Review

PREVENTION OF EXPOSURE TO MYCOTOXINS FROM FOOD AND FEED

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Mycotoxins are metabolites of moulds that may be found in food and feed of plant and animal origin. This paper gives a short review of the agronomical methods and food and feed storage recommendations for the prevention of mould contamination. It describes the practical methods of feed decontamination and the use of feed additives where mycotoxin contamination prevention has failed. However, these methods should be avoided as much as possible because they may increase the cost of production, reduce the nutritional value of feed, and leave residues of mycotoxins or their toxic metabolites. Since there is no universal and reliable method of feed decontamination for all mycotoxins, the paper stresses the importance of preventive measures.

KEY WORDS: *aflatoxins, decontamination, ergot alkaloids, fumonisins, ochratoxins, trichothecenes*

According to the evaluation of the Food and Agriculture Organization (FAO), 25% of the world cereal production is contaminated by mycotoxins (1). Food and feed are usually contaminated by more than one mycotoxin, because a certain strain of moulds may produce different mycotoxins. Residues of mycotoxins may also be found in food of animal origin (meat, milk, eggs, and cheese) as the consequence of feed contamination. Mycotoxins are toxic compounds, and some of them are also mutagenic, genotoxic, carcinogenic, or teratogenic. In mild climates, the most frequent toxicogenic moulds in food and feed are the species of genera *Fusarium* and *Penicillium*. Some *Fusarium* species produce fumonisins (fumonisin B_1 – FB_1, fumonisin B_2 – FB_2, and fumonisin B_3 – FB_3), trichothecenes (T–2 toxin, nivalenol – NIV, deoxynivalenol – DON, diacetoxyscirpenol – DAS), and zearalenone (ZEA). Ochratoxins (the most important is ochratoxin A – OTA), citrinin, and penicillic acid are produced by some *Penicillium* and *Aspergillus* moulds (2). Ergot alkaloides (ergoatamine and ergocristine) are the products of fungi of genus *Claviceps* and some strains of *Penicillium, Aspergillus,* and *Rhizopus* that contaminate cereals (mainly rye, barley, and wheat) (3).

Aflatoxins (aflatoxin B_1 – AFB_1, aflatoxin B_2 – AFB_2, aflatoxin G_1 – AFG_1, and aflatoxin G_2 – AFG_2) are the metabolic products of *Aspergillus* species that contaminate cereals and other commodities mostly in tropical countries. The prevention of mycotoxin production should include all phases of food and feed production, because the mould contamination may occur in the field, during storage, as well as in transport (4).

PREVENTION OF MYCOTOXIN PRODUCTION

The most important arable crops in the Republic of Croatia are wheat and maize. They are frequently contaminated by *Penicillium* and *Fusarium* moulds which, in favourable conditions, may produce mycotoxins (5). This contamination may be avoided

by the use of mould–resistant wheat cultivars and maize hybrids (6–8).

Agrotechnical measures may also help to combat the maize and wheat ear diseases. The sowing time determines the harvesting time, which can have a significant influence on the appearance of disease. This is particularly important when late FAO maize hybrids are used, because they are readily contaminated by moulds in wet autumns. Mould contamination is more pronounced if wheat is sown after maize or vice versa. Multi–field crop rotation in which rape, sugar beet, sunflower or soya–beans are present reduces the infection. Fertilization with nitrogen increases plants' sensitivity to moulds (9), and balanced fertilization based on nutrition analysis is required. Climatic conditions, such as temperature and humidity, are not under human control, but they may be crucial in contamination with moulds. Fungicides applied before blossoming decrease contamination with *Fusarium* and the related production of mycotoxins (10). Delayed harvest particularly favours contamination with *Fusarium*. Mechanically damaged and shrivelled grains are regularly contaminated by moulds, and mouldy grains can partially be removed by separators (11). The humidity of grain and the relative air humidity are very important in processing and transport (12). The kernel must be desiccated as soon as possible, and optimal humidity maintained. It is particularly important not to allow increases in humidity after desiccation, as they strongly favour contamination. During long–term storage, the kernel is exposed to oscillations in temperature and humidity, and insects of species *Sithohilus, Tribolium, Trogodernma, Oryzaephilus* can be vectors for mould contamination (13). Adequate storage with optimal temperature and humidity of grains and relative humidity and the hygiene in silos may decrease the growth of toxicogenic moulds (14). It should be emphasised that at the end of the storage period grains can not be less contaminated with mycotoxins than at the beginning. The entrance of mycotoxins in the nutritional chain can be avoided by storing only high quality products in silos and by strictly observing good agricultural practice. Mycotoxin decontamination in later stages of food production is difficult; it increases the cost of production and the results are not always satisfactory. According to FAO (15), the decontamination process must:

