihumate as an aflatoxins-adsorbing agent in male broiler chickens exposed to aflatoxin-contaminated feed was also assessed. Oxihumate was effective in diminishing the adverse effects caused by aflatoxin on BW of broilers, and also showed protective effects against liver damage, stomach and heart enlargement, as well as some of the haematological and serum biochemical changes associated with aflatoxin toxicity.

## 5.2. Relationship between *in vitro* and *in vivo* studies for biotransforming agents

An alternative strategy to deactivate mycotoxins in animal feeds is the application to contaminated feedstuffs of microorganisms or enzyme systems having the capability to detoxify mycotoxins by metabolisation or degradation prior to their resorption in the gastro-intestinal tract. Indeed, this approach can be a specific, irreversible and environmentally friendly way of detoxification because it leaves neither toxic residues nor undesirable by-products. Biotransformation is not really a new issue. The first attempts to biotransform and degrade aflatoxins were already made a few years later the discovery of aflatoxins and the beginning of the modern mycotoxicolgy. A lot of detoxification studies on OTA, trichothecenes and ZEA were performed in the eighties and nineties. Published information on this area has been recently reviewed by Kabak and Wu et al.(Kabak, 2009; Wu et al., 2009), and is detailed on the **chapter 3** of the present document. However, although the first reports on *in vitro* studies regarding microbial detoxification of mycotoxins can be datable on sixties, up to now only few biotransforming agents, essentially microorganisms, have been tested for their efficacy *in vivo*. Main results of these studies with animals are summarized below.

### 5.2.1. Aflatoxin-biotransforming agents

There have been many studies of aflatoxin degradation carried out in laboratory conditions, but no biological system exists to be used in the full commercial sphere currently.
As mentioned in **chapter 3**, interesting results have been obtained by *Nocardia corynebacteroides* application. This soil bacterium is supposed to remove aflatoxins B, G, and M1 from a variety of food products, including milk, oil, peanut butter, peanuts, and maize, without leaving any toxic by-products (Wu et al., 2009). Recently, the effectiveness of this bacterium in irreversibly removing AFB1 from aflatoxin-contaminated chick feed has been confirmed by Tejada-Castaneda et al. The authors observed that it was able to decrease the accumulation of aflatoxin in the liver and alleviate the toxic effect of aflatoxins on liver, intestine and kidneys of poultry chicks (Tejada-Castaneda et al., 2008).

### 5.2.2. Trichothecenes-biotransforming agents

A lot of experiments on trichothecenes have been performed *in vitro* in the past 20 years using rumen fluid and intestinal contents. Rumen fluid was chosen because ruminants are known to be very resistant against toxic effects of trichothecenes, like DON. As mentioned in **chapter 3**, mixed cultures of anaerobic microorganisms are capable of detoxifying DON into de-epoxydeoxynivalenol (DOM-1), which was first described by Yoshizawa et al. (Yoshizawa et al., 1983). DOM-1 appeared to be non-toxic in toxicity studies (Kollarczik et al., 1994). However, no pure culture of the DON-biotransforming strain could be isolated. Binder and Binder through variation of medium components (energy source, minerals, antibiotics) and subsequent subcultivation in dilution series and highly active enriched cultures, were the first who isolated a pure bacterial strain, BBSH 797, that is able to bio-transform DON to DOM-1 (Binder and Binder, 1998). For the use of BBSH 797 as a feed additive, the fermentation and stabilization processes were optimized with respect to fast growth of the microbe and high biotransformation activity of the resulting product. For enhancement of stability during storage and within the gastro-intestinal tract, a three-step encapsulation process was

implemented. So far, strain BBSH 797 is the only microorganism that has been developed into a commercial product designed for detoxifying trichothecenes in animal feed. The results of animal trials showed that a BBSH 797-based product could significantly reduce the adverse effects of DON on sows and dairy cows, and T2-toxin on growing broilers. The positive effect of a BBSH 797-based on dairy cows may be due to the enhanced activity of rumen flora by the product (Hochsteiner et al., 2000). This biotransforming agent has been found effective in counteracting the toxic effect of DON on intestinal glucose transport (Awad et al., 2004), as well as to alleviate the histological lesions caused by DON in broiler chickens at the gut level (Awad, 2006).
Recently, several bacterial isolates have been successfully identified from chicken digesta which are able to transform DON through de-epoxidation into its derivative DOM-1 (Young et al., 2007).
An animal trial with starter pigs was subsequently conducted to test the effect of the "fermented" moldy maize on pig growth performance. The pigs were fed a basal diet containing DON contaminated maize with a concentration of DON equivalent to 5 µg/g diet or a basal diet containing the "fermented" moldy maize (detoxified-DON diet). While the pigs given a DON diet showed significant adverse effects, including a decrease in daily feed intake, weight gain and feed efficiency, the pigs fed given a detoxified-DON diet showed no difference in growth performance compared to those given a microbial control diet or given a toxin-free maize diet (Li et al., 2008).

### 5.2.3. Ochratoxin A-biotransforming agents

Pitout described that the enzyme carboxypetidase A was able to cleave OTA to phenylalanine and the metabolite ochratoxin-α (Pitout, 1969). The latter metabolite has been shown to be non-toxic or less toxic than the parent compound. Microorganisms capable of detoxifying OTA are *Phenylobacterium immobile* (Wegst and Lingens, 1983) and *Acinetobacter calcoaceticus* (Hwang and Draughon, 1994). Some reports on the OTA detoxification capabilities of *Aspergillus* and *Rhizopus* fungi were published (Varga et al., 2000). However, none of these strains were suited for the development of an OTA-deactivating feed additive.
Recently, a new yeast species named *T. mycotoxinivorans*, isolated from the hindgut of termites, was shown to have the best prerequisites for becoming an OTA-deactivating feed additive (Molnar et al., 2004). The inclusion of *T. mycotoxinivorans* at different levels ($10^4$ to $10^6$ CFU/g) to a diet containing OTA at 500 µg/kg reduced the detrimental effect of OTA on broiler chicks (Politis et al., 2005). In this study, in accordance with the *in vitro* tests, *T. mycotoxinivorans* completely blocked all the negative effects of OTA on the immune system of broilers.

### 5.2.4. Zearalenone- biotransforming agents

El-Sharkawy and Abul-Hajj reported that the fungus *Gliocladium roseum* is able to open the lactone ring of ZEA resulting in detoxification. (Duvick and Rood Jr, 1998) patented *Rhodococcus erythropolis* and *Nocardia globulera* as ZEA-degrading strains. There are no reports stating that these microbes were used for a practical application in feed (El-Sharkawy and Abul-Hajj, 1988).
Recently, it was found that *T. mycotoxinivorans* was also able to degrade ZEA to a metabolite (Molnar et al., 2004).

## CONCLUDING REMARKS

- Mycotoxin-detoxifying agents have been assessed using both *in vitro* and *in vivo* models. Studies were regarded in order to draw a possible, rough correlation between *in vitro* and *in vivo* models.

### ADSORBING AGENTS

- Good correlation between the *in vitro* and *in vivo* efficacy of HSCAS: adsorption of AFB1 with high affinity and reduction of its bioavailability in poultry. Several *in vivo* studies confirm that HSCAS is selective in its "chemisorption" of aflatoxins in the gastro-intestinal tract.

- Good correlation between the *in vitro* and *in vivo* efficacy of smectite clays in adsorbing aflatoxins: smectite clay can consistently suppress the impact of AFB1 in the diets of many types of animals.

- Activated charcoal is a relatively non-specific sequestering agent. The great variability in the results of long-term exposure experiments and its potential for also sequestering important nutrients diminish its overall practical effectiveness for routine dietary inclusion.

- The chemical complexity of mycotoxins means that the effectiveness of a compound in sequestering one mycotoxin does not mean equal ability to sequester other mycotoxins.

- *In vitro* evidence of toxin adsorption by an adsorbing agent in water provides little (if any) scientific proof of effectiveness in animals.

- A potential mycotoxin-adsorbing agent should be adequately tested not only for its *in vitro* binding capabilities, but also for its *in vivo* ability, because there is great variability in the efficacy of adsorbing agents *in vivo*, even though the compounds may show potential for toxin binding *in vitro*.

### BIOTRANSFORMING AGENTS

- Although there are many publications on biological transformation of mycotoxins by microorganisms, their application in detoxification of animal feeds have been limited. This may be due to lack of information about mechanisms of transformation, toxicity of transformation products, effects of the transformation reactions on nutritional values of the feeds.

- Further studies may lead to identification of more efficient and more applicable mycotoxin-detoxifying agents. Detoxifying mycotoxins in contaminated feed before feeding animals can be an alternative approach to feed additives, which will avoid adaptability limitations.

- In general, biotransforming agents to be used in practice as animal feed additives must rapidly degrade mycotoxins into non-toxic metabolites, under different oxygen conditions and in a complex environment. They must be safe for animals and stable in the gastrointestinal tract.

- *N. corynebacteroides* was shown to significantly remove aflatoxins from several substrates, including animal feeds, and was found safe for chicks.

- Only two microorganisms show the potential to be used for bio-detoxification of trichothecene-contaminated animal feeds.

- So far *T. mycotoxinivorans* is the only microorganism able to degrade OTA and which meet the prerequisites for use as animal feed additive. It also shows a good potential to degrade ZEA.

# 6. BENEFIT AND RISK ASSESSMENT OF MYCOTOXIN-DETOXIFYING AGENTS

## 6.1. Benefits

### 6.1.1. Specific benefits related to the reduction of mycotoxin contamination

Some mycotoxin-detoxifying agents have been tested *in vivo* to evaluate their effectiveness to counteract the adverse effects of mycotoxin-contaminated feeds on animal health (see **chapter 4**).

Benefits could be identified not only for animal health but also in an indirect way for human consumption due to the improvement of hygiene and safety of animal products. The occurrence of mycotoxins in feeds could pose sanitary problems for human consumers if these toxins and/or their metabolites can stay and/or be excreted in animal products. Along toxicokinetic pathways, residues and/or metabolites of mycotoxins can be found in some tissues and body fluids: intestine membrane, liver, bile, blood, and kidneys. For most animal species and mycotoxins, the half life of metabolites is short, a few days, except for OTA in pigs. So, the occurrence of OTA in pork kidneys could raise a human health concern, as could residues of others mycotoxins in liver. Even though the half life is short, contaminated feeding over a long period or through the animal's life could generate the continuous presence of residues and/or metabolites of mycotoxins in animal products. However, in general, mycotoxins are not accumulated in muscles. For all species, the metabolism is oriented towards excretion in urine and faeces, but also to eggs for poultry and into milk for mammals. Regarding lactating ruminants, a great majority of mycotoxins found in feeds do not cause problems either because they are not excreted into milk such as the majority of *Fusarium* toxins or they are excreted as a less toxic metabolite such as ochratoxin α from OTA. This is the reason why the majority of studies have been done on AFM1, the metabolite excreted in milk when contaminated AFB1 feedstuffs are ingested by ruminants (**see chapter 1 and 4**).

Regarding absorbing agents, on the basis of the toxicokinetic principle (see definition in **chapter 4**), since they can reduce mycotoxin absorption by the gastro-intestinal tract, they can reduce the further steps such as toxin distribution and metabolism in organs and tissues: for example, the more mycotoxins such as AFB1 and OTA are sequestred, the less residues could be found in liver and kidneys. The consequence is also the decrease of residue and/or metabolite excretion in eggs for poultry and in milk for mammals and in particular mycotoxins: for example the more AFB1 is sequestred, the less AFM1 could be found in milk (see below data from farm trials).

Since biotransforming agents are capable of modifying the molecular structure of some mycotoxins, the new conpound is supposed to be less toxic than the parent mycotoxin. If molecular structure(s) of new compound(s) is not modified during the toxicokinetic steps, the minimized toxicity is respected. Consequently, the action of biotransforming agents can improve the sanitary safety of some animal products.

### 6.1.2. General benefits

Some detoxifying agents, such as adsorbing agents, have shown also other health benefits than those of counteracting the direct and indirect effects due to mycotoxins. Some of them were even used at the beginning for these other health benefits and then tested and used for counteracting the effects due to mycotoxins.

*Saccharomyces cerevisiae* was initially used as a performance promotor in the early 1990s (Galvano et al., 2001). The cell wall of *Saccharomyces cerevisiae* is composed of beta-D-glucans which have, besides their high binding properties towards ZEA and AFB1 and to a lesser extent to FB1 and DON, a well-known ability to stimulate the immune system (Yiannikouris et al., 2004). Consequently, their application to animals may strengthen their resistance to infectious diseases.

Clays have been used in both human and animal nutrition for a long time (Phillips et al., 2008). Trckova et al. reviewed the advantages and risks related to feeding kaolin, bentonite and zeolites as supplements to animals (Trckova et al., 2004). Kaolin is considered to be a simple and effective means of preventing adserve effects exerted by many toxic agents, not only those from the environment but also those from living organisms. Kaolin combined with pectin is commonly used as a palliative for diarrhea and digestive problems in humans. In this review also, many papers are cited regarding the ability of kaolin when given to animals in their diet, to bind, in addition to aflatoxins, heavy metals, plant metabolites, poisons, diarrhea-causing enterotoxins, and pathogenic microrganisms. Bentonites have been also found to be excellent adsorptive materials of heavy metals and bacteria. Bentonites including in particular montmorillonites, when used in animal diets, act as gut protectants. Zeolites such as aluminosilicates, including clinoptolites, have been used not only as adsorbing agents of toxic compounds, but also to remove of excess ammonia both in the digestive tract (feed supplement) and bedding.

## 6.2. Risks

Some aspects should be considered on whether or not the use of mycotoxin-detoxifying agents could generate some adverse effects directly to animals or indirectly to humans and environment and specifically:

1. The consequence on nutritional aspects through interactions with some dietary compounds
2. The possible risks for animals and consequently humans consuming animal products and/or environment.

### 6.2.1. Consequence on nutritional aspects through interactions with dietary compounds

Two kinds of interactions could be considered. One is the possible long-term undesired adsorption of essential nutrients (i.e. vitamins and minerals) due to a lack of specificity of the adsorbing agents. Secondly, some nutrients (such as vitamins) or other feed additives (such as antioxidants) could also interact *in vivo* with mycotoxins by preventing their absorption or bioactivation or by enhancing their metabolisation or excretion and, as such, they could act as mycotoxin-detoxifying agents. In the later case there is no real interaction between nutrients (or other chemicals) with mycotoxin-detoxifying agents but rather an effect of the nutrient on the fate of the mycotoxins themselves.

#### 6.2.1.1. Lack of selectivity of physical sorbents used as mycotoxin-detoxifying agents

Very few *in vivo* trials have reported the possible interactions of the mycotoxin-detoxifying agents and other nutrients or feed compounds, and most of them were focused on vitamin or minerals availabilities (first kind of interaction).

Papaioannou et al. assessed the effect of the inclusion of clinoptilolite in feed (2%) on the levels of certain nutrients (Vitamin A and E, K, Na, P, Ca, Mg, Cu and Zn) in blood, liver and kidneys of sows under long term dietay use of the adsorbing agent. According to this study, there was no effect of clinoptilolite on nutrient uptake and their distribution in the body (Papaioannou et al., 2002).

Pimpukdee et al. assessed the minimal effective dose of a calcium montmorillonite clay, required to protect broiler chicks from AFB1 and the depletion of hepatic vitamin A levels. In this study no significant alteration in the hepatic concentration of vitamin A was observed at all HSCAS levels (0.125, 0.25, and 0.5%) tested against 5 mg AFB1/kg. These results suggest that the overall health and immune defenses of aflatoxin-challenged broilers were maintained and not compromised by a vitamin A deficiency (Pimpukdee et al., 2004).

One of the most comprehensive studies about the possible interactions of a mycotoxin-detoxifying agent with other micronutrients was performed by Afriyie-Gyawu et al. to assess the potential interference of HSCAS, with vitamins A and E and minerals (15 nutrient and 15 non-nutrient minerals) in humans, measuring them in serum samples (Afriyie-Gyawu et al., 2008). Results from this study clearly showed that administration of HSCAS over a 3-month period did not affect the serum concentrations of vitamins A and E and nutrient minerals and non-nutrient minerals, except for strontium levels that were higher in humans that consumed HSCAS at high doses compared to humans from placebo group. The main risk of aluminosilicates in animal feeding lies in binding some feed minerals, decreasing their utilization by animal (CAST, 2003). This phenomenon depends on the aluminosilicate and may be counted by increasing trace element content in feed. So the risk seems to be weak. It is not known if modified aluminosilicates may bind more or different trace elements than raw aluminosilicates.

In conclusion, it can be said that the available information about lack of selectivity of the mycotoxin-absorbing agents (first kind of interaction) is very limited and deals only with mineral adsorbing agents such as clay minerals. For example, no data has been found regarding activated charcoals.

#### 6.2.1.2. Other interactions involving nutrients and/or other feed additives

Some feed components, nutritional supplements or additives have themselves protective properties against mycotoxin toxicity. Several examples and cases could be mentioned:

- Additives and supplements

Since some mycotoxins such as AFB1, FB1, OTA and T2-toxin are known to produce membrane damage through increased lipid peroxidation, some antioxidant substances such as selenium and vitamin A, C and E, act as superoxide anion scavangers. In their review, Galvano et al. mentioned several papers on *in vitro* and *in vivo* studies showing that selenium inhibits AFB1-DNA binding and adduct formation and sodium selenite and selenium-enriched yeast extract protect cells from AFB1 cytotoxicity (Galvano et al., 2001). Several studies dealing with the effects of Vitamin A and E as well as Butylated Hydroxy Toluene (BHT) -an antioxidant considered as "Generally Recognised As Safe (GRAS)" according to the FDA assessement classification- can be found in the literature. BHT (4000 ppm) was tested in turkeys which are the most sensitive species to toxic effects of AFB1. BHT treatment significantly reduced the hepatocellular necrosis, biliary hyperplasia and elevated serum enzymes commonly caused by AFB1. One ppm of AFB1 decreased weight gain of the bird, but this effect was counteracted by BHT. It seems, indeed, that vitamins A/E and BHT can influence the fate of mycotoxins *in vivo* by modulating their bioavailability, their bioactivation, their metabolization etc, and, hence, they are able to decrease the deleterious effects caused by some mycotoxins such as aflatoxins and trichothecenes (DAS and DON).

Other compounds that were shown to be effective as aflatoxin-detoxiyfing agents are ammonia solutions (Allameh et al., 2005), calcium propionate (Bintvihok and Kositcharoenkul, 2006) and polyvinylpolypyrrolidone (Celik et al., 2000).

- Plant components

Plant components such as phenolic compounds, flavonoids, coumarins and even chlorophyll and its derivatives (chlorophyllin) have chemoproptective properties against carcinogenic substances including AFB1. For example, chlorophyllin acts as an interceptor molecule by forming a strong non-covalent complex with AFB1, reducing consequently AFB1-DNA adduct formation and hepatic toxicity such as liver tumors (Galvano et al., 2001).

In conclusion, this kind of interaction must not be confounded with possible side effects due to the use of mycotoxin-detoxifying agents but rather as information on a possible new way to use nutrients and other food/feed additives.

