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Mycotoxin contamination in food- and feedstuffs

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Budapest, Hungary

2013

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Summary

Mycotoxins are secondary metabolites of various molds of high importance in animal nutrition, food production, veterinary medicine and human health. As mold species, producing mycotoxins, are ubiquitous, mycotoxins are present everywhere in the environment and, thus, can cause various problems when their levels increase. Mycotoxins are often highly toxic and cause disadvantageous biological effects, including severe diseases in both humans and animals. In common veterinary practice mycotoxins are "frequent players" as they can cause serious nutritional problems and animal diseases causing loss for farmers and animal keepers. The role of mycotoxins is especially important in food and feed hygiene. If the foodstuff contaminated with mycotoxins, or the animals suffering mycotoxicosis, are integrated into the food market, it will have serious consequences on human health as well. The present thesis summarizes basic information on mycotoxins with special regard to food and feed hygiene and with emphasis on common veterinary aspects.

Introduction

Mycotoxins are the toxic secondary metabolites of various fungal species. Although the problems caused by mycotoxins have been known for a very long time, the identification of mycotoxins as pathogenic agents has only a half-a-century history (FORGÁCS, 1962). In 1962 in the London area an unusually large-scale loss of turkey poults took place (over 100.000

turkeys died) and the cause of their death was mysterious and unexplained at first instance. Meticulous further investigations linked the mortality to peanut feed imported from South America contaminated with secondary metabolites, called aflatoxins, of the mold *Aspergillus flavus* (BLOUT, 1961). It was the first event when researchers could scientifically demonstrate that certain mold metabolites might be deadly.

By today research has identified numerous secondary mold metabolites, mycotoxins, which can contaminate food and feed and by this way causing diseases called mycotoxicosis (or mycotoxoses in plural). Research indicates that several food and feed products, produced in the world, are in large quantities contaminated by mycotoxins. Consequently, mycotoxin exposure to both humans and animals has become a serious problem worldwide, resulting in diseases both in humans and animals and causing significant financial damage to crop producers, animal keepers and the human health system alike.

Mycotoxins in general

Mycotoxins are toxic secondary metabolites produced by some fungal species that readily colonize crops and contaminate them with toxins in the field or after harvest. One mold species may produce many different mycotoxins, and the same mycotoxin may be produced by several species. Mycotoxins are of low molecular weight (~700 Dalton) and are as small as 0.1 microns (compared to mold spores which are between 1 and 20 microns).

The name comes from the Greek μύκης (mykes, mukos) "fungus" and τοξικόν (toxikon) "poison"). The term was coined in 1962 by British researchers who identified contaminated groundnut-based feed as cause of the unusually devastating poultry crisis in the London area, resulting in the mysterious death of over 100.000 turkeys (BLOUT, 1961). The peanut meal was contaminated with secondary metabolites of a mold, A*spergillus flavus*, and the scientists could later demonstrate that these secondary metabolites, called aflatoxins, might under certain circumstances be deadly.
Soon after this discovery researchers had realized that a large number of previously known fungal toxins (for instance the so called ergot alkaloids), and even certain compounds that had originally been isolated and identified as antibiotics (e.g., patulin) belong to mycotoxins. Up until 1975 approximately 400 compounds had been recognized as mycotoxins. Of this larger group a tenfold lead-groups receive regular attention due to their evident threats to human and

animal health (COLE and COX, 1981). However, recent estimates, based on the genetic variety of mycotoxin producing molds, indicate that the possible number of mycotoxins may be in the range of 300.000 (WHITLOW and HAGLER, 2006).

Whereas all mycotoxins are of fungal origin, not all toxic compounds produced by fungi are called mycotoxins:

(i) Fungal products which are primarily toxic to bacteria are usually called antibiotics. Penicillin is a prime example of this category and its history well demonstrates that the physiological effects of certain secondary metabolites of molds had already been known before the "mycotoxin era" (Alexander Fleming, 1928; Nobel Prize: 1945).

(ii) Fungal metabolites that are toxic to plants are called phytotoxins. They can be pathogenic or virulence factors, for instance they can cause a plant disease or they can play a role in exacerbating various plant diseases. In example, the phytotoxins made by fungal pathogens of Cochliobolus and Alternaria have a well-established role in disease development. Other mycotoxins made by *Fusarium* species contribute to plant pathogenesis (e.g. Desjardins et al., 1989).

(iii) Finally, fungal products that are toxic to vertebrates and other animal groups in low concentrations are called mycotoxins.

On the other hand, various other low molecular weight fungal metabolites, including ethanol, which are toxic only in high concentrations, are not considered mycotoxins. Also, though mushroom poisons are definitely fungal metabolites that can cause disease and death in humans and animals, are excluded from the category of mycotoxins despite the fact that based on formal definitions they should have been included in the group.

In spite of intensive research, the rationales and biological objectives of the production of mycotoxins by molds (i.e., microfungi) are not clearly understood as mycotoxins are not essential for the growth and development of the fungi. As long as fungi enjoy optimal living conditions, they proliferate into colonies and produce high levels of mycotoxins. One possible interpretation behind the reason for the production of mycotoxins is that because mycotoxins weaken the receiving host, the molds may use them as a strategy to "improve" the

environment for further fungal proliferation. From the point of view of the environment, if this is an animal or a human, this process can lead to health problems, weakened immune systems, diseases and even death.

According to traditional distinction, of the kingdom of fungi, molds make mycotoxins, whereas mushrooms and other macroscopic fungi make mushroom poisons. Whereas mushroom poisoning in humans is either intentional (intentional poisoning, murdering or seeking psychedelic-hallucinogenic effects), mycotoxin exposure is almost always accidental.

The study of mycotoxins is a sub-discipline called mycotoxicology, whereas the animal and human diseases caused by mycotoxins are called mycotoxicoses.

The practical importance of mycotoxins from a medical point of view

Due to the every-presence of fungi in nature, mycotoxins are also ubiquitously present and, consequently, affect their living environment everywhere on earth. They are contaminating food and feed crops as well as their products and, consequently, affect the health status of both humans and animals, consuming them. The ingestion of contaminated food or feed results in health problems, including severe disease conditions which can even result in cancer and death. Mycotoxin poisoning of feed products results in farm animal breeding losses, and that of human food products of plant or animal origin (cereals, vegetables, meat, milk, egg, etc.) may cause very significant financial damage to the livestock industry: only in North America this is in the range of 5 billion USD annually (www.fao.org).

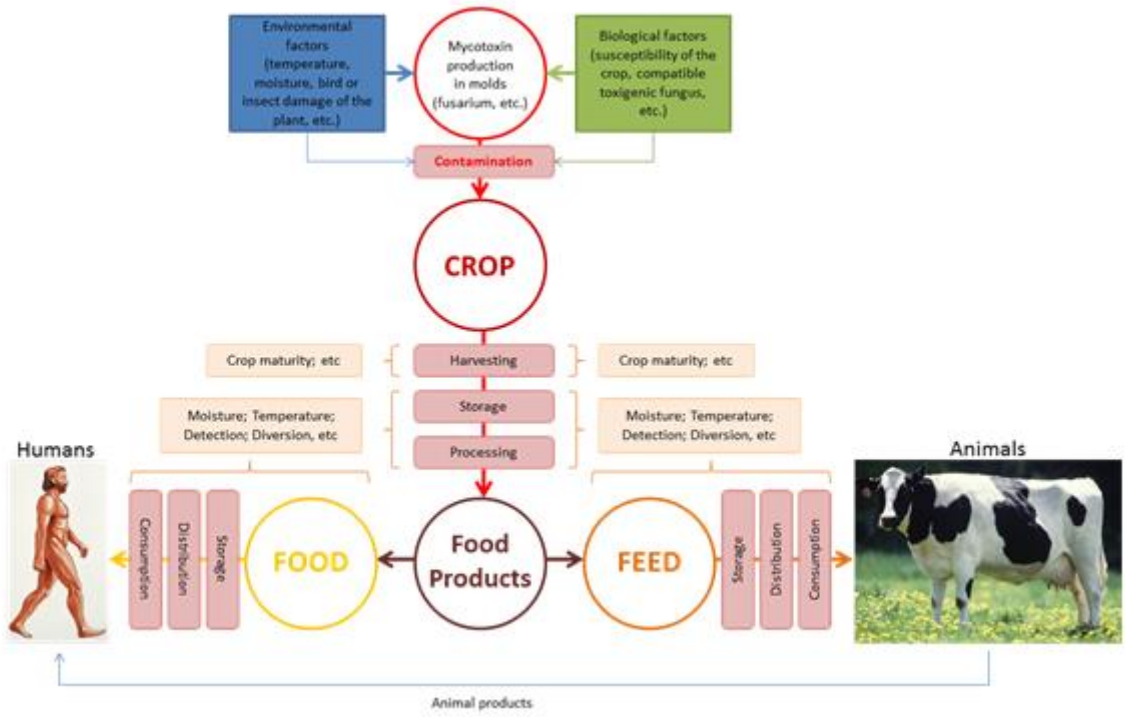
Mycotoxins can cause low growth, birth defects, liver and nervous tissue damage, as well as, among many other symptoms and disorders, cancer. To our recent knowledge, there is no treatment for mycotoxin poisoning. It is extremely difficult to destroy them in livestock and, consequently, consumption of contaminated food products almost always results in negative biological effects.

In human history several well documented large scale tragedies took place which, according to our recent knowledge, can be traced back to mycotoxins. For instance, in 944 AD over 40.000 people died in France due to ergot poisoning, caused by the *Claviceps purpurea* fungus. The resulting disease, called ergotism or Saint Anthony's Fire, was recurrently present in Europe and America and caused episodes of social bewilderment, as ergot poisoning causes convulsive symptoms as well as gangrene and the convulsive symptoms, often associated with mental symptoms such as mania, hysteria and psychosis, made many people believe that the patients were bewitched and, consequently, should be executed as witches. Such an episode was the fact behind, among others, the famous Salem Witchcraft Trials in 1692. But other mycotoxins can also cause large-scale poisoning, often affecting large territories and populations. These cases may even be interpreted as events of chemical warfare. For instance, in the early 1980s during the Cold War, small, powdery yellow deposits were said to rain down from the sky – the so called "yellow rain" – and was found on surfaces such as leaves in Southeast Asia and Afghanistan and, according to the first interpretations of the US military, they were associated with chemical weapon, containing T-2 mycotoxins, disseminated by air by the Soviet Army, in order to destroy enemy lines. Research has identified that the compound was containing the fungal toxin tricothecene, a product of *Fusarium tricinctum* and other *Fusarium* molds (TUCKER, 2001).

The production and circulation of mycotoxins

The production of mycotoxins in fungi, and their presence in food and feed, animals and humans, depend on several biological and environmental factors, which can significantly influence the resulting effects, including the severity of mycotoxicosis. The process is displayed in Figure 1.

