Different methods to counteract mycotoxin production and its impact on animal health

Verschillende methoden om mycotoxineproductie en de impact op de diergezondheid tegen te gaan

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ABSTRACT

Mycotoxins can cause serious adverse effects on animal health. This may lead to great economic losses in animal husbandry. In this review, the most common methods to counteract mycotoxins are presented, including several pre- and post-harvest strategies as well as an overview of the different mycotoxin detoxifying agents. The current legislation regarding maximum, guidance or action levels of mycotoxin contamination in various feedstuffs is also mentioned. It allows the agricultural industry to interpret feed analysis results and to decide whether to undertake actions or not.

SAMENVATTING

De aanwezigheid van mycotoxines in veevoeder kan aanzienlijke schade aan de diergezondheid toebrengen. Dit kan tot beduidende economische verliezen leiden voor de veehouderij. In dit overzichtsartikel worden de belangrijkste maatregelen weergegeven om mycotoxineproductie tegen te gaan en haar effect op de diergezondheid te verminderen. Zowel de mogelijke maatregelen vóór als na de oogst en een overzicht van de verschillende mycotoxine-detoxificerende producten worden besproken. De huidige wetgeving met betrekking tot maximum toegelaten gehaltes of indicatieve waarden van mycotoxines in verschillende voeders wordt eveneens vermeld. Deze wetgeving stelt de landbouwindustrie in staat om de resultaten van voederanalysen te interpreteren en te beslissen om al dan niet maatregelen te treffen.

METHODS TO COUNTERACT MYCOTOXINS

Mycotoxins are secondary metabolites produced by several fungal species on feed and foodstuffs. They can exert distinguished toxic effects on several animal species, and may lead to great economic losses in animal husbandry. Because of their detrimental effects, a number of strategies have been developed to 1. reduce the growth of mycotoxigenic fungi and mycotoxin production, 2. detoxify contaminated feed and 3. lower the systemic availability once mycotoxins are ingested by the animal.

Pre- and post-harvesting strategies

Mycotoxin contamination may occur in the field, pre-harvesting period, or during storage and processing, the post-harvesting period. Methods for preventing mycotoxicosis in animals may therefore be divided into pre- or post-harvesting strategies. Certain methods have been found to significantly reduce specific mycotoxins. However, the complete eradication of mycotoxin contamination is currently not achievable (Kabak et al., 2006).

The most important strategy for pre-harvesting is the application of Good Agriculture Practices (GAP). Appropriate GAP includes crop rotation, tillage, irrigation and the proper use of chemicals. Crop rotation is important and is focused on breaking the chain of infectious material, for example by wheat-legume rotations. Including maize in the rotation should be avoided, as maize is very susceptible to *Fusarium spp*. infestations. Any cultivation process that includes destruction and the removal of the burial of the infected crop is regarded as good soil cultivation. The deeper the soil is inverted (ploughing), the less plausible fungi growth will be on the following crop (Edwards, 2004). Irrigation is also valuable to prevent fungi infestation

by reducing plant stress. All plants in the field need an adequate water supply; however, excess irrigation during flowering (anthesis) makes conditions favorable for *Fusarium* infection (Codex Alimentarius, 2002). Another factor which is known to increase the susceptibility of agricultural commodities to mold invasion is damage due to birds, lepidopteran insects or rodents. Insect damage and consequent fungal infection must be controlled by the appropriate use of insecticides and fungicides. This should be integrated with an adequate pest management control (Codex Alimentarius, 2002).

All these parameters can be controlled; however, environmental conditions can not. Relative humidity and temperature are known to have an important onset on mold infection and mycotoxin production. Drought damaged plants are shown to be more susceptible to infection, so crop planting should be timed to avoid high temperatures and drought (Kabak et al., 2006). For *Fusarium spp*. infection however, sufficient moisture conditions at anthesis are critical for the onset of *Fusarium* head blight (FHB) (Aldred and Magan, 2004).