1. destroy, inactivate or remove the mycotoxin;
2. not produce or leave toxic, carcinogenic or mutagenic residues in final products or in food products obtained from animals fed decontaminated feed;
3. retain the nutritive value and acceptability of the product;
4. not significantly alter important technological properties, and
5. destroy fungal spores and mycelia which could, under favourable conditions, proliferate and form new toxins.

The US Food and Drug Administration requires additional data on the environmental impact of the method (15).

This paper gives an overview of methods used in industrial decontamination of food and feed. It also addresses the possibilities of the use of adsorbens as farm animal feed additives. We have not addressed specific methods characteristic for certain foods, such as the decontamination of trichothecenes or OTA in brewing barley for beer production (16) or the effect of milk processing on the aflatoxin M_1 (AFM_1) concentration (17).

PHYSICAL METHODS OF DECONTAMINATION

There are several physical methods of decontamination of agricultural products known to us such as the removal of damaged grains or of a part of contaminated crop, washing procedures, radiation, ultrasound and extraction with organic solvents.

The removal of damaged parts of a crop (usually mould–contaminated) is possible when contamination is uneven or partial. Physical removal of discoloured, damaged, or inadequately developed peanut kernels significantly decreases the concentration of aflatoxins, fumonisins and ergot alkaloids (15). Although this is the most widely used decontamination technique in the peanut and pistachio industry (18), it is not practical for maize and cottonseed (15). Blanching and electronic eye colour sorting of raw peanuts contaminated by *Aspergillus flavus*, and damaged peanut kernels decrease the concentration of aflatoxins down to under 5 μg/kg (19). Fluorescent sorting can be used for maize, cottonseed and dried figs, but it is ineffective for the decontamination of peanuts (15). Fluorescent properties of kojic acid, the metabolic product of *Aspergillus flavus* and

other fungi, are used for sorting maize contaminated by aflatoxins. False negative results obtained using this method are possible when the maize is contaminated by aflatoxins, but kojic acid is not present (15). Sieving could decrease the concentration of fumonisins in maize, because damaged maize has a ten times higher concentration of fumonisins than undamaged maize (20). The removal of maize kernels smaller than 3 mm may reduce the fumonisin level by 70% (21).

Flotation may lower high concentrations of aflatoxins in contaminated maize and peanuts by as much as 90%, because contaminated seeds float on water (22). Rinsing grain with water or sodium carbonate water solution could lower the concentration of mycotoxins DON, ZEA and fumonisins in wheat and maize (23). These methods are limited by the cost of seed drying, and they are used only before wet milling and brewing.

Most mycotoxins are heat resistant, and high temperatures are not used in the decontamination of cereals and other agricultural products.

Different types of radiation (γ, X–ray, UV, VIS, microwave) were tested for the detoxification of mycotoxins. In wheat, γ–radiation successfully reduces the concentration of T–2 toxin, ZEA, DON (15). Unfortunately, radiation produces AFB_1 metabolites, and the radiation is effective in decontamination only when applied to a thin layer of grain. It is found that sun light is the most effective detoxifier of AFB_1 and could be used in tropics to detoxify coconuts, peanuts and maize (24).

Organic solvents (ethanol, isopropanol, methoxymethane) effectively remove aflatoxins from different types of food products. In addition to the limiting high cost of organic solvents, these compounds are not practical for industrial use because they themselves are removed from the treated products with difficulty (22, 25).

Table 1 summarises the physical methods of decontamination.

