

The occurrence of mycotoxins in feed and food

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Abstract

Mycotoxins are secondary fungal metabolites, toxic to humans and animals. Toxigenic fungi often grow on edible plants, thereby contaminating food and feed. The most common mycotoxins are produced by the genera *Fusarium*, *Aspergillus* and *Penicillium*. While *Fusarium* species are plant pathogens producing mycotoxins (trichothecenes, fumonisins and zearalenone), before or after harvest, species of the genera *Aspergillus* and *Penicillium* are frequent contaminants of food during processing and storage. The most toxic mycotoxins produced by the genera *Penicillium* and *Aspergillus* are aflatoxins and ochratoxin A. Their occurrence is not only associated with plant commodities, but they are also found in products of animal origin. Plants, as living organisms, can alter the chemical structure of mycotoxins as part of their defence against xenobiotics. The extractable conjugated or non-extractable bound mycotoxins formed remain present in the plant tissue, but are currently neither routinely screened for in food nor regulated by the legislation, for which reason they may be considered masked. *Fusarium* mycotoxins (deoxynivalenol, zearalenone, fumonisins, nivalenol, fusarenon-X, T-2 toxin, HT-2 toxin, fusaric acid) are prone to metabolisation or binding by plants, though the transformation of other mycotoxins by plants (ochratoxin A, patulin, destruxins) has also been described. A potential threat to consumer safety can occur from these substances, particularly due to the possible hydrolysis of masked mycotoxins back to their toxic parents during mammalian digestion. The occurrence of masked mycotoxins in food and analytical aspects for their determination and toxicology must be studied in detail. This review focuses on the types and occurrence of various kinds of mycotoxins in food and feed associated with risks to humans and livestock as well as their significance.

Aflatoxins, fumonisins, mycotoxins, ochratoxin A, trichothecenes, zearalenone

Introduction

Mycotoxins represent a heterogenic group of secondary metabolites of moulds. The most frequent source of mycotoxins are plant products contaminated by moulds which, as part of the food chain, may consequently cause mycotoxicological contamination of food of animal origin. Microscopic filamentous fungi colonise crops during growth, ageing, harvesting, storage and processing. The occurrence of mycotoxins is influenced by many factors including moisture, temperature, pH, concentration of oxygen, substrate type and presence of competitive microflora. Under proper growth conditions, toxicogenic mould species can accumulate mycotoxins in crops to a level threatening human and animal health. Mycotoxins are the greatest hazard for most livestock (bovine and porcine animals and poultry) because they consume large amounts of plant-based feed. The toxicity of mycotoxins depends mainly on the type of mycotoxin, the amount and length of exposure, the sex and age of the animal, its health and immunological and nutritional condition, and also environmental factors (zoo hygiene and animal husbandry) (Binder 2007).

Diseases caused by mycotoxins are called mycotoxicoses. They can be manifested in three forms – acute, chronic and secondary. Nowadays, acute mycotoxicosis is extremely rare in the human and animal population and occurs mainly after ingestion of evidently contaminated food. Chronic primary mycotoxicosis is frequent in livestock. The diagnostics of this form are not always clear, mainly because the symptoms are easily confused with manifestations of infectious diseases or nutritional deficiencies. Diseases currently caused by mycotoxicosis are mostly connected with secondary mycotoxicosis.

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Intoxication is characterised by delayed consequences, mainly because of their negative impact on animal and human immunity. It is caused by intake of mycotoxins over a longer duration (Piecková 2009).

Mycotoxins are a heterogenic group of metabolites produced by microscopic filamentous fungi. They differ in terms of chemical structure, metabolism, toxicity and affinity to a target organ. This study presents the most important mycotoxins occurring in feed and food and their possible negative effects. The most frequent causes of mycotoxicoses are mycotoxins produced by the genera *Aspergillus*, *Penicillium* and *Fusarium*. The genus *Fusarium spp.* is a destructive plant pathogen capable of producing mycotoxins before or after harvest. The genera *Penicillium spp.* and *Aspergillus spp.* are more often contaminants of commodities and foods during drying and storage.