Figure 1. The production and circulation of mycotoxins and the various factors influencing their amount in crop and food products.



The optimal conditions for mycotoxin production depend on various factors. For instance, during the food storage process temperatures between 4 and 32ºC, relative humidity values over 70 %, 22-23 % moisture content in the grain and 1-2 % oxygen levels appear to be the optimal conditions (Figure by GULYÁS, B., after PPT file by FORSYTH, D.M., without date).

Classification of mycotoxins

Due to their very diverse chemical structures, biosynthetic origins, biological effects, and production by different mold species, it is rather difficult and challenging to make a universally acceptable classification of mycotoxins, satisfying the various facets and expectations of mycotoxicology. Available classifications are based upon various criteria. A few approaches are shown in Table 1.

Approach	Main aspect of the classification	Divisions / examples
Pathology	Effected organ	hepatotoxins, nephrotoxins, neurotoxins, immunotoxins, hemotoxins, cardiotoxins, etc.
Cell biology	Generic group	teratogens, mutagens, carcinogens, allergens, etc.
Organic chemistry	Chemical structure	lactones, coumarins, etc
Biochemistry	Biosynthetic origin	polyketides, amino acid-derived, etc.
Clinical	Diseases they cause	St. Anthony's fire, stachybotryotoxicosis, etc.
Mycology	Fungi producing the toxins	*Aspergillus* toxins, *Penicillium* toxins, etc.

Table 1. A possible way of classifying mycotoxins.

Despite these efforts, no classification is fully satisfactory as the same toxin may be placed in different categories. For example, aflatoxin is a hepatotoxic, mutagenic, carcinogenic, difuran-containing, polyketide-derived *Aspergillus* toxin or zearalenone is a *Fusarium* metabolite with potent estrogenic activity, hence, it is also labeled a phytoestrogen, a mycoestrogen and a growth promotant.

Others simply enlist the major groups, using their established names in alphabetic order, as shown in Table 2.

Mycotoxin	Acronym	Species producing
Aflatoxins B1, B2, G1, G2	AFB1	*Aspergillus flavus*
	AFB2	
	AFG1	
	AFG2	
Alternariol	AOH	*Alternaria alternate*
Alternariol monomethyl	AME	*Alternaria alternata*

ether		*Alternaria solani*
Tenuazonic acid	TeA	*Alternaria alternata*
Altertoxins	ALTs	*Alternaria tenuissima*
Altenuene	ALT	*Alternaria alternata*
		Alternaria alternata
Beauvericin	BEA	*Fusarium sporotrichioides*
		Fusarium poae
		Fusarium langsethiae
		Fusarium section Liseola
		Fusarium avenaceum
Enniatins	ENNs	*Fusarium avenaceum*
		Fusarium tricinctum
Fusaproliferin	FUS	*Fusarium poae*
		Fusarium langsethiae
		Fusarium sporotrichioides
		Fusarium proliferatum,
		Fusarium subglutinans
Moniliformin	MON	*Fusarium avenaceum*
		Fusarium tricinctum
		Fusarium section *Liseola*
Ergot alkaloids	EAs	*Claviceps purpurea*
		Claviceps fusiformis
		Claviceps africana
		Neotyphodium spp
Fumonisins B1, B2	FB1,	*Fusarium* section *Liseola*
	FB2	
Ochratoxin A	OTA	*Aspergillus* section *Circumdati*
		Aspergillus section *Nigri*
		Penicillium verrucosum
		Penicillim nordicum
Patulin	PAT	*Penicillim expansum*
		Bysochlamis nívea
		Aspergillus clavatus
HT-2 and T-2 toxin (type A trichothecenes)	HT-2	*Fusarium acuminatum*
		Fusarium poae
	T-2	*Fusarium sporotrichioides,*

	DON	*Fusarium langsethiae*
Deoxynivalenol (type B trichothecenes)		*Fusarium graminearum*
		Fusarium culmorum
		Fusarium cerealis
Zearalenone	ZEN	*Fusarium graminearum*
		Fusarium roseum
		Fusarium culmorum
		Fusarium equiseti
		Fusarium cerealis
		Fusarium verticillioides
		Fusarium incarnatum

Table 2. An incomplete list of mycotoxins and the fungi species which produce them (based on: MARIN et al., 2013).

A further possible way of classifying mycotoxins can be based on the number of notifications by national or international authorities. For instance mycotoxin notifications in the EU during 2008-2012 indicates that aflatoxins are the most important category of mycotoxins, followed by ochratoxin A, deoxynivalenol and fumosinins (Table 3).

Nr	Mycotoxin	2008	2009	2010	2011	2012	Total
1	Aflatoxins	902	638	649	585	484	3258
2	Ochratoxin A	20	27	34	35	32	148
3	Deoxynivalenol	4	3	2	11	4	24
4	Fumonisins	2	1	3	4	4	14
5	Zearalenone	2	-	-	-	4	6
6	Patulin	3	-	-	-	-	3
Total		**933**	**669**	**688**	**635**	**525**	**3450**

Table 3. Mycotoxin notifications in the EU during 2008-2012 (after MARIN et al., 2013).

Based on Table 3, the most common notifications in the EU are related to the following mycotoxin groups:

Aflatoxins are produced by *Aspergillus* species of fungi, such as *Aspergillus flavus* and *Aspergillus parasiticus*. There are four major categories of aflatoxins, labeled as B_1, B_2, G_1, and G_2. Aflatoxin B_1 is the most toxic one and is a carcinogen. Exposure to Aflatoxin B_1 has been directly correlated to adverse health effects, including liver cancer, in various animal species. Aflatoxins are mainly associated with commodities produced in the tropics and subtropics, such as cotton, peanuts, spices, pistachios and maize.

Ochratoxin is produced by *Penicillium* and *Aspergillus* species and appears in three secondary metabolite forms, A, B, and C. *Aspergillus ochraceus* is often found as a contaminant in commodities such as beer and wine. *Aspergillus carbonarius* is found on vine fruit, which releases its toxin during the juice making process. Ochratoxin A has been labeled as a carcinogen and a nephrotoxin, and has been linked to, among others, tumors in the human urinary tract.

Deoxynivalenol is a mycotoxin produced by various species of fungi belonging to the Tricothecene family. It has many toxic effects in animals, including diarrhea and weight loss as well as other alimentary and hematological toxicities.

Fusarium toxins are produced by more than 50 species of *Fusarium*. They are infecting the grain of developing cereals such as wheat and maize. They include various mycotoxins, including fumonisins, trichothecenes, zearalenones, beauvercins, enniatins, butenolides, equisetins and fusarins.

Zearalenone is a potent estrogenic metabolite produced by some *Fusarium* and *Gibberella* species causing infertility, abortion or other breeding problems, especially in swine. Zearalenone is heat-stable and is found worldwide in a number of cereal crops, such as maize, barley, oats, wheat, rice as well as in bread.

Patulin is a mycotoxin produced by the *Penicillium expansum*, as well as other *Penicillium*, *Aspergillus* and *Paecilomyces* fungal species and is especially associated with a range of moldy fruits and vegetables, for instance rotting apples and figs as well as in juices. It has been reported to damage the immune system in animals.

Further possible classifications can be based upon on their chemical structures. Such a classification was originally made by Bérdy and modified by Betina (BETINA, 1989) (Table 4).

Code number	Compounds	Representative
2	macrocyclic lactones	
2.3.53	Brefeldin type	Zearalenone
3	quinone and similar compounds	
3.1.3.2	dianthraquinone derivatives	Rugolosin
3	amino acid, peptide compounds	
4.1.3.2	diketopiperazine derivatives	
4.1.3.2.1	Gliotoxin type	Gliotoxin
6	oxygen-containing heterocycles	
6.1	furan derivatives	
6.1.2.1	Aflatoxin type	Aflatoxin B_1
6.2	pyran derivatives	
6.2.3.2	Citreoviridin type	Citreoviridin
6.3	benzo[g]pyran derivatives	
6.3.4.1	dibenzo[g]pyrone derivatives	Secalonic acid D
6.4.	small lactones	
6.4.2.1	small lactones condensed with hetero- or alicycles	Patulin
6.4.2.5	isocoumarin derivatives	Ochratoxin A
7	alicyclic compounds	
7.3	oligoterpenes	
7.3.3.1	Trichodermin type	T-2 toxin
8	aromatic compounds	
8.2.1.1	Griseofulvin type	Griseofulvin

Table 4. Chemical classification of mycotoxins according to Bérdy, modified by BETINA (1989).

Finally, mycotoxins can also be classified according to the basis of which mold species produce them. For instance, various *Aspergillus* species can produce different mycotoxins as shown in Table 5.

Fungus	Mycotoxin produced
Aspergillus aculeatus	Secalonic acid D
Aspergillus albertensis	Ochratoxin A, Ochratoxin B
Aspergillus alliaceus	Ochratoxin A, Ochratoxin B
Aspergillus auricomus	Ochratoxin A, Ochratoxin B
Aspergillus bombycis	Aflatoxin B_1, Aflatoxin G
Aspergillus brevipes	Viriditoxin
Aspergillus caespitosus	Fumitremorgin A
Aspergillus candidus	Citrinin, Acetylisoneosolaniol
Aspergillus carneus	Citrinin
Aspergillus clavatus	Patulin, Tryptoquivaline A (C), Cytochalasin E
Aspergillus flavipes	Citrinin
Aspergillus flavus	Aflatoxin B_1, Aflatoxin B_2, Aflatoxin M_1, Cyclopiazonic acid, Aflatrem (indole alkaloid), 3-Nitropropionic acid, Sterigmatocystin, Versicolorin A, Aspertoxin
Aspergillus fresenii	Xanthomegnin
Aspergillus fumigatus	Fumitremorgin A, Verruculogen, Gliotoxin, Fumagillin, Helvolic acid, Sphingofungins, Brevianamide A, Phthioic acid, Fumigaclavin C, Aurasperone C
Aspergillus giganteus	Patulin
Aspergillus melleus	Ochratoxin A, Viomellein, Xanthomegnin
Aspergillus microcysticus	Aspochalasin
Aspergillus nidulans (Emericella nidulans)	Sterigmatocystin, Dechloronidulin, Emestrin
Aspergillus niger	Malformin, Ochratoxin A, Fumonisin B_2
Aspergillus nomius	Aflatoxin B_1, Aflatoxin B_2, Aflatoxin G_1, Aflatoxin G_2

Aspergillus ochraceoroseus	Aflatoxin B_1, Sterigmatocystin
Aspergillus ochraceus	Ochratoxin A, Ochratoxin B, Ochratoxin C, Viomellein, Penicillic acid
Aspergillus oryzae	Cyclopiazonic acid, Maltoryzine, 3-Nitropropionic acid
Aspergillus ostianus	Ochratoxin A
Aspergillus parasiticus	Aflatoxin B_1, Aflatoxin B_2, Aflatoxin G_1, Aflatoxin G_2, Aflatoxin M_1, Versicolorin A
Aspergillus petrakii	Ochratoxin A
Aspergillus pseudotamarii	Cyclopiazonic acid, Aflatoxin B_1
Aspergillus restrictus	Restrictocin
Aspergillus sclerotiorum	Ochratoxin B
Aspergillus sulfureus	Ochratoxin A, Ochratoxin B
Aspergillus terreus	Territrem A, Citreoviridin, Citrinin, Gliotoxin, Patulin, Terrein, Terreic acid, Terretonin, Itaconic acid, Aspulvinone, Asterric acid, Asterriquinone, butyrolactone I, Emodin, Geodin, Itaconate, Lovastatin, Questin, Sulochrin, Terrecyclic acid.
Aspergillus ustus	Austdiol, Austin, Austocystin A, Sterigmatocystin
Aspergillus variecolor	Sterigmatocystin
Aspergillus versicolor	Sterigmatocystin, Cyclopiazonic acid, Versicolorin A
Aspergillus viridinutans	Viriditoxin

Table 5. A variety of mycotoxins produced by *Aspergillus* molds (source: BRÄSE et al., 2009).