Post-harvest storage conditions are essential in preventing mold growth and mycotoxin production (Schrodter, 2004). For example, grains should be stored with less than 15% moisture content to avoid hotspots with high moisture, favorable for mold growth. Before storage, visibly damaged or infected grains should be removed. However, this method is not exhaustive or very specific (Jard et al., 2011), and multiple reduction strategies should be combined.

Several chemical detoxification methods have also been described. In all cases, they should destroy or inactivate mycotoxins, generate non-toxic products, warrant the nutritional value of the food and feed, and should not induce modification to the technical properties of the product (Jard et al., 2011). The wide variety of chemical decontamination processes include radiation, oxidation, reduction, ammonization, alkalization, acidification and deamination (Kabak et al., 2006). These chemical methods are not allowed in the European Union (European Commission, 2001) as chemical transformation might lead to toxic derivatives. In the United States of America, only ammonization is licensed for detoxifying aflatoxins.

Mycotoxin detoxifying agents

The use of many of the previously described methods for the detoxification of agricultural commodities is restricted due to associated problems. An alternative approach to reduce the exposure to mycotoxins in feed is to decrease the bioavailability by the inclusion of mycotoxin detoxifying agents (mycotoxin detoxifiers) in the feed. This method is the most commonly used today (Jard et al., 2011; Kolosova and Stroka, 2011). These detoxifiers can be divided into two different classes, namely mycotoxin binders and mycotoxin modifiers. An overview of the different products covered by both classes is given in Table 1. These two classes have different modes of action. Mycotoxin binders adsorb the toxin in the gut, resulting in the excretion of a toxin binder complex in the feces, whereas mycotoxin modifiers transform the toxin into non-toxic metabolites (EFSA, 2009). In 2009, the extensive use of these additives led to the establishment of a new group of feed additives: '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' (European Commission, 2009). It should be pointed out that the use of such products does not mean that animal feed exceeding maximal regulatory limits may be used. Their use should rather improve the quality of the feed which is lawfully on the market, providing additional guarantees for animal health safety (Kolosova and Stroka, 2011).

Mycotoxin binders

Mycotoxin binders (adsorbing or sequestering agents) are large molecular weight compounds that are able to bind the mycotoxins in the gastrointestinal tract of the animal. In this way, the toxin binder complex passes through the animal, and is eliminated via the feces. This prevents or minimizes the exposure of animals to mycotoxins. Mycotoxin binders are mainly divided in silica-based inorganic compounds or carbon-based organic polymers (EFSA, 2009).

Inorganic binders

The efficacy of inorganic binders depends on the chemical structure of both the adsorbent and the mycotoxin. The most important feature is the physical structure of the adsorbent, i.e. the total charge and charge distribution, the size of the pores and the accessible surface area. On the other hand, the properties of the adsorbed mycotoxins, such as polarity, solubility, shape and charge distribution, also play a significant role. Generally speaking, the binding capacity increases with surface area and chemical affinities between adsorbent and mycotoxin (Avantaggiato et al., 2005; Huwig et al., 2001; Kabak et al., 2006).

Aluminosilicate minerals (clays) are the largest class of mycotoxin binders, and most of the studies on the alleviation of mycotoxicosis by the use of adsorbents have been focused on these clays. Within this group, there are two different subclasses: the phyllosilicate subclass and the tectosilicate subclass. Phyllosilicates include bentonites, montmorillonites, smectites, kaolinites and illites. The tectosilicates include zeolites (EFSA, 2009). Montmorillonite is primarily a layered phyllosilicate composed of layers of octahedral aluminum and tetrahedral silicon coordinated with oxygen atoms. Bentonite is generally an impure clay consisting mostly of montmorillonite. Zeolites are composed of tetrahedrons of SiO_4 and AlO_4 possessing an infinite three-dimensional cage-like structure. In these minerals, some of the tetravalent silicon

Table 1. Overview of the different classes and subclasses of mycotoxin detoxifiers (adapted from EFSA (2009)).