### 6.2.2. Possible risks for animals, consumers and the environment

This chapter only deals with the risks related to the function of mycotoxin detoxification of agents, and not related to the nature of these products. All the risks, related to the use of bacteria, enzymes, and other products, such riks associated with the selection and/or transfer of resistance to antimicrobials are not mentioned.

#### 6.2.2.1. Possible risks for animals

- Blending and particle size

Inorganic adsorbing agents may be administered finely ground in order to increase the surface with mycotoxins. Ultra fine particles represent a hazard because the smaller the particles size is, the more likely the pigs are to develop gastric ulcers (Hedde et al., 1985).

As shown in **chapter 2**, most mycotoxin-detoxifying agents are a blend of products, and often mixtures of several additives which have already been authorised, but for another use. Some mixtures of products can modify the physical structure of the initial additive. This is the case for a mycotoxin-detoxifying agent produced by OLMIX (TX+). By modifying the clay with a seaweed extract, OLMIX created a new product. The ulvans contained in the seaweeds have the ability, under specific technology, to modify the structure of montmorillonite. The seaweed extract acts as pillars between the layers, creating a ten-fold increase of the interlayer space and a much wider range in the absoption capacity of the clay. This newly developed nanostructure was named “Amadéite”. This new product has a new physico-chemical property. The production process and the final composition of the product were modified and could be assessed.

The Commission indicated in September 2008 (summary of meeting of Standing commitee on the food chain and animal health): “*Since this product (montmorillonite and 10 to 20% of algae) is derived from a manufacturing process, it was considered as a new product not covered by the old authorisation. If a company wants to market it as feed additive, it should submit an application under Article 4 of Regulation (EC) No 1831/2008 accompanied with related information under Article 7 of the same Regulation.*

In conclusion, particular attention should be paid to mixtures of products in particular those whose physical properties are changed by manufacturing processes.

Furthermore, considering scientific discussions and recent evolution of regulations at the European level in the field of nanotechnology in food area, the feed regulation should specifically consider the question of nanomaterials.

- Mistake in mixing proportion

A hazard could be generated by feed mixing mistakes; but the authors Miles and Henry using a HSCAS with up to eight times the recommended content in the feed observed no difference in blood parameters (Miles and Henry, 2007). So the risk associated with this hazard appears to be low.

There are not many references with modified clays but Lemke et al. have observed that a modified montmorillonite clay increased the effect of ZEA instead of reducing it (Lemke et al., 2001).

- Release of toxic bound agents

Another risk is the release of toxic components, molecules or elements bound to the clay and which would be released in the intestinal gut of animals and accumulate in animal organs. Among these subtances, heavy metals or dioxins found in clays are of concern.

Horii et al. provided new evidence for the natural formation of dioxins in ball clay, explaining the occurrence of concentrations of PCDDs found not only in American ball clay but also in kaolin clay from Germany and Japan. Dioxin profiles are characteristically dominanted by the congener OCDD. This pattern differs from the profile found for anthropogenic sources of contamination in river or surface sediments for example. Several hypotheses have been put forward to explain this occurrence: one of them is the formation of pyrolytic compounds from vegetation fires in sediments deposited before the Tertiary era (Horii et al., 2008).

As a result of a study to control feedstuff, the contamination caused by dioxins (PCDDs and PCDFs) in different feed ingredients of mineral origin was evaluated by Eljarrat et al. 15 samples of feed additives used as binder and anticaking agents such as bentonite, damoline, kaolin, magnesite, sepiolite and zeolite were randomly selected. The levels ranged from 0.05 to 460.59 pg WHO-TEQ/g. The highest concentrations were observed for the kaolin samples (Eljarrat et al., 2002).

A preliminary study on the influence of sepiolite in dioxin content in feedingstuffs of laying hens diet was undertaken by Abad et al. (Abad et al., 2002). The 3% sepiolite diet had an average dioxin content of 75 pg WHO-TEQ/kg and the analyses of egg samples collected over 8 months gave an average dioxin content of 345 pg WHO-TEQ/kg. (Parera, 2008) feeding chickens with a control diet, or a diet with a clay (3% in the feed) contaminated by PCDD and PCDF or a diet with added dioxins showed that dioxins from the clay were not available for the animals and were not found in liver contrarly to the dioxins added.

However, the release of toxic substances is possible: in the review by Trckova et al. on the advantages and risks related to feeding kaolin, bentonite and zeolites as supplements to animals several papers are cited indicating that the presence of dioxins was detected in animal meat samples and then related to the natural dioxin contamination of kaolin added in animal diets in 1997 (USA) and in 1999 (Europe) (Trckova et al., 2004). In 2004 an unacceptable dioxin level found in farm milk in The Netherlands was related to potato peels entering the feed chain after contamination through clay used as floating agent during the sorting process of potatoes.

Despite of a low risk for animals, this possible release of contaminants is a concern for consumers (see below “possible risk for consumers”).

- Risk of contamination with mycobacteria

In their review, Trckova et al. mentioned, based on several papers mostly published in 2003 and 2004, that the risk of contamination of kaolin by pathogenic mycobacteria must be considered: “They are

often found in surface water used for levigation during kaolin processing. If the final product is humid and the temperature used for drying does not reach the values necessary for conditionally pathogenic devitalisation, the risk of induction of tuberculosis lesions in pig and cattle lymph nodes is high" (Trckova et al., 2004).

- Enzymatic biotransformation and reversibility of degradation

Some biotransforming agents are capable of modifying the molecular structure of some mycotoxins. If the new compound is non toxic or less toxic than the parent mycotoxin, on the basis of the toxicokinetic principle, there are likely to be two possibilities:

1. The transformation is stable: the non or minimized toxicity of the transformed mycotoxin is kept until its excretion. For instance, most of the degradation reactions through oxidation and hydrolysis are not reversible.
2. The transformation is unstable: the non or minimized toxicity of the transformed mycotoxin is modified again before its excretion. For instance, glycosylation and glucuronation can still generate the parent compound through chemical or enzymatic reactions.

In the case of the second option, a reversibilty of toxicity could be expected and consequently a reversibility of risks for animals and for consumers through the consumption of some organs (liver, kidneys) and others animal products (milk, eggs, blood).

However no data has been found on this kind of possibility.

Enzymes cutting mycotoxins raise two questions: are these enzymes safe and are products from degradation of mycotoxins safe? Consequently, if a detoxifying agent containing an enzyme is proposed to avoid mycotoxin effects, the detoxifying agent must be subject to the same authorisation procedure as for any enzyme.

#### 6.2.2.2. Possible risks for consumers

The consumption of unsafe animal products could cause hazards and risks for consumers. The most likely origins would be through the release of trace elements and toxic substances such as dioxins and the reversibility of mycotoxin degradation (see above "risk for animals").

- Release of toxic bound agents

As reported by Trckova et al., the FDA detected in 1997, a contamination of poultry meat by dioxins. The source of the natural contamination was clays used as anticaking agents in diets (Trckova et al., 2004).

In 1999 dioxin contamination was detected in kaolin from Germany used in animal feed all over Europe. The maximum content was around 1600 pg/g and mean content was several hundred pg/g in kaolin samples. Animal species in France (laying hens and rabbits) on a diet containing such contaminated samples showed dioxin contents in their product above the permitted European levels. In its opinion issued in August 1999, AFSSA recommended a maximal limit of 2.5 pg dioxins/g of total feedstuffs, taking into account the possible ingestion of dioxin contamination in clay from the field (Afssa, 1999).

In 2004, an unacceptable dioxin level was detected in farm milk during routine testing in the Netherlands. As a result of the investigation launched by the Food and Consumer Product Safety Authority and the General Inspection Service, the contamination was found to have been caused by

potato industry by-products used as animal feed. More than 160 dairy farms in the Netherlands and Belgium have been closed after dioxin was found in dairy products. The potato industry by-product had been contaminated by marly clay used in the potato washing and sorting process. The marly clay used at the plant had been supplied by a German company and had a contamination level of 910 ng TEQ per kg of clay (Minister of Agriculture, 2004).

Arsenic and heavy metals are other examples of harmful chemicals that could be released from clays and for which it is necessary to check possible contamination of the ingredients used for the production of feed additives.

- Reversibility of mycotoxin degradation

If the non or minimized toxicity of the transformed mycotoxin is modified again before its excretion, a reversibilty of toxicity could be expected and consequently a reversibility of risks for animals and for consumers through the consumption of some organs (liver, kidneys) and other animal products (milk, eggs, blood).

However no data has been found on this kind of possibility (see above "Possible risks for animals").

#### 6.2.2.3. Possible risks for the environment

Two situations can be mentioned: the release of mycotoxins and the release of detoxifying agents.

- Release of mycotoxins

The first kind of hazard is the release of mycotoxins (or part of mycotoxins) in the litter and subsequently in the field. This risk is difficult to assess but is probably low. Some experimental researches have shown that mycotoxins are destroyed by gut microflora (Kollarczik et al., 1994) while others have shown that mycotoxins are destroyed by soil microflora (Mortensen et al., 2006), even though the degradation rate (days) depends on the nature of the soil (Angle, 1986; Mortensen et al., 2006). On cereals, it has been shown that spraying DON or NIV during the flowering stage increases the risk of *Fusarium* attacks (Maier et al., 2006), but this situation is experimental and not practical because litter is not spread on cereals or grass during flowering.

The second kind of hazard could also be due to the release of mycotoxins (or part of mycotoxins) in feces and subsequently in the environmental dust with the consequence of altering the air quality of in-door breeding facilities. In poultry production for instance, where environmental dust is a real problem, it consists of vegetation and animal particles, notably feed, feces, feathers, squams, microorganisms (moulds, bacteria, and virus) and litter particles. Even without using detoxifying agents, mycotoxins have been found in dust samples: NIV (20-50 µg/kg), DON (20-320 µg/kg) and ZEA (45 µg/kg) (Michel et al., 2007). The same mycotoxins have also been found in faeces (40-105 µg/kg) and feedstuffs (50-470 µg/kg). Their presence in dust would be due to the presence of dry faeces and feed. Also straw and straw dust may be contaminated by some mycotoxins. The amounts of mycotoxins found in dust appear to be low, and the calculated content of mycotoxins in the volume of air inhaled by a farmer during one working day remain inferior to the acceptable daily intake. However, as no data about the toxicity of mycotoxins by air route are available in human or animals, it is difficult to express an opinion about their impact on the farmer's health.

- Release of detoxifying agents

The second situation is the release of detoxifying agents in the litter and subsequently in the animal unit and in the field.

In the case where the detoxifying agent is an enzyme, the risk appears low because the enzyme will probably be destroyed by soil microflora.

In the case where the detoxifying agent is a persistent material (e.g. synthetic polymer), the risk is its accumulation in the soil. The consequence of this accumulation depends on the quantity of detoxifying agents brought to the soil by soil volume unit (considering not the total volume but the worked volume), the nature of soil (sandy, sandy clay, loam, silt…). On the other hand, aluminosilicates may be a hazard for people working in animal units, because they increase the risk of slipping on concrete floors.

## CONCLUDING REMARKS

Inorganic absorbing agents such as clays have been used both in human and animal nutritions for a long time: they seem to be an effective mean of preventing adserve effects exerted by many toxic agents, not only from the environment but also from living organisms. Organic absorbing agents such as yeasts have a well-known ability to stimulate the immune system. Consequently, a better resistance to infectious diseases may be expected.

Regarding mycotoxins, if the efficacy of a detoxifying agent is proven, it may benefit not only animal health but also in an indirect way human consumption due to improvements in hygiene and safety of animal products: on the basis of the toxicokinetic principle, since detoxifying agents can reduce mycotoxin absorption by the gastro-intestinal tract, they can reduce the subsequent steps such as toxin distribution and metabolism in organs and tissues.

However some possible risks or adverse effects have to be considered due to the facts that:

- Mycotoxin-adsorbing agents can interact with other nutrients or feed compounds, and particularly vitamin or mineras. In this case, biological availabilities and consequently health benefits of these nutriments could be reduced.
- Some feed components, nutritional supplements or additives have their own protective properties against mycotoxin toxicity such as antioxydant substances. This kind of interaction must not be confounded with possible side effects due to the use of mycotoxin-detoxifying agents but rather seen as information on a possible new way to use nutrients and other food/feed additives.
- The non or minimized toxicity of the modified mycotoxin due to the action of biotransforming agents could be modified again before its excretion, a reversibilty of toxicity may be expected and consequently a reversibility of risks for animals and for consumers through the consumption of some organs (liver, kidneys) and others animal products (milk, eggs, blood). However no data has been found on this kind of possibility.
- The release of toxic bound agents such as heavy metals, dioxins: despite a low risk for animals, this possible release of contaminants is a concern for consumers through the occurrence of these environmental contaminants in animal products (meat, fat, eggs, milk, etc.).
- The risk for animals of contamination of kaolin by pathogenic mycobacteria.

For all these reasons it is difficult to assess the benefit/risk balance because it must be determined case by case, i.e. mycotoxin by mycotoxin (even those not included in Directive 2002/32 because Directive 2002/32 will perhaps concern other mycotoxins in the future), animal species by animal species and detoxifying agent by detoxifying agent. On the whole, it seems that numerous situations may occur.

Consequently, recommendations or proposals to revise established guidelines could be useful for assessing risk in the agreement on how the product should be used (**see chapter 7**).

# 7. DISCUSSION AND RECOMMENDATIONS

This chapter is a discussion about **chapters 2 to 6** and points out the lack of knowledge, and the benefits and risks of using mycotoxin-detoxifying agents. It concludes with some recommendations for the assessment of mycotoxin-detoxifying agents.

## 7.1. Mycotoxin issues in animal feed: uncertainties and lack of knowledge

The identification of mycotoxin hazards is based on mostly incomplete toxicological data. Furthermore, some experimental studies highlight toxic effects following administration via routes which are far removed from the actual conditions of human and animal exposure. Even though some mycotoxins have been studied more thoroughly than others as regards their toxicological properties and effects on human and animal health, a more precise characterisation of hazards can only be achieved with new studies.

A list of mycotoxins of interest for the safety of animal feed in the European Union was given in an EU SCAN report (2003). It is well known that most mycotoxins can cause chronic diseases in animals. However, the different animal species do not react in the same way. It should be noted that toxicity may vary considerably within a structural group of mycotoxins and that the hazard or adverse effect may not always be due to the toxin itself but to its metabolites and possible synergistic modes of action in cases of multiple contamination.

Chronic intoxication can adversely affect on animal health and also lower zootechnical performance. One of the main bio-indicators of such effects is that animals ingest less feed which of course slows down their weight gain and results in a loss of income. Mycotoxin levels in feedstuffs which lower zootechnical performance depend on the animal species in question, the level of contamination and on the mycotoxin itself. This means that each mycotoxin at a given level of contamination can cause a specific sensitivity depending on the animal species: for example, pigs are more sensitive to fumonisins than poultry; also DON and ZEA are more toxic to pigs than poultry. Due to their economic importance, broiler chickens are the most frequently used animal model; in addition pigs and rats are used on a routine basis. This finding makes it more difficult to assess mycotoxin-detoxifying agents.

It should be noted that the available toxicological data are mainly related to individual toxins and not the effects resulting from a combination of mycotoxins although they may be found simultaneously in the same feedstuff. These co-contaminations could alter toxicity doses, thus making it difficult to characterise the hazard. This means that specific experiments should be undertaken as currently available data are incomplete.

The regulations and recommendations for mycotoxins in feed only concern a few mycotoxins, and their maximum acceptable levels, but these levels do not appear to have been based on pivotal studies.

Very few studies have investigated the effect of mycotoxins at levels lower that the maximum levels. Thus this observation is yet another difficulty to be overcome when evaluating the efficacy of products used in feed contaminated with low mycotoxin levels.

“Masked-mycotoxins”are an emerging concern but both analytical problems, and lack of *in vivo* toxicological data limit an accurate assessment of their importance.

## 7.2. Description and inventory of mycotoxin-detoxifying agents

### 7.2.1. Definition of the two categories

The Commission Regulation (EC) 386/2009 of 12 May 2009 defines a new functional group of feed additives as "*substances for reduction of the contamination of feed by mycotoxins: substances that can suppress or reduce the absorption promote the excretion of mycotoxins or modify their mode of action*".

It is important to clarify that the phrase "modify the mode of action" of mycotoxins could cover other feed additives than those which have been defined as detoxifying agents such as antioxidants which interact with the action of mycotoxins. This kind of substance is mentioned as being "responsible of interactions" or "interacting agents" of whose mode of action should not be confounded with the mode of action of detoxifying agents. These cases are mentioned in the section on "risk and benefit assessment".

Depending on their mode of action, these feed additives may reduce the bioavailability of the mycotoxins or degrade them or transform them into less toxic metabolites.

We can thus define a general category of "**detoxifying agents**", including two sub-categories as follows:

- **Adsorbing agents:** these decrease the bioavailability of mycotoxins thus causing a reduction of mycotoxin uptake and decreased distribution to the blood and target organs. Adsorbing agents are also called binding agents, adsorbents, binders, etc.
- **Biotransforming agents**: these have the ability to degrade mycotoxin molecules into non-toxic metabolites.

### 7.2.2. Inventory

An inventory of the products used or being developed as mycotoxin-detoxifying agents shows that:

- The products used in studies or listed are essentially binders. Very few studies (published, on websites or as confidential data) concern biotransforming agents;
- Most of adsorbing agents, especially aluminosilicates, have been tested for their ability to bind aflatoxins. The mycotoxins targeted by yeast cell wall, bacteria and other adsorbing agents are more diverse. Concerning mycotoxin-detoxifying agents, as anticipated by their mode of action, their spectrum in term of targeted mycotoxins, are narrower;
- The companies did not participate as expected;
- Most of the commercial products are compounds of additives belonging to the sub-categories: adsorbing agents and biotransforming agents.

## 7.3. Assessment of mycotoxin-detoxifying agents and *in vitro* studies

As mentioned in **chapter 3** (*in vitro* studies), very few studies concern biotransforming agents. Thus, the recommendations are on the whole based on adsorbing agents. New *in vitro* studies are needed to increase our knowledge of biotransforming agents.

### 7.3.1. What do these tests prove about the efficacy of mycotoxin-detoxifying agents?

First of all, if a mycotoxin-detoxifying agent does not detoxify a mycotoxin *in vitro*, it has little or no chance of doing so *in vivo*. *In vitro* laboratory techniques (as shown in **chapter 3**) can be very useful for identifying and ranking potential mycotoxin-detoxifying agents and for helping to determine favourable mechanisms and conditions for detoxification to occur. If an agent is not performing well *in vitro*, it must be concluded that it does not belong to the functional class of mycotoxin-detoxifying agents.

*In vitro* studies for assessing the efficacy of mycotoxin-detoxifying agents in binding/degrading mycotoxins can be performed using a simulated gastro-intestinal model, which is useful for identifying physiological conditions that are important for detoxification process. Several *in vitro* approaches, using so called "static" and "dynamic gastro-intestinal" models, have been developed to test the efficacy of mycotoxin-detoxifying agents.