But other molds than *Aspergillus* also produce a wide variety of mycotoxins. A few examples are shown in Table 6.

Mold species	Mycotoxin produced
Alternaria alternata	tenuazonic acid, alternatiol, alternatiol monomethyl ether, alterotoxins
Chaetomium globosum	chaetoglobosins, chaetomin
Memnoniella echinata	trichodermol, trichodermin, dechlorogriseofulvins, memnobotrins A and B, memnoconol, memnoconone
Penicillium aurantiogriseum	auranthine, penicillic acid, verrucosidin, nephrotoxic glycopeptides
Penicillium brevicompactum	mycophenolic acid
Penicillium chrysogenum	roquefortine C, meleagrin, chrysogin
Stachybotrys chartarum	satratoxins, verrucarins, roridins, atranones, dolabellanes, stachybotrylactones and lactams, stachybotrydialis

Trichoderma harzianum	alamethicins, emodin, suzukacillin, trichodermin
Wallemia sebi	walleminols A and B

Table 6. Some examples of mycotoxins produced by other than *Aspergillus* molds (source: DOBRANIC et al., 2006).

The most common mycotoxins of high pathogenic value: aflatoxins

Aflatoxins are a group of mycotoxins. Regarding the number of notifications by national agencies (cfr. Table 3), aflatoxins are most frequently responsible for mycotoxicoses and for this reason a few words have to be dedicated here to aflatoxins. Aflatoxins stood behind the famous Turkey X disease in London in 1962, but also behind a huge outbreak of a mysterious disease affecting first dogs, followed by humans, in India in 1974 (in which 397 disease cases were recorded, whereof 108 deaths) and an outbreak in Kenya in 2004 in which 125 people died. It is also noted by several researchers that where aflatoxin contamination levels are high, the occurrence of hepatitis B is also very high.

Aflatoxins are produced by *Aspergillus* species molds, mainly *Aspergillus flavus* and *Aspergillus parasiticus* (GROOPMAN et al. 1988). As the first aflatoxins have been linked to *Aspergillus flavus*, their name comes from this mold (**A**spergillus **fla**vus **toxin**).

The production of aflatoxins, just as that of any other mycotoxins, is dependent upon the temperature, humidity, host plant type, and the strain of fungus; high humidity usually required for growth. In the US it is most common in the South and South East, as it prefers high temperatures and humidity values (optimum: 30ºC and 83 % humidity). For this reason, aflatoxins most frequently and most commonly occur in tropical circumstances, as warm and humid climate promotes the proliferation of the *Aspergillus fungi*, producing aflatoxins. *Aspergillus* molds grow ubiquitously on plants and crops from tropical and subtropical areas: peanuts, figs, spices, corn, maize, Brazil nuts, pecans, walnuts, soybeans, pistachios, wheat and grains may contain them in large quantities.

Until today more than a dozen different types of aflatoxins have been identified, of which the most important ones are termed as B_1, B_2, G_1 and G_2. Chemically, they are difuranocoumarin derivatives, produced by a polyketide pathway. In the milk producing animals , e.g. dairy

cattle, that are fed with grains contaminated with aflatoxins, the aflatoxin M_1 and M_2 can be formed, which both are toxic hydroxylated metabolites and might be present in the milk of the animals. Therefore dairy products in human nutrition can also cause a threat to our health (BENNETT and KLICH, 2003).

Regarding its potency as a carcinogen, Aflatoxin B_1 is the major compound in this group (Figure 2).

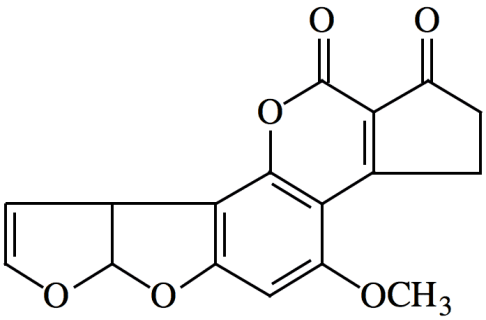


Figure 2. The chemical structure of Aflatoxin B_1.

The other members of the aflatoxin group are chemically similar to Aflatoxin B_1 (Figure 3), but regarding their cancerogenic and other effects, they are less potent than Aflatoxin B_1.

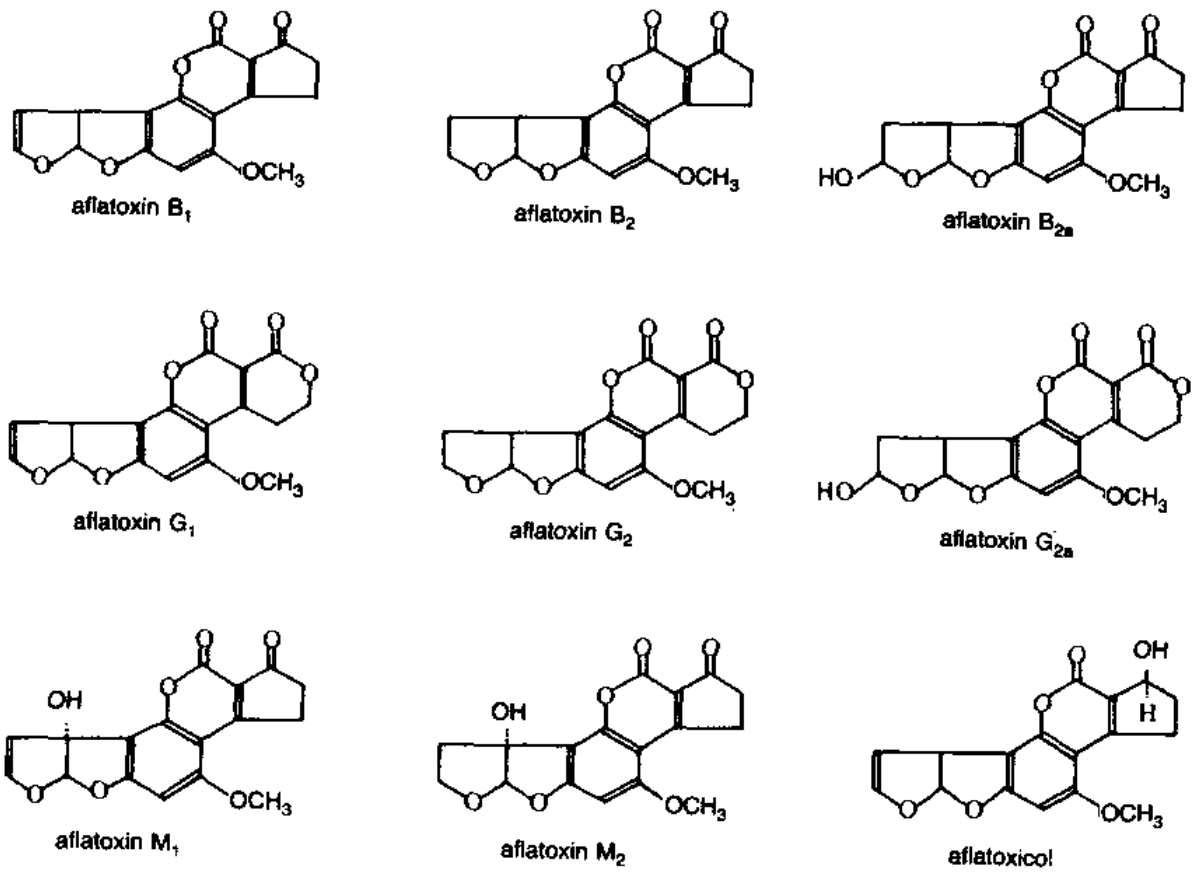


Figure 3. Chemical structures of some members of the aflatoxin group.

Aflatoxins, including aflatoxin B_1, are metabolized in the liver and various metabolites appear in the blood as well as are excreted by the kidneys (Figure 4). Due to their metabolism in the liver, they often have fatal hepatotoxic effects. Furthermore, due to their interactions with the most important oxidative biochemical pathways in the cell they, as well as their metabolites, have a strong carcinogen effect.

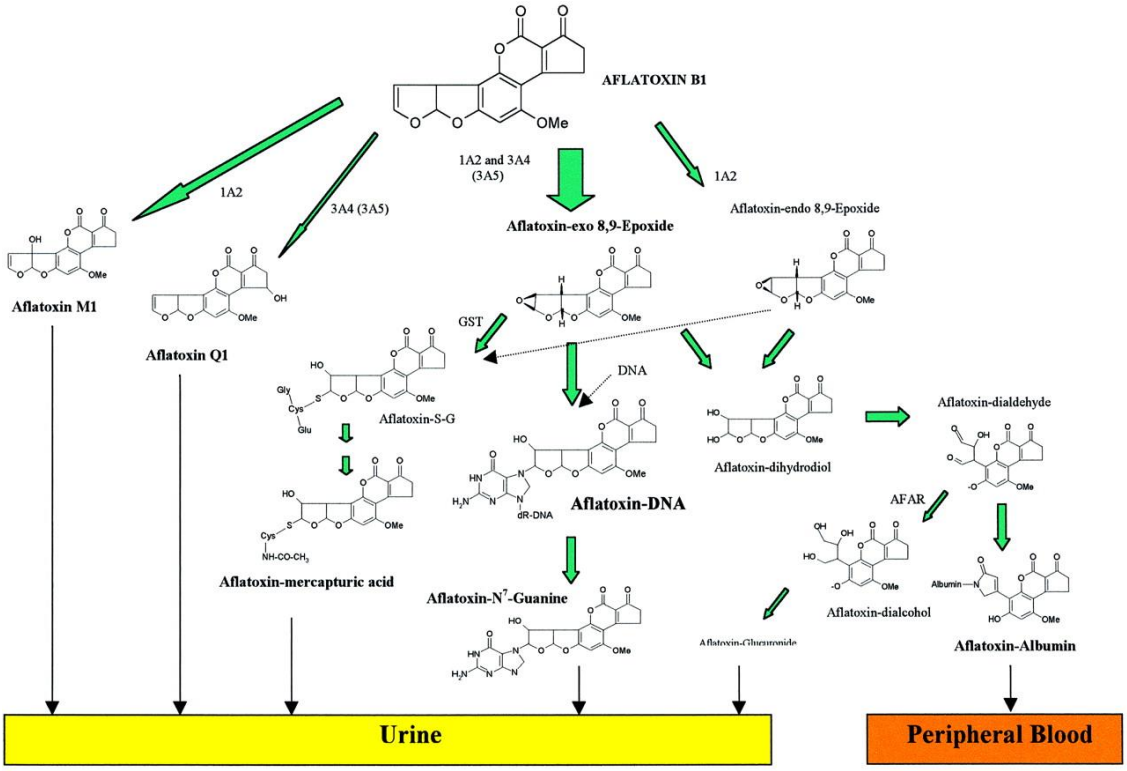


Figure 4. Principle metabolism of aflatoxin B1 leading to reactive metabolites and biomarkers. 1A2, CYP1A2; 3A4, CYP3A4; 3A5, CYP3A5; GST, glutathione S-transferase; AFAR, aflatoxin aldehyde reductase; Aflatoxin-S-G, aflatoxin–glutathione conjugate. (From WILD and TURNER, 2002).