Mycotoxin binders	Inorganic	Aluminosilicates	
		Phylosilicates	
		Bentonites	
		Montmorillonites	
		HSCAS	
		Smectites	
		Kaolinites	
		Illites	
		Tectosilicates	
		Zeolites	
		Activated charcoal	
		Synthetic polymers	
		Dietary fibre	
		Polyvinylpyrrolidone	
		Cholestyramine	
	Organic	*Saccharomyces cerevisiae*	
		Live yeast	
		Yeast cell wall components	
		Glucomannans	
		Lactic acid bacteria	
		Lactococcus	
		Lactobacillus	
		Leuconostoc	
		Pediococcus	
Mycotoxin modifiers	*Bacteria*	*Eubacterium* BBSH 797	
		Nocardia asteroides	
		Corynebacterium rubrum	
		Mycobacterium fluoranthenivorans	
		Rhodococcus erythropolis	
		Flavobacterium aurantiacum	
		Pseudomonas fluorescens	
		…	
	Yeast	*Trichosporon mycotoxinivorans*	
		Phaffia rhodozyma	
		Xanthophyllomyces dendrorhous	
		Saccharomyces cerevisiae	
		…	
	Fungi	*Aspergillus sp.*	*A. flavus*
			A. niger
		Rhizopus sp.	*R. stolonifer*
			R. oryzae
			R. microsporus
		Penicillium raistrickii	
		Exophalia spinifera	
		Rhinocladiella atrovirens	
		…	
	Enzymes	Epoxidase	
		Lactonohydrolase or Lactonase	
		Carboxypeptidase A	
		α-Chymotrypsin	
		Carboxylesterase	

are replaced by trivalent aluminum giving rise to a deficiency of positive charge, which is balanced by inorganic cations, such as sodium, calcium and potassium ions. Hydrated sodium calcium aluminosilicate (HSCAS) contains calcium ions and protons, which are exchanged against the naturally occurring sodium ions (Huwig et al., 2001). This HSCAS is a heat processed and purified montmorillonite clay. It was deve-

loped by Phillips et al. (1988) and commercialized as NovaSil®. Clay products, including bentonites, zeolites and HSCAS, are the most common feed additives effective in binding aflatoxins in vitro as well as in vivo (Kabak et al., 2006). Because of their fairly non-polar properties, they lack the ability of adsorbing *Fusarium* mycotoxins, such as fumonisins, zearalenone (ZON) and trichothecenes, as well as ochratoxin A (OTA) (Avantaggiato et al., 2005; Kabak et al., 2006; Phillips et al., 2008). HSCAS has a lamellar interlayer structure in which the planar aflatoxin B1 (AFB1) can be bound. The interaction is based on the negative charge of the clay with the partly positive charged dicarbonyls of AFB1 (Phillips et al., 2008). Although the mentioned clays have proven to be effective in preventing aflatoxicosis in various animal species, several disadvantages should be considered. They do not exert any binding potential towards other mycotoxins, they can adsorb vitamins and minerals, and the risk of natural clays to be contaminated with dioxins should also be considered (Huwig et al., 2001; Jouany, 2007).

Another inorganic sorbent of interest is activated charcoal, also called active carbon (AC). AC is a non-soluble powder formed by pyrolysis of several organic compounds. It is manufactured by an activation process to develop a highly porous structure (Galvano et al., 2001). The sequestrant properties of AC depend on many factors, including pore size, surface area, structure of the mycotoxin and dose. The surface-to-mass ratio of AC varies from 500 to 3500 m^2/g. AC has been shown to be an effective binder of a wide variety of drugs and toxic agents. It has been commonly used as a medical treatment for severe intoxications since the 19th century (Huwig et al., 2001). AC has been proven an effective adsorbent of deoxynivalenol (DON), ZON, AFB1, fumonisin B1 (FB1) and OTA (Avantaggiato et al., 2004; Devreese et al., 2012; Huwig et al., 2001). Nevertheless, its unspecific binding is the major drawback in the practical use of AC as a feed additive. It diminishes nutrient absorption, such as vitamins and minerals, and consequently impairs the nutritional value of feed (Avantaggiato et al., 2004; Ramos et al., 1996).