Gastro-intestinal models are rapid and physiologically relevant methods for assaying the efficacy of mycotoxin-detoxifying agents and can be used in pre-screening studies to select the most promising materials for use as potential mycotoxin-detoxifying agents.

In general, all *in vitro* studies, ranging from simple single-concentration studies to complex studies simulating gastro-intestinal conditions, should be considered as key elements of an optimal prescreening strategy for selecting and ranking promising mycotoxin-detoxifying agents. Such a multi-tiered approach is highly desirable as it can limit the number of animal studies.

### 7.3.2. What do they not prove? What are their limits?

The fact that a mycotoxin-detoxifying agent does detoxify a mycotoxin in certain conditions *in vitro* is not a proof that it will also be effective in other conditions or *in vivo*. For example the presence of food matrix is likely to strongly reduce the apparent affinity and capacity of the adsorbing agent for the mycotoxin, so that experiments in the presence of food matrix should be part of the tiered approach. However, not all of the differences between *in vitro* experiments and actual *in vivo* conditions can be addressed experimentally, and even the most elaborate experimental gastro-intestinal models can still be too far from the *in vivo* conditions.

In the *in vivo* gastro-intestinal tract, small molecular weight compounds are transported across the intestinal epithelium into the body, thereby keeping the unbound compound concentration low in the chyme in the intestine. In beakers or batch agitated vessels used in the so called "static gastrointestinal models", the compounds are not removed from the chyme during simulated digestion. Consequently, the binding capacity may be overestimated when saturation of the compound occurs in the chyme in the beaker (Versantvoort et al., 2005). In addition, static *in vitro* methods do not really mimic the kinetic physiological conditions of the animal gastro-intestinal tract, including secretion of saliva, gastric juice, bile and pancreatic juice combined with peristaltic mixing and transit, and absorption of ingested compounds. An exception is the dynamic, multi-compartmental, computer-controlled *in vitro* gastro-intestinal model (TIM). However, this very sophisticated model cannot account for all environmental factors that can indirectly influence the severity of mycotoxicoses under field conditions, and shows also limitations. There are no real feedback mechanisms in TIM, except for pH and intestinal water absorption, there are no mucosal cells inside the model, and there is no immune system.

Few examples (see **chapter 3**) show how results from *in vitro* and *in vivo* studies may be compared quantitatively, using the BC50 and EC50 respectively.

### 7.3.3. How can the results of *in vitro* studies be expressed and compared quantitatively?

There are several different ways to define and express the affinity of an adsorbing agent for a toxin, depending on the model and equations used (Freundlich, Langmuir, etc). Fortunately the "distribution coefficient" $K_d$ (mL/g), defined as the ratio of bound toxin (µg/g) to free toxin (µg/mL) does not depend on any assumption or model and provides a universal basis for quantitative comparison of affinity. Furthermore it can be given an intuitively useful meaning, by interpreting it as equal to (or, in non-ideal cases, as approximately equal to) the inverse of the "BC50" (g/mL), i.e. the adsorbing agent concentration that will bind 50% of the toxin present. This "BC50" is also readily comparable with the various "EC50" endpoints that may be derived from *in vivo* studies.

## 7.4. Assessment of mycotoxin-detoxifying agents and *in vivo* studies

In the *in vivo* assessment of mycotoxin-detoxifying agents as adsorbing agents and/or biotransforming agents of mycotoxins, the following **specific parameters** based on the toxicokinetic to be measured in the trials could be:

- Bio-availability of the toxin by analysis of the mycotoxin and/or its metabolites
- Excretion/absorption of the toxin
- Transfer of toxins to animal products.

Nevertheless in most trials **non-specific parameters** are measured to estimate the negative impacts of mycotoxins and the beneficial effects of mycotoxin-detoxifying agents, such as:

- Productive parameters: body weight gain, feed intake, feed conversion ratio
- Mortality/morbidity
- Weight (and relative weight to body weight) of target organs: liver, kidney, spleen, heart and gizzard (in poultry)
- Physiological samples that need to be justified for the target-mycotoxin evaluated
- Blood serum parameters: total protein, albumins, globulins, key enzymatic activities
- Histologial evaluation of liver, or other target tissues.

Regarding the parameters to be used for assessing the efficacy of detoxifying agents, most of the reviewed papers have considered some but not all of them. So, the choice of relevant endpoints could be listed as follows.

### 7.4.1. General efficacy including relevance of endpoints and zootechnical parameters

Since absorbing agents which are capable of binding mycotoxin molecules, according to the toxicokinetic principle, can reduce toxin absorption by the gastro-intestinal tract, they also can reduce subsequent steps such as toxin distribution and metabolism in organs and tissues. This results in a reduction of toxic effects and metabolite excretions in urine and in milk for mammals and for particular mycotoxins, and an increase in toxin excretion in faeces. Also, the *in vivo* efficacy of absorbing agents can be assessed in several ways:

(1) By studying ADME in lab animals and/or monitoring the concentration of mycotoxins in farm animal excreta,
(2) By assessing the effects on animal health based on zootechnical performance,
(3) By considering other parameters and combination of them with those considered in (1) and/or (2).

#### 7.4.1.1. Concentration of mycotoxins in excreta

The most precise method evaluates the decrease in concentration of mycotoxins and/or their metabolites in different biological fluids by comparing the excretion balance between faeces and urine or by testing the transfer reduction into milk. The latter is the most commonly reported. A decrease in excretion via milk implies that mycotoxin absorption has been impaired which may protect the animal as well as consumers. Consequently, toxicokinetic studies and particularly those taking into account milk carry-over for female mammals provide reliable and **specific** results that can be satisfactorily interpreted. As mentioned in the **chapters 1 and 4**, the greatest concern is the carry-over of AFB1 into milk as AFM1.

#### 7.4.1.2. Zootechnical parameters

This way of evaluating the efficacy of detoxifying agents based on animal toxicity and zootechnical performances with and without the feed detoxifying agents is indirect and consequently less precise. These criteria are unspecific and differences obtained between treated and untreated animals cannot be solely attributed to the effect of the detoxifying agents. Indeed there may be some confounding negative and/or positive side effects, e.g. immuno-modulating activity of β-glucans, antioxidant action of other additives and feed components, as mentioned above.

#### 7.4.1.3. Consideration of other parameters and their combination

Several papers reporting experimental *in vivo* and farm studies are increasingly considering and combining other parameters than solely zootechnical ones (i.e. body weight and feed consumption) and/or the mycotoxin concentration in some excreta.

These parameters are based not only on the toxicokinetic principle but also on effects such as:

- The weight of organs (liver, kidney, gizzard) and concentration of mycotoxin(s) in them;
- The concentrations of mycotoxin adduct(s) and metabolite(s) in serum and bile;
- Serum chemistry, e.g. total protein, albumin, globulins, glucose, uric acid, cholesterol, bilirubin, inorganic phosphate, magnesium, glutamate dehydrogenase, γ-glutamyl transferase, aspartate aminotranferase, alanine aminotransferase, lactate dehydrogenase, creatine kinase, α-hydroxybutyrate dehydrogenase;
- Haematology measurements, e.g. red blood cell count, haematocrit and haemoglobin;
- The analysis of immunoglobulins in serum and bile: IgA, IgG, and IgM in serum and IgA in bile;
- Determination of antibody titers for an infectious disease specific to a given species;
- The proportion of sphinganine to sphingosine (SA/SO) which has been proposed specifically for fumonisins as a valid biomarker in rats;
- Brain transmitters concentrations (norepinephrine, 3,4-dihydroxyphenylacetic acid, dopamine, homovanillic, tryptophan, 5-hydroxytryptamine, 5-hydroxyindoleacetic acid) which are directly affected by DON and which affect in turn the feed intake.

The choice of the accurate parameters to measure is mainly based on the nature of the mycotoxins studied and the species of the target animal. But the impact of mycotoxins on these parameters depends also of the mycotoxin dietary concentration, the duration of exposure, the animal species the animal age, the animal health status, etc. As consequence, it is necessary to measure a sufficient number of the parameters previously cited in order to have a chance to observe an effect of the studied detoxifying agent. In addition, these tests can prove the efficacy of mycotoxin-detoxifying agents, but

they may also reveal some benefits related to the use of detoxifying agents without demonstrating that they actually act as detoxifying agents (confounding effects).

## 7.5. Discussion on the relationship between *in vitro* and *in vivo* studies: evaluation of *in vitro/in vivo* efficacy

Mycotoxin detoxifying agents have been evaluated using both *in vitro* and *in vivo* models. Most of these studies investigate feed additives acting as mycotoxin adsorbing products. Studies were regarded in order to draw a possible, rough correlation between *in vitro* and *in vivo* models.

### 7.5.1. Adsorbing agents

#### 7.5.1.1. Aflatoxins-adsorbing agents

- Hydrated sodium calcium aluminosilicate (HSCAS)

Results of studies indicate good correlation between the *in vitro* and *in vivo* efficacy of hydrated sodium calcium aluminosilicate (HSCAS). They show that HSCASclay can adsorb AFB1 with high affinity and high capacity in aqueous solutions (including milk) and in the meantime it can markedly reduce the bioavailability of aflatoxins in poultry; it can greatly diminish the effects of aflatoxins in young animals, i.e., rats, chicks, turkey poults, ducklings, lambs, and pigs; and it can decrease the level of AFM1 in milk from lactating cows and goats.

- Smectite clays (montmorillonites/bentonites)

Results of studies indicate good correlation between the *in vitro* and *in vivo* efficacy of hydrous aluminium phyllosilicate clay (smectite clays) in adsorbing aflatoxins, and show that there is clear evidence that smectite clay consistently suppresses the impact of AFB1 in the diets of many types of farm animals (CAST, 2003). This means that smectite clays with proven effectiveness as adsorbing agents of aflatoxin can be added to feeds containing aflatoxins below the action levels established by the EU or other regulatory agencies for the specified types of animals consuming the feeds.

It should be noted that the effectiveness of smectite clays as feed additives sequestering mycotoxins in/on feeds must be demonstrated by reliable and standardised *in vitro* assays. As pointed out by (Dixon et al., 2008), smectite clays occur in nature in large deposits yet they are impure and sometimes vary in composition and properties. The authors analysed many smectites from the United States and Mexico and found a ten-fold range in sorption maxima (Kannewischer et al., 2006). Thus, thorough analysis and reliable adsorption tests are required before they can be approved as feed additives.

- Tectosilicates (zeolites, clinoptilolites)

The results of the reviewed papers show that, when compared with smectite clays (montmorillonite/bentonite), zeolite minerals showed much lower adsorption properties with respects to aflatoxins. Results of the studies on zeolite minerals show some correlation between *in vitro* and *in vivo* efficacy of these minerals in adsorbing aflatoxins. However, there is clear evidence that these products do not adsorb aflatoxins equally and can even be toxic to animals. Therefore they should be thoroughly evaluated for efficacy and safety in sensitive animals before being included in animal diets.

#### 7.5.1.2. Mycotoxins other than aflatoxins / silicate materials adsorbing agents

The assumption that HSCAS is selective in its "chemisorption" of aflatoxins in the gastro-intestinal tract has been confirmed by several *in vivo* studies. Therefore, predictions about the ability of clays or zeolite materials to protect animals against the adverse effects of mycotoxins other than aflatoxins should be approached with caution and should be confirmed *in vivo*, while paying particular attention to the potential for nutrient interactions.

#### 7.5.1.3. Aflatoxins and other mycotoxins-adsorbing agents

- Charcoals

As reported in **chapter 3**, *in vitro* studies of charcoals as well as activated charcoals have shown higher affinities for all toxins than any other adsorbing agents, and so far they are the best adsorbing agents for the hydrophilic trichothecenes DON and NIV. However, charcoal-based products are not as selective in binding mycotoxins as clays; consequently, their efficacy in adsorbing *in vitro* mycotoxins can be dramatically reduced in more complex *in vitro* simulations mimicking the digestion process of contaminated feed. This may explain why the effectiveness of charcoal-based products in sequestering *in vitro* mycotoxins has not been confirmed in most animal studies, unless high dosages have been used.

Activated charcoal is a relatively non-specific sequestrant which is probably more suited for counteracting toxicity due to the accidental ingestion of high doses of mycotoxins. The great variability in the results of long-term exposure experiments and its potential for also sequestering important nutrients diminish its overall practical effectiveness for inclusion in routine diets.

- Organic polymers

Some complex indigestible carbohydrates (cellulose, polysaccharides in the cell walls of yeast and bacteria such as glucomannans, peptidoglycans, and others), synthetic polymers (such as cholestyramine and polyvinylpyrrolidone), humic acid and vegetable fibres can sequester mycotoxins.

The adsorptive capacity of the carbohydrate complexes in the yeast cell wall offers an interesting alternative to inorganic adsorbing agents. Modifications in manufacturing techniques have enabled the production of specifically modified yeast cell wall preparations with the ability to adsorb a range of mycotoxins. Several reports indicate the possibility of there being more than one target for mycotoxin binding in cell wall preparation. However, it is too early to interpret the mechanistic aspects and more basic studies are needed on the interaction of individual mycotoxins with different components of *S. cerevisiae* cell wall. More studies are needed on the chemistry of binding and stability of the complex, especially under the harsh conditions of the gastro-intestinal tract. Moreover, several studies suggest that yeasts or esterified glucomannan products may not be effective in reducing AFM1 concentrations. Further *in vivo* studies are needed to confirm the effectiveness of yeasts and derivative products in suppressing absorption of aflatoxins in ruminants. Results on the efficacy of synthetic polymers or vegetable fibres in sequestering mycotoxins are highly promising, although this field is still in its infancy and further research is needed.

#### 7.5.1.4. Conclusion and recommendations

There is excellent potential for the use of mycotoxin-detoxifying agents to counter mycotoxins. However, as noticed by several authors the following recommendations should be taken into account (Diaz et al., 2005; Phillips, 1999).

The addition of HSCAS results in almost total protection against aflatoxicosis, however its efficacy against other mycotoxins is very limited.

Clay and zeolite minerals are structurally and functionally diverse; they vary considerably from source to source and do not have equal affinities and capacities for aflatoxins and other mycotoxins, thus they should be rigorously tested one by one and thoroughly characterised *in vivo*, paying particular attention to their effectiveness and safety for sensitive animal models and their potential for harmful interactions. Similarly, generalisations should be avoided for all potential mycotoxin-detoxifying agents, as adsorbing compounds can differ in efficacy even within the same category.

The chemical complexity of mycotoxins means that a compound's effectiveness in sequestering one mycotoxin does not mean an equal ability to sequester other mycotoxins.

Some adsorbing agents have been reported to chemically sequester various mycotoxins (*in vitro*), and thus, would be considered to be non-selective in their action (for instance, activated carbon), since they could incur significant hidden risks due to interactions with critical nutrients in the diet.

Based on the available scientific literature, *in vitro* evidence of toxin adsorption by an adsorbing agent in water provides little (if any) scientific proof of effectiveness in animals. A potential mycotoxin adsorbing agent should be adequately tested not only for its *in vitro* binding capabilities, but also for its *in vivo* ability, because results in the past have indicated that there is great variability in the efficacy of adsorbing agents *in vivo*, even though the compounds may show potential for toxin binding *in vitro*.

### 7.5.2. Biotransforming agents

Although there are many publications on biological transformation of mycotoxins by microorganisms, their application in detoxification of animal feeds have been limited. This may be due to lack of information about mechanisms of transformation, toxicity of transformation products, effects of the transformation reactions on nutritional values of the feeds, and safe towards animals. Therefore, further study of these aspects may lead to identification of more efficient and more applicable mycotoxins detoxifying agents. Detoxifying mycotoxins in contaminated feed before feeding animals can be an alternative approach to feed additives, which will avoid adaptability limitations. Structures, stability and toxicity of transformation products and potential side-effects of the transformations should be investigated. Without this knowledge, no real advantage can be taken of these transformation reactions in the human/animal food chains.

In general, biological agents to be used in practice as animal feed additives must rapidly degrade mycotoxins into non-toxic metabolites, under different oxygen conditions and in a complex environment. They must be safe for animals and stable in the gastrointestinal tract. So far, few microorganisms fulfil these requirements.
Despite the efficacy of some of these transforming agents to alleviate the adverse effects of toxins on animals, it is necessary to confirm that the mode of action of biotransforming agents is due to the degradation and/or modification of mycotoxins and not by another mechanism that counterbalance the negative effects of toxins at the cellular or systemic level.

***Aflatoxins*:** Interesting results have been obtained by *Flavobacterium aurantiacum* application. This soil bacterium was shown to significantly remove aflatoxins from several substrates, including animal feeds, and was found safe for chicks, thus it may be used to partly detoxify chicken feed contaminated with aflatoxin.

***Trichothecenes:*** Although there are many publications on biological transformation of trichothecenes, their applications in detoxification have been limited. Only two microorganisms (*Eubacterium*: BBSH 797 and LS100) show the potential to be used for bio-detoxification of trichothecene-contaminated animal feeds.

***Ochratoxin A:*** So far *T. mycotoxinivorans* is the only microorganism that shows the potential to degrade OTA and meets the prerequisites for use as animal feed additive.

***Zearalenone:*** Although the efficacy of *T. mycotoxinivorans* in degrading ZEA to a metabolite needs to be assayed by animal experimentation, it shows a good potential to be used as animal feed additive.

## 7.6. Benefit/risk assessment

Inorganic absorbing agents such as clays have long been used both in human and animal nutrition: they seem to be an effective means of preventing the adserve effects of many toxic agents, substances from the environment but also living organisms. Organic absorbing agents such as yeasts have a well-known ability to stimulate the immune system. Consequently, better resistance to infectious diseases may be expected.

Regarding mycotoxins, although the efficacy of detoxifying agent has been proven, there may be benefits not only for animal health but also indirectly for human consumption due to the improvement in the safety of animal products: based on the toxicokinetic principle, since they can reduce mycotoxin absorption by the gastro-intestinal tract, they can subsequently reduce the further steps toxin distribution and metabolism in organs and tissues.

However some possible risks or adverse effects have to be considered due to the fact that:

- Mycotoxin-adsorbing agents can interact with other nutrients or feed compounds, particularly vitamins or minerals. In this case, biological availability and consequently the health benefits of these nutriments could be reduced.
- Some feed components, nutritional supplements or additives have their own protective properties against mycotoxin toxicity such as antioxidant substances. This kind of interaction must not be confounded with possible side effects due to the use of mycotoxin-detoxifying agents but rather considered as a possible new way of using nutrients and other food/feed additives.
- The non- or minimised toxicity of the modified mycotoxin due to the action of biotransforming agents could be modified again before its excretion, a reversibility of the toxicity could be expected and consequently a reversibility of the risks for animals and consumers through the consumption of some organs (liver, kidneys) and other animal products (milk, eggs, blood). However no data has been found on this possibility.
- The release of toxic bound agents such as heavy metals, and dioxins: despite a low risk for animals, this possible release of contaminants is of concern for consumers due to the occurrence of these environmental contaminants in animal products.
- The risk for animal of contamination of kaolin by pathogenic mycobacteria.