Both metabolized and unmetabolized aflatoxin is excreted mostly in urine. It is also excreted in milk, stool, faeces, and saliva, which may be swallowed and re-enter the gastrointestinal tract.

The pathogenic effects of aflatoxins are multifold and they are behind the highest number of poisonings and diseases caused by mycotoxins. The mechanisms of the pathogenesis of aflatoxins include various biochemical processes at various levels of the cell's reproductive and metabolic mechanisms. A most common metabolite of aflatoxin B_1, the Aflatoxin B_1 8,9-epoxide (see Figure 3), has highly toxic effects in the mitochondrion: it binds to amino acids of the mitochondrial DNA (for instance, to guanine), thereby hindering both ATP production and various enzymatic functions important for the oxidative mechanisms of the cell. The consequence is mitochondrial directed apoptosis. Aflatoxins and their metabolites can also result in uncoupling of metabolic processes, leading to lipid peroxidation and cell membrane defects. The aflatoxin B_1 8,9-epoxide metabolite can also react with the amino acids in the

DNA and thereby it can prevent DNA repair, which is an important mechanism to repair gene mutations and the consequent development of cancer. Furthermore, aflatoxin can also inactivate the p53 tumor suppressor gene, resulting in uncontrolled cell proliferations and, consequently, tumor genesis. Other mechanisms may include a reduced lipid transport in the liver and reduced levels of oxidative mechanisms, leading to lipid accumulation and, later on, liver function failure, with the resulting symptoms of jaundice, ascites, portal hypertension and liver necrosis. Last but not least, aflatoxins can affect negatively several enzymatic functions as well as impair normal immune functions and affect normal growth rates. In the case of other mycotoxins than aflatoxin, similar cytotoxic effects are present and underlie the pathology.

Acute toxicity of aflatoxin B_1 has been widely studied. The LD_{50} (median lethal dose) values for aflatoxin B_1 after a single oral administration are shown in Table 7.

Species	**LD_{50} (mg/kg bodyweight)**
Rabbit	0.30
Duckling (11 day old)	0.43
Cat	0.55
Pig	0.60
Rainbow trout	0.80
Dog	0.50 - 1.00
Sheep	1.00 - 2.00
Guinea pig	1.40 - 2.00
Baboon	2.00
Chicken	6.30
Rat (male)	5.50 - 7.20
Rat (female)	17.90
Macaque (female)	7.80
Mouse	9.00
Hamster	10.20

Table 7. Acute toxicity of aflatoxin B1 expressed as a single oral dose LD50 (http://www.icrisat.org/aflatoxin/health.asp)

The contamination of animals by mycotoxins

Livestock animals, such as cattle, are most often contaminated by mycotoxins by feed, however, they can also be exposed to, and contaminated by, mycotoxins through inhalation (e.g. during grazing) or by skin contact (e.g. via contaminated bedding). The biological effects of contamination with mycotoxins may vary from mild symptoms, such as irregular body temperature, through impaired gastrointestinal functions to fatal outcomes (Figure 5).

Furthermore, what is highly important in the case of livestock animals is that their products (meat, milk, etc.) may also contain mycotoxins and by eating these food products humans can also be contaminated with mycotoxins.

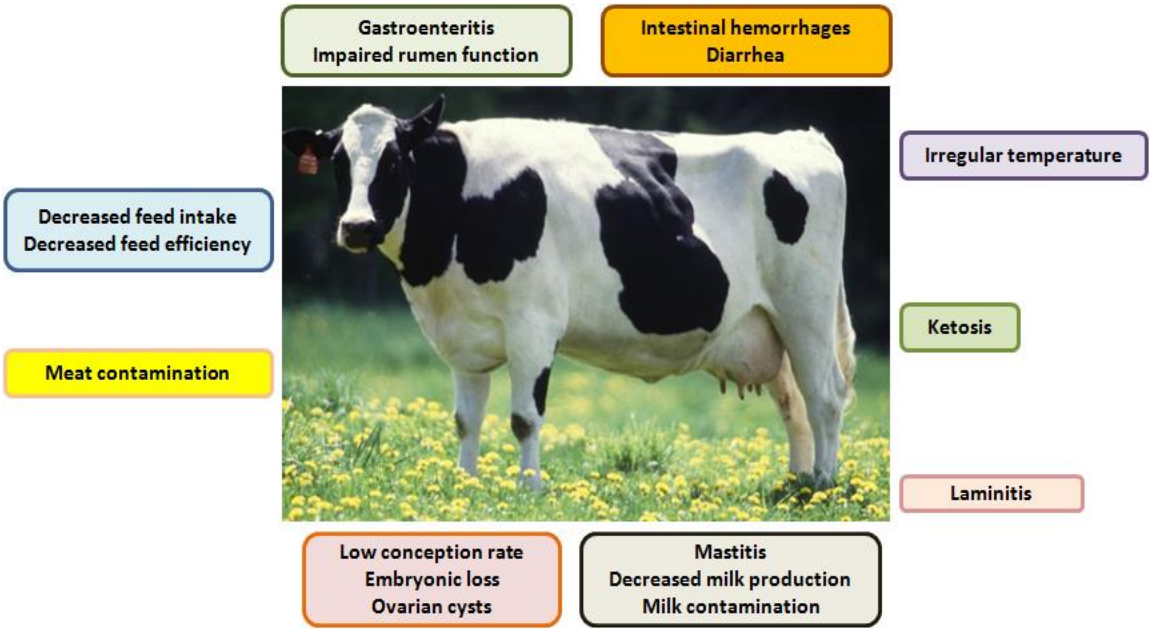


Figure 5. Consequences of livestock animals with mycotoxins.

Mycotoxicoses

The diseases called mycotoxicoses are basically poisoning by natural means and, consequently, their pathologies are in many respects similar to those caused by exposure to

pesticides or heavy metal residues. As mycotoxicoses do not need to involve the toxin-producing fungus, they are abiotic hazards with biotic origin.

Mycotoxins can appear in the food chain because of fungal infection of crops. They are either consumed directly by humans or used as livestock feed for animals. The metabolism of ingested mycotoxins could result in mycotoxin accumulation in different organs or tissues, entering into the food chain through meat, milk, or eggs. The consumption of animal products with mycotoxin infection can, consequently, poison humans indirectly.

Mycotoxins can injure humans or animals upon ingestion, inhalation, or skin contact. The symptoms of a mycotoxicosis depend on various factors: (i) the type of the mycotoxin responsible for the poisoning, (ii) the amount of the exposure, (iii) the duration of the exposure, (iv) the age of the exposed animal or individual, (v) its health status, (vi) its dietary status, and (vii) several other confounding factors, including less well understood or unknown factors including vitamin deficiency, caloric deprivation, alcohol abuse, concurrent infectious diseases, gender and genetic predisposition.

Mycotoxicoses can increase vulnerability to microbial diseases, they can worsen the effects of malnutrition and synergistically enhance the efficacy of other toxins.

The number of animals or people affected by mycotoxicoses is unknown. Although researchers estimate that the total number is smaller than the number afflicted with bacterial, protozoan, and viral infections, mycotoxicoses are a major source of serious international health problems, especially in underdeveloped countries.

An important feature of mycotoxicoses is that it is not a communicable form of disease, i.e. it is not transmissible from animal to animal or person to person. Other important features are that drug and antibiotic treatments have little or no effect, outbreaks are often seasonal, the outbreaks are usually associated with a specific foodstuff and examination of the suspected food or feed often reveals signs of fungal activity.

The pathological effects of mycotoxins on animals

The most common mycotoxins, aflatoxins, can cause acute or chronic toxicity, nowadays more commonly known as acute or chronic aflatoxicosis. In some cases it might even lead to

death in mammals, as well as in fish and birds. The lethal dose – LD_{50} – differs between species but has been estimated to be somewhere between 0.5 – 10.0 mg/kg body weight in animals (see Table 7, page 18).

The aflatoxins are carcinogenic, mutagenic and teratogenic in several species. The target organ in aflatoxicosis is the liver with typical signs of cirrhosis and acute necrosis, but in some studies done post mortem in individuals that died of aflatoxicosis, a high level of the toxin has also been proven to accumulate in other organs, such as the brain, kidney, myocardium and the lung (http://www.mycotoxins.org/).

Poisoning with aflatoxins can occur in many different animal species and also in humans. Clinical signs of the toxicosis in animals can be one or several of the following: decreased production, diarrhea, incoordination, haemorrhages due to the liver damage causing decreased synthesis of clotting factors, anorexia, edema, jaundice and sudden death. The aflatoxins leads to immunosuppression, therefore they enhance the risk of infections which can usually be seen as mastitis among the farm animals.

In acute cases of the toxicosis, which is less frequent than chronic cases, there is sudden death with usually no signs of illness pre-death except, in some cases, were reluctance to eat can be a symptom. Postmortem investigations can reveal haemorrhages, icterus and other pathophysiological landmarks. For instance, histopathologically; enlarged, necrotized liver with fatty infiltration (http://www.merckmanuals.com/vet/toxicology/mycotoxicoses/aflatoxicosis.html).

Acute toxicity with aflatoxins has been demonstrated in a wide range of animals, including mammals, fish, birds, rabbits, dogs and primates. In animals, ducks, turkeys and trout are especially highly susceptible (http://www.mycotoxins.org/). Young animals are also more susceptible to aflatoxicosis than adults. However, in the well-developed countries, acute poisoning by aflatoxins is not known to have occurred in man and is very rare in animals.