Polymers are another group of inorganic mycotoxin binders. Several agents belong to this group, such as dietary fibre and polyvinylpyrrolidone (highly polar amphoteric polymer), but the most well-known is cholestyramine. Cholestyramine is an insoluble, quaternary ammonium anion exchange resins, which strongly binds anionic compounds (Underhill et al., 1995). It has been used as drug in humans for absorbing bile acids in the gastrointestinal tract in order to reduce cholesterol. This compound has been proven to be an effective binder for FB1, OTA and ZON in vitro (Avantaggiato et al., 2003; Avantaggiato et al., 2005; Döll et al., 2004; Ramos et al., 1996). The cost of polymers is high, limiting their practical use in animal feed (Kolosova and Stroka, 2011).

Organic binders

Organic mycotoxin binders which are commonly used are cell wall components from *Saccharomyces cerevisiae* yeasts. By using only yeast cell walls (composed of β-glucans and mannan oligosaccharides) instead of the whole cell, mycotoxin binding can be enhanced. The fact that dead cells do not lose their binding ability shows that the interaction of such products with mycotoxins is by adhesion to cell wall components rather than by covalent binding or by metabolism (Shetty and Jesperson, 2006). It has recently been demonstrated that the β-D-glucan fraction of yeast cell wall is directly involved in the binding process with ZON, and that the structural organization of β-D-glucans modulates the binding strength. Hydrogen and van-der-Waals bonds have been evidenced in the glucans-mycotoxin complexes (Jouany, 2007; Shetty and Jespersen, 2006; Yiannikouris et al., 2004; Yiannikouris et al., 2006). Based on in vitro assays, this glucomannan (GMA) binder has shown to effectively bind DON, T-2 toxin (T-2), ZON, OTA and AFB1 (Bejaoui et al., 2004; Freimund et al., 2003; Yiannikouris et al., 2004; Yiannikouris et al., 2006). Protective effects of GMA against the detrimental consequences of mycotoxins on animal production parameters have been demonstrated in several studies: Raju and Devegowda (2000) demonstrated that GMA has beneficial effects in broilers when included in feed contaminated with AFB1 (0.3 mg/kg), OTA (2 mg/kg) and T-2 (3 mg/kg). In the study, individual and combined effects of these mycotoxins were examined. Significant interactions were observed between any two mycotoxins, such as additive effects on body weight or feed intake, or antagonistic effects on serum protein and serum cholesterol content. The GMA incorporation increased body weight and feed intake, decreased liver weight, and improved some serum biochemical and hematological parameters which were negatively influenced by the mycotoxins in the feed (Raju and Devegowda, 2000). These binders also alleviate the adverse effects of AFB1 (1 mg/kg) on performance, liver weight and mortality in broiler chickens (Kamalzadeh et al., 2009). GMA counteracts most of the plasma parameter alterations caused by a DON contaminated diet (3 mg/kg) in chickens (Faixova et al., 2006). Aravind et al. (2003) showed a protective effect of GMA against antioxidant depletion in chicken livers caused by the intake of T-2 contaminated (8 mg/kg) diet. Some positive effects of these products have also been demonstrated in pigs. In a study by Diaz-Llano and Smith (2007), GMA was able to counteract the alterations of serum biochemical parameters induced by DON (5.5 mg/kg) in sows. However, no positive effect on feed intake and body weight gain was seen. Furthermore, Dänicke et al. (2007) did not observe improved performance in pigs due to GMA addition in a DON contaminated feed (4.4 mg/kg). However, Nesic et al. (2008) did observe improved performance of pigs when GMA was included in the diet compared to a diet only contaminated with ZON at 3.8 and 5.2 mg/kg.