For all these reasons, it is difficult to assess the benefit/risk trade-off since it must be carried out case by case, i.e. mycotoxin by mycotoxin (including those not covered in Directive 2002/32 because Directive 2002/32 may cover other mycotoxins in the future) and detoxifying agent by detoxifying agent. In addition benefit/risk balance must be done for each targeted species. On the whole, it would appear that there are many possible cases.

## 7.7. Recommendation

### 7.7.1. How should *in vitro* studies be designed?

When designing studies, a certain number of rules should be followed.
For *in vitro* assessment of the efficacy of mycotoxin-adsorbing agents, isotherm adsorption studies (including the more complex modified isotherm studies, with the addition of a feed matrix), should be preferred to single concentration studies as they give a much more complete and reliable picture.
The key parameters for the set-up of these isotherm/modified isotherm studies are as follows:

- The inclusion level of adsorbing material should be high enough to ensure the binding of at least 20%, and preferably 50%, of the toxin present;
- The range of toxin concentrations should cover at least one, and preferably more, orders of magnitude;
- Isotherms should consist of a reasonable number of data points (at least 6-7 points) spread over the widest possible concentration range;
- The pH(s) and temperature(s) should be recorded. The temperature(s) tested should include a temperature of around 37 °C. In particular if the toxin is an ionisable compound (e.g. a weak acid), the pH's tested should include one pH above and one pH below the pKa of this toxin;
- As the accuracy of the analytical method can influence the accuracy of adsorption measurements, the reliability of the analytical method has to be checked. A robust analytical method with appropriate accuracy, precision, and detection/quantification limits has to be used;
- Detection/quantification limits of the analytical method should be 2-5 orders of magnitude below mycotoxin concentration of working solutions. This threshold will safeguard the ability to perform accurate measurements even when strong adsorption occur (90%), and allow determining later the adsorption isotherms;
- In most cases the quantity actually measured is the free toxin concentration, after which the bound fraction is calculated by subtraction. Since losses of free toxins may occur due to causes other than adsorption on the binding agent, e.g. adsorption on filters or centrifugation tubes, the bound fraction should always be calculated by comparison with binder-free procedure controls rather than with nominal concentrations. The recovery rate of these binder-free controls (in proportion to the concentration measured in solutions prepared in the same way as the binder-free controls but not subjected to the incubation procedure) should also be recorded. If this recovery rate is significantly different from 100%, bound and free fractions (in %) can be translated into bound and free concentrations (in µg/kg and µg/ml) by multiplying the percentages either by the nominal concentrations or by the apparent concentration in the binder-free blanks. The first choice is more appropriate if the low recovery rate is due to losses during the analytical steps (e.g. by adsorption in centrifugation tubes), the second if it reflects actual concentrations during incubation (e.g. lower than nominal due to degradation). Whichever choice is made should be clearly stated.

- All experiments should be performed at least in duplicate. The averages of the %ads, the SD or the RSD, and the $K_d$ value should be reported for each set of results.

Concerning the *in vitro* assessment of the efficacy of mycotoxin-degrading agents, a multi-tiered, interdisciplinary approach is required for their characterization. Dealing with biotransformation requires:

- microbiological methods and microbial enrichment methods for investigating (selection/isolation) mycotoxin-degrading agents,
- appropriate analytical methods for quantification and characterization of degradation processes (if they occur),
- toxicity assays for assessment of residual toxicity.

Physiologically relevant models simulating gastro-intestinal digestion of animals, as well as absorption of digested materials, are highly desirable and should be considered as a means of achieving a multi-tiered approach for *in vitro* testing of either mycotoxin-adsorbing agents or mycotoxin-biotransforming agents.

In addition, novel *in vitro* technology combining gut simulation models with specific bioassays can help to select proficient products at adsorbing/degrading mycotoxins and at reducing the relevant toxic effects. These new technologies should include, as a first step, a gut simulation procedure, in order to assess the binding efficiency as well the mycotoxin-degrading efficacy of the assayed compounds, in a system designed to mimic the animal gastrointestinal tract. As a second step, they should comprise specific cytological assays, in which the liquid phase of the artificial "chyme" is tested for toxin-specific effects in cell cultures. By means of these sophisticated systems a number of relevant factors occurring *in vivo*, such as interactions of toxins with feed components and the effect of digestive enzymes, which may affect the effectiveness of feed additives in detoxifying mycotoxins, can be reflected in the *in vitro* studies. Moreover, the coupled biological assays can be considered a valuable addition to the common chemical analysis of the unbound/undegraded fraction of mycotoxins, as they will allow identifying possible toxic reaction products as well as significant toxic effects of the adsorbing agents as such. They would also detect unpredictable tenside-like activities of mycotoxin-detoxifying agents affecting the permeability of cell membranes and resulting in an increased cellular uptake and increase toxicity of the tested mycotoxins.

### 7.7.2. Reporting and choice of end-points for *in vitro* studies:

In the case of mycotoxin-adsorbing agents, the distribution coefficients, which rely on no underlying assumption or model, should be recorded for each toxin concentration tested.

Besides this, the results of isotherm studies should be plotted as log Cads vs log Caq, and:

- if the results are close to a straight line (constant slope) : the Freundlich model should be applied and the values of KF and 1/n should be recorded;
- if saturation is observed (decreasing slope):
    - the Freundlich model should be applied to the initial linear region of the results, if possible; the values of KF and 1/n should be recorded;
    - the Langmuir model should be applied to the whole curve; the K1 and K2 (=Csat) values should be recorded (normally the product K1.K2 should be close to the KF evaluated earlier);
- if a sigmoid shape is observed:
    - the Hill model can be applied and the $K_d$ and n values recorded;

- the Freundlich and Langmuir models are not applicable; since Hill's $K_d$ and n parameters cannot be used for quantitative comparisons with the KF values of binders with classical behaviour, these comparisons should rely on the range of $K_d$ values (below and above the "trigger" toxin concentration).

Affinities and capacities measured in buffer solutions should be regarded as "best cases", and the differences with *in vivo* conditions must not be overlooked:

- Parameters such as the presence of digestive secretions, the succession of pH, etc. may affect the efficiency of the adsorbing agents.
- Selectivity of mycotoxin-adsorbing agents has rarely been studied experimentally, even though the few available results suggest that this might be the most important limitation on the efficacy of adsorbing agents in practice.

Microbiological agents to be used for deactivation of mycotoxins in animal feed have to fulfill several requirements as listed below.

- Biotransformation must lead to metabolites having a much lower toxicity or being non-toxic;
- Biological agents have to degrade mycotoxin rapidly, because the time available for detoxification in the gastro-intestinal tract is very limited;
- Biological agents should be resistant to mycotoxins. Investigations of several bacteria and yeast show that differences may be found both in sensitivity and selectivity of microbial agents against mycotoxins;
- The mechanism involved in mycotoxin resistance (e.g. selective uptake through membranes, decomposition by selective enzymes, blocking by complex formation, etc.) should be well displayed, as it may be helpful in finding applicable microbes;
- They must be safe, and do not cause structural damage to the digestive tract or to interfere with digestive function. Several *in vitro* tests are available for assessing their safety, such us screening for transferable antibiotic resistance genes, virulence genes, toxic producing genes, etc.;
- It has to be proven that detoxification can take place in a complex environment and is not inhibited by the presence of other nutrients. Thus, the final proof that biological agents are capable of detoxifying mycotoxins has to be done in feeding trials;
- Stabilized and applied as lyophilized powders in feed, the mycotoxin-degrading microorganisms have to regain activity in the gastro-intestinal tract very rapidly. Therefore, it is necessary to optimize growth and mycotoxin-degradation activity of these strains;
- They must be stable in pelleted or extruded feeds.

### 7.7.3. Recommendations on recording, choice of end-points for *in vivo* studies and design of studies

#### 7.7.3.1. Design of *in vivo* studies

When only non-specific parameters are evaluated (biological effects), and in order to evaluate the effects of detoxifying agents, even in absence of mycotoxins, the design of the trial should include at least the following dietary treatments:

- Non contaminated diet (negative control);
- Mycotoxin-contaminated diet (positive control);
- Non contaminated diet with the mycotoxin detoxifying agent;
- Mycotoxin-contaminated diet with the mycotoxin detoxifying agent.

Any attempt to evaluate results across animal studies should consider that many factors/conditions can affect the outcomes of the studies. These factors may be related to the detoxifying agents (type and source of detoxifier, mode of action, inclusion rate and method of inclusion into feed, etc.), the mycotoxins (single or multiple contaminations, natural or artificial contaminations, etc), the animals (species, ages, genders, health conditions, animal husbandry, etc) and even to the lots of product batches used for the study.

#### 7.7.3.2. Concentration of mycotoxins in excreta and ADME studies

- Comparison of the excretion balance between faeces and urine or by testing the reduction of the amount transfered into milk
- Make a clear distinction between specific and non-specific biological effects. In the first case, laboratory animal (rats) models should be used in order to performe ADME studies and to ensure the general safety of the detoxifying agents whilst zootechnical parameters must be applied to the targeted farm animals on a case-by-case basis.

#### 7.7.3.3. Zootechnical parameters

Zootechnical parameters that should be considered for the assessment of mycotoxin-detoxifying agents in *in vivo* studies are:

- Body weight gain,
- Feed intake,
- Feed conversion ratio.

#### 7.7.3.4. Consideration of other parameters and their combination

- These parameters are based not only on the toxicokinetic principle but also on effects such as:
    - The weight of organs (liver, kidney, gizzard) and concentration of mycotoxin(s) in these organs,
    - The concentration of mycotoxin adduct(s) and metabolite(s) in serum and bile;
    - The serum chemistry, e.g. total protein, albumin, globulins, glucose, uric acid, cholesterol, bilirubin, inorganic phosphate, magnesium, glutamate dehydrogenase, γ-glutamyl transferase, aspartate aminotranferase, alanine aminotransferase, lactate dehydrogenase, creatine kinase, α-hydroxybutyrate dehydrogenase;
    - Haematology measurements, e.g. red blood cell count, haematocrit and haemoglobin;
    - Analysis of immunoglobulins in serum and bile: IgA, IgG, and IgM in serum and IgA in bile
    - Determination of antibody titers for an infectious disease specific to a considered species
    - Ratio of sphinganine to sphingosine (SA/SO) which has been proposed specifically for fumonisins as a valid biomarker in rats
    - Brain transmitters concentrations (norepinephrine, 3,4-dihydroxyphenylacetic acid, dopamine, homovanillic, tryptophan, 5-hydroxytryptamine, 5-hydroxyindoleacetic acid) which are directly affected by DON and which affect in turn the feed intake.
- Simulation of gastro-intestinal conditions in order to gain information on bioaccessibility throughout the gastro-intestinal tract (use of gastric juices, presence of food, etc.)
- Performing *in vitro* tests using (cell) models/tools in order to mimic *in vivo* conditions

- Performing studies on laboratory animals such as rats in order to improve the knowledge of toxicokinetic (ADME) studies and demonstrate that the agent is really working by reducing mycotoxin absorption.

### 7.7.4. Other recommendations

It is also recommended that the possible occurrence of xenobiotics such as contaminants (chemical and microbial) in some inorganic agents of this new class of additives be considered given the objective that animal feedstuffs should satisfy current regulations with regard to contaminants.

# Annexes

**Annex 1: EU regulation on aflatoxin B1 in feedstuffs, in µg/kg, (Directive 2002/32/CE, amended by Directive 2003/100)**

| Feedstuffs | Maximum content in µg/kg relative to a feedstuff with a moisture content of 12%) |
|---|---|
| All feed materials | 20 |
| Complete feedstuffs for cattle, sheep and goats<br>with the exception of :<br>- dairy cattle<br>- calves and lambs | 20<br><br>5<br>10 |
| Complete feedstuffs for pigs and poultry (except young animals) | 20 |
| Other complete feedstuffs | 10 |
| Complementary feedstuffs for cattle, sheep and goats (except complementary feedstuffs for dairy animals, calves and lambs) | 20 |
| Complementary feedstuffs for pigs and poultry (except young animals) | 20 |
| Other complementary feedstuffs | 5 |

**Annex 2: Mycotoxin syndromes in farm animals through consumption of contaminated feed (FAO 2007)**

| Fungi | Mycotoxin | pre or post harvest | Climatic requirements | food commodities contaminated | COMMENT |
|---|---|---|---|---|---|
| *Fusarium verticillioides (moniliforme), F. proliferatum* | Fumonisin | Pre-harvest | Cosmopolitan | Wheat, maize and products, hay | Very stable, can survive cooking, soluble can be removed by wet milling and nixtamalisation |
| F. graminearium | Deoxynivalenol (DON or vomitoxin) | Pre-harvest | Cosmopolitan | Wheat, maize, millet, hay (zearalenone) | Very stable, can survive cooking, soluble can be removed by wet milling; not transferred to milk, meat or eggs |
| F. graminearium | Zearalenone | | | | |
| *F. graminearium, F. culmorum, F. poae* | Trichothecenes | Pre-harvest | Temperate to cold | Wheat, maize (overwintered), hay | Very stable, can survive cooking |
| *F. sporotrichioides* | T-2 toxin | | | | |
| *Aspergillus ochraceus* | Ochratoxin A | Post-harvest | Warm, humid | Cereals and cereal products, pulses, dried vine fruit, beverages, spices, groundnuts | Has been found in pig meat |
| *Penicillium verrucosum* | Ochratoxin A | Post-harvest | Temperate | | |
| *Penicillium sp., Aspergillus sp.* | Citrinin | | | | |
| *Penicillium sp., Aspergillus sp.* | Cyclopianzonic acid | | | | |
| *Aspergillus sp.* | Sterigmatocystin | | | | |
| Claviceps purpurea | Ergot | Pre-harvest | Mainly temperate | cereals | |
| Various | Patulin | Post-harvest | | Mouldy fruit, vegetables, cereals, other foods. | Destroyed by fermentation |
| *Aspergillus flavus, A. parasiticus* | Aflatoxins (B1, B2, G1, G2, M) | Mostly post-harvest | Tropical and sub-tropical | Maize, wheat, groundnuts, other edible nuts, figs, spices, soya bean, cotton seed, oil palm kernels, copra, coconut oil, cassava | Moulds do not grow in silage but aflatoxins can survive ensiling process. |

*Fusarium* toxins are apparently not carried over into milk, meat and eggs (M. E. Doyle (1997): http://www.wisc.edu/fri/fusarium.htm :5). Although fumonisins and DON are not the most toxic of the *Fusarium* toxins they are the most frequently detected and therefore the most associated with human and animal illness (this applies to US in particular and maybe also Europe, but may not be relevant for Africa).

**Annex 3: Toxic effects of major mycotoxins and mechanisms of action**

| Toxin | Toxic effects | Cellular and molecular mechanisms of action |
|---|---|---|
| **Aflatoxin** | Hepatotoxicity<br>Genotoxicity<br>Oncogenicity<br>Immunomodulation | Formation of DNA adducts<br>Lipid peroxidation<br>Bioactivation by cytochromes P450<br>Conjugation to GS-transferases |
| **Ochratoxin A** | Nephrotoxicity<br>Genotoxicity<br>Immunomodulation | Effect on protein synthesis.<br>Inhibition of ATP production<br>Detoxification by peptidases |
| **Patulin** | Neurotoxicity<br>*In vitro* mutagenesis | Indirect enzyme Inhibition |
| **Trichothecenes (Toxin T-2, DON, …)** | Hematotoxicity<br>Immunomodulation<br>Skin toxicity | Induction of apoptosis in haemopoietic progenitor cells and immune cells.<br>Effect on protein synthesis<br>Abnormal changes to immunoglobulins |
| **Zeralenone** | Fertility and Reproduction | Binding to oestrogen receptors<br>Bioactivation by reductases<br>Conjugation to glucuronyltransferases |
| **Fumonisin B1** | Central nervous system damage<br>Hepatotoxicity<br>Genotoxicity<br>Immunomodulation | Inhibition of ceramide synthesis<br>Adverse effect on the sphinganin/sphingosin ratio<br>Adverse effects on the cell cycle. |

**Annex 4: Toxic effects of major mycotoxins**

- **AFB1**

**Ruminants**: First effects were observed at levels of 1.2-2.23 AFB1/kg of feed such as lower feed ingestion quantity and milk yield in adult cattle and dairy cattle, respectively and at levels higher than 50 mg AFB1/kg of feed in small ruminants (Miller and Wilson, 1994). However, it was previously observed that a level of 0.6 mg/kg in complete feedstuffs generates lower ingestion quantities in fattening cattle (Helferich, 1984; Helferich et al., 1986; Helferich et al., 1986). This level is in accordance with the level of 0.7 mg AFB1/kg observed almost 20 years earlier by (Garrett et al., 1968) on ingestion modification. During the same experiment reported by the same author, toxic effects were observed in cattle livers (Garrett et al., 1968). The 2004 EFSA opinion concluded that the current maximum levels of aflatoxin B1 in animal feeds (see table 2) *"provide an adequate protection from adverse health effects in dairy animals, in particular dairy cattle"*.

**Pigs**: The (Meissonnier et al., 2005) review summaries aflatoxicosis characteristics in swine: during acute aflatoxicosis episodes almost all animals died within days following the consumption of feed contaminated at levels of 10-20 mg AFB1/kg of feed and some mortalities and liver diseases were observed within one month after ingestion of contaminated feeds at 0.8-3 mg AFB1/kg. During chronic experimental aflatoxicosis, the first signs are significant reductions in feed intake following the consumption of feed contaminated at levels of 0.14-1 mg AFB1/kg of feed.

**Poultry**: During chronic experimental aflatoxicosis in chickens, the first signs are reductions of weight gain for 30% of the animals following the consumption of feed contaminated at levels of 0.03 mg AFB1/kg of feed. Biochemical alterations are observed at the minimal level of 0.62 mg AFB1/kg of feed (Hamilton, 1984).

- **OTA**

**Ruminants**: Polygastric animals seem to be resistant to OTA exposure: only one case of ochratoxicosis found in the literature was reported by (Lloyd, 1980) and (Lloyd and Stahr, 1980) with kidney disease following the consumption of feed contaminated at levels up to 20 mg OTA/kg of feed and 2-10 mg of citrinin/kg of feed.

**Pigs**: Acute ochratoxicosis episodes mention kidney diseases (nephropathy) and were firstly described in 1928 and confirmed by (Krogh et al., 1974). During chronic ochratoxicosis, the compiled data indicate that the first signs are reductions of feed consumption and weight gain at the level of 1-1.4 mg OTA/kg of feed.

**Poultry**: Nephropathy is reported to occur for all poultry species from level of 2 mg OTA/kg of feed. The first signs of chronic ochratoxicosis were noticed at a minimal level of 0.5 mg OTA/kg of feed in laying hens and chickens.

- **TCTs**

**Ruminants**: Polygastric animals seem to be resistant to TCT exposure. No report on adverse effects due to T2 toxin was found in the literature except for calves in which feed refusal, diarrhea and weight loss were noticed following the consumption of a contaminated diet at level of 20 mg T2 toxin/kg (Osweiler et al., 1985). Regarding effects due to DON, the first signs of zootechnical effects such as reduction of feed intake were observed after consumption by post lactating cows during 10 weeks of a wheat concentrate at level of 6.4 mg DON/kg (Trenholm, 1985).