The subacute cases are characterised by less prominent but still evident hepatic changes, anorexia, diarrhea, decreased growth, immunosuppression and premature death. The liver is somewhat enlarged and firmer than usual (CARDWELL, 2001).

The most common of toxicosis with aflatoxins is chronic forms. Regarding food safety issues, the chronic toxicosis by aflatoxins is the most important one of all mycotoxicoses. Chronic aflatoxicosis can cause liver and bile duct carcinoma, immunosuppression and metabolic disorders. As Aflatoxin B_1 belongs to one of the most potent carcinogens affecting the liver,

and, as shown by research, it is also mutagenic in a wide variety of animals, it has to be considered as potentially very harmful to humans, as well. Consequently, aflatoxin B_1 should not be ingested even in low levels over a longer time period, because it can have serious negative effects on human health. Research has shown that ingesting smaller amounts of aflatoxin B_1 over a longer time might cause primary liver cancer, preceeded by jaundice, chronic hepatitis and/or liver cirrhosis, as well as decreased metabolism and impaired uptake of nutrients by the gastrointestinal tract(http://www.mycotoxins.org/). According to the FDA (Food and Drug Administration) (1992), a daily consumption of 55 µg aflatoxin in humans for a longer period of time can be fatal. In ruminants fed with fodder containing high amounts of aflatoxins, their ruminal contractions can decrease.

There is not much information yet about the effects of chronic toxicity when it comes to aflatoxin G_1 and M_1, but they are considered to be carcinogenic, similar to the aflatoxin B_1, and even more potent as kidney carcinogens, although, slightly less potent as liver carcinogens (National Toxicology Program, Department of Health and Human Services, 2011).

The various effects of the mycotoxin groups in different livestock and other animals are surveyed in the next tables (Table 8 - 12).

The effects of mycotoxins in horses

Mycotoxin	*Effects*	*Clinical Signs & Symptoms*
Aflatoxins	Hepatotoxic effects	Liver damage
	Hematopoietic effects	Haemorrhages

		Anaemia
Ergot alkaloids	Reproductive effects	Reproductive abnormalities
Fumonisins	Neurotoxic effects	Equine leukoencephalomalacia (ELEM) Decreased feed consumption, lameness, ataxia Oral and facial paralysis, head pressing, recumbency
Ochratoxin A	Hepatotoxic effects	Liver failure
	Nephrotoxic effects	Kidney failure
Trichothecenes	Immunosuppression	Decreased resistance to environmental and microbial stressors Increased susceptibility to diseases
	Decreased performance	Decreased feed intake Feed refusal Reduced weight
Zearalenone	Reproductive effects	Infertility Enlargement of the uterus Abortions Vaginal prolapse

Table 8. The effects of mycotoxins in horses

(Table after data from http://www.mycotoxins.info, 2013.08.08)

The effects of mycotoxins in ruminants

Mycotoxin	*Effects*	*Clinical Signs & Symptoms*
Aflatoxins	Carcinogenic effects	Higher incidence of cancer in exposed animals
	Immunosuppression	Decreased resistance to environmental and microbial stressors Increased susceptibility to diseases
	Decreased performance	Decreased feed intake and milk production (dairy) Weight loss and reduced weight gain (beef)
	Pathological changes	Increased liver and kidney weight
	Hepatotoxic effects	Liver damage
	Gastro-intestinal effects	Impaired rumen function: - Decreased cellulose digestion - Volatile fatty acid formation - Proteolysis and rumen motility Diarrhea
	Residues	Residues (aflatoxin M_1) present in milk
	Reproductive effects	Decreased breeding efficiency Birth of smaller and unhealthy calves Acute mastitis
Ergot alkaloids	Neurotoxic effects	Anorexia Occasional convulsions Reduced feed intake
	Decreased performance	Low milk production Reduced growth
	Reproductive effects	Abortions Decreased pregnancy rates Decreased calving rates Weak testicular development Low sperm production
	Pathological changes	Lameness Necrosis of abdominal fat Diarrhea
Trichothecenes	Immunosuppression	Decreased resistance to environmental and microbial stressors Increased susceptibility to diseases
	Decreased performance	Reduced milk production Reduced feed intake
	Gastro-intestinal effects	Gastroenteritis Inflammation of the rumen
	Hematopoietic effects	Haemorrhages
	Dermal effects	Inflammation of mouth, lesions
	Neurotoxic effects	Restlessness
Zearalenone	Reproductive effects	Infertility Decreased conception rates Abortions Teat enlargement Udder secretion
	Decreased performance	Decreased milk production

Table 9. The effects of mycotoxins in ruminants

(Table after data from http://www.mycotoxins.info, 2013.08.08)

The effects of mycotoxins in poultry

Mycotoxin	*Effects*	*Clinical Signs & Symptoms*
Aflatoxins	Hepatotoxic effects	Jaundice
	Teratogenic effects	Birth defects of the offspring
	Carcinogenic effects	Higher incidence of cancer in exposed animals
	Pathological changes	Weight variation of the internal organs: - Enlargement of the liver, spleen and kidneys (fatty liver syndrome) - Bursa of Fabricius and thymus reduction Change in the texture and coloration of the organs (liver, gizzard)
	Decreased performance	Decreased feed intake (anorexia) Decreased daily weight gain Decreased slaughtering weight Decreased egg production Inhomogeneous flocks
	Hematopoietic effects	Haemorrhages Anaemia
	Immunosuppression	Decreased resistance to environmental and microbial stressors Increased susceptibility to diseases
	Neurotoxic effects	Nervous syndrome (abnormal behavior)
	Dermal effects	Impaired feathering
		Paleness of the mucous membranes and legs (Pale Bird Syndrome)
	Residues	Residues present in liver, meat and eggs
	Decreased performance (parental stock)	Decreased hatchability of eggs
Ergot alkaloids	Neurotoxic effects	Reduced feed intake Respiratory difficulties Reluctance to move
	Decreased performance	Poor feathering Poor growth Decreased egg production
	Pathological changes	Gangrenous lesions on toes, beaks and claws
	Gastro-intestinal effects	Diarrhea Death
Fumonisins	Decreased performance	Reduced weight gain Impaired FCR
	Pathological changes	Increased liver and kidney weight Liver necrosis
	Gastro-intestinal effects	Diarrhea
	Residues	Residues in liver and kidneys
Ochratoxin A	Poultry	Immunosuppression: Decreased resistance to environmental and microbial stressors Increased susceptibility to diseases
		Decreased performance: Reduced egg production Reduced egg weight Reduced weight gain
		Residues: Residues present in liver, meat and eggs
		Decreased: Retarded growth Decreased feed conversion

	Turkeys, chickens	performance	Higher mortality rates
		Nephrotoxic effects	Increased water consumption Renal dysfunction
	Turkeys	Decreased performance	Feed refusal
	Layers	Decreased performance	Decreased egg production Decreased egg shell quality
	Broilers	Hepatotoxic effects	Liver damage
Trichotecenes	Immunosuppression		Decreased resistance to environmental and microbial stressors Increased susceptibility to diseases
	Decreased performance		Reduced feed intake Reduced weight gain Decreased egg shell quality Impaired FCR Feed refusal Inhomogeneous flocks
	Dermal toxicity		Oral and dermal lesions
	Pathological changes		Necrosis of the lymphoid and hematopoietic tissues
	Neurotoxic effects		Lack of reflexes Abnormal wing positioning Impaired feathering
	Hematopoietic effects		Haemorrhages Blood pattern disorders
	Gastro-intestinal effects		Diarrhea
Zearalenone	Reproductive effects		Enhanced secondary sex characteristics Vent enlargement

Table 19. The effects of mycotoxins in poultry

(ducklings, broilers, breeders, layers, parental stock, turkeys, quails)

(Table after data from http://www.mycotoxins.info, 2013.08.08)

The effects of mycotoxins in pigs

Mycotoxin	*Effects*	*Clinical Signs & Symptoms*
Aflatoxins	Carcinogenic effects	Higher incidence of cancer in exposed animals
	Immunosuppression	Decreased resistance to environmental and microbial stressors Increased susceptibility to diseases
	Decreased performance	Reduced feed intake Feed refusal Impaired FCR
	Hepatotoxic effects	Toxic hepatitis
	Nephrotoxic effects	Kidney inflammation
	Hematopoietic effects	Systemic haemorrhages
	Residues	Residues and metabolites in liver and milk
Ergot alkaloids	Neurotoxin effects	Low prolactin production Low colostrum production Agalactia
	Decreased performance	Reduced weight gain
	Reproductive effects	Shrunken udders Signs of estrus Stillbirths Reduced pregnancy rate Abortions
	Pathological changes	Vasoconstriction Necrosis of the extremities
Fumonisins	Immunosuppression	Decreased resistance to environmental and microbial stressors Increased susceptibility to diseases
	Pulmonary & cardiovascular effects	Porcine pulmonary edema (PPE)
	Pathological changes	Pancreatic necrosis
	Hematopoietic effects	Hematological disorders
	Hepatotoxic effects	Liver damage
	Residues	Residues in kidneys and liver
Ochratoxin A	Decreased performance	Reduced weight gain Impaired FCR Increased mortality
	Nephrotoxic effects	Kidney damage (porcine nephropathy) Increased water consumption Kidney and bladder dysfunction Altered urine excretion (wet beds)
	Hepatotoxic effects	Liver damage
	Gastro-intestinal effects	Diarrhea
	Immunosuppression	Decreased resistance to environmental and microbial stressor Increased susceptibility to diseases
	Residues	Residues present in liver, kidneys and meat
	Gastro-intestinal effects (**DON**)	Vomiting Diarrhea
	Immunosuppression	Decreased resistance to environmental and microbial stressors

Trichotecenes		(**T-2 toxin**)	Increased susceptibility to diseases Affects immune cells and modifies immune response
		Decreased performance	Feed refusal Decreased weight gain Impaired FCR
		Hematopoietic effects	Haemorrhages Hematological disorders
		Teratogenic effects	Splaylegs
		Dermal effects	Oral and dermal lesions Necrosis
Zearalenone	Female swine	Reproductive effects	Affected reproduction cycle, conception, implantation and ovulation. Pseudopregnancy, abortion, anoestrus. Embryonic death, inhibition of fetal development, reduced litter size. Enlargement of mammary glands. Swelling and reddening of vulva. Rectal and vaginal prolapse.
		Pathological changes	Atrophy of ovaries, uterus hypertrophy
	Male swine	Reproductive effects	Feminization Enlargement of mammary glands Impaired semen quality Testicular atrophy Swollen prepuce
	Piglets	Reproductive effects	Reddened teats (females). Swelling and reddening of vulva (females).
		Teratogenic effects	Splaylegs