Aflatoxins

Aflatoxins are among the most well-known, the most toxic and the most widespread mycotoxins produced by the toxicogenic species *Aspergillus flavus*, *A. parasiticus*, *A. nomius* and *A. pseudotamari* (IARC 2002; Bintvihok et al. 2002; Mishra and Das 2003; Manning et al. 2005; Williams et al. 2009; Khan et al. 2010 and Arana et al. 2011). Aflatoxins belong to a group of structurally related difuranocoumarinic derivatives. Eighteen types of aflatoxins have been identified; the most frequent in foods are B1, B2, G1, G2, M1 and M2. The well-defined species *A. flavus* produces only B aflatoxins and sometimes the mycotoxin cyclopiazonic acid (CPA), while *A. parasiticus* produces both B and G aflatoxins, but not CPA. Aflatoxin M1 is a metabolite of aflatoxin B1 that can occur in milk and milk products from animals (Quillien 2002). The International Agency for Research on Cancer (IARC 1993) has classified aflatoxin B1 in group 1 – carcinogenic.

These secondary metabolites contaminate a number of oil seed crops during growth of the fungus and this can result in severe negative economic and health impacts (Cary et al. 2009). High levels of aflatoxins have been found in cotton and maize seeds, peanuts and other nuts. The presence of aflatoxins is less frequent in grains such as wheat, rice, rye and barley. 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 metabolism by living plants, fungi and mammals or after food processing. 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). A variety of studies have been conducted in order to understand the process of crop contamination by aflatoxins.

Aflatoxins are dangerous metabolites that are often carcinogenic, teratogenic, mutagenic and immunotoxic, and represent a serious threat to both animal and human health (Reverberi et al. 2010). Aflatoxins have chronic and, in some cases, even late toxic effects – these toxic effects develop after long-term consumption of low doses of aflatoxins (Laciaková et al. 2011). Aflatoxin B1 is the most toxic and is associated with liver cancer and immune suppression (Sheppard 2008). Exposure to large doses (> 6 000 mg) of aflatoxin may cause acute toxicity with lethal effect, whereas exposure to small doses for prolonged periods is carcinogenic. There may be an interaction between chronic mycotoxin exposure and malnutrition, immune suppression, impaired growth and disease (Williams et al. 2004). However, the presence of mycotoxins in food is often overlooked due to public ignorance about their existence, lack of regulatory mechanisms, dumping of food products and the introduction of contaminated commodities into the human food chain during chronic food shortages due to drought, wars and political and economic instability. The largest mycotoxin poisoning epidemic in the last decade occurred in Kenya in 2004. Aflatoxin poisoning was associated with the eating of home-grown maize stored under damp conditions (Lewis et al. 2005).

Aflatoxin contamination in crops is a worldwide food safety concern due that are compound carcinogenic highly and mutagenic in animals of all species, including fish and birds (Frisvad et al. 2006). Aflatoxins have a negative impact on animal production (egg production, milk production and weight gain) (Miller and Wilson 1994). The main manifestations of chronic aflatoxicosis in laying hens are reduced egg production and weight and an increase in liver fat levels (Rosmaninho et al. 2001). A toxic amount of aflatoxins in broilers influences the metabolism of poultry, reducing the activity of enzymes that digest starch, proteins, lipids and nucleic acids, decreases blood protein, total cholesterol and urea, and increases the activity of serum enzymes that indicate liver damage. The consequence is loss of body weight, bad colouration, malfunctions of blood coagulation and the appearance of macroscopic and histological lesions in the liver. These losses are pronouncing in meat and eggs in terms of quality and quantity, and also threaten the health safety of animal products (Bintvihok et al. 2002 and Farombi 2006).

Aflatoxin M1 is an important hepatocarcinogen mycotoxin frequently found in milk and dairy products. Milk products such as cheese may be contaminated by aflatoxin M1 when dairy cattle have been fed with aflatoxin B1-contaminated feeds. When ingested by dairy animals, the metabolite is bio-transformed at the hepatic level into aflatoxin M1. It is then excreted in this form in the milk used for human consumption and, thanks to its affinity for casein, is also present in dairy products (Richard 2007). This affinity for casein causes an increased concentration of this mycotoxin principally in cheeses, and its content can increase by a factor of from 1.7 to 8 compared to the original concentration in milk (Cattaneo et al. 2008; Motawee and McMahon 2009). The presence of mycotoxin M1 has also been confirmed in whey and products thereof (Cattaneo et al. 2013). A problem is presented by the fact that aflatoxin M1 is relatively resistant to heat treatments such as pasteurisation of milk and to treatments used during cheese production.