Another group of organic mycotoxin binders, which have recently become of interest, are lactic acid bac-

teria (LAB). LAB are gram-positive, catalase-negative, non-sporulating, usually non-motile rods and *cocci* that utilize carbohydrates fermentatively and form lactic acid as major end product (Gerbaldo et al., 2012). These bacteria are mainly divided into four genera: *Lactococcus, Lactobacillus, Leuconostoc* and *Pediococcus*. They have been used in the food processing industry for decades because of their fermentative and food preserving abilities. They have also displayed mycotoxin binding abilities (Dalie et al., 2010). The interaction mechanism between LAB and mycotoxins is thought to be similar to the interactions involved in adsorption by GMA. It appears that the polysaccharide components (glucans and mannans) are common sites for binding, with different toxins having different binding sites. Several authors have concluded that the strength of the mycotoxin-LAB interaction is influenced by the peptidoglycan structure and, more precisely, by its amino acid composition (Dalie et al., 2010). The most extensively investigated mycotoxin binding LAB are strains of *Lactobacillus rhamnosus. L. rhamnosi* strains have a demonstrated in vitro binding capability of DON, T-2, ZON, FB1, AFB1 and OTA (El-Nezami et al., 1998; El-Nezami et al., 2002a; El-Nezami et al., 2002b; Niderkorn et al., 2006; Piotrowska and Zakowska, 2005). However, the in vitro adsorption capacity is strain and dose dependent, and it is a reversible process balancing between adsorption and desorption (Kankaanpaa et al., 2000; Lee et al., 2003). All the available literature on LAB-mycotoxin interactions is based on in vitro results. To date, no in vivo trials have been conducted to effectively demonstrate their mycotoxin binding potential, and therefore caution regarding their effectiveness is recommended.

Mycotoxin modifiers

Another strategy to control mycotoxicoses in animals is the application of microorganisms and their enzymes, called mycotoxin modifiers or mycotoxin biotransforming agents. These products biodegrade or biotransform mycotoxins into less toxic metabolites. They can be divided into four classes: bacteria, yeasts, fungi and enzymes. They act in the intestinal tract of animals prior to the absorption of mycotoxins. It has to be pointed out that for the effective use of mycotoxin modifiers as feed additives, certain prerequisites should be fulfilled. Those include rapid degradation, degradation into non-toxic (or far less toxic) metabolites under different oxygen conditions and in a complex environment, preserve the organoleptic and nutritive properties of the feed, safety of use and stability along the intestinal tract at different pH levels. In addition, the choice of the biodegradation approach depends on its practical and economical feasibility (Awad et al., 2010; Kolosova and Stroka, 2011). Anaerobic microorganisms isolated from animal gut contents are generally suitable for developing feed additives, which act in the animals' intestines (Zhou et al., 2008). Survival and adaptation of the microorganisms in the animal gut are key factors for successful detoxification (Zhou et al., 2008).

Bacteria

Mycotoxin degrading bacteria have been isolated from diverse matrices, such as rumen and intestinal microbiota, soil and even water. The most extensively investigated mycotoxin degrading microorganism is the *Eubacterium* BBSH 797 strain, originally isolated from bovine rumen fluid. This bacterial strain produces enzymes (de-epoxidases) that degrade trichothecenes by selective cleavage of their 12,13-epoxy group, which is important for the toxicity of these mycotoxins. This detoxification has been investigated for several trichothecenes (Fuchs et al., 2002), and the mode of action has been proven in vitro and in vivo (Schatzmayr et al., 2006). During its manufacture, BBSH 797 is stabilized by freeze-drying and embedding into protective substances (mainly organic polymers) to guarantee stability when passing through the acidic gastric tract of animals (Heidler and Schatzmayr, 2003). *Eubacterium* BBSH 797 is currently the only microorganism available for commercial purposes (He et al., 2010).

A variety of other bacterial strains has shown mycotoxin degrading abilities in vitro. For example, *Nocardia asteroides, Corynebacterium rubrum, Mycobacterium fluoranthenivorans, Rhodococcus erythropolis, Flavobacterium aurantiacum* and *Pseudomonas fluorescens* (EFSA, 2009). However, none of them have been investigated in vivo.