Table 11. The effects of mycotoxins in pigs

(Table after data from http://www.mycotoxins.info, 2013.08.08)

The effects of mycotoxins in pet animals

Mycotoxin	*Species*	*Effects*	*Clinical Signs & Symptoms*
Aflatoxins	Dog, Cat & Pet birds	Gastro-intestinal effects	Vomiting
		Hepatotoxic effects	Hepatitis Jaundice
		Neurotoxic effects	Anorexia Lethargy Depression
		Nephrotoxic effects	Polydipsia Polyuria
		Hematopoietic effects	Disseminated intravascular coagulation Death
Fusaric acid	Dog	Hematopoietic effects	Gastro-intestinal, hepatic and pneumonic bleeding
		Gastro-intestinal effects	Reduced appetite Vomiting
		Neurotoxic effects	Hypotension
		Decreased performance	Suppressed weight gain
Ochratoxin A	Dog, Cat & Pet birds	Nephrotoxic effects	Kidney damage
		Gastro-intestinal effects	Vomiting Intestinal haemorrhages Dehydration
		Neurotoxic effects	Anorexia Tenesmus
		Decreased performance	Weight loss Prostration
		Immunosuppression	Tonsillitis
		Pathological changes	Epithelial degeneration of the kidney Muco-haemorrhagic enteritis (caecum, colon, rectum) Necrosis of the lymphoid tissues
Trichothecenes	Dog, Cat	Gastro-intestinal effects	Vomiting
		Neurotoxic effects	Feed refusal
Zearalenone	Dog	Reproductive effects	Pathological changes in the reproductive system Arrested spermatogenesis Edema and hyperplasia in oviducts and uterus Pyometra

Table 12. The effects of mycotoxins in pet animals

(Table after data from http://www.mycotoxins.info, 2013.08.08)

The pathological effects of mycotoxins on humans

A number of human diseases, demonstrated to be caused by mycotoxins, are displayed in Table 13.

Disease	Substrate	Mold	Toxin	Symptoms
Akakabi-byo	wheat, barley, oats, rice	*Fusarium* spp.	*Fusarium* metabolites	Gastrointestinal syndromes, Weakness
Alimentary toxic aleukia	cereal grains (toxic bread)	*Fusarium* spp.		necrosis in lymphoid and haemopoetic tissue
Balkan nephropathy	cereal grains	*Penicillium*		interstitial nephritis
Cardiac beriberi	rice	*Aspergillus* spp., *Penicillium* spp.		pedal edema, anasarca, cardiac failure
Celery harvester's disease	celery (pink rot)	*Sclerotinia*		various, including bullous, erythematous, nonpruritic, discrete rash
Dendrodochiotoxicosis	fodder (skin contact, inhaled fodder particles)	*Dendrochium toxicum*		acute poisoning
Ergotism	rye, cereal grains	*Claviceps purpurea*	Ergot alkaloids	convulsive syndromes, gangrene
Esophageal tumors	corn heterocycles	*Fusarium moniliforme*		dysphagia, odynophagia
Hepatocarcinoma (acute aflatoxicosis)	cereal grains, peanuts	*Aspergillus flavus, A. parasiticus*		jaundice, nausea, emesis, fatigue, bloating from ascites, easy bruising from blood clotting abnormalities, loss of appetite, weight loss, abdominal pain
Kashin Beck disease (Urov disease)	cereal grains	*Fusarium*	*Fusarium* metabolites	joint pain, joint stiffness, disturbances of flexion and extension in the elbows, enlarged inter-

				phalangeal joints
Kwashiorkor	cereal grains	*Aspergillus flavus, A. parasiticus*	Aflatoxins	edema, irritability, anorexia, ulcerating dermatoses, enlarged liver
Onyalai	millet	*Phoma sorghina, Fusarium* sp.	*Fusarium* metabolites	haematoma on oral mucous membranes, hemorrhagic lesions, haematuria, melena, epistaxis, petechiae, ecchymoses, menorrhagia.
Reye's syndrome	cereal grains	*Aspergillus*		rash, vomiting, liver damage, death
Stachybotryotoxicosis	rye, cereal grains, fodder (skin contact, inhaled rye dust)	*Stachybotrys atra*	Trichothe-cenes	skin rash, pharyngitis, leukopenia
Kodua poisoning		*Aspergillus* sp.; *Penicillium* sp.	Cyclopiazo-nic acid	hepatotoxicity

Table 13. Some diseases caused by mycotoxins (after BRÄSE et al., see above).

In addition to the those diseases for which rigorous scientific research has postulated mycotoxins as primary cause of the disease, there are several diseases for which mycotoxins are hypothesized as possible causes, but further research should unequivocally validate this hypothesis (Table 14).

Disease	Mycotoxin	Source
Gout / Hyperuricemia	Cyclosporin Penicillin Multiple Multiple Ergotamine	Moldy Corn Barley Beer/Wine/Bread Meat Products Rye
Atherosclerosis	Cyclosporin	
Hyperlipidemia	Cyclosporin	
Hypertension	T-2 Toxin	Alcohol

Multiple Sclerosis	Ergot	
Scleroderma	Amanita	
Diabetes	Cryptococcus, Alloxan	
Crohn's Disease	S.cerversisae	Fermentation
Lung Cancer	*Fusarium*	Tobacco
Esophageal carcinoma	*Fusarium*	
Breast Cancer	*S.cerversisae*	Fermentation
Endometriosis	*Fusarium*	
Colon Cancer	*Fusarium*	
Hepatocellular carcinoma	*Aspergillus*	Cereal grains, peanuts
Hepatoma	Aflatoxin	Food
Cardiomyopathy	Alcohol	Fermentation
Osteoporosis	Alcohol	Fermentation
Alimentary toxic aleukia (ATA or septic angina)	*Fusarium trichiodes*	Cereal grains (toxic bead)
Dendrodochiotoxicosis	*Dendrodochium toxicum*	Fodder (skin contact, inhaled fodder particles)
Kashin Beck Disease, "Urov Disease"	*Fusarium trichiodes*	Cereal grains
Stachybotryotoxicosis	*Stachybotris atra*	Hay, cereal grains, fodder (skin contact, inhaled haydust)
Cardiac beriberi	*Fusarium*	Rice
Ergotism	*Claviceps purpurea*	Rye, cereal grains
Kwashiorkor	Aflatoxins	Food
Balkan-nephropathy	*Penicillium*	Cereal grains
Reye's Syndrome	*Aspergillus*	Cereal grains
Pink rot	*Sclerotenia Sclerotiorium*	Celery
Onyalai	*Phoma sorgina*	Millet
Chronic Intestinal Inflammatory Diseases	*Aspergillus, Fusarium, Penicillium species*	Food

Hormono-sensitive cancers	Zearalenone	Food
IgA-related nephropathy	Deoxynivalenol	Food
Chronic Fatigue Syndrome	Aflatoxin, ochratoxin, etc.	Household dust, air conditioning, ventillation, etc.

Table 14. Some diseases probably caused by mycotoxins (sources: BUCHE, 2013; BREWER et al., 2013; MARESCA and FANTINI, 2010).

Mycotoxicoses can especially be harmful, even fatal, in patients with suppressed immune status, for instance in cancer patients undergoing immunosuppressice therapies. In such cases the fungi can grow in various organs of the body, causing serious pathological reactions (inflammation, necrosis, blood clotting, etc.) (Figure 6).

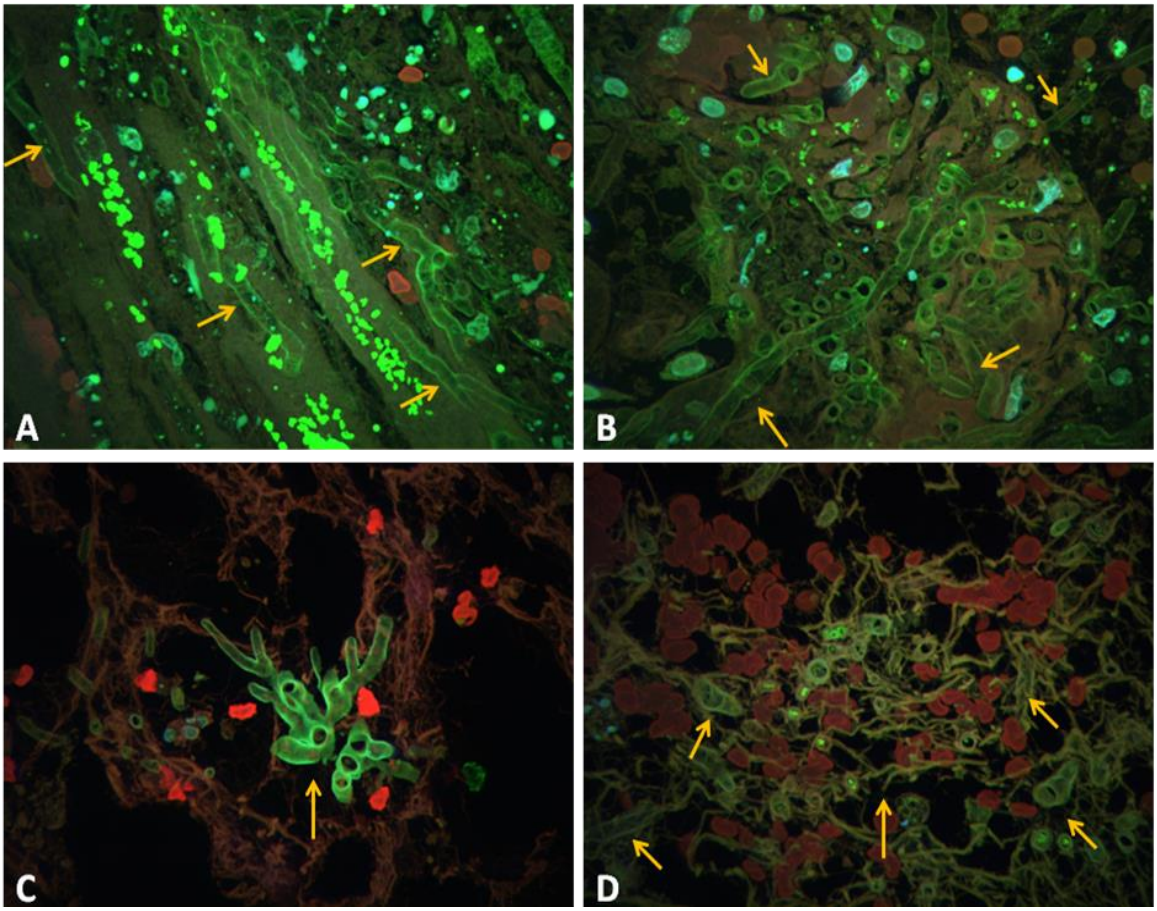


Figure 6. Aspergillus fumigatus (fusarium) infection of the human heart (A), kidney (B) and lung (C, D). Omnifluor Bright (OFB) staining. By courtesy of Professor László Székely,

Karolinska Institute, Stockholm (http://laszlo.mtc.ki.se/Aspergillus/). Yellow arrows indicate typical examples of mold growth. In C and D the red structures are red blood cells.