Ochratoxin A

Ochratoxin A (OTA) has been a mycotoxin of increasing interest in foods and feeds in recent years. Ochratoxin A is produced by a single *Penicillium* species, *P. verrucosum*, by *A. ochraceus* and several related *Aspergillus* species, and by *A. carbonarius*, with a small percentage of isolates of the closely related *A. niger*. Ochratoxigenic species belonging to the *Aspergillus* section Nigri have been reported in regions with a warmer and tropical climate. They are able to grow on various substrates and to tolerate diverse conditions of moisture, pH and temperature (Abarca et al. 2001 and Rosa et al. 2002). *P. verrucosum* produces OTA in cereals, whereas *P. nordicum* can produce this toxin in meat products and cheeses (Samson and Frisvad 2004). *A. ochraceus* belongs among isolates derived from dried and stored foods, e.g. smoked and salted fish, soy, spices, nuts and dried fruits. *A. carbonarius* is the main producer of OTA in grapes and grape products (Heenan et al. 1998). Recent studies have shown that, in addition to the abovementioned species, *A. westerdijkiae*, *A. steynii* and *A. ochraceus* are also responsible for the production of OTA in coffee (Noonim et al. 2008). The International Agency for Research on Cancer (IARC 1993) has classified it in the group 2B – possible carcinogen.

Biosynthetically, OTA is a pentaketide derived from the dihydrocoumarin family coupled to β -phenylalanine. OTA is a weak organic acid with a molar mass of 403.8 g·mol⁻¹. With a crystalline structure varying from colour less to white, this molecule displays an intense green fluorescence under UV light in an acid medium and blue fluorescence in alkaline conditions. OTA is a naturally occurring mycotoxin soluble in organic solvents and in an aqueous solution of sodium bicarbonate. It is slightly soluble in water. OTA is efficiently absorbed in the gastrointestinal tract, mainly in the small intestine. Information from a number of species shows that it is distributed via the blood, mainly to the kidneys, with lower concentrations found in the liver, muscles and fat (Sorrenti et al. 2013).

OTA has nephrotoxic, hepatotoxic, neurotoxic, teratogenic and immunotoxic effects and can cause kidney and liver tumours in rats and mice. Its toxicity is variable depending on sex, species and cell type in tested animals. OTA has an affinity mainly to the kidneys and liver. It has been shown that OTA is a potential nephrotoxic for all mammals – non-ruminants (El-Khoury and Atoui 2010). Over the last few decades, studies aimed at elucidating the modes of action implicated in OTA toxicity and carcinogenicity have been published. There has been considerable debate for many years over the genotoxicity of OTA and its actual role in carcinogenicity. Several authors and expert groups have concluded that OTA is genotoxic. However, other authors indicate that OTA is unlikely to act through a direct genotoxic mechanism and that its carcinogenicity is due to an indirect mechanism, such as induction of oxidative stress (Sorrenti et al. 2013).

Epidemiologic studies in Denmark, Hungary, Scandinavia and Poland show that OTA has an important place in the aetiology of nephropathy in pigs. OTA is nephrotoxic and is suspected of being the main etiological agent responsible for human Balkan endemic nephropathy (BEN) and associated urinary tract tumours. Striking similarities between OTA-induced porcine nephropathy in pigs and BEN in humans are observed. This is an interstitial chronic disease affecting the population in southeast Europe (Croatia, Bosnia, Bulgaria and Romania) and also considered to be the main cause of Tunisian nephropathy (TCIN) (Leszkowicz and Manderville 2007).