Yeast

Only one yeast, *Trichosporon mycotoxinivorans*, has been thoroughly investigated regarding its mycotoxin degrading abilities, which has resulted in its commercial use. This yeast, derived from the hindgut of the termite *Mastotermes darwiniensis*, was isolated and characterized previously by Molnar et al. (2004). This yeast is able to modify ZON and OTA into non-toxic metabolites. ZON is detoxified by opening the macrocyclic ring at the ketogroup at C-6. The metabolite shows no estrogenic effect in a yeast bioassay, and does not interact with the estrogen receptor in an in vitro assay. The detoxification of OTA occurs by the cleavage of the phenylalanine moiety from the isocoumarin derivate, producing OTα (Schatzmayr et al., 2006). The detoxification of OTA occurs fast. After 2.5 hours, a conversion of almost 100% is observed in vitro. For ZON on the other hand, only after 24 hours of incubation, the mycotoxin is completely metabolized. This questions its practical use towards this mycotoxin, because detoxification should occur fast after ingestion (<8 hours). The use of *T. mycotoxinivorans* as a mycotoxin modifier against OTA is promising. A study by Politis et al. (2005) demonstrated that the inclusion of this yeast (10^5 CFU/g) in the diet alleviated the immunotoxic effects of OTA (0.5 mg/kg) in broiler chickens.

Other potential OTA degrading yeasts are *Phaffia rhodozyma* and *Xanthophyllomyces dendrorhous* (Peteri et al., 2007), but they have not been well cha-

racterized, and their practical application at present is limited. Styriak and Conkova (2002) reported that two out of several tested *Saccharomyces cerevisiae* strains are able to degrade FB1, but only for 25 or 50% after five days of incubation, which is therefore unusable in practice.

Fungi

Fungi can not only produce mycotoxins, some of them are also able to degrade them. The fungal strains *Aspergillus niger, A. flavus, Eurotium herbariorum* and *Rhizopus sp*. are able to convert AFB1 to aflatoxicol (AFL) by reducing the cyclopentenone carbonyl of AFB1 (Wu et al., 2009). AFL has been reported to be 18 times less active than the parent compound, but it still has carcinogenic properties (Pawlowski et al., 1977), raising the question if this is an appropriate detoxification strategy. Other fungal strains have shown AFB1 metabolizing properties as well, such as *Penicillium raistrickii*, although their metabolization products have almost similar toxicity (AFB2) or have not yet been identified (Wu et al., 2009).

Next to their AFB1 degrading potential, *Rhizopus* isolates have also shown ZON detoxifying abilities. The selected isolates include strains of *R. stolonifer, R. oryzae* and *R. microsporus* (Varga et al., 2005). Further studies are needed to identify the ZON degrading enzymes in the isolates. A preliminary study has been performed to screen twelve black *Aspergillus* strains for their ZON transformation activity by the incubation in contaminated culture medium. Analyses have shown that ZON is removed after 48 hours of incubation by two *A. niger* strains (EFSA, 2009).

Aspergillus niger is also able to degrade OTA to the less toxic compound ochratoxin alpha (OTα). It is subsequently degraded into an unknown compound (Varga et al., 2000).

Fumonisin degrading fungi have been identified as well. *Exophalia spinifera* and *Rhinocladiella atrovirens* extensively metabolize fumonisin B1 to HFB1 and free tricarballylic acid via esterases (Blackwell et al., 1999).

Enzymes

An attractive alternative to the use of live microbes to counteract mycotoxins in animal feed is the application of enzymes responsible for the degradation of mycotoxins. Enzymatic reactions offer a specific, often irreversible, efficient and environmentally friendly way of detoxification that leaves neither toxic residues nor undesired by-products (Kolosova and Stroka, 2011). These mycotoxin degrading enzymes are primarily produced by microorganisms.

Epoxidases are enzymes which are able to detoxify trichothecenes by transforming their epoxy group into diene groups (Schatzmayr et al., 2006). For example, DON can be detoxified to its de-epoxy form, DOM-1.

Takahashi-Ando et al. (2002) reported that ZON is converted into a less estrogenic product by the cleavage of the lactone structure. The responsible enzyme is a lactonohydrolase, originating from the fungus *Clonostachys rosea* IFO 7063.

Pitout (1969) presented the first in vitro hydrolysis of OTA by carboxypeptidase A and, in lower amounts, by α-chymotrypsin. Abrunhosa et al. (2006) reported the ability of several commercial proteases to hydrolyze OTA into OTα. After an incubation period of 25 hours, a significant hydrolytic activity is detected for protease A (87.3%) and for pancreatin (43.4%).