**Pigs**: The first signs of reductions of feed consumption are observed at the levels of 1 mg T2/kg, are significant from level of 3 mg T2/kg and feed refusal is noticed from level of 16 mg T2/kg in feed. Signs of reductions of feed consumption are observed at levels of 2-10 mg or 1-3 mg of DAS or DON /kg feed, respectively. Above 10 mg or 3 mg DAS or DON /kg, feed refusal syndrome is noticed. However data compiled from several experiments performed by different authors, show that feed ingestion is again at normal quantities after 1-2 weeks when contaminated diets are at a level below 3 mg of T-2 or DON. Despite that DON is also called "vomitoxin", vomiting syndrome is observed at a level of 20 mg DON/kg feed.

**Poultry**: Compiled data were available for only T-2 and DON. Effects vary according to the species. However it can be said that levels below 2 mg of T-2 or DON do not generate any effects. Reduction of feed consumption is observed for chickens at levels of 2-16 mg of T-2 /kg feed ingested during 2-4 weeks and for laying hens at a level of 8 mg of T-2 /kg feed ingested during 8 weeks. Egg production decreases proportionally to doses of 1-10 mg T-2/kg in feed.

- **ZEA**

**Ruminants**: Some infertility cases associated with the presence of ZEA in forages were reported. Based on surveys in the USA, (Linn and Chapman, 2002) observed a reduction of fertility in dairy cattle for levels of ZEA above 0.5 mg/kg feed.

**Pigs**: First signs of estrogenic syndrome appear from 3-7 days on a ZEA contaminated diet at levels 1.5-2 mg/kg feed. These signs disappear within 7-14 days after removal of the contaminated feed.

**Poultry**: Among the livestock species of interest, poultry seems to be the most resistant to ZEA. Based on experimental studies, levels above 100 mg ZEA /kg feed are needed to get the first signs of intoxication.

- **FB1**

**Ruminants**: First signs of reduction of feed intake were noticed during an experimental study on young bulls by (Osweiler et al., 1993).

**Pigs**: Based on several studies, levels above 100 mg FB1/kg feed are needed to get first signs of zootechnical disturbance.

**Poultry**: Minimal levels generating reductions of feed consumption and consequently weight gain vary according to the species: from 10-20 mg FB1/kg feed for ducks to 25-50 for turkeys and 100-500 for chicks.

**Annex 5: Recommendations on some mycotoxins in feedstuffs (in µg/kg) (Commission recommendation 2006/576/CE)**

| Toxins | Feedstuffs | Guidance value in µg/kg relative to a feedstuff with a moisture content of 12% |
|---|---|---|
| **OTA** | Feed materials (*) : cereals and cereal products (**) | 250 |
| | Complete and complementary feedstuffs:<br>- for pigs<br>- for poultry | <br>50<br>100 |
| **DON** | Feed materials (*):<br>- cereals and ceral products (**), with the exception of maize by-products<br>- maize by-products | <br>8000<br>12000 |
| | Complementary and complete feedstuffs<br>with the exception of:<br>- Complementary and complete feedstuffs for pigs<br>- Complementary and complete feedstuffs for calves (< 4 months), lambs and kids | 5000<br><br>900<br>2000 |
| **FB1+ FB2** | Feed materials (*) : maize and maize by-products (***) | 60000 |
| | Complementary and complete feedstuffs for:<br>- pigs, horses (Equidae), rabbits and pet animals<br>- fish<br>- poultry, calves (< 4 months), lambs and kids<br>- adults ruminants (>4 months) and mink | <br>5000<br>10000<br>20000<br>50000 |
| **ZEA** | Feed materials (*):<br>- cereals and cereal products (**), with the exception of maize by-products<br>- maize by-products | <br>2000<br>3000 |
| | Complementary and complete feedstuffs:<br>- for piglets and gilts (young sows)<br>- for sows and fattening pigs<br>- for dairy cattle, sheep (including lambs) and goats (including kids) | <br>100<br>250<br>500 |

*(*) Particular attention has to be paid to cereals and cereal products fed directly to animals that their use in a daily ration should not lead to animals being exposed to an higher level of these mycotoxins than the corresponding levels of exposure where only the complete feeding stuffs are used in a daily ration.*
*(**) The term "cereals and cereal products" includes not only the feed materials under heading "cereal grains, their products and by-products" but also other feed materials derived from cereals in particular cereal forages and roughages.*
*(***) The term "maize and maize by-products" includes not only the feed materials under heading "cereal grains, their products and by-products" but also other feed materials derived from maize in particular maize forages and roughages.*

**Annex 6: Regulations for some animal-derived products**

| Toxins | Food | Level (µg/kg) |
|---|---|---|
| **AFM1([1])** | Milk | 0,05 |
| | Infant formulae and follow-on formulae including infant milk and follow-on milk | 0,025 |
| **OTA([2])** | Pork Kidney | 10*<br>25** |

*[(1)]Commission regulation 1881/2006/CE,*

*[(2)]National regulation in Denmark*

*viscera condemned; visibly damaged kidneys are analysed chemically ;

**whole carcass condemned; visibly

damaged kidneys are analysed chemically

**Annex 7: Details related to analytical methods**

Standardized methods are the most accurate and reliable methods recommended for monitoring food and feed (including for raw materials) at industrial levels and for officials controls. However, those physico-chemical protocols are time-consuming and costly; they are not adapted for instance for screening the large batches of raw materials for which rapid ELISA-type techniques would be more pragmatically applied, even if the analytical results may only be indicative (when producing only qualitative or semi-quantitative results). Data given by screening tests can be confirmed by one of the standardized methods if needed. For a review on commercial or non-commercial rapid tests and kits see www.mycotoxins.org.

Regarding analysis of mycotoxins which should be done for evaluation studies of adsorbing agents in feed, the choice of the protocol would depend on the materials to be analysed: in feed (cereals), the methods described above may be used to check the mycotoxin content before and after the application of adsorbing agents in the lot. Usually, the ratio of the remaining mycotoxin amounts measured after application of adsorbing agents to the estimated previous mycotoxin content of the lot studied will give an indication of the efficiency of the adsorbing agents (this may be expressed as a simple percentage of the initial mycotoxin amount). Nevertheless, one should previously check that the adsorbing agents are not interfering with the analytical process. If the materials to be analyzed are biological fluids (urine, blood), there are no standardized methods for this and the analyst should validate the methods used internally and keep the validation data for control purposes or for publishing results.

To confirm the reliability of his analysis, the analyst should use certified reference materials if necessary (delivered by IRMM, Geel, B, http://www.irmm.jrc.be) but there are only two available for mycotoxins in feed (BCR 375 and 376 for Aflatoxins in compound feed (blank and high level). Others are available for cereals: Aflatoxins in peanut meals (BCR 262 to 264), DON in maize (BCR 377) and wheat (BCR 396) flours, Ochratoxin A in wheat (BCR 471) and Zearalenone in maize (ERM-BC716 and 717). But again, there are no reference materials for mycotoxins in biological matrices.

However, for most mycotoxin studies for adsorbing agent assessment, i.e. AFB1, ZEA, OTA, DON and Fumonisins, the analyst can employ and if necessary validate internally (in particular with regard to the scope, i.e. the materials to be analysed) the methods already described. But for the analysis of other mycotoxins of interest in feed (DAS, NIV, T2 and HT2 Toxins), careful attention should be paid by the analyst to the development and in-house validation of suitable protocols. The analyst should also be prepared, especially when using rapid methods, to scrutinise false results, mainly false negative results in the same way as for positive ones, by using a confirmatory method (i.e. standardized or at least validated physico-chemical protocols), which may help when assessing the true level of mycotoxins in the sample. In any event, for evaluation studies of detoxifying agents where toxic metabolites of mycotoxins may be produced, a suitable method should be properly developed, or if a published protocol is used, it should be validated internally before hand. The analytical situation may become tricky as often feed lots are multi-contaminated (thus the need to analyze several mycotoxins in one analytical shot) and multiple metabolites may be produced and interact so that only toxicological tests would help to determine the safety of the detoxifying agents studied.

Finally to demonstrate the efficiency of adsorbing agents, they should be applied on a large scale. In this respect, it should be remembered that mycotoxins are often unequally distributed in cereal lots and an appropriate sampling plan should be carefully defined to reinforce the demonstration.

**Annex 8: Excerpt from Strategies for detoxification of mycotoxins in maize (FAO 1999)**

**FAO 1999, Issue N° 23. "Mycotoxins prevention and decontamination:** A case-study on maize » by Riley RT. and Norred WP.

| | | |
|---|---|---|
| **Physical methods** | ***Cleaning*** | *Simple but incomplete*<br>*Screening out fine materials reduces fumonisins and other mycotoxins* |
| | ***Segregation and sorting*** | *Simple but misleading "black light" test for aflatoxins* |
| | ***Colour sorting technology*** | *unproven with maize, but promising* |
| | ***Density segregation and washing*** | *Non-specific and incomplete, but suitable for wet milling and alkaline processing of maize for fumonisins, deoxynivalenol, zearalenone* |
| | ***Thermal degradation*** | *Incomplete for most mycotoxins* |
| | ***Microwave treatment*** | *High levels destroy trichothecenes* |
| | ***Solar degradation*** | *Results in maize oil encouraging for countering aflatoxins* |
| | ***Extrusion cooking*** | *Very promising for fumonisins: temperature- and screw speed-dependent destruction* |
| | ***Wet milling*** | *Produces starch free, or almost free for zearalenone, fumonisins and aflatoxins, but T-2 toxin is increased in maize germ* |
| | ***Hydrated sodium calcium aluminosilicates*** | *Bind aflatoxins with high affinity and capacity - demonstrated efficacy in vivo when added to diets. Attention should be paid for non-selective aluminosilicates which may pose significant risks and should be avoided (Mayura et al., 1998)* |
| | ***Activated charcoal*** | *Reduces dietary conversion of aflatoxin $B_1$ to aflatoxin $M_1$ in cows* |
| **Chemical methods** | ***Thermal treatment plus reducing sugars*** | *Promising but toxicology and stability uncertain for fumonisins* |
| | ***Nixtamalization / alkaline hydrolysis*** | *Not an effective method; toxicity remains. reversible degradation of aflatoxins and partial degradation of fumonisins; reduced zearalenone and deoxynivalenol* |
| | ***Bisulphite*** | *Bisulphite is a common food additive which destroys aflatoxin $B_1$, reduces deoxynivalenol in maize (the DON sulphonate is unstable in alkali)* |
| | ***Ammoniation*** | *Approved method for aflatoxin in maize in Mexico, South Africa and several states in the United States - may not be effective in detoxifying fumonisins in maize* |
| | ***Hydrogen peroxide/sodium bicarbonate*** | *Destroys fumonisin in maize* |
| | ***Ozonation*** | *A promising method degrades and detoxifies aflatoxins in naturally contaminated maize* |
| **Microbiological methods** | ***Ethanol fermentation*** | *Does not break down aflatoxin $B_1$, zearalenone or fumonisin $B_1$; toxins may actually be increased in spent grain used in animal feeds.*<br>*Probiotic mixtures: Lactobacillus and Propionibacterium may reduce bio-availability of dietary aflatoxins* |
| | ***Dietary interventions*** | *Choline, methionine, vitamins, protein, dietary fat, antioxidants and inducers of metabolizing enzymes: addition to animal feeds can lower toxicity caused by mycotoxins in maize* |

**Annex 9: List of companies contacted**

| Company | Address |
|---|---|
| 1. **ABAC R&D AG** | Wagistrasse 23,<br>CH-8952 Schlieren<br>SWITZERLAND |
| 2. **Agil** | Calleva Park, Aldermaston, Reading, RG7 8DN,<br>ENGLAND |
| 3. **Agri-Growth International Inc.** | HEAD OFFICE<br>Agri-Growth International Inc.<br>18024 - 107 Avenue, Edmonton, Alberta,<br>CANADA |
| 4. **Adisseo** | Adisseo France S.A.S.<br>42, rue Aristide Briand - B.P. 100<br>92164 Antony Cedex<br>FRANCE |
| 5. **Agri-Tec** | Agri-Tec<br>240 S.E. 58th St.<br>Amarillo, TX 79118<br>USA |
| 6. **Agrimex** | Achterstenhoek 5<br>2275 Lille,<br>BELGIUM |
| 7. **Alltech** | ZI Belle Etoile – 25, Allée des Sapins<br>44470 Carquefou<br>FRANCE |
| 8. **Alvetra u. Werfft AG** | Alvetra u. Werfft AG<br>A-1090 Wien Boltzmanngasse 11,<br>Vienna<br>AUSTRIA |
| 9. **BASF Nutrition Animale** | Zone Industrielle de Bellitourne - AZE<br>53200 CHATEAU-GONTIER<br>FRANCE |
| 10. **Béghin Meiji** | BEGHIN-MEIJI – SYRAL<br>Z.I. et Portuaire BP 32<br>67390 Marckolsheim<br>FRANCE |
| 11. **BIOCHEM GmbH** | 10, rue Jules Vernes<br>44700 Orvault<br>FRANCE |
| 12. **BIOMIN France Sarl** | Parc Technologique du ZOOPOLE<br>Rue Irène Joliot-Curie<br>22440 Ploufragan<br>FRANCE |
| 13. **Borregaard LignoTech** | Borregaard France<br>86, Avenue de Saint-Ouen<br>F-75018 Paris<br>FRANCE |
| 14. **CALCIALIMENT** | Zone artisanale de la gare<br>22690 Pleudihen-sur-Rance<br>FRANCE |
| 15. **CCPA (Conseil et Compétences en Productions Animales)** | Z.A. Nord Est du Bois de Teillay<br>35150 Janzé<br>FRANCE |
| 16. **CELTIC Nutrition Animale** | Parc d'Activités de Ferchaud - BP 10<br>35320 Crevin<br>FRANCE |

| | |
|---|---|
| 17. **CENTRALYS** | 9-11 avenue Arago – CS 90508<br>78197 Trappes Cedex<br>FRANCE |
| 18. **CEVA Santé Animale** | Z.I. de la Ballastière - B.P. 126<br>33501 Libourne Cedex<br>FRANCE |
| 19. **Delacon Phytogenic Feed Additives** | Delacon International - Delacon Biotechnik Ges.m.b.h<br>Weissenwolffstraße 14<br>A-4221 Steyregg<br>AUSTRIA |
| 20. **DSM Nutritional Products France – Nutrition et Santé Animales** | Le Véronèse - 19-21, avenue Dubonnet<br>92400 Courbevoie<br>FRANCE |
| 21. **EVIALIS Nutrition** | B.P. 234<br>56006 Vannes Cedex<br>FRANCE |
| 22. **FIS Feed Industry Service** | F.I.S. s.r.l.<br>Via dell'Industria 11,<br>26900 Lodi<br>ITALY |
| 23. **GUYBRO CHEMICAL** | GUYBRO CHEMICAL<br>A 201, Maruti Chambers,<br>Fun Republic Lane, Link Road,<br>Andheri (W), Mumbai 400 053,<br>INDIA |
| 24. **IDENA** | Parc d'Activités du Moulin<br>21, rue du Moulin B.P. 30<br>44880 Sautron<br>FRANCE |
| 25. **INVE Nutri-Ad** | Kloosterstraat 1 Bus 7<br>2460 Kasterlee<br>BELGIUM |
| 26. **INVE France S.A.** | 244, rue du Vert Touquet BP2<br>62350 Calonne-sur-la-Lys<br>FRANCE |
| 27. **INZO° - UNION IN VIVO** | 1, rue de la Marébaudière - B.P. 96669<br>35766 St Gregoire Cedex<br>FRANCE |
| 28. **Impextraco** | Impextraco<br>Wiekevorstsesteenweg 38<br>B-2220 Heist-Op-Den-Berg<br>BELGIUM |
| 29. **JEFO Europe** | 6, allée des Sapins<br>44483 Carquefou cedex<br>FRANCE |
| 30. **Kemin Europa** | Kemin Pharma bvba<br>Atealaan 4H<br>2200 Herentals<br>BELGIUM |
| 31. **Kemira** | Kemira Chimie Fontainebleau-Avon<br>79 Avenue Franklin Roosevelt<br>77210 Fontainebleau-Avon<br>FRANCE |
| 32. **LALLEMAND Nutrition Animale** | 19, rue des Briquetiers<br>31702 Blagnac<br>FRANCE |

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|---|---|
| 33. **LESAFFRE Feed Additives** | 90, rue de Lille<br>59520 Marquette-lez-Lille<br>FRANCE |
| 34. **LUCTA SA – Flavours, Fragrances & Feed Additives** | Carretera de Masnou a Granollers<br>08170 Montornes des Vallés<br>Barcelona<br>SPAIN |
| 35. **Rio Tinto Minerals-Luzenac Operations** | Luzenac Europe<br>2, place Edouard Bouillères<br>BP 33662<br>31036 Toulouse Cedex 1<br>FRANCE |
| 36. **M.G.2 MIX** | Zone de la Basse Haie<br>35220 Chateaubourg<br>FRANCE |
| 37. **NOVUS International** | Novus France S.A.<br>9, rue Fabert<br>44000 Nantes<br>FRANCE |
| 38. **Oil-Dri Corporation** | Oil-Dri Corporation of America<br>410 Nord Michigan Avenue, Suite 400<br>Chicago, Illinois 60611<br>USA |
| 39. **Olmix** | SA Olmix - ZA du Haut du Bois,<br>56580 Brehan<br>FRANCE |
| 40. **Optivite Ltd** | Unit 5<br>Manton Wood Enterprise Park<br>Worksop<br>Notts, S80 2RS<br>UNITED KINGDOM |
| 41. **ORFFA France S.A.** | 38 rue de Bassano<br>75008 Paris<br>FRANCE |
| 42. **PATENT CO.** | 65 Zahumska St.<br>11000 Belgrade<br>SERBIA |
| 43. **Peak Minerals - Azomite, Inc.** | AZOMITE Mineral Products, Inc.<br>7406 NE 84th Terrace<br>Kansas City, MO 64157<br>USA |
| 44. **PHYTOSYNTHESE** | 57 avenue Jean Jaurès<br>ZA Mozac / Volvic - B.P 50100<br>63203 Riom Cedex<br>FRANCE |
| 45. **ANDRES PINTALUBA, S.A.** | Prudenci Bertrana,<br>5 · Agro-Reus ·<br>43206 REUS ·<br>P.O.BOX:1002/43200<br>SPAIN |
| 46. **Prince Agri Products, Inc.** | Prince Agri Products, Inc.<br>229 Radio Road<br>P. O. Box 1009<br>Quincy, IL 62306<br>USA |
| 47. **PRIMEX S.A.** | La Gare de Baud - B.P. 21<br>56440 Languidic<br>FRANCE |

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|---|---|
| 48. **PRISMA et SNPS** | Prisma - BP 394<br>56009 Vannes Cedex<br>FRANCE |
| 49. **QUALIMAT** | 11 Boulevard de la Paix<br>56000 Vannes<br>FRANCE |
| 50. **REALDYME** | REALDYME<br>ZI La Haute Epine<br>28700 Garancières-en-Beauce<br>FRANCE |
| 51. **ROQUETTE Nutrition Animale (Roquette Frères)** | Roquette Frères<br>62136 Lestrem<br>FRANCE |
| 52. **SANDERS** | Sanders Nutrition Animale<br>Saint-Gérand - B.P. 61<br>56302 Pontivy Cedex<br>FRANCE |
| 53. **Süd-Chemie (Germany)** | Société Francaise des Bentonites et Dérivés S.A.S.<br>Choisy-le-Roi, Distribution BAA<br>6, rue Louise Michel<br>94603 Choisy-le-Roi<br>FRANCE |
| 54. **TECHNA** | Les Landes de Bauche - Route de St Etienne de Montluc - BP 10<br>44220 Coueron<br>FRANCE |
| 55. **Tolsa S.A.** | Siège social (France)<br>Zone Portuaire – Quai de Saint Wandrille<br>76490 Saint Wandrille – Rançon<br>FRANCE |
| 56. **Trouw Nutrition** | Trouw Nutrition International B.V.<br>Nijverheidsweg 2<br>3881 LA Putten<br>THE NETHERLANDS |
| 57. **Ultra Bio-Logics Inc.** | International Export Division<br>24 Sequin Rigaud QC,<br>CANADA J0P 1P0 |
| 58. **VETAGRI** | Rue Arthur Enaud - B.P. 572<br>22605 Loudeac Cedex<br>FRANCE |
| 59. **ZEO Inc.** | P.O. Box 2353 / McKinney,<br>TX 75070<br>USA |
| 60. **Special Nutrients, INC** | Special Nutrients, INC.<br>2766 Douglas Road,<br>Cocunut Grove, FL, 33133<br>USA |

**Annex 10: Questionnaires sent to the companies producing/using mycotoxin-detoxifying agents**

- **Questionnaire for producers of mycotoxins detoxifying agents**

1-Did you develop or are you in the course of development of products or agents having a possible action on the mycotoxin detoxification of cereals, raw materials, feed and food?
2- In which category these detoxifying agents or binders are classified: i) biological agents, ii) adsorbents or other chemicals agents, iii) unclassifiable in the 2 first categories? Can you give their mode of action?