Table 1 *Physical methods of mycotoxin decontamination applied in food industry*

Procedure	Mycotoxin	Product	Efficiency	Reference
automated removal of damaged kernels	aflatoxins	peanuts	+++	15
		pistachio	+++	18
fluorescence sorting	aflatoxins	maize, cottonseed, dried figs	+++	15
sieving	fumonisins	maize	++	21
flotation	aflatoxins	maize, peanuts	+++	22
rinsing	DON, ZEA	wheat, maize	++	23
	fumonisins	wheat, maize	++	23
wet–milling	aflatoxins	maize	+/–	26
	ZEA	maize	+/–	26
roasting	aflatoxins	coffee, maize, peanuts	+ or –	27
	OTA	coffee	++	28
	OTA	coffee	–	29
heat processing	OTA	flour	++	30
	trichothecenes	all food	–	31
	DON	all food	–	31
	FB_1	maize–based food	–	32
γ–radiation	T–2 toxin, ZEA,	wheat	++	15
	DON	wheat	++	15
	aflatoxins	wheat	++*	24
sunlight	aflatoxins	wheat	+++	24

+++ elimination 90–100%; ++ elimination 50–90%; + elimination 10–50%, +/– mycotoxin is eliminated from certain fractions, but concentrated in others; + or – mycotoxin elimination depends on how the procedure is carried out; * procedure leaves behind residual metabolites

CHEMICAL METHODS OF DECONTAMINATION

Compounds such as acids (formic and propionic acids), alkaline compounds (ammonium, sodium hydroxyde), oxidizing compounds (hydrogen peroxide, ozone), reducing compounds (bisulphite) and chlorinating (chloride) compounds were tested for their efficacy in mycotoxin decontamination. Chemical detoxification is very effective, but it does not meet the FAO requirements, because some compounds leave behind their toxic metabolites and others reduce the nutritional value of treated food and feed.

Propionic acid is used to inhibit mould growth. Its disadvantage is that it is a corrosive, which makes it dangerous for handling (33). Hydrogen peroxide can destroy a large amount of FB_1 in maize (34) and detoxify aflatoxins containing peanut (35). Hydrogen peroxide and ammonia are mostly used to remove aflatoxins from feed. It has been shown that these methods do not leave toxic metabolites of mycotoxins in feed, but the ammonia reduces its nutritional value by decreasing lysine and sulphur–containing amino acids (15). The animal readily accepts the ammoniated product, if adequate aeration is allowed to remove residual ammonia. The concentration of AFM_1, metabolic product of AFB_1, is considerably reduced in milk of lactating cows fed ammoniated peanut meals naturally contaminated by AFB_1 (36). Ammoniation is considered safe and practical for the decontamination of aflatoxins in feed, and it is used in some states of the USA, Mexico, France, Senegal, Sudan, and Brazil (27, 37). Ammoniation under increased pressure (60 psi) with ambient temperature, or under normal pressure with increased temperature reduces the concentration of FB_1 in wheat by 79% (38). The disadvantages of ammoniation are the relatively long period of aeration and its cost which can increase the price of the product by 5–20% (39). Ammoniation is not recommended for detoxifying OTA–contaminated grains and feeds (40). Monomethylamine or ammonia solutions with calcium hydroxide used at 96 °C were shown to decompose OTA in swine feed (41). Sodium bisulphite is a common food additive that can significantly reduce DON and AFB_1 in maize–based pig feed (42). Sodium chloride reduces the concentration of aflatoxins in unshelled peanuts cooked under pressure (31).

The efficiency of the described methods in mycotoxin removal is shown in Table 2.

Table 2 *Chemicals for mycotoxin decontamination applied in food industry*

Chemicals	Mycotoxin	Product	Efficiency	Reference
calcium hydroxide for tortilla preparation	FB_1	maize	++*	43
	ZEA	maize	++	44
	DON	maize	++	44
hydrogen peroxide	aflatoxins	peanut	+++	15
hydrogen peroxide/sodium bicarbonate	FB_1	maize	+++	12
sodium bisulphite	DON, AFB_1	feed	+++	42
sodium chloride	aflatoxins	peanuts	+++	31
ammonia	aflatoxins	maize	+++	37
	aflatoxins	peanut meal	+++	36
	fumonisins	maize	+	45
ammonia wih calcium hydroxide (at 96 ^{0}C)	OTA	swine feed	+++	40
ammonia with increased pressure and ambient temperature	aflatoxins	cottonseed	+++	46
	aflatoxins	maize, peanut meal	+++	15
	fumonisins	maize	++	38
ammonia with atmospheric pressure and increased temperature	aflatoxins	peanut meal	+++	47

+++ elimination 90–100%; ++ elimination 50–90%; + elimination 10–50%; * procedure leaves behind equally toxic metabolites

THE USE OF FEED ADDITIVES

Feed additives have been introduced recently, and their purpose is to reduce mycotoxin bioavailability by binding them in the gastrointestinal system (48). Although a number of adsorbents are shown to be active *in vitro*, this is not predictive for their activity *in vivo* (49, 50). Hydrated sodium calcium aluminosilicates (HSCAS), zeolites, bentonite, active charcoal, clays (such as kaolin and sepiolitic clay), synthetic anion exchange resins (cholestyramine), and alfalfa fibre have already established their efficiency.