Recently, two genes of *Sphingopyxis sp*. MTA 144 responsible for the detoxification of FB1 have been identified, and recombinant enzymes have been produced (Heinl et al., 2010). The degradation of FB1 consists of two consecutive pathways. FB1 is first metabolized to HFB1 by a carboxylesterase, followed by an aminotransferase, which deaminates HFB1, leading to an even less toxic compound.

EC REGULATIONS ON MAXIMUM LEVELS OF MYCOTOXINS IN ANIMAL FEED

The growing awareness that mycotoxins are a great concern to animal health has led to regulations of maximum allowed contamination levels in feed in many countries (van Egmond et al., 2007). In Europe, these levels have been defined by European Commission (EC) Regulations and Recommendations. The maximum levels set are influenced by several factors. One of the most important factors is species susceptibility. As mentioned, pigs are the most susceptible species for several major mycotoxins, including DON, ZON and OTA. As a result, the maximum levels in feed for pigs are lower than for less susceptible species, such as poultry. Maximum levels may also differ within one species. Piglets and gilts are more susceptible to the estrogenic effects of ZON than sows and fattening pigs, resulting in lower maximum ZON contamination levels for those subcategories. The toxicity of the mycotoxin itself is a key determining factor. AFB1 has long been known to be acutely toxic and even carcinogenic after chronic exposure, resulting in very low guidance values. Next to the toxicodynamic properties, the kinetic properties of mycotoxins also play a role. Fumonisins for example have a very low oral bioavailability, ± 3.5% in pigs (Martinez-Larranaga et al., 1999), and consequently, only low concentrations reach the target tissues. Hence, this allows higher contamination levels in feed. Maximum limits have been established for complete feed as well as for main feed materials (i.e. maize and cereals). In general, maximum set levels are higher for feed materials than for complete feedingstuffs. Nevertheless, it should be taken into account that if a component's proportion in the daily ration of animals is higher than in common practice, this should not lead to the animal being exposed to a higher level of these mycotoxins than normal.

EC Regulations for AFB1 in feed have been established by Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on un-

Table 2. The guidance values on the presence of deoxynivalenol, zearalenone, ochratoxin A and fumonisins in products intended for animal feeding as determined in the Commission Recommendation of 17 August 2006 (2006/576/EC), maximally allowed values on the presence of aflatoxin B1 in products intended for animal feeding as determined by EC Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 (2002/32/EC).

Mycotoxin	Products intended for animal feed	Guidance value in mg/kg (ppm) relative to a feedingstuff with a moisture content of 12%
Deoxynivalenol	Feed materials (*)	
	- Cereals and cereal products (**) with the exception of maize by-products	8
	- Maize by-products	12
	Complementary and complete feedingstuffs with the exception of:	5
	- Complementary and complete feedingstuffs for pigs	0.9
	- Complementary and complete feedingstuffs for calves (<4 months), lambs and kids	2
Zearalenone	Feed materials (*)	
	- Cereals and cereal products (**) with the exception of maize by-products	2
	- Maize by-products	3
	Complementary and complete feedingstuffs	
	- Complementary and complete feedingstuffs for piglets and gilts (young sows)	0.1
	- Complementary and complete feedingstuffs for sows and fattening pigs	0.25
	- Complementary and complete feedingstuffs for calves, dairy cattle, sheep (including lambs) and goat (including kids)	0.5
Ochratoxin A	Feed materials (*)	
	- Cereals and cereal products (**)	0.25
	Complementary and complete feedingstuffs	
	- Complementary and complete feedingstuffs for pigs	0.05
	- Complementary and complete feedingstuffs for poultry	0.1
Fumonisin B1+B2	Feed materials (*)	
	- Maize and maize products (***)	60
	Complementary and complete feedingstuffs for:	
	- Pigs, horses (*Equidae*), rabbits and pet animals	5
	- Fish	10
	- Poultry, calves (<4 months), lambs and kids	20
	- Adult ruminants (>4 months) and mink	50
Aflatoxin B1	All feed materials (*)	0.02
	Complete feedingstuffs for cattle, sheep and goat with the exception of:	0.02
	- Complete feedingstuffs for dairy animals	0.005
	- Complete feedingstuffs for calves and lambs	0.01
	Complete feedingstuffs for pigs and poultry (except young animals)	0.02
	Other complete feedingstuffs	0.01
	Complementary feedingstuffs for cattle, sheep and goats (except complementary feedingstuffs for dairy animals, calves and lambs)	0.02
	Complementary feedingstuffs for pigs and poultry (except young animals)	0.02
	Other complementary feedingstuffs	0.005