3-Can you give the components of the products, their physicochemical characteristics, their origin, their purity?

4-Do this(ese) product(s) has(ve) an authorization? For which utilization? In Europe? In other countries in the world? In third countries?

5- Do this(ese) product(s) has(ve) a use or a specific protective effect on pig, poultry, bovines, sheep, goat, rabbit, horse, fish and pet ? What are the quantities and the frequency of products used in each production?

6-Did you carry out *in vitro* tests to determine their effectiveness? Did you determine the mode(s) of actions? On which mycotoxins the studies were carried out?

7-Did you carry out *in vivo* tests? On which animal species? What were the measured parameters to assess the efficacy?

8-Do you have some data on the potential risks of these products for the animal health, the consumers or users of these products (contaminants, impurities, virulence determinants for micro-organisms, adverse effects on micronutrient absorption, possible metabolites of toxicological relevance, irritation/sensitization potential) and on the environment?

9- Do you accept to forward your data for the compilation to be done in the present proposal (with or without a level of confidentiality)?

- **Questionnaire for users of mycotoxin detoxifying agents**

1- Which mycotoxins have the most relevant concern in animal production: the most frequent feed contaminants; those having the highest level of contamination, or the highest deleterious effect on animals, or the highest risk of transfer in animal products intended in human nutrition?
2- What are the frequency and the average levels of the mycotoxins contaminations found in the main feed or ingredients (by animal species: pig, poultry, bovine, sheep, goat, rabbit, horse, fish and pet)?

3- Which techniques are used to reduce the levels of these mycotoxins in feed or in raw materials: physical, chemical or biological techniques…?

4- What products have you already used? What results did you get on feed and on animals? Do you use associations of products? What is the interest of their association? What is the frequency of use of these products (by animal species: pig, poultry, bovine, sheep, goat, rabbit, horse, fish and pet)?

5- How do you measure the efficacy of these detoxifying agents? What are your waitings on this type of product?

6-What adverse effects have you been observed on animals (by species: pig, poultry, bovine, sheep, goat, rabbit, horse, fish and pets) or feed could you identify following the use of these products? What can be the causes for them?

7- Do you accept to transmit your data for the compilation to be done in the present proposal (with or without a level of confidentiality)?

**Annex 11: Examples of calculations and verification of BC50**

The experimental results of Ramos et al (1996) on the binding of zearalenone by magnesium trisilicate and crospovidone provide neat examples of these interpretations and uses of the numerical values of $K_d$ or of $K_F$ (reminder : $K_F$ is the value taken by $K_d$ when $C_{aq}$ = 1 µg/mL) as equal to $1/BC_{50}$ :

- The $K_F$ value for magnesium trisilicate is estimated at 23 mL/g. As expected:

1) the amount of toxin bound is 24 µg/g (i.e. almost equal to the estimated $K_F$ of 23, when the free zearalenone concentration is 1 µg/mL (it rises up to c. 90 ùg/g for $C_{aq}$ = c. 6 µg/L)
2) a $K_F$ of 23 mL/g gives a $BC_{50}$ around 44 g/L, and it happened that the binder concentration in this study was 50 g/L, slightly above the $BC_{50}$ value; accordingly, the fraction of zearalenone bound is slightly above 50% in the first part of the curve .

- The $K_F$ value for crospovidone is much higher, 314 mL/g:

1) the amount of toxin bound reaches 183 µg/g for 0.58 µg/L, the highest free zearalenone concentration reached; linear extrapolation to 1 µg/mL gives 318 µg/g, very close to the estimated $K_F$ of 314
2) a $K_F$ of 314 mL/g gives a $BC_{50}$ around 3.2 g/L; since the binder concentration in this study was c. 15 times higher, the ratio $C_{ads}/C_{aq\ is}$ is also around 15, in other words it is expected that 95% of the zearalenone will be bound: this is very close to the actual result, as can be verified in the detailed data reported;

Another example is the article of (Döll et al., 2004), in which the authors give the "C50", analogous to the $BC_{50}$ discussed here but expressed as binder:toxin ratios rather than binder:solvent ratios. The authors tested a series of binders at 0.82 g/L in their GI-tract model, and afterwards carried out a full dose-response study (from 0.02 to 10 g/L binder) with the same model in order to determine this C50. The $BC_{50}$ we estimated from the results at 0.82 g/L range from 0.05 to 5 g/L and are in good agreement with the $BC_{50}$ calculated by the authors from their dose-response study. Furthermore the authors then studied, at constant binder:toxin ratio (equal to the C50 measured previously) , the influence of the amount of buffer, in other words of the binder concentration. As we expected, the percentage of toxin bound depended on binder concentration: it rose to c. 70% when the amount of buffer was halved (in other words, the $C_{ads}/C_{aq}$ ratio was doubled because the binder concentration was doubled). Inversely it decreased to c. 40% ($C_{ads}/C_{aq}$ = c. 2/3) when the amount of buffer was multiplied by 1.5 (binder concentration multiplied by 2/3). These observations confirm that the binder concentration rather than the binder:toxin ratio is the critical parameter (as long of course as the binder:toxin ration is far from saturation levels, which was the case here)

**Annex 12: Summary of mycotoxin-detoxifying agents (adsorbing or biotransforming agents) in poultry, swine and ruminants**

**Table 1: Summary of mycotoxin-detoxifying agents (adsorbing or biotransforming agents) in poultry[7]**

| Mycotoxin | Mycotoxin levels (mg/kg) | Product | Product inclusion (g/kg) | Number and details on animals | Duration | Design | Parameters evaluated | Reference |
|---|---|---|---|---|---|---|---|---|
| AF | 0.5 | Cell wall yeast *Saccharomyces cerevisiae* | 0.1% | 320 male 1-day-old broilers | 42 days | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod -<br>• Myco+ / prod + | Feed intake, BW, FCR<br>Relative weight gain of liver and kidneys | Santin, 2003 |
| AF | 100 µg/kg | Clinoptilolite | 0.5-1% | 300 day-old broiler chicks | 6 weeks | • Myco- / prod –<br>• Myco+ / prod -<br>• Myco+ / prod + | Feed intake, BW, FCR<br>Mortality<br>Dressing percentage<br>Bursal body weight ratio<br>Organ (liver, heart and gizzard) weight | Pasha, 2007 |
| AF | 0.1 | MMN (Modified montmorillonite nanocomposite) | 0.30% | 160 one-day-old Avian broiler chicks | 6 weeks | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, feed consumption<br>Mortality<br>Weight of liver, kidney, spleen, pancreas, bursa of Fabricius<br>Serum concentrations of TP, ALB, GLOB<br>Serum activities of AST, GGT and ALP | Shi, 2006 |
| AF | 2 | Yeast glucomannan | 0.5-1 | 240 male broiler chicks | 21 days | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | Pathologic changes in liver : enlargement and paleness, hydropic degeneration and/or fatty changes in hepatocytes, bile duct proliferation and periportal fibrosis, lymphoid depletion in bursa of Fabricius… | Karaman, 2005 |
| AF | 4 | Superactivated charcoal | 0.50% | 216 broiler chicks | 21 days | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | Feed consumption, BW, FCR<br>Serum concentrations of ALB, TP, urea nitrogen, glucose, cholesterol, triglycerides, creatinine, uric acid, calcium<br>Activities of ALT, AST, GGT, LDH, ALKP, | Edrington, 1997 |

[7] Trials highlighted in green were performed with mycotoxins present under the maximum recommended levels.

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| | | | | | | | CK<br>Weight of liver, kidney, spleen, pancreas, heart… | |
| AF | 100 µg/kg | Mg K aluminosilicate | 0.5-1% | 300 day-old broiler chicks | 6 weeks | • Myco- / prod –<br>• Myco+ / prod -<br>• Myco+ / prod + | Feed intake, BW, FCR<br>Mortality<br>Dressing percentage<br>Bursal body weight ratio<br>Organ (liver, heart and gizzard) weight | Pasha, 2007 |
| AF | 3 | Live yeast culture residue | 0.1% | 120 broiler breeder hens, 35-week-old | 3 weeks | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | Mean percentage of fertility, hatchability, hen-day egg production, egg weight, chick weight at hatch, embryonic mortality<br>Serum protein, GLOB, and ALB | Stanley, 2004 |
| AF | 2.5 | *Saccharomyces cerevisiae* | 0.1% | 40 growing Japanese quail chicks | 5 weeks | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, food consumption, FCR | Oguz, 2001 |
| AF | 1 | Sodium bentonite | 2.5-5 | 72 one-day-old breed broiler chicks | 45 days | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod -<br>• Myco+ / prod + | Activity of SOD, GPx, CAT activities and LPO levels in the liver, kidney | Eraslan, 2004 |
| AF | 168 ppb | Esterified glucomannans | 0.05% | One-day-old broiler chicks | 35 days | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, feed consumption, feed efficiency<br>Hematology<br>Serum TP, cholesterol, urea nitrogen<br>Enzyme activities: GGT, ALT, AST | Aravind, 2003 |
| AF | 5 | *Saccharomyces cerevisia*e | 0.05-0.1% | 360 day-old broiler chicks | 4 weeks | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | BW<br>Weight of liver, proventiculus, pancreas, heart<br>Serum concentrations of ALB, TP, cholesterol, uric acid, triglycerides<br>Enzyme activities: ALT, AST, LDH, CK | Stanley, 1993 |
| AF | 100 µg/kg | Sodium bentonite | 0.5-1% | 300 day-old broiler chicks | 6 weeks | • Myco- / prod –<br>• Myco+ / prod -<br>• Myco+ / prod + | BW, feed intake, FCR<br>Mortality<br>Dressing percentage<br>Bursal body weight ratio<br>Organ (liver, heart and gizzard) weight | Pasha, 2007 |
| AF | 100 µg/kg | Sodium bentonite + | 0.5-1% | 300 day-old broiler chicks | 6 weeks | • Myco- / prod -<br>• Myco+ / prod – | BW, feed intake, FCR<br>Mortality | Pasha, 2007 |

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| | | gention violet | | | | • Myco+ / prod + | Dressing percentage<br>Bursal body weight ratio<br>Organ (liver, heart and gizzard) weight | |
| AF | 100 µg/kg | Sodium bentonite + acetic acid | 0.5-1% | 300 day-old broiler chicks | 6 weeks | • Myco- / prod -<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, feed intake, FCR<br>Mortality<br>Dressing percentage<br>Bursal body weight ratio<br>Organ (liver, heart and gizzard) weight | Pasha, 2007 |
| AFB1 | 7.5 | HSCAS | 0.50% | Day old male leghorn chicks and broiler chicks | | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, feed consumption<br>Organ weight | Phillips, 1988 |
| AFB1 | 1-5 (µg/kg) | *Nocardia corynebacteriodes* | 6.00E+10 | 100 1-day old chicks | 21 days | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | Histopathologic analysis of liver, kidney, bursa of Fabricius, pancreas, and small intestine (duodenum, jejunum, and ileum)<br>Analysis by scanning electron microscopy of the 3 sections of the intestine | Tejada-Castaneda, 2008 |
| AFB1 | 5 | HSCAS | 0.125-0.5% | One-day-old male broiler chickens | 3 weeks | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, feed consumption<br>Chick mortality<br>Hepatic vitamin A determination<br>Liver and kidney weights | Pimpukdee, 2004 |
| AFB1 | 2.5 | Synthetic crystalline aluminosilicate | 1% | 80 day-old male chicks | 42 days | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, FCR<br>Liver weight<br>Histopathology of liver | Miazzo, 2000 |
| AFB1 | 0.3 | Esterified glucomannans from *Saccharomyces cerevisiae* | 0.10% | 960 broiler chickens from 1 to 35 d of age | 5 weeks | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, feed intake, FCR<br>Morphology of liver, kidney, gizzard, adrenals<br>Serum biochemistry: TP, cholesterol, urea nitrogen<br>Activities of GGT, ALT, AST<br>Haematology | Raju, 2000 |
| AFB1 | 5 | Argentinean sodium bentonite | 0.30% | Thirty-day-old male chickens | 3 weeks | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, FCR<br>TP, ALB, GLOB concentrations<br>Histopathology (liver) | Rosa, 2001 |
| AFB1 | 2.5 | Sodium | 0.30% | 160 Ross male | | • Myco- / prod - | BW, FCR | Miazzo, 2005 |

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| | | bentonite | | broiler chicks | | • Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | TP, ALB, GLOB concentrations<br>Histopathology (liver) | |
| Cyclopiazonic acid | 45 | Acidic phyllosilicate | 1% | One-day-old chicks | 3 weeks | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, feed consumption<br>Weight of liver, kidney, spleen, pancreas, proventriculus, gizzard, bursa of Fabricius, heart<br>Serum concentrations of TP, ALB, glucose, cholesterol, triglycerides, uric acid, blood nitrogen, inorganic phosphorus<br>Activities of LDH, ALKP, GGT, AST, ALT, CK<br>Mean corpuscular hemoglobin concentrations | Dwyer, 1997 |
| Cyclopiazonic acid | 45 | HSCAS | 1% | One-day-old chicks | 3 weeks | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, feed consumption<br>Weight of liver, kidney, spleen, pancreas, proventriculus, gizzard, bursa of Fabricius, heart<br>Serum concentrations of TP, ALB, glucose, cholesterol, triglycerides, uric acid, blood nitrogen, inorganic phosphorus<br>Activities of LDH, ALKP, GGT, AST, ALT, CK<br>Mean corpuscular hemoglobin concentrations | Dwyer, 1997 |
| Cyclopiazonic acid | 45 | Zeolite | 1% | One-day-old chicks | 3 weeks | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, feed consumption<br>Weight of liver, kidney, spleen, pancreas, proventriculus, gizzard, bursa of Fabricius, heart<br>Serum concentrations of TP, ALB, glucose, cholesterol, triglycerides, uric acid, blood nitrogen, inorganic phosphorus<br>Activities of LDH, ALKP, GGT, AST, ALT, CK<br>Mean corpuscular hemoglobin concentrations | Dwyer, 1997 |
| DAS | 1-2 | *Eubacterium* | 0.75-1.5 | 140 male growing broiler | 21 days | • Myco- / prod -<br>• Myco- / prod + | BW, feed intake, FCR<br>Weight of liver, heart, spleen, proventriculus, | Diaz, 2002 |

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| | | | | chickens | | • Myco+ / prod –<br>• Myco+ / prod + | and gizzard | |
| DAS | 31 | Zeolite | 0.5% | 40 broiler chickens | 28 days | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, feed consumption<br>Morphology of liver, kidney, spleen; thymus, pancreas, heart, gizzard<br>Serum concentration of TP, ALB, glucose, triglycerides, cholesterol, ALT, AST, creatinine and uric acid | Curtui, 2000 |
| DON | 0.1-3.97 | NSP-ENZYME (Xylanase) | 0.2 | 384 1-day-old male turkeys | 5 weeks | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, FCR, feed intake<br>Weights of liver, spleen, bursa of Fabricii, heart<br>Activities of GGT, glutamate deshydrogenase | Danicke, 2007 (2) |
| DON | 0.14-9.2 | Esterified glucomannans from *Saccharomyces cerevisiae* | 0.20% | 360 1-d-old male broiler chicks | 56 days | • Myco- / prod -<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, feed consumption<br>Serum concentrations of TP, ALB, globulins, glucose, uric acid, cholesterol, bilirubin, calcium, and phosphorus<br>Activities of amylase, LDH, AST, GGT, CK<br>Analysis of immunoglobulins in serum and bile<br>Evaluation of natural anti-rabbit erythrocyte response in chicken sera<br>Determination of antibody titers to infectious bronchitis<br>Determination of muscle color | Swamy, 2002 (2) |
| DON | 10 | *Eubacterium* | $2.5.10^8$ CFU/kg | 277 1-d-old broiler chicks | 6 weeks | • Myco- / prod -<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, feed consumption, feed efficiency<br>Weights of gizzard, heart, liver, pancreas, spleen, duodenum, jejunum, cecum, and colon<br>Intestinal lesions and morphology<br>Measurement of Villus height and width | Awad, 2006 |
| DON | 14 | Polyvinylpolypyrrolidone | 0.3% | 60 1-d-old broiler chicks | 21 days | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | Immunological parameters: ANAE positivity, splenic plasma cell counts | Celik, 2000 |
| FB1 | 200 | Sodium bentonite | 0.3% | 160 Ross male broiler chicks | | • Myco- / prod -<br>• Myco- / prod + | BW, FCR<br>TP, ALB, GLOB concentrations | Miazzo, 2005 |