Protective effects of mycotoxins

As referred to earlier, certain mycotoxins can have advantegous biological effects in both humans and animals. The more, some are widely used in treatment of diseases and even synthetic versions of the lead molecule are produced as drugs. A few examples are listed in Table 15.

Mycotoxin	Diseases, for which it can be used for curative purposes
Allopurinol	Sarcoidosis Oxalate Nephrolithopathy Idiopathic Respiratory Distress Syndrome/Newborns Duchenne's Muscular Dystrophy
Colchicine	Acute Gouty Arthritis Alcoholic Cirrhosis Familial Mediterranean Fever Mollaret's Meningitis Bechet's Syndrome Psoriasis Thrombocytopenic Purpura Chronic Lymphocytic Leukemia Amyloidosis North African Leukocytoclastic Vasculitis Sarcoid Arthritis Rheumatoid Arthritis (some) Calcium Pyrophosphatopathy Hyperlipidemia Inflammatory Bowel Disease
Griseofulvin	Atherosclerosis (Angina) Systemic Sclerosis Raynaud's Syndrome/Disease Shoulder-Hand Syndrome
Ketocinazol	Inflammatory Bowel Diseases Disseminated Vascular Coagulation Idiopathic Female Infertility Precocious Puberty in Boys Hyper-Low-Density-Lipoproteinemia Hyperaldosteronism aldosteronism Prostate Carcinoma
Nystatin	Psoriasis Inflammatory Bowel Disease Hyperactivity Syndrome

	Multiple Sclerosis

Table 15. The therapeutic use of certain mycotoxins (source: BUCHE, 2013).

A special case is penicillin which was discovered already in 1928 as an antibiotic.
It – and its several "siblings" – are produced by *Penicillium* fungi. These include penicillin G, procaine penicillin, benzathine penicillin, and penicillin V. Other mycotoxins can also have antibiotic properties, however it is important to note here, that not all antibiotics are mycotoxins, i.e. are produced by fungi.
Some examples of the most common mycotoxins with antibiotic properties are shown in Table 16.

Antibiotics	Mold species	Mainly used in
Penicillin	*Penicillium*	Staphylococci and streptococci infections
cephalosporins	*Acremonium*	Gram positive infections
Fusafungine	*Fusarium lateritium*	Nasal and throat infections
Fumagillin	*Aspergillus fumigatus*	Myxozoa parasite infections
Alamethicin	*Trichoderma viride*	
Fusidic acid	*Fusidium coccineum*	Gram positive infections
Brefeldin A	*Eupenicillium brefeldianum*	
Nigrosporin B	*Nigrospora*	Mycobacterial infections

Table 16. Some examples of mycotoxins with antibiotical characteristics and medical use.

In addition to antibiotical features, some mycotoxins have cytostatic and anticancer features which can be used in medical practice. Anticancer drugs can be based either directly on fungi-produced mycotoxins or synthetic molecules for which the lead molecule is a mycotoxin.

A few examples are shown in Table 17. In some cases these compounds are not only used as anticancer agents but also used as antifungal agents.

Anticancer compound	Mold species
Aurantiamine	*Penicillium aurantiogriseum*
Griseofulvin	*Penicillium griseofulvum*
Neoxaline	*Aspergillus japonicus*

Oxaline	*Penicillium oxalicum*
Podophyllotoxin	*Podophyllum*
Vinblastine	*Vinca rosea*
Vincristine	*Vinca rosea*
Verrucarin A	*Fusarium*

Table 17. Some examples of mycotoxins with cytostatic characteristics.

Food and feed contamination by mycotoxins

Mycotoxins are present everywhere in the world in agricultural commodities TAJKARIMI et al., 2011). Mycotoxins may enter in the food chain as a result of fungal infection of crops. They can be eaten directly by humans or by being used as livestock feed. In the latter case humans can consume them by livestock food consumption.

Mycotoxins greatly resist decomposition or being broken down in digestion, so they remain in the food chain in meat and dairy products for a long time. Even temperature treatments, such as cooking and freezing, do not destroy some mycotoxins. Mycotoxins can remain toxic for several years. According to experts, for instance the mycotoxin of Trichothecenes is so stable and long lasting that even ultraviolet light or freezing temperatures have no effect on trichothecene mycotoxin decomposition.
Food contamination by mycotoxins, especially by aflatoxins, is a rather frequent problem in several countries (WILLIAMS et al., 2004), as shown in Table 18.

Country	Commodity	Frequency of aflatoxin-positive samples (%)	Contamination rate (ppb)
Argentina	Maize	19.6	(positive)
Bangladesh	Maize	67	33.0 (mean)
Brazil	Corn	38.3	0.2-129.0
	Peanut products	67	43.0-1099.0
	Peanuts	27	43.0-1099.0

	Sorghum	12.8	7.0-33.0
China	Corn	76	>20.0
Costa Rica	Maize	80	>20.0
Cyprus	Peanut butter	56.7	>10.0
Egypt	Hazelnut	90	25.0-175.0
	Peanut and watermelon seeds	82	(positive)
	Soybean	35	5.0-35.0
	Spices	40	>0.250
	Walnut	75	15.0-25.0
Gambia	Groundnut sauce	(no data)	162.0
Guatemala	Incaparina (mixture of corn and cottonseed flour)	100	3.0-214.0
Ghana	Peanut	12.8-31.7	(positive)
India	Chilies	18	>30.0
	Dry slices of quince	23.14	96.0-8164.0
	Groundnut	21	>30.0
	Maize	26	>30.0
Korea	Barley food	12	26.0 (mean)
	Corn food	19	74.0
Kuwait	Milk	6	>0.2
Portugal	Yogurt	18.8	19.0-98.0
Malaysia	Wheat	1.2	>25.62
Mexico	Kerneled corn	87.8	5.0-465.0
Nigeria	Corn	45	25.0-770.0
	Maize-based gruels	25	0.002-19.716
Qatar	Pistachio	8.7 to 33	>20.0
Senegal	Peanut oil	85	40.0 (mean)
Turkey	Cheese	12.28	Positive[1]
Uganda	Maize	29	1-100

	Peanut, cassava	12	

Table 18. Examples of market sample contamination frequencies and concentrations (source: WILLIAMS et al., 2004).

Prevention of mycotoxins

According to the Food and Agriculture Organization of the United Nations (FAO) approximately 25% of the cereals produced in the world are contaminated by mycotoxins. Other foodstuffs can also be contaminated by mycotoxins.

Prevention of mycotoxicoses should include pre- and post-harvest strategies. The most optimal way to reduce their content in food and feed is the prevention of mycotoxin formation in the field. However, this is often not sufficient. For feed decontamination and/or detoxification the most prevalent approach is the inclusion of sorbent materials in the feed. This results in a selective removal of toxins by adsorption during passage through the gastrointestinal tract. Another widely used approach is to add enzymes or microorganisms to the feed, capable of detoxifying some mycotoxins.

It is of great importance to try to prevent the formation of mycotoxins on crop, especially if the field crops are already free of any fungal infestations or if we want to stop their further spreading. To develop preventative methods, one has to have knowledge about the different fungal species that can produce mycotoxins, and to understand how, when and where they can grow.

The fungal invasion can occur at any time of the processing of the field grains; before, during or after the harvesting. The toxicogenic fungi affecting the crop can be classified into three categories (Table 19):

Category	Species
Field fungi	Fungi pathogenic to plants (e.g. the *Fusarium genus)*
Storage fungi	*Penicillium* and *Aspergillus genera*
Advanced deterioration fungi	(These fungal infestations usually only occur in already non-intact crops with species from e.g. the *Rhizopus, Mucor, Absidia* and *Aspergillus genera)*

Table 19. Categorisation of toxicogenic fungi, affecting crop.

We have to keep in mind that although we can manage to minimize the risk of mycotoxin contamination by preventative methods, we can never entirely eliminate all the fungal growth that can be present on the field products. According to the Food and Agricultural Organisation (FAO), the prevention of mycotoxin contamination and infection can be classified into three main levels:

1. ***Primary prevention***

The primary prevention is the most important out of these three levels. The point of this step is that it should be applied before there is any fungal infestation at all on the crop by setting up conditions which are unfavorable for fungi to exist in. This might be difficult due to the fact that the conditions which are good for fungal growth are usually also good for the growth of field plants. But there are some techniques such as:

- Using crop breeds which have been developed specifically as genetically resistant, or at least less susceptible, to fungal infections. (No such plant has been developed yet, although, a number of researchers have been trying to develop corn and peanut hybrids resistant to *Aspergillus flavus).*
- Reduce the density of the crop, rotate crop regularly, and have regular weed control on the fields.
- Applying fungicides.
- Reduce the damage to the plants (both on the field and during storage) made by e.g. mechanical injuries, birds, rodents, insects etc. by using adequate methods while harvesting and a proper amount of insecticides and rodenticides. The usage of chemical fertilizers should be enough to give proper protection, but also limited because they can cause physiological stress on the plants, decreasing their natural resistance against fungi.
- It is also of great importance to harvest at the right time of the year and to try to reduce the amount of moisture in the plants stored post-harvest.
- If possible, the storage place should have a cooling system so that the optimal temperature (25-40^{o}C) for fungal growth can be avoided.

2. Secondary prevention

If there is an early, milder contamination of mycotoxin causing fungi, their growth should be stopped before they cause further spreading, or if possible all fungi should be eliminated by:

- Controlling of the seeds and remove the ones which are contaminated.
- Dry the harvested products again to inactivate the fungal growth.
- Disinfect the equipment used at harvest and remove all the plant residues from the machines to prevent any further contamination.
- Applying the techniques of storing harvest in conditions unfavorable for fungi mentioned above in "primary prevention".

3. Tertiary prevention

This level should be applied when the products are already heavily contaminated with fungi and the other two levels would not be helpful any more. Here, the complete termination of fungal growth and their mycotoxin production is no longer possible, but the aim is to prevent the spreading of fungi and the mycotoxins from contaminating further food and feed products.

The Food and Agricultural Organization (FAO) gives an example here about the extracted peanut oil. According to them, the peanut seeds which are poorly graded are always heavily infested with aflatoxins and since the toxin is soluble in oil, it has to be subtracted during the oil-refining procedure by alkalization and absorption. Very few techniques are recommended in this level:

- All of the infested crops should be completely eradicated
- Attempts should be made to reach the minimal level of mycotoxins in the products by their nullification or detoxification.