As with the other mycotoxins, there is also a potential risk in this case of contamination of animal products with OTA mycotoxin (Garies and Scheuer 2000; Castella et al. 2002; Lund and Frisvad 2003; Matrella et al. 2006 and Pietri et al. 2006). One of the ways of contamination is intake of mycotoxin by food-producing animals in feed and its transfer into animal products, or contamination of these products by toxinogenic species of micromycetes during processing or storage. The results have pointed to the fact that sub-chronic pig exposure leads to the accumulation of OTA in raw materials (kidney, liver, fatty and muscle tissues) and consequently in meat products whose level of contamination is directly dependent on the OTA content in the raw materials used in their production (Perši et al. 2014).

P. verrucosum, *P. nordicum* and *A. ochraceus*, as primary producers of OTA, are able to grow on the surfaces of foods such as salami, dried hams, meat and other meat products during ageing and storage (Gareis and Scheuer 2000 and Pietri et al. 2006).

Fusarium mycotoxins

Fusarium mycotoxins are produced by fungi of the genera *Fusarium*. These mycotoxins occur in cereals, mainly in wheat, barley and maize planted in the temperate zone of America, Europe and Asia. *Fusarium* infects crops during growth and flowering in the field and significantly affects the amount of mycotoxins in the final product. Many toxinogenic species of *Fusarium* are able to produce a variable range of mycotoxins. The main types are fumonisins A and B, trichothecenes and zearalenone (Bennett and Klich 2003).

Fumonisin (B1, B2) are carcinogenic metabolites of *Fusarium proliferatum* and *Fusarium verticillioides*. *Fusarium verticillioides* (formerly *Fusarium moniliforme*) is a common fungal contaminant of maize. Fumonisin inhibit ceramide synthase, causing accumulation of bioactive intermediates of sphingolipid metabolism (sphinganine and other sphingoid bases and derivatives) as well as depletion of complex sphingolipids which interferes with the function of some membrane proteins, including the folate-binding protein. Fumonisin are especially widespread in the tropical and subtropical zone, but due to global warming they are even produced in south and central Europe (Marasas et al. 2004). Higher toxicity was proven for fumonisin B1 which has been classified by the International Agency for Research on Cancer (IARC 1993) in group 2B – optional carcinogen.

Some correlation studies have suggested a link between the consumption of maize with a high incidence of *F. verticillioides* and fumonisins and the high incidence of human oesophageal carcinoma in certain parts of South Africa, America and Asia (Yoshizawa et al. 1994 and Marasas et al. 2004). The toxic effects of fumonisin B1 have also been proven in animals and have been shown to promote tumours in rats and cause equine leukoencephalomalacia and porcine pulmonary oedema (Marasas et al. 2004).

Trichothecenes (TCT) comprise a vast group of over 100 fungal metabolites with the same basic structure. Several fungal genera are capable of producing TCT; most of them have been isolated from *Fusarium* spp., though also from species of the genera *Myrothecium*, *Trichoderma*, *Trichothecium*, *Cephalosporium*, *Verticimonosporium* and *Stachybotrys* (Wiedenborner 2001). All trichothecenes contain an epoxide at the C^{12,13} positions which is responsible for their toxicological activity. At the cellular level, the main toxic effect of TCT mycotoxins appears to be a primary inhibition of protein synthesis. TCT affect actively dividing cells, such as those lining the gastrointestinal tract, the skin, and lymphoid and erythroid cells. The toxic action of TCT results in extensive necrosis of the oral mucosa and skin in contact with the toxin, an acute effect on the digestive tract and decreased bone marrow and immune function (Schwarzer 2009).

They are divided into several groups according to similarity in chemical structure. The most important are the first two groups A and B. T-2 toxin, HT-2 toxin and diacetoscirpenol belong to group A. In experimental animals, type A compounds such as HT-2 toxin (HT-2) and T-2 toxin (T-2) have been shown to be significantly more toxic than type B trichothecenes, e.g. deoxynivalenol (DON), nivalenol (NIV), fusarenon-X (Fus-X), 15-acetyldeoxynivalenol (15ADON) and 3-acetyldeoxynivalenol (3ADON) (Wiedenborner 2001).