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| | | | | | | • Myco+ / prod –<br>• Myco+ / prod + | Histopathology (liver) | |
| Fusaric acid | 18-20 | Esterified glucomannans from *Saccharomyces cerevisiae* | 0.20% | 360 1-d-old male broiler chicks | 56 days | • Myco- / prod -<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, feed consumption<br>Serum concentrations of TP, ALB, globulins, glucose, uric acid, cholesterol, bilirubin, calcium, and phosphorus<br>Activities of amylase, LDH, AST, GGT, CK<br>Analysis of immunoglobulins in serum and bile<br>Evaluation of natural anti-rabbit erythrocyte response in chicken sera<br>Determination of antibody titers to infectious bronchitis<br>Determination of muscle color | Swamy, 2002 (2) |
| Nivalenol | 78 | Zeolite | 0.50% | 40 broiler chickens | 28 days | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, feed consumption<br>Morphology of liver, kidney, spleen; thymus, pancreas, heart, gizzard<br>Serum concentration of TP, ALB, glucose, triglycerides, cholesterol, ALT, AST, creatinine, uric acid | Curtui, 2000 |
| OTA | 2 | HSCAS | 0.25% | 288 broilers | 42 days | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | Feed intake, BW, FCR<br>Relative weights of the liver, kidneys, and bursa<br>Serum levels of Ca, P, TP, AST, GGT | Santin, 2002 |
| OTA | 0.5-1 | Combination of *Eubacterium* BBSH 797 with dried yeasts and clays | 1-2 | 270 one-day-old broiler chicks | 42 days | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | Weights of kidney, liver, spleen and bursa of Fabricius<br>Serum activities of LDH, GGT, AST<br>Histological examination: kidney, liver, spleen and bursa of Fabricius | Hanif, 2008 |
| OTA | 2 | Diatomaceous earth | 5 | 28 laying hens | 5 weeks | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | Egg production<br>Egg weight<br>Egg shape index<br>Feed consumption, FCR<br>Concentrations of TP, uric acid, cholesterol, calcium, posphorus, triglycerides<br>Activities of ALP, AST, GGT, ALT in serum | Denli, 2008 |
| OTA | 8.4 ppb | Esterified | 0.05% | One-day-old | 35 days | • Myco- / prod - | BW, feed consumption, feed efficiency | Aravind, 2003 |

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| | | glucomannans | | broiler chicks | | • Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | Hematology<br>Serum TP, cholesterol, urea nitrogen<br>Enzyme activities: GGT, ALT, AST | |
| OTA | 0.5 | *Trichosporon mycotoxinivorans* | $10^4$-$10^6$ | 300 1-d-old commercial broiler chicks | 42 days | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, feed intake, FCR<br>Determination of total cell-associated u-PA activity, of membrane-bound u-PA activity, of free u-PA binding sites on the cell membrane and of superoxide production | Politis, 2005 |
| OTA | 2 | Esterified glucomannans from *Saccharomyces cerevisiae* | 0.10% | 960 broiler chickens from 1 to 35 d of age | 5 weeks | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, feed intake, FCR<br>Morphology of liver, kidney, gizzard, adrenals<br>Serum biochemistry: TP, cholesterol, urea nitrogen<br>Activities of GGT, ALT, AST<br>Haematology | Raju, 2000 |
| OTA | 4 | Charcoal | 1% | 1-day-old growing chicks | 7 days | • Myco- / prod –<br>• Myco- / prod+<br>• Myco+ / prod –<br>• Myco+ / prod + | Feed consumption, BW | Rotter, 1989 |
| T2-toxin | 2 | Aluminosilicate | | 180, 1-d-old male meat-type chickens | 28 days | • Myco- / prod -<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, feed intake, feed efficiency<br>Serum activity of AST and LDH<br>Weight of liver, heart, spleen, proventriculus, gizzard, and bursa of Fabricius | Diaz, 2005 |
| T2-toxin | 2 | *Eubacterium* | | 180, 1-d-old male meat-type chickens | 28 days | • Myco- / prod -<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, feed intake, feed efficiency<br>Serum activity of AST and LDH<br>Weight of liver, heart, spleen, proventriculus, gizzard, and bursa of Fabricius | Diaz, 2005 |
| T2-toxin | 2 | Esterified glucomannans | | 180, 1-d-old male meat-type chickens | 28 days | • Myco- / prod -<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, feed intake, feed efficiency<br>Serum activity of AST and LDH<br>Weight of liver, heart, spleen, proventriculus, gizzard, and bursa of Fabricius | Diaz, 2005 |
| T2-toxin | 6 | Superactivated charcoal | | 216 broiler chicks | 21 days | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, feed intake, feed efficiency<br>Serum concentrations of ALB, TP, urea nitrogen, glucose, cholesterol, triglycerides, creatinine, uric acid, calcium<br>Activities of ALT, AST, GGT, LDH, ALKP, CK | Edrington, 1997 |

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| | | | | | | | Weight of liver, kidney, spleen, pancreas, heart… | |
| T2-toxin | 2 | Aluminosilicate | | 180, 1-d-old male meat-type chickens | 28 days | • Myco- / prod -<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, feed intake, feed efficiency<br>Serum activity of AST and LDH<br>Weight of liver, heart, spleen, proventriculus, gizzard, and bursa of Fabricius | Diaz, 2005 |
| T2-toxin | 32 ppb | Esterified glucomannans | | One-day-old broiler chicks | 35 days | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, feed intake, feed efficiency<br>Hematology<br>Serum TP, cholesterol, urea nitrogen<br>Enzyme activities: GGT, ALT, AST | Aravind, 2003 |
| T2-toxin | 3 | Esterified glucomannans from *Saccharomyces cerevisiae* | | 960 broiler chickens from 1 to 35 d of age | 5 weeks | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, feed intake, FCR<br>Morphology of liver, kidney, gizzard, adrenals<br>Serum biochemistry: TP, cholesterol, urea nitrogen<br>Activities of GGT, ALT, AST<br>Hematology | Raju, 2000 |
| T2-toxin | 16 | Zeolite | 0.5% | 40 broiler chickens | 28 days | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, feed consumption<br>Morphology of liver, kidney, spleen; thymus, pancreas, heart, gizzard<br>Serum concentration of TP, ALB, glucose, triglycerides, cholesterol, ALT, AST, creatinine and uric acid | Curtui, 2000 |
| ZEA | 0.1-0.56 | Esterified glucomannans from *Saccharomyces cerevisiae* | 0.2% | 360 1-d-old male broiler chicks | 56 days | • Myco- / prod -<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, feed consumption<br>Serum concentrations of TP, ALB, globulins, glucose, uric acid, cholesterol, bilirubin, calcium, and phosphorus<br>Activities of amylase, LDH, AST, GGT, CK<br>Analysis of immunoglobulins in serum and bile<br>Evaluation of natural anti-rabbit erythrocyte response in chicken sera<br>Determination of antibody titers to infectious bronchitis<br>Determination of muscle color | Swamy, 2002 (2) |
| ZEA | 1-51 (µg/kg) | | | 384 1-day-old male turkeys | 5 weeks | • Myco- / prod -<br>• Myco- / prod + | BW, FCR, feed intake<br>Weights of liver, spleen, bursa of Fabricii, heart | Danicke, 2007 (2) |

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| | | | | | | • Myco+ / prod –<br>• Myco+ / prod + | Activities of GGT, glutamate deshydrogenase | |
| ZEA | 54 ppb | Esterified glucomannans | | One-day-old broiler chicks | 35 days | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | BW, feed consumption, feed efficiency<br>Hematology<br>Serum TP, cholesterol, urea nitrogen<br>Enzyme activities: GGT, ALT, AST | Aravind, 2003 |

**Table 2: Summary of mycotoxin-detoxifying agents (adsorbing or biotransforming agents) in swine**

| **Mycotoxin** | **Mycotoxin levels** (mg/kg) | **Product** | **Product concentration (g/kg)** | **Number and details on animals** | **Duration** | **Design** | **Parameters evaluated** | **Reference** |
|---|---|---|---|---|---|---|---|---|
| DON<br>ZEA | 8.6 ppm<br>1.2 ppm | Organophil modified montmorillonite | 0.4% | 20 weaned piglets<br>initial weight: 10.5 kg | 35 days | • myco -/prod –<br>• myco+/prod –<br>• myco+/prod +<br>• myco -/prod + | ZEA in bile fluids<br>α-ZOL and β-ZOL in bile fluids<br>DON in serum | Döll, 2005 |
| FB1 | 30 ppm | Activated carbon | 1% | 56 piglets<br>initial weight: 6.9 kg | 42 days | • myco -/prod –<br>• myco+/prod –<br>• myco+/prod +<br>• myco -/prod + | Serum free sphingosine, sphinganine and free Sa/free So<br>Serum sphinganine 1-phosphate, sphingosine 1-phosphate<br>Serum total sphinganine and sphingosine<br>Urinary free sphingosine, sphinganine and free Sa/free So | Piva, 2005 |
| AF | 0.5-0.6 ppm | HSCAS-1 and HSCAS-2 | 0.05% | Growing pigs | | • myco -/prod –<br>• myco+/prod –<br>• myco+/prod + | Concentrations of AFB1, AFB2 and AFM1 in liver , muscle, kidney and adipose tissue | Beaver, 1990 |
| DON | 5 ppm | Probiotic (Contents of large intestine of hens) | 5 ml/g corn before feed production | 30 piglets<br>Initial weight: 11.6 kg | 5 + 5 days | • myco -/prod –<br>• myco+/prod –<br>• myco+/prod +<br>• myco -/prod + | Dietary DON concentration | He, 1993 |
| DON<br>ZEA<br>T-2 toxin<br>HT2-toxin | 6620 ppb<br>49 ppb<br>< 4 ppb<br>< 10 ppb | Combination of *Eubacterium* BBSH 797 with dried yeasts and clays | 0.25% | 12 growing pigs<br>Initial weight: 23 kg | 98 days | • myco -/prod –<br>• myco+/prod –<br>• myco+/prod +<br>• myco -/prod + | Urine DON and de-epoxy DON<br>Faecal DON<br>Total urine and faeces DON recovery/DON intake<br>Serum DON | Danicke, 2004 |

**Table 2: Summary of mycotoxin-detoxifying agents (adsorbing or biotransforming agents) in swine**

| Mycotoxin | Mycotoxin levels (mg/kg) | Product | Product concentration (g/kg) | Number and details on animals | Duration | Design | Parameters evaluated | Reference |
|---|---|---|---|---|---|---|---|---|
| ZEA | 0.84 ppm | Modified clinoptilolite | 0.2% | 15 piglets initial weight: 14.4 kg | 31 days | • myco -/prod –<br>• myco+/prod –<br>• myco+/prod + | Daily feed intake, daily gain and feed efficiency | Sinovec, 2006 |
| FB1 | 30 ppm | Granulated activated carbon | 1% | 3 or 4 piglets | 42 days | • myco+/prod +<br>• myco -/prod + | Blood CD4 and CD8 (T lymphocyte)<br>Blood CD14 (monocyte) | Cabassi, 2005 |
| DON<br>ZEA | 8.6 ppm<br>1.2 ppm | Organophil modified montmorillonite | 0.4% | 20 weaned piglets initial weight: 10.5 kg | 35 days | • myco -/prod –<br>• myco+/prod –<br>• myco+/prod +<br>• myco -/prod + | Feed intake, daily gain and feed efficiency<br>Vulva swelling<br>Cervix volume<br>Weight of uterus, ovaries, urinary bladder, small intestine, stomach, oesophagus, heart, kidney and liver<br>Serum concentration of FSH, cholesterol, triglycerides, protein and ALB<br>Serum activities of ASAT, GLDH, GGT<br>Liver concentrations of retinol, retinyl oleate, retinyl palmitate, retinyl stearate<br>Serum concentration of retinol, retinyl palmitate and alfa-tocopherol | Döll, 2005 |
| DON<br>ZEA | 8.6 ppm<br>1.2 ppm | Organophil modified montmorillonite | 0.4% | 12 weaned piglets initial weight: 9.7 kg | 35 days | • myco -/prod –<br>• myco+/prod –<br>• myco+/prod +<br>• myco -/prod + | Feed intake, daily gain, feed efficiency | Döll, 2005 |
| AF | 0.8 ppm | 4 different clays | | 9 weaned piglets | 28 days | • myco -/prod –<br>• myco+/prod –<br>• myco+/prod + | Daily gain, feed intake, feed efficiency<br>Serum ALB | Schell, 1993 |

| **Mycotoxin** | **Mycotoxin levels** (mg/kg) | **Product** | **Product concentration (g/kg)** | **Number and details on animals** | **Duration** | **Design** | **Parameters evaluated** | **Reference** |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Serum γ-GT and ALP activities | |
| ZEA<br><br>Trichothecenes<br>AFB1 | 158-1550 ppb<br>140-310 ppb<br>< 4.5 - 9 ppb | Clinoptilotite | 2.00% | 120 sows | 115 days | • myco+/prod –<br>• myco+/prod + | Anoestrus, return to oestrus, farrowing rate, inappetence, pyrexia, mastitis, vaginal discharge, pregnancy interval, lactation interval, number of piglets born alive, number of piglets weaned… | Papaioannou, 2002 |
| ? | ? | Clinoptilotite | 2% | 12 sows | One reproductive cycle | • myco -/prod –<br>• myco -/prod + | Serum vitamins E and A<br>Serum K, Na, P, Ca, Mg, Cu, Zn<br>Liver or kidney vitamins E and A<br>Liver or kidney , Na, P, Ca, Mg, Cu, Zn | Papaioannou, 2002 |
| FB1 | 30 ppm | Activated carbon | 1% | 56 piglets<br>Initial weight: 6.9 kg | 42 days | • myco -/prod –<br>• myco+/prod –<br>• myco+/prod +<br>• myco -/prod + | Daily gain, feed intake, feed efficiency<br>Weight of liver, kidney and pancreas<br>Serum enzymatic activities: glutamic oxaloacetic transaminase; GGT<br>Serum concentrations: bilirubin, glucose, cholesterol, protein, urea, albumin, creatinine, Ca, P, Mg<br>Organ abnormalities: lung, heart, liver, pancreas, kidney, intestine, spleen, lymph nodes, brain<br>Blood CD4, CD8, CD14 and CD4/CD8 | Piva, 2005 |
| AF | 922 ppb | Sodium bentonite | 1% | 96 weaned piglets<br>Initial weight; 8.8 kg | 42 days | • myco -/prod –<br>• myco+/prod –<br>• myco+/prod +<br>• myco -/prod + | Daily gain, feed intake, feed efficiency<br>Serum enzymatic activities: γ-GT, ALP, AST<br>Serum Cu, Fe, Ca, Mg, P, Na, K, Zn, urea, glucose, albumin, total protein<br>Liver and kidney weight | Schell, 1993 |

| **Mycotoxin** | **Mycotoxin levels** (mg/kg) | **Product** | **Product concentration (g/kg)** | **Number and details on animals** | **Duration** | **Design** | **Parameters evaluated** | **Reference** |
|---|---|---|---|---|---|---|---|---|
| AF | 922 ppb | Sodium bentonite | 1% | 24 barrows<br>Initial weight: 38.2 kg | 14 days | • myco -/prod –<br>• myco+/prod –<br>• myco+/prod +<br>• myco -/prod + | Digestibility of dry matter and crude proteins %<br>Absorption and retention % for Ca, P, Mg, Na, Zn, Fe<br>Serum enzymatic activities: γ-GT, AST<br>Serum Cu, Fe, Ca, Mg, P, Na, K, Zn, urea, glucose, albumin, total protein<br>Liver and kidney weight | |
| AF | 0.5 ppm | 7 different clays | ? | 9 weaned piglets | 35 days | • myco -/prod –<br>• myco+/prod –<br>• myco+/prod + | Daily gain, feed intake, feed efficiency<br>Serum ALB and TP<br>Serum enzymatic activities: γ-GT, AST | Schell, 1993 |
| AF | 0.8 ppm | Treated calcium bentonite | 0.25, 0.5, 1, 2% | 9 weaned piglets | 28 days | • myco -/prod –<br>• myco+/prod –<br>• myco+/prod + | Daily gain, feed intake, feed efficiency<br>Serum enzymatic activities: γ-GT, AST | Schell, 1993 |
| ZEA<br><br>Trichothecenes | 0.16-1.55 ppm<br>0.14-0.31 ppm | Natural zeolite: clinoptilolite-rich tuff mined from Greece | 2% | 120 gilts and sows | Compete reproductive cycle | • myco+/prod -<br>• myco+/prod + | Appetite<br>Ppyrexia, mastitis, vaginal discharge, oestrus behavior<br>Teratogenic effect<br>Number of piglets born alive and weaned<br>Piglet body weight at birth, at weaning<br>Piglet body weight gain during lactation<br>Anoestrus, return to oestrus… | Kyriakis, 2002 |
| ZEA<br><br>Trichothecenes | 0.16-1.55 ppm<br>0.14-0.31 ppm | Natural zeolite: clinoptilolite-rich tuff mined from Greece | 2% | 12 sows | Compete reproductive cycle | • myco+/prod -<br>• myco+/prod + | Serum vitamins E and A<br>Serum K, P, Zn, Cu | Kyriakis, 2002 |
| AF | 200 µg/kg | Bentonite clay | 4 and 5% | 22 piglets | 41 days | • myco-/prod - | BW, feed intake, feed efficiency | Thieu, 2008 |

| **Mycotoxin** | **Mycotoxin levels** (mg/kg) | **Product** | **Product concentration (g/kg)** | **Number and details on animals** | **Duration** | **Design** | **Parameters evaluated** | **Reference** |
|---|---|---|---|---|---|---|---|---|
| | | | | Initial weight: 10.7 kg | | • myco+/prod -<br>• myco+/prod + | Erythrocyte, hemoglobin, hematocrite<br>Serum leucocyte, albumin, globulin, total protein, SOT, GPT, GGT, ALP, LDH | |
| DON | 5 ppm | Polyvinylpyrrolidone | 1.5% | 10 barrows and gilts<br>Initial weight: 35 kg | | • myco-/prod -<br>• myco+/prod –<br>• myco-/prod+<br>• myco+/prod + | Feed intake, daily gain, FCR<br>Weight of heart, stomach, liver, kidney, spleen<br>Esophageal abnormality | Friend, 1984 |
| DON | 5 ppm | Ammonium carbonate | 1.5% | 10 barrows and gilts<br>Initial weight: 35 kg | | • myco-/prod -<br>• myco+/prod –<br>• myco+/prod + | Feed intake, daily gain, FCR<br>Weight of heart, stomach, liver, kidney, spleen<br>Esophageal abnormality | Friend, 1984 |
| DON | 3.5-6.8 ppm | HSCAS | 1 | 6 or 8 piglets<br>Initial weight: 8-8.5 kg | 7-14 days | • myco-/prod -<br>• myco+/prod –<br>• myco-/prod+<br>• myco+/prod + | Daily gain, feed intake | Patterson and Young, 1993 |
| DON | 2-2.5 ppm | Toxan | 0.4% | 12 growing pigs<br>Initial weight: 30 kg | 110 days | • myco-/prod -<br>• myco+/prod –<br>• myco+/prod + | Daily gain | Wetscherek et al., 1998 |
| NIV<br>DON | 8.2 ppm<br>2.2 ppm | Bentonite clay | 0.2 and 0.5% | 6 growing pigs<br>Initial weight: 23 kg | 29 days | • myco-/prod -<br>• myco+/prod –<br>• myco+/prod + | Daily gain<br>Feed intake | Williams, 1994 |
| DON<br><br>ZEA | 2.5-3.3 ppm<br>0.35-0.7 ppm | Aluminosilicate | 0.4% | 20 growing pigs<br>Initial weight: 9.7-10.5 kg | 35 days | • myco-/prod -<br>• myco+/prod –<br>• myco+/prod +<br>• myco-/prod+ | Daily gain, feed intake<br>Uterus weight | Döll, 2004 |
| ZEA | 0.18-0.36 ppm | Zeolite | 2% | 8 piglets | 67 days | • myco-/prod -<br>• myco+/prod –<br>• myco+/prod + | Uterus weight | Coenen, 2001 |
| AF | 3 ppm | HSCAS | 0.5 and 2% | 5 growing barrows | 28 days | • myco-/prod -<br>• myco+/prod –<br>• myco+/prod + | Daily gain<br>Serum GGT and ALP activity<br>Serum prothrombin time | Harvey, 1989 |