The best aflatoxin adsorbent seems to be HSCAS, which not only prevents aflatoxicosis in domestic animals (51), but also reduces the concentration of AFM_1 in cow and goat milk (52). Unfortunately, HSCAS poorly absorbs other mycotoxins such as fumonisins, DON, T–2 toxin, and OTA (48, 53). Zeolites are hydrated alkalinic aluminosilicates that adsorb AFB_1 and ZEA from feed (48, 54). The experimental use of clinoptilolite, a zeolite variety, produced rather different results. Some authors found that, in experimental conditions, clinoptilolites reduce the accumulation of AFB_1 in the liver of chickens (55), while others found that they have a synergistic toxic effect with AFB_1 in the liver of female rats (56). A recent study, however, has confirmed the beneficial effect of clinoptilolites in sows fed ZEA–contaminated feed (57). Bentonites are adsorbents of natural origin used in the production of pelletted feed. Their adsorbing properties depend on whether they contain sodium or calcium; more effective are those which contain sodium (58). Bentonites effectively adsorb aflatoxins (59), but not NIV and ZEA (53). Mannanoligosaccharides, the extracts of yeast cell walls, are very effective in the adsorption of aflatoxins, ZEA, and FB_1 *in vitro*, and do not affect the absorption of minerals and vitamins (53). Their effect on OTA and toxins of *Fusarium* moulds is less pronounced. Polyvinylpyrrolidone is a synthetic resin which reduces the absorption of fumonisins in the gastrointestinal system of experimental animals (60). Active charcoal is not widely used because it is not known whether its long–term use might lead to mineral and vitamin deficiency in domestic animals. Cholestyramine adsorbs ZEA, OTA and FB_1 from feed (50, 53), and reduces the nephrotoxic effect of OTA (61). High cost of active charcoal and cholestyramine limits their use on farms. It was also found that Fuller earth effectively absorbs AFB_1 from peanut oil. In India, this method is successfully applied in industry (27). So far, no single adsorbent has been proven effective against most types of mycotoxins (49).

DECONTAMINATION IN FOOD PREPARATION

The content of mycotoxins may be reduced in the preparation of food, or concentrated in certain parts of food. The fate of mycotoxins during food preparation depends on the way of contamination (natural or experimental spiking), their concentration, on the type of food, humidity and temperature. The sampling of mycotoxins for analysis is a complex problem, and it gets even more complex in double sampling: before and after food preparation.

Although most mycotoxins are resistant to heat, some ergot alkaloids are completely destroyed when bread is baked. Others, like OTA, are more heat–resistant, and the reduction of its concentration by baking is not significant (30). However, the reduction of OTA concentrations correlates with the baking temperature, and inversely correlates with the content of moisture in bread (62). It has been shown that heating flour at 250 °C for 40 minutes lowers OTA concentration by 76% (31). Coffee roasting raised a controversy about its effect on OTA concentrations. While some authors believe that this procedure considerably reduces the OTA concentration (28), others disagree (29). Processing coffee, maize, and peanuts at high temperatures seems to reduce aflatoxin contamination only partially (27). FB_1 is heat resistant, and it takes baking or frying at temperatures >150 °C to reduce it substantially.

Water solution of calcium hydroxide $\{Ca(OH)_2\}$ is used to soften the shells of maize kernels prior to further processing into maize flour for tortillas, but it also happens to remove FB_1. This procedure partially hydrolyses FB_1 into aminopentol and tricarboxilic acid (43), and partially converts it to hydroxy–FB_1 whose toxicity is equal to that of FB_1 (20). The removal of FB_1 is more rapid and extensive in alkaline or acid environments than at pH neutral (63). Calcium hydroxide effectively reduces ZEA (59–100 %) and DON (72–82 %) (44).

Fumonisins proved resistant to baking, and frying did not significantly reduce FB_1 in artificially contaminated maize muffins (32). However, the reduction was significantly greater at the surface than in the core of the muffins. The reaction between fumonisins and reducing sugars (glucose or fructose) yielded products that were non–toxic (64).