T-2 and HT-2 toxins

T-2 toxin and HT-2 toxin are mycotoxins and are members of a large group of fungal sesquiterpenes, commonly denoted as trichothecenes. They are produced by various *Fusarium* species, including *F. sporotrichoides*, *F. poae*, *F. equiseti* and *F. acuminatum*, as well as species of the genera *Myrothecium*, *Cephalosporium*, *Verticimonosporium*, *Trichoderma*, *Trichothecium* and *Stachybotrys*.

Generally, the *Fusarium* species grow and invade crops, and may produce T-2 and HT-2 toxins under cool moist conditions prior to harvest. T-2 toxin and HT-2 toxin and other trichothecenes are predominantly found in cereal grains (particularly in oats) and products thereof. Consequently, T-2 toxin and HT-2 toxin are toxic to all animal species as well as to humans. Historical cases of human intoxications associated with the consumption of overwintered, mouldy grains are described as Alimentary Toxic Aleukia (ATA), characterised by sepsis and haemorrhages and a general pancytopenia. The toxic effects exerted by T-2 toxin and HT-2 toxin include the inhibition of protein synthesis, also affecting the synthesis of immunoglobulins and, in turn, the humoral immunity. Alteration of cell membrane functions and lipid peroxidation account for many of the acute effects of T-2 and HT-2 toxins, including the necrotic lesions observed at the contact sites. Apoptosis of proliferating cells, including bone marrow cells (inhibition of haematopoiesis) and cells of the immune system (lymphoid depletion), account for the systemic toxicity following dietary exposure (Scientific Opinion on the risks for animal and public health related to the presence of T-2 and HT-2 toxin in food and feed 2011).

Results of experiments performed on dairy cattle and broilers confirm that transfer of fusarium mycotoxin into animal products is negligible. In the case of broilers, approx. 80% of 3H-marked T-2 toxin was metabolised into many polar derivatives secreted in faeces in 48 hours. Only a low amount of T-2 toxin, HT-2 toxin, neosolaniol and T-2 tetraol (0.06 – 1.13% of total dose) was detected 48 hours after administration (Yoshizawa et al. 1980).

The European Commission asked EFSA for a scientific opinion on the risk to human and animal health related to the presence of T-2 and HT-2 toxin in food and feed. A total of 20 519 results for the sum of T-2 and HT-2 toxins in food, feed and unprocessed grains, collected in years 2005 – 2010 in 22 European countries, were used in the evaluation. The highest mean concentrations for the sum of T-2 and HT-2 toxins were observed in grains and grain milling products, notably in oats and oat products. Grains and grain-based foods, in particular bread, fine bakery wares, grain milling products and breakfast cereals, made the largest contribution to the sum of T-2 and HT-2 toxin exposure in humans. T-2 toxin is rapidly metabolised into a large number of products, HT-2 toxin being a major metabolite. Pigs are among the animals most sensitive to the effects of T-2 toxin, the most sensitive endpoints being immunological and haematological effects (Scientific Opinion on the risks for animal and public health related to the presence of T-2 and HT-2 toxin in food and feed 2011).

Thirteen countries were asked to provide information on the exposure of the population to *Fusarium* toxins in their country. Data has been provided by the participating institutes on the following trichothecenes: Deoxynivalenol (DON), Nivalenol (NIV), 3-Acetyldeoxynivalenol (3-AcDON), 15-Acetyldeoxynivalenol (15-AcDON), Fusarenon-X (FUS-X), T-2 toxin, HT-2 toxin, T2-triol, Diacetoxyscirpenol (DAS), Neosolaniol (NEOSOL), Monoacetoxyscirpenol (MAS) and Verrucarol (VOL). Twelve countries provided data on trichothecenes, 9 countries each on zearalenone and fumonisins. The database covers a total of 16 *Fusarium* mycotoxins and 44 959 analyses. Positive samples ranged from 0% (verrucarol) to 57% (deoxynivalenol) of all samples. In all positive samples, Type B trichothecenes represented the highest amount of Deoxynivalenol (57%) and Nivalenol (16%), Type A trichothecenes T-2 Toxin (20%), HT-2 Toxin (14%), Zearalenone 32%, and fumonisins Fumonisin B1 (46%), Fumonisin B2 (42%), Fumonisin B3 (36%).