4. Fungal growth inhibition

Because a large number of the existing research regarding mycotoxins have been done on the most common toxin, the aflatoxin, the preventative methods have been developed to protect

the plants from growth of the *Aspergillus fungi*. Therefore, this text is mainly about the precautions applied to prevent aflatoxin contamination on crops.

In the agricultural sector, the inhibition of pathogenic fungal growth is extremely important to avoid the products from becoming contaminated with mycotoxins. With the different treatments we also have to remember that the detoxification and inactivation processes cannot be harmful for humans and animals when the treated products end up in the food and feed industry and also they have to keep their nutritive values. There are some physical, biological and chemical methods suitable for these purposes:

Physical treatment: the crops should be dried and stored properly as soon as possible after the harvest and be protected from conditions which are favorable for the fungal growth, like high humidity, high moisture and a temperature of about 25-40^{o}C. The optimal moisture levels for mycotoxin prevention should be < 9% for peanut kernel and < 13,5% for corn. Rodent and insect infestation should also be avoided in the storage area to protect the crops from being mechanically injured. If there are seeds contaminated with fungi, they can be picked by hand and removed from the healthy kernels, but this technique requires a considerable effort, and is expensive and time consuming.

Chemical treatment: chemical treatment is the most effective way to eliminate fungi in agricultural products and the usage of some organic solvents, such as acetone, chloroform, hexane and methanol have been applied to remove the aflatoxins. These solvents are mainly used in the processing of vegetable oil refining. When using chemical compounds, the goal is to sufficiently detoxify the products by reforming the toxins to nontoxic derivatives without modifying the raw products too much. In this case, the mutagenic capacity of the products treated has to be evaluated by using for example animal trials. Except for the previously mentioned chemicals, other substances have been tested for their sufficiency to prevent fungal growth: acetic acid, ammonia gas or ammonium salts, calcium hydroxide, formaldehyde, hydrogen peroxide, methylamine, phosphoric acid, sodium bicarbonate, sodium bisulfate, and sodium hypochlorite.

Aflatoxins are quite resistant to high temperatures (up to 260^{o}C) but in an experiment performed by Coomes et al. in 1966, it was stated that heating and boiling rice under pressure would destroy almost 70% of the aflatoxins, while under atmospheric pressure just 50% would be destroyed (COOMES et al., 1966). The negative effects of heat and pressure treatment

of food in such high temperatures is that a longer time of cooking and heating would not just decrease the amount of mycotoxins, but unfortunately also destroy essential amino acids and vitamins of the nutrients. Research has proved that ionizing radiation, like gamma irradiation can inhibit the growth of organisms spoiling food; this includes yeast, mold and bacterial organisms as well as parasites and insects. Gamma-irradiation can also reduce the amount of aflatoxin in food products, but it cannot destroy the toxins entirely, nor their mutagenic capacity.

Biological method: a new discovery showed that a great number of plants contain enzymes like chitinase and B-1, 3-glucanase which have antifungal properties. These enzymes can protect the plants from fungal infections by hydrolyzing the polysaccharides (chitin and glucan) of the fungal cell wall resulting in the damage and destruction of the mycelia and spores of the fungi. To decrease the mycotoxin producing fungi in crops, land owners could use seeds high in these antifungal enzymes on their fields.

Detection and measurement of mycotoxins

Due to the versatility of chemical structures of mycotoxins, it is not possible to develop a single unified standard technique for their detection and measurements in the food, feed or body. Most commonly HPLC (high-performance liquid chromatography) and mass spectroscopy techniques (MS) are used to measure their quantity in food commodities or organs.

Various international agencies try to achieve a universal standard for regulatory limits of mycotoxins. Recently more than 100 countries have introduced regulatory measures with regard to mycotoxins in the feed industry. Countries between which trade agreement exists, aim at coordinating their regulations with each other. Furthermore, for instance the European Committee for Standardization (CEN) set up the standards for the method of quantitative and qualitative analysis of mycotoxins in food commodities in Europe.
In the EU the European Commission, whereas in the US the U.S. Food and Drug Administration (FDA) have made the relevant regulations and are responsible for their enforcement and the two authorities coordinate their activities.

Worldwide regulations of mycotoxins

Natural contamination of mycotoxins can occur in food and feedstuff. Natural contamination means that an unwanted substance is added to food or feed products, not on purpose, but due to environmental infections, or at harvesting, processing, treatment of products etc.

In each country, there is a legislative framework which states that food which contains a level above the limited amount of the contaminating substance, especially a level that might be toxic to humans, is not allowed to be marketed because it poses a risk to public health.

The ideal would be to have no mycotoxin contamination at all in the foodstuff but since it is almost impossible to most farmers, food- and feed factories, sellers, and exporters to provide completely mycotoxin free products, the limits have been set to be as low as possible that can be accomplished by Good Practice.

In the field of mycotoxins, many countries worldwide have created regulations that encompass the maximum amount of mycotoxins allowed for different food- and feedstuff. These regulations include the most significant mycotoxins which can cause a threat to human and animal health; aflatoxins, fumonisins, patulin, ochratoxin A and some trichothecenes such as deoxynivalenol.

Table 20 shows the maximum limit of total aflatoxins in peanuts intended for further processing on an international level:

Australia / New Zealand	15 µg/kg
Hungary	15 µg/kg
India	30 µg/kg (!!!)
South-Africa	15 µg/kg
USA	20 µg/kg

Table 20. The maximum limit of total aflatoxins in peanuts in a number of selected countries (based upon http://services.leatherheadfood.com/eman/FactSheet.aspx?ID=79 ; modified by the author, N. GULYÁS).

While some countries have very strict regulations for the significant mycotoxins in foodstuff, other countries do not have any specific limits at all. Here are some examples:

- Australia, New Zealand, Kenya and South Africa → no limits for deoxynivalenol (DON).
- Australia, New Zealand, Canada, Mexico → no limits for patulin.
- China, Japan, India, Russia, Canada and some countries in Latin America → no limits for fumonisins.
- USA, Canada, Japan, Australia, New Zealand and some African countries → no limits for zearalenone.

Although neither deoxynivalenol, fumonisins nor zearalenone is limited in Australia and New Zealand, these countries have imposed limits for ergot alkaloids in some foodstuff, which not a lot of other countries have imposed.

Interestingly, except for Russia not a single country has so far set a maximum limit for T-2 toxin (http://services.leatherheadfood.com/eman/FactSheet.aspx?ID=79).

Regulations of aflatoxins in the European Union

The regulations of aflatoxins can be divided into three classes; one class is the aflatoxin B_1 (the most toxic aflatoxin) and then another class, called Total aflatoxins, which includes the sum of the aflatoxin B_1, B_2, G_1 and G_2. The class of aflatoxin M_1 has been set for regulations concerning milk- and dairy products. The limitations for aflatoxins in food products are the most frequently based limits of mycotoxins in the world since it is of biggest concern out of the mycotoxins in the food industry.

Within all the member countries of the European Union the limits for the maximum amount of aflatoxin B_1 and Total aflatoxins for a variety of foodstuff have been determined in the Commission Regulation (EC) No 1881/2006 of 19 December 2006 Setting Maximum Levels

for Certain Contaminants in Foodstuffs. The maximum amount is given in µg/kg foodstuff and varies between 0,10 – 8,0 in case of aflatoxin B1, and 4,0 – 10,0 in case of Total aflatoxins between the different foodstuffs.

This include - amongst others - the limits in spices (e.g. chillies, paprika and cayenne), all cereals and all products derived from cereals, dried fruits and their processed products, groundnuts and nuts. It also covers the limits of aflatoxin M_1 for raw milk, heat-treated milk and milk for the manufacture of milk-based products (all with a maximum level of 0,050 µg/kg), and aflatoxin M_1 in infant milk formulae (0,025 µg/kg).

In addition to these limitations, some member states like Austria, Spain, Denmark, Finland, Germany and Sweden have applied further regulations in their national legislation by setting limits for every food product which is not included in the Commission Regulation.
There are a couple of non-EU member countries, e.g. Turkey, Switzerland and Bosnia and Herzegovina that also applies the EU regulations in their control of aflatoxins.

Regulations of other mycotoxins in the European Union

Like in the case of aflatoxins, the Commission Regulation (EC) No 1881/2006 of 19 December 2006 imposes the specific maximal amount of other mycotoxins in foodstuffs as well. Some of the feedstuff regulated on EU level and their maximum allowed amount of mycotoxins will be presented in the following text;

Ochratoxin A: regulated in e.g. unprocessed cereals and their products, dried vine fruits (e.g. raisins), roasted coffee beans, soluble coffee, wine, grape juice, processed cereal-based foods and baby foods for infants and young children. Among these foodstuff, the dried vine fruits and soluble coffee have the highest maximal limit with 10,0 µg/kg while wine and wine products, together with grape juice have the lowest maximal limit at 2,0 µg/kg (if we do not take the products for infants into consideration – 0,50 µg/kg). Denmark, Hungary, Italy and Germany have applied additional regulations for ochratoxin A.

Patulin: fruit juices, spirit drinks and cider can all contain a maximum of 50 µg/kg, while solid apple products such as apple compote or puree have a limit of 25 µg/kg and apple juice of 10 µg/kg patulin. The Swedish National Food Agency also have regulations for fruit

products and berry products which are not included in the previously mentioned Commission Regulation. The maximum limit of patulin in fruit and berry products is 50 µg/kg (LIVSFS 2004:7).

Deoxynivalenol: the maximum limits of deoxynivalenol ranges between 200 and 1750 µg/kg foodstuff and is regulated in e.g. unprocessed cereals, (including unprocessed durum wheat, oats and maize), pasta, bread, cereals intended for direct human consumption and cereal flour.

Fumonisins (the sum of Fumonisin B_1 + B_2) and Zearalenone: the maximal amounts of zearalenone (limits between 20 and 200 µg/kg) are mostly established for unprocessed cereals but also for bread and maize snacks. Fumonisins are regulated in maize products such as e.g. maize flour and refined maize oil and their maximal limit is up to 2000 (!) µg/kg in unprocessed maize.

Regulation of mycotoxins in feedstuffs in the European Union

In 2004 and 2005, the European Commission requested the European Food Safety Authority (EFSA) to evaluate and form an opinion about the maximum limits for some specific mycotoxins (deoxynivalenol, zearalenone, ochratoxin A, T-2, HT-2 and fumonisins) in feedstuffs.

Based on the investigations of EFSA, the recommendation which should be applied in the European Union member countries has been established in the Commission Recommendation of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding (2006/576/EC).

The European Commission states that countries have to take into account that the limits (given in mg/kg feedstuff) found in the Annex have been determined for the most tolerant animal species, and feed given to animal species less tolerant to mycotoxins should contain lower levels of mycotoxins than the recommended maximum limits.

The EU recommendations for mycotoxins in feedstuffs intended for animal feed contains the following (Table 21):

Mycotoxin	Products intended for animal feed	*
Deoxynivalenol	