| **Mycotoxin** | **Mycotoxin levels** (mg/kg) | **Product** | **Product concentration (g/kg)** | **Number and details on animals** | **Duration** | **Design** | **Parameters evaluated** | **Reference** |
|---|---|---|---|---|---|---|---|---|
| | | | | | | • myco-/prod+ | | |
| AF | 3 ppm | HSCAS-1 and HSCAS-2 | 0.5 % | 8 growing barrows | 28 days | • myco-/prod -<br>• myco+/prod –<br>• myco+/prod +<br>• myco-/prod+ | Daily gain | Harvey, 1994 |
| AF | | HSCAS | 0.5 % | Growing pigs | | • myco-/prod -<br>• myco+/prod –<br>• myco+/prod + | Daily gain, feed intake | Colvin, 1989 |
| ZEA | 0.84 ppm | Esterified glucomannan | 0.1% | 15 piglets | 31 days | • myco-/prod -<br>• myco+/prod –<br>• myco+/prod + | Daily feed intake, daily gain, feed efficiency | Sinovec, 2006 |
| DON<br>ZEA | 3.1 ppm<br>0.06 ppm | Apple pommace | 8% | 56 piglets<br>Initial weight : 9.9 kg | 35 days | • myco-/prod -<br>• myco+/prod –<br>• myco+/prod +<br>• myco-/prod+ | BW, feed intake, feed efficiency<br>Energy conversion ratio<br>Carcass yield<br>Vaccin response<br>Gastric mucosa abnormality<br>Uterus weight<br>Vulva and teast swelling | Gutzwiller, 2007 |
| DON<br>15-acetyl DON<br>ZEA | 5.5 ppm+<br>0.5 ppm+<br><br>0.3 ppm | Polymeric glucomannan mycotoxin adsorbing agent | 0.2% | 12 pregnant gilts<br>Initial weight : 180 kg | 6 months | • myco-/prod -<br>• myco+/prod –<br>• myco+/prod + | Daily gain, feed intake, feed efficiency<br>Stillbirth, born alive, number of mummies, total piglets born per litter, litter weight at birth<br>Serum beta-hydroxybutyrate<br>Serum haptoglobin, TP, ALB, GLOB, ALB / GLOB, urea, creatinine, glucose, cholesterol,<br>Serum enzymatic activities: ALP, GGT, AST, CK, LDH<br>Serum total, free and conjugated bilirubin<br>Serum Ca, P, Mg, Na, K, Cl | Diaz, 2006 |
| DON<br>ZEA | 5.5 ppm<br>0.5 ppm | Polymeric glucomannan | 0.2% | 150 piglets<br>Initial weight: | 22 days | • myco-/prod -<br>• myco+/prod – | Daily gain, feed intake, feed efficiency | Swamy, 2003 |

| **Mycotoxin** | **Mycotoxin levels** (mg/kg) | **Product** | **Product concentration (g/kg)** | **Number and details on animals** | **Duration** | **Design** | **Parameters evaluated** | **Reference** |
|---|---|---|---|---|---|---|---|---|
| | | extracted from the cell wall of Saccharomyces cerevisiae | | 9.3 kg | | • myco+/prod + | Liver, spleen and kidney weights<br>Serum TP, ALB, GLOB, ALB/ GLOB, urea, conjugated bilirubin<br>Serum enzymatic activites: GGT, CK<br>Blood total lymphocytes, CD4, CD8, CD4+CD8, blood IgM<br>Contact hypersensitivity response to dinitrochlorobenzene<br>Antibody response to sheep red blood cells | |
| DON<br><br>Fumonisin<br><br>ZEA<br><br>15-ADON | 4600-6000 ppb<br>23400-29700 ppb<br>300-400 ppb<br>500 ppb | Polymeric glucomannan extracted from the cell wall of Saccharomyces cerevisiae | 0.05, 0.1 and 0.2% | 35 piglets<br>Initial weight: 10 kg | 21 days | • myco-/prod -<br>• myco+/prod –<br>• myco+/prod +<br>• | Daily gain, feed intake, feed efficiency<br>Hypothalamus NE, DOPAC, DA, HVA, TRP, HT, HIAA<br>Pons NE, DOPAC, DA, HVA, TRP, HT, HIAA<br>Cortex NE, HVA, TRP, HT, HIAA<br>Serum IgA, IgG, IgM<br>Serum L-galactonolactone dehydrogenase and GGT activity<br>Serum glucose, Ca, P, Cl<br>Mean corpuscular volume<br>Weight of liver, kidney and spleen | Swamy, 2002 |
| AFB1 | 482-1912 ppb | E-GM | 0.2% | 50 piglets<br>Initial weight: 13.8 kg | 28 days | • myco-/prod -<br>• myco+/prod –<br>• myco+/prod +<br>• myco-/prod+ | Weight gain<br>Serum ALB<br>Serum enzymatic activities: ALP, GGT<br>Liver abnormality<br>Antiovalbumin immune response<br>Global acquired immune response<br>Liver total cytochrome P450<br>Liver EROD activity<br>Liver 6beta-testosterone hydroxylation activity Liver phase I | Meissonnier 2009 |

| **Mycotoxin** | **Mycotoxin levels** (mg/kg) | **Product** | **Product concentration (g/kg)** | **Number and details on animals** | **Duration** | **Design** | **Parameters evaluated** | **Reference** |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | N-demethylation activities<br>Liver phase II transferase activities | |
| T2-toxin | 540-2100 ppb | E-GM | 0.2% | 50 piglets<br>Initial weight: 11.3 kg | 28 days | • myco-/prod -<br>• myco+/prod –<br>• myco+/prod +<br>• myco-/prod+ | Weight gain<br>Serum ALB<br>Serum enzymatic activities: ALP, GGT, transaminase<br>Intestinal and spleen abnormality<br>Antiovalbumin immune response<br>Global acquired immune response<br>Liver total cytochrome P450<br>Liver EROD activity<br>Liver benzphetamine N-demethylation activity<br>Liver phase I N-demethylation activities<br>Liver phase II transferase activities | Meissonnier, 2009 |
| DON | 2-2.5 ppm | Extracted from the cell wall of yeast | 1% | 12 growing pigs<br>Initial weight; 30 kg | 110 days | • myco-/prod -<br>• myco+/prod –<br>• myco+/prod + | Daily gain | Wetscherek, 1998 |
| DON | 1200 µg/kg | *Eubacterium* sp, isolated from bovine rumen | 5 x 108 cfu/kg feed recommended dose | 19-20 piglets<br>Initial weight: 8 kg | 42 days | • myco+/prod –<br>• myco+/prod + | Daily gain, feed efficiency | EFSA 2005 |
| DON | 1200 µg/kg | *Eubacterium* sp, isolated from bovine rumen | 2.5 x 109 cfu/kg feed > recommended dose | 19-20 piglets<br>Initial weight: 8 kg | 42 days | • myco+/prod –<br>• myco+/prod + | Daily gain, feed efficiency | EFSA 2005 |
| DON | 1200 µg/kg | *Eubacterium* sp, isolated from bovine rumen | 2.5 x 1010 cfu/kg feed 11 x recommended dose | 19-20 piglets<br>Initial weight: 8 kg | 42 days | • myco+/prod –<br>• myco+/prod + | Daily gain, feed efficiency | EFSA 2005 |
| - | - | *Eubacterium* sp, | 2-6 x 109 | 20 piglets | 42 days | • myco-/prod – | Daily gain, feed efficiency | EFSA 2005 |

| Mycotoxin | Mycotoxin levels (mg/kg) | Product | Product concentration (g/kg) | Number and details on animals | Duration | Design | Parameters evaluated | Reference |
|---|---|---|---|---|---|---|---|---|
| | | isolated from bovine rumen | cfu/kg feed 10 x recommended dose | Initial weight: 6.88 kg | | • myco-/prod + | Mortality<br>Duodenum, jejunum or ascending colon total aerobes count | |
| DON | 2500 µg/kg | *Eubacterium* sp, isolated from bovine rumen | 1.24 x 109 cfu/kg feed 10 x recommended dose | 3 or 12 piglets Initial weight: 6.7 kg | 44 days | • myco+/prod –<br>• myco+/prod + | Daily gain, feed efficiency<br>Ileal or caecal lactobacilli count | EFSA 2005 |
| DON | 2500-3500 µg/kg | *Eubacterium* sp, isolated from bovine rumen | 1.7x109 cfu/kg feed10 x recommended dose | 3 or 20 fattening pigs Initial weight: 29 kg | 121 days | • myco+/prod –<br>• myco+/prod + | Daily gain, feed efficiency<br>Ileal or caecal lactobacilli count | EFSA 2005 |
| DON | 5 ppm | Contents of large intestine of hens | 5 ml/g corn before feed production | 30 piglets Initial weight: 11.6 kg | 5 + 5 days | • myco-/prod -<br>• myco+/prod –<br>• myco+/prod +<br>• myco-/prod+ | Daily gain, feed intake, feed efficiency | He, 1993 |
| ZEA | 50 ppm | Alfafa | 15 and 25% | 12 piglets Initial weight: 7 kg | 28 days | • myco-/prod -<br>• myco+/prod –<br>• myco+/prod +<br>• myco-/prod+ | Daily gain<br>Uterus weight | Smith, 1980 |
| ZEA | 10-40 ppm | Alfafa | 15 and 25% | 12 piglets Initial weight: 7 kg | 28 days | • myco-/prod -<br>• myco+/prod –<br>• myco+/prod +<br>• myco-/prod+ | Daily gain<br>Uterus weight | James, 1982 |
| OTA<br>ZEA | 500 µg/kg<br>200 µg/kg | *Eubacterium* BBSH 797 + *T. mycotoxinivorans* | 0.05 and 0.1% | 24 weaning piglets | 42 days | • myco-/prod -<br>• myco+/prod –<br>• myco+/prod + | Daily gain, feed intake, feed efficiency<br>Swollen vulva, swollen prepuce, rectum prolapsed, vomiting and diarrhea, frequent urination<br>Damages on kidneys | Hofstetter, 2006 |
| T2-toxin | 5-9 ppb+ | Thymol + micronised | 0.1% | 25 weaned | 42 days | • myco+/prod – | Daily gain, feed efficiency | Jaunet, 2006 |

| **Mycotoxin** | **Mycotoxin levels** (mg/kg) | **Product** | **Product concentration (g/kg)** | **Number and details on animals** | **Duration** | **Design** | **Parameters evaluated** | **Reference** |
|---|---|---|---|---|---|---|---|---|
| ZEA<br><br>Fumonisin | 29-37 ppb+<br>90-120 ppb | yeast | | piglets | | • myco+/prod + | Lungs + kidney + liver abnormality | |
| T2-toxin<br>ZEA<br><br>Fumonisin | 5-9 ppb+<br>29-37 ppb+<br>90-120 ppb | Enzymes + plant extracts | 0.1% | 25 weaned piglets | 42 days | • myco+/prod –<br>• myco+/prod + | Daily gain, feed efficiency<br>Lungs + kidney + liver abnormality | Jaunet, 2006 |
| DON<br>ZEA<br>T2-toxin<br>HT-2 toxin<br>NIV | 6620 ppb<br>49 ppb<br>< 4 ppb<br>< 10 ppb<br>12-38 ppb | Combination of *Eubacterium* BBSH 797 with dried yeasts and clays | 0.25% | 12 growing pigs<br>Initial weight : 23 kg | 98 days | • myco-/prod -<br>• myco+/prod –<br>• myco+/prod +<br>• myco-/prod+ | Feed intake, daily gain, feed efficiency<br>Digestibility of organic matter, crude protein and crude fat<br>N-free extracts digestibility<br>Metabolisable energy<br>Serum TP<br>Serum glutamate deshydrogenase and GGT activity | Danicke, 2004 |
| DON | 2-5 ppm | Combination of *Eubacterium* BBSH 797 with dried yeasts and clays | 1% | 12 growing pigs<br>Initial weight : 30 kg | 110 days | • myco-/prod-<br>• myco+/prod –<br>• myco+/prod + | Daily gain | Wetscherek, 1998 |
| T2-toxin<br>ZEA<br><br>Fumonisin | 5-9 ppb+<br>29-37 ppb+<br>90-120 ppb | Thymol + micronized yeast + inorganic sorbent | 0.1% | 25 weaning piglets | 42 days | • myco+/prod –<br>• myco+/prod + | Daily gain, feed efficiency<br>Lungs+kidney+liver abnormality | Jaunet, 2006 |
| DON<br>ZEA<br>FB1 | 0.2 ppm<br>0.6 ppm<br>0.8-1.1 ppm | Combination of *Eubacterium* BBSH 797 with dried yeasts and clays | 0.25% | 22 piglets<br>Initial weight : 9 kg | 56 days | • myco-/prod-<br>• myco+/prod –<br>• myco+/prod + | Daily gain | Pietri, 1999 |
| DON<br>ZEA | 5.3-7.3 ppm | Combination of *Eubacterium* BBSH 797 with dried yeasts | 0.25% | 13 growing pigs<br>Initial weight : | 85-93 days | • myco-/prod-<br>• myco+/prod –<br>• myco+/prod + | Daily gain, feed intake | Weiss, 1999 |

| **Mycotoxin** | **Mycotoxin levels** (mg/kg) | **Product** | **Product concentration (g/kg)** | **Number and details on animals** | **Duration** | **Design** | **Parameters evaluated** | **Reference** |
|---|---|---|---|---|---|---|---|---|
| | | and clays | | 37-40 kg | | | | |
| DON<br><br>ZEA | 1.8-7.5 ppm<br>0.1-0.3 ppm | Combination of *Eubacterium* BBSH 797 with dried yeasts and clays | 0.25% | 70 growing pigs<br>Initial weight : 28-33 kg | 105-116 days | • myco-/prod-<br>• myco+/prod –<br>• myco+/prod + | Daily gain, feed intake | Hoppenbrock, 2002 |

**Table 3: Summary of mycotoxin-detoxifying agents (adsorbing or biotransforming agents) in ruminants**

| **Mycotoxin** | **Mycotoxin levels** (mg/kg) | **Product** | **Product concentration / inclusion (g/kg)** | **Number and details on animals** | **Duration** | **Design** | **Parameters evaluated** | **Reference** |
|---|---|---|---|---|---|---|---|---|
| AFB1 nat | 174 | Commercial sequestering agent | 22 | 8 multiparous Holstein Friesian lactating cows | | • Myco- / prod -<br>• Myco- / prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | AFB1 concentration<br>AFM1 concentration in milk<br>Particle size (g/100 g)<br>Pellet durability and resistance<br>Specific weight (kg/l) | Masoero, 2009 |
| AFB1 art | 100 | HSCAS | 0.56% | | | | | Kutz, 2009 |
| AFB1 art | 100 | HSCAS | 0.56% | | | | | Kutz, 2009 |
| AFB1 art | 100 | AFB1100 | 0.56% | | | | | Kutz, 2009 |
| AFB1 art | | Atox | | Twenty-four Holstein lactating cows | | | Recovery rate of AFB1 excreted in milk<br>AFM1 excretion | Moschini, 2008 |
| AFB1 art | | HSCASPlus | | Twenty-four Holstein lactating cows | | | Recovery rate of AFB1 excreted in milk<br>AFM1 excretion | Moschini, 2008 |
| AFB1 art | | Polymeric glucomannan | | Twenty-four Holstein lactating cows | | | Recovery rate of AFB1 excreted in milk<br>AFM1 excretion | Moschini, 2008 |
| AFB1 | 55 | Sodium bentonite | 1.20% | 16 Lactating cows | 11 days | • Myco+ / prod –<br>• Myco+ / prod + | Measure of AFM1 concentration in milk | Diaz, 2004 |
| | | Sodium bentonite | 1.20% | 16 Lactating cows | 11 days | • Myco+ / prod –<br>• Myco+ / prod + | Measure of AFM1 concentration in milk | |
| | | Sodium bentonite | 1.20% | 16 Lactating cows | 11 days | • Myco+ / prod –<br>• Myco+ / prod + | Measure of AFM1 concentration in milk | |
| | | Calcium bentonite | 1.20% | 16 Lactating cows | 11 days | • Myco+ / prod –<br>• Myco+ / prod + | Measure of AFM1 concentration in milk | |
| | | E-GM | 0.05% | 16 Lactating cows | 11 days | • Myco+ / prod –<br>• Myco+ / prod + | Measure of AFM1 concentration in milk | |
| | | Activated carbon | 0.25% | 16 Lactating cows | 11 days | • Myco+ / prod –<br>• Myco+ / prod + | Measure of AFM1 concentration in milk | |
| AFB1 nat | 11 | AC1 | 2% | | | | | Galvano, 1996 |
| AFB1 nat | 11 | AC2 | 2% | | | | | Galvano, 1996 |

| **Mycotoxin** | **Mycotoxin levels** (mg/kg) | **Product** | **Product concentration / inclusion (g/kg)** | **Number and details on animals** | **Duration** | **Design** | **Parameters evaluated** | **Reference** |
|---|---|---|---|---|---|---|---|---|
| AFB1 nat | 11 | HSCAS | 2% | | | | | Galvano, 1996 |
| AFB1 pure | 100 | Na-bentonite | 1% | 9 lactating crossbred goats | 14 days | • Myco- / prod -<br>• Myco+ / prod –<br>• Myco+ / prod + | Dry matter intake and daily milk yield<br>AFM1 concentration and total excretion in milk<br>Carry-over of aflatoxins in milk<br>Composition of milk | Nageswara, 2001 |
| AFB1 pure | 100 | AC | 1% | 9 lactating crossbred goats | 14 days | • Myco- / prod -<br>• Myco+ / prod –<br>• Myco+ / prod + | Dry matter intake and daily milk yield<br>AFM1 concentration and total excretion in milk<br>Carry-over of aflatoxins in milk<br>Composition of milk | Nageswara, 2001 |
| AFB1 | 200 | HSCAS | 4% | Lactating dairy goats | 8 days | • Myco- / prod –<br>• Myco -/ prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | Milk production<br>Determination of milk composition<br>Analysis for AFM1 by HPLC | Smith, 1994 |
| AFB1 | 100 | HSCAS | 1 and 2% | Lactating dairy goats | 12 days | • Myco- / prod –<br>• Myco -/ prod +<br>• Myco+ / prod –<br>• Myco+ / prod + | Residue of AFM1 in the milk | Smith, 1994 |

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