Cereals ranking first in the food categories most frequently contaminated with *Fusarium* mycotoxins, among them corn and wheat, showed the highest level of contamination with *Fusarium* mycotoxins. The main food items contaminated by *Fusarium* mycotoxins are, in Type B trichothecenes, Deoxynivalenol in corn (89%) and wheat (61%), Nivalenol in corn (35%), oats (21%) and wheat (14%), in Type A trichothecenes, T-2 Toxin in corn (28%), wheat (21%) and oats (21%), HT-2 Toxin in oats (41%), corn (24%) and rye (17%), Zearalenone in corn (79%), corn milling fractions (51%), corn-based products (53%), Fumonisin B1 in corn (66%), corn flour (79%), corn-based products (31%), corn flakes (46%) and wheat (79%), and Fumonisin B2 in corn (51%) (Schothorst and Van Egmond 2004).

Deoxynivalenol

Deoxynivalenol (DON), also called vomitoxin, is produced mainly by *Fusarium graminearum*, *F. culmorum*, *F. sporotrichoides*, *F. poae*, *F. tricinctum* and *F. acuminatum* (Pittet 1998). DON and other trichothecenes bind to the 60S ribosomal subunit which inhibits translation and activates a signalling pathway known as the ribotoxic stress response. DON ingestion causes acute gastrointestinal effects in animals including vomiting, feed refusal and growth retardation (Pestka and Smolinski 2005). There are clear species differences in susceptibility, with swine and rodents being the most sensitive and poultry and ruminants the least. These variations may in part reflect species differences in intestinal detoxification to de-epoxy DON (Yoshizawa et al. 1986). A recent small study examining faeces suggests that humans lack this putative detoxification route (Sundstøl-Eriksen and Pettersson 2003). Epidemiological data from food poisoning incidents in China between 1961 and 1991 imply that DON may play a role in acute human toxicity (Luo 1994). A food poisoning incident in 1987, affecting up to 50 000 individuals from the

Kashmir Valley, India who consumed contaminated wheat (Bhat et al. 1989), leads further support to this hypothesis.

Random collections of ears of winter wheat cultivars grown in the Czech Republic were made during 2004 – 2013. This 10-year survey reveals a significant adverse effect from maize as the preceding crop on the accumulation of DON. The study revealed prevailing occurrence of *Fusarium poae* and *Fusarium graminearum* in the Czech Republic. The occurrence of *F. poae* increased greatly in 2012 (with 93.3% of samples infected) in association with relatively warmer and drier weather. In the survey as a whole, an average content of DON in wheat of 0.45 mg·kg⁻¹ was determined. The results obtained during analysis of all factors affecting the amount of DON detected showed a significant risk of the cultivation of wheat after maize (Chrpová et al. 2014). Schaafsma and Hooker (2007) claim that the content of DON is influenced by environmental conditions (48%), variety (27%) and pre-commodity (14 – 28%), and less than 5% by method of tillage.

The results of the study by Vojsová et al. (2014) claim a risk of the presence of DON in Slovak foods of plant origin and feeds. This study presents analytical data collected in the period 2010 – 2013, and the number of positive samples ranged from 54 to 65%. However, the percentage of samples exceeding the maximum permitted limits were much lower at 1% in 2010 and 5% in 2011. The main products contaminated by DON were cereals, bakery products, feeds, flour and oats.

Prelusky et al. (1987) presented a study of the metabolism of DON in lactating sheep. DON was rapidly metabolised and excreted mainly in the urine (91%) and the bile (6%) in the form of glucuronid conjugates of DON (54%) and metabolite de-epoxid-DON, DOM-1 (13%). Excretion of non-metabolised DON represented 11%. In milk, in respect to dose, only traces of DON and its metabolites were detected. This fact was confirmed by Keese et al. (2008) after experimental administration of DON to lactating cows. Laying hens and adult roosters were assigned to a feeding trial to study the effect of increasing concentrations of deoxynivalenol (DON) in the diet (0, 5, 10 mg·kg⁻¹). Mycotoxin levels in eggs were too low to cause food safety concerns (Ebrahim et al. 2014).