AFB_1 is completely eliminated by the refinement of oil (31), and wet milling of maize eliminates starch together with fumonisins, ZEA, and aflatoxins (15).

PREVENTION OF MYCOTOXIN TOXICITY AND FOOD DECONTAMINATION ON THE EXPERIMENTAL LEVEL

In addition to the described methods that can be applied on the industrial level, there is a number of interesting experimental methods devised to decontaminate food and feed containing mycotoxins and to protect humans and animals from mycotoxin toxicity.

Some mycotoxins damage the lipid layer of the cell membrane through increased lipid peroxidation. This is why antioxidants such as selenium and vitamins were tested for their protective efficiency in experimental conditions. The results of these investigations as well as the effect of various food components have been reviewed by Galvano and co–workers (53).

Protection targeted at specific mycotoxins was also investigated. Creppy and co–workers (65) have found that amino acid phenylalanine protects experimental animals from OTA, as it increases the urinary and hepatobiliary route of OTA excretion. The effect of artichoke extracts on vaccinal immunity and on the health of broilers chickens was studied by Stoev and co–workers (66). These methods of protection from OTA toxicity are not in industrial use.

Some authors found that *Fusarium subglutinans* and *Fusarium graminearum* were competitive, which led to a decrease in trichothecenes production by *Fusarium graminearum* (67). However, *Fusarium subglutinans* itself may produce other types of mycotoxins. Other authors tested the efficiency of antimicrobial food additives in the inhibition of moulds *Aspergillus sulphureus* and *Penicillium viridicatum* and their production of OTA (68). They found that potassium sorbate, sodium propionate, methyl paraben, and sodium bisulphite efficiently reduced their growth and the production of OTA. Except for parabens, this effect was pH–dependent; the efficiency of antimicrobial food additives generally increases with lower pH.

CONCLUSION

Mycotoxins are widespread toxins in cereals produced all over the world. In order to protect crops from contamination by mycotoxins, it is of the utmost importance to follow preventive agrotechnical measures that counteract mould growth. There is no ideal method of mycotoxin decontamination of food and feed, one which would destroy all mycotoxins without leaving their residues or metabolites and without changing the nutritional value of food and feed. All methods of decontamination increase the cost of production and should be used only in cases when preventive measures have failed.

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Sažetak

SPREČAVANJE IZLOŽENOSTI MIKOTOKSINIMA IZ HRANE I KRMIVA

Mikotoksini su metaboliti plijesni koji se nalaze u namirnicama biljnog i životinjskog podrijetla i u stočnoj hrani. Najčešće plijesni koje kontaminiraju žitarice u umjerenoj klimatskoj zoni iz rodova su *Fusarium* i *Penicillium*. U krajevima s umjerenom klimom, s toksikološkog su gledišta najvažniji mikotoksini fumonizini, trihoteceni i zearalenon koje proizvode neki biotipovi vrsta *Fusarium* i okratoksini, citrinin i penicilinska kiselina koje proizvode neki biotipovi *Penicilliuma* i *Aspergillusa*. U tropskim i suptropskim krajevima čest je nalaz aflatoksina, metaboličkih produkata nekih biotipova vrsta *Aspergillus*. Zbog međunarodne trgovine hranom postoji mogućnost izloženosti ljudi i životinja aflatoksinima i izvan tropskih područja. Iznesene su agronomske metode i preporuke za skladištenje hrane biljnog podrijetla i krmiva koje su nužne za sprečavanje kontaminacije plijesnima i njihovim produktima. Opisane su metode dekontaminacije krmiva kao i uporaba dodataka krmivu koje se mogu rabiti kada zakažu metode prevencije onečišćenja mikotoksinima. Ove metode treba izbjegavati koliko god je to moguće jer povećavaju cijenu proizvodnje i mogu smanjiti prehrambenu vrijednost krmiva. Metodama dekontaminacije mikotoksini se ne mogu potpuno ukloniti, a primjenom nekih metoda mogu nastati njihovi toksičniji metaboliti. Budući da nema jedinstvene i pouzdane metode za dekontaminaciju mikotoksina u krmivu, naglašava se važnost preventivnih mjera.

KLJUČNE RIJEČI: *aflatoksini, alkaloidi snijeti, dekontaminacija, fumonizini, okratoksini, trihoteceni*

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