(*) Particular attention has to be paid to cereals and cereal products fed directly to the animals that their use in a daily ration should not lead to the animal being exposed to a higher level of these mycotoxins than the corresponding levels of exposure where only the complete feedingstuffs are used in a daily ration.

(**) The term 'Cereals and cereal products' includes not only the feed materials listed under heading 1 'Cereal grains, their products and by-products' of the non-exclusive list of main feed materials referred to in part B of the Annex to Council Directive 96/25/EC of 29 April 1996 on the circulation and use of feed materials (OJ L125, 23.5.1996, p. 35) but also under feed materials derived from cereals in particular cereal forages and roughages.

(***) The term 'Maize and maize products' includes not only the feed materials derived from maize listed under heading 1 'Cereal grains, their products and by-products' of the non-exclusive list of main feed materials referred to in the Annex, part B of Directive 96/25/EC but also other feed materials derived from maize in particular maize forages and roughages.

Table 3. Indicative levels for the sum of T-2 and HT-2 toxin in cereals and cereal products, as determined in the Commission Recommendation of 27 March 2013 (2013/165/EC).

	Indicative levels for the sum of T-2 and HT-2 (µg/kg) from which onwards/above which investigations should be performed, certainly in case of repetitive findings (*)
1. Unprocessed cereals (**)	
1.1. barley (including malting barley) and maize	200
1.2. oats (with husk)	1000
1.3. wheat, rye and other cereals	100
2. Cereal products for feed and compound feed (***)	
2.1. oat milling products (husks)	2000
2.2. other cereal products	500
2.3. compound feed, with the exception of feed for cats	250

(*) The levels referred to in this Annex are indicative levels above which, certainly in the case of repetitive findings, investigations should be performed on the factors leading to the presence of T-2 and HT-2 toxin or on the effects of feed and food processing. The indicative levels are based on the occurrence data available in the EFSA database as presented in the EFSA opinion. The indicative levels are not feed and food safety levels.
(**) Unprocessed cereals are cereals which have not undergone any physical or thermal treatment other than drying, cleaning and sorting.
(***) The indicative levels for cereals and cereal products intended for feed and compound feed are relative to a feed with a moisture content of 12%.

desirable substances in animal feed (European Commission, 2002). The guidance values for the presence of DON, ZON, OTA and fumonisins in products intended for animal feeding have been determined in the Commission Recommendation of 17 August 2006 (European Commission, 2006). These limits are presented in Table 2.

In 2011, the European Food Safety Authority (EFSA) determined a tolerable daily intake (TDI) for the sum of T-2 and its major metabolite, HT-2, of 100 ng/kg BW (EFSA, 2011a). Just recently, the EC has made a proposition regarding these toxins in grains and complete feedingstuffs (European Commission, 2013) (Table 3). For the *Fusarium* mycotoxins, BEA and ENNs, which have recently become of interest, no maximum levels nor TDIs have been put forward. The EFSA is currently establishing its opinion on the risks to human and animal health related to the presence of BEA and ENNs in food and feed. The results are expected at the latest in September 2014.

CONCLUSION

In this review, several methods to counteract mycotoxin production and its impact on animal health are described. These include pre- and post-harvest strategies and the use of mycotoxin detoxifiers. In order to reduce the risk of mycotoxicosis in animals, several methods should be applied simultaneously.

The current legislation regarding maximum, guidance or action levels of mycotoxin contamination in various feedstuffs is given. This allows the agricultural industry to interpret feed analysis results and to decide whether to undertake actions or not.

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