Zearalenone

Zearalenone (ZEA) is a non-steroid oestrogenic mycotoxin produced mainly by the *Fusarium* species of fungi which is present in cereals cultivated all over the world, such as wheat, corn, oats, barley, rice and products made from them. Huge amounts of Zearalenone are produced by the *Fusarium* species during cereal storage under high humidity and temperature. ZEA is produced mainly by *Fusarium graminearum* and some other species such as *F. culmorum* and *F. sporotrichoides*. Zearalenone is easily absorbed from the alimentary canal and metabolised into α -Zearalenol and β -Zearalenol through 3-hydroxysteroid dehydrogenase in the cytosol or hepatocyte organelles. Zearalenone and its metabolites are conjugated through glucuronic acid. Zearalenone attaches to receptors for oestrogens present in the uterus, breast gland, adrenal gland and pituitary gland and affects oestrogen-dependent transcription in the cell nucleus (Ouanes-Benothem et al. 2008). The oestrogenic properties of Zearalenone are greater than those of Zearalenone – α -Zearalenol is 17 times stronger oestrogen than β -Zearalenol and 4 times stronger than Zearalenone. ZEA causes severe morphological and functional disorders of the reproductive organs in livestock, especially female swine. In feeding experiments with female swine, clear clinical symptoms (e.g. reduced feed efficiency, organ weight changes and reduced fertility) were observed (Takahashi-Ando et al. 2004).

According to the Opinion of the Scientific Committee on Food on *Fusarium* Toxins (2000), ZEA has been found worldwide in a number of cereal crops such as maize, barley, oats, wheat, rice and sorghum and also in bread. It has been implicated in numerous mycotoxicoses in humans and also in farm animals, especially in pigs. ZEA was measured

in endometrial tissue from 49 women. There were 27 endometrial adenocarcinoma, 11 endometrial hyperplasia and 11 normal proliferative endometria.

In respect to average concentrations of ZEA in feeds, transfer of this mycotoxin and its metabolites into the tissues and milk of dairy cows and pigs is low and consumption of their meat and milk is not a health risk for humans (Opinion of the Scientific Committee on Food on Fusarium Toxins Part 2: Zearalenone (ZEA) 2000).

Masked forms of mycotoxins

Mycotoxin derivatives that are undetectable by conventional analytical techniques because their structure has been changed in the plant are designated masked mycotoxins.

Chemical transformations that generate masked mycotoxins are catalysed by plant enzymes, most commonly by enzymes involved in detoxification processes. Plants have versatile detoxification systems to counter a wide variety of non-natural as well as natural phytotoxic chemical compounds. Among these compounds, mycotoxins are a target of plants' detoxification metabolic processes since they can interact with vital cell functions. Plants are endowed with two major detoxification mechanisms: chemical modification and compartmentation. Plants are most likely to try to lower the toxicity of mycotoxins by increasing polarity (water solubility) binding polar molecule (e.g. β -D-glucose) which makes it easier to excrete the conjugate into the vacuole.

Food processing, on the other hand, can also chemically alter mycotoxins, however most food-processing compounds are less toxic than their precursors. Microorganisms used in fermentation processes may transform mycotoxins into products that are also not detected by analytical methods conventionally used in mycotoxin monitoring. These derivatives resulting from the enzymatic activities of microbial cultures used for fermentation. The chemical changes of mycotoxins involve the formation of conjugates with aminoacids (cysteine, lysine) or with albumins and can be called detoxification. Bound mycotoxins can be considered as detoxicated as long as they are released from the matrix during food processing or in the digestive system. The fact that these forms can be a health risk has been shown by recent research. Masked mycotoxins can, in some cases, be particularly released by the action of the digestive enzymes of mammals, by bacteria present in the gastrointestinal tract or by enzymes of yeast (Berthiller et al. 2005).

The group of masked mycotoxins comprises both extractable conjugated and bound (non-extractable) varieties. Bound mycotoxins are covalently or non-covalently attached to polymeric carbohydrate or protein matrices (Berthiller et al. 2009). The most well-known masked forms of mycotoxins are produced by micromycetes of the genus *Fusarium*. The presence of modified mycotoxins has been revealed in cereal-based food and feed for trichothecenes, mycoestrogens and fumonisins. More specifically, deoxynivalenol-3-glucoside (DON-3G), zearalenone-14-glucoside (ZEN-14G), zearalenone-14-sulphate (ZEN-14S), α -Zearalenol-14-glucoside (α -ZEL-14G), β -Zearalenol-14-glucoside (β -ZEL-14G), T-2-toxin-3-glucoside (T-2-3G), HT-2-toxin-3-glucoside (HT-2-3G), diacetoxyscirpenol-3-glucoside (DAS-3G), nivalenol-3-glucoside (NIV-3G), mono-acetoxyscirpenol-3-glucoside (MAS-3G), neosolaniol-3-glucoside (NEO-3G) and fumonisin esters have been detected and confirmed (De Boevre et al. 2012).

The appearance of conjugated forms of mycotoxins has been proven in cereals and cereal products, and also in other mainly fermented foods in which bound mycotoxins can be released. Berthiller et al. (2007) reported the occurrence of DON in raw material and final products produced by fermentation processes. Analysis of the results confirmed a larger amount of DON in cereal products. It is well known that cereal crops exposed to deoxynivalenol (DON) infection are capable of detoxifying this mycotoxin through the plant metabolism. In this context, one major pathway is the conjugation of DON to a glucose moiety giving rise to 3- β -D-glucopyranosyl-4-deoxynivalenol (D3G).

Though no longer toxic for plants, this metabolite may potentially be hydrolysed in the digestive tract of humans and animals, thereby releasing the toxic precursor (DON). The co-occurrence of DON and D3G in cereal-based products has already been reported, but data on their absolute and relative concentrations is still insufficient. In order to contribute to a better understanding of the significance of D3G, the quantitative determination of DON and D3G was carried out by liquid chromatography-tandem mass spectrometry (LCMS/MS) in 22 cereal samples and 4 malt-based products collected from 9 countries. DON was detected in all cereal samples (median 176 $\mu\text{g}\cdot\text{kg}^{-1}$) (Desmarchelier and Seefelder 2011).

Twenty-five samples of retail corn flakes (from 15 lots) were analysed for fumonisin B(1) (FB(1)) and fumonisin B(2) (FB(2)). After extraction of the corn flake residue with 1% sodium dodecyl sulphate (SDS) solution and hydrolysis with potassium hydroxide, hidden (protein-bound) fumonisin was determined as HFB(1) which was found in residues from all the corn flake samples, even those containing no detectable FB(1). The results therefore showed an average of 2.6 times more FB(1) present in bound form than as determined by conventional analysis (Kim et al. 2003).

Masked mycotoxins might also be less toxic than their parent compounds if the hydrolysis of glucosides during digestion is incomplete. Finally, masked mycotoxins might also be more toxic than their parent compounds when, for example, they are more bioavailable. Currently, it is impossible to perform a proper risk assessment for masked mycotoxins in food due to the lack of data on exposure and toxic properties. Various forms of toxins clearly contribute to the toxicity of a given food and should be taken into consideration in setting future maximum residue limits. There is a clear need for more toxicological studies, preferably comparing the masked mycotoxin with its parent. Another possibility might be to use contaminated food plant commodities containing parent and masked mycotoxins and perform comparison toxicity studies with the pure mycotoxin (Berthiller et al. 2013).

Conclusions

The occurrence of mycotoxins in agricultural products is a worldwide problem that requires constant attention. The presence of mycotoxins in grains and other staple foods and feedstuffs has serious implications for human and animal health. It is important to realise that mycotoxins are often present in foods and feeds in the form of a mix or “cocktail” where their co-operation can have an additive or even synergetic effect. For this reason, the EFSA collects data extremely intensively about the presence of a wide spectrum of mycotoxins in foods to form an appropriate opinion leading to measures taken from the perspective of food safety and the reduction of health risks. Finally, mycotoxin considerations should be a component of an integrated commodity management programme focusing on the maintenance of commodity quality from the field to the consumer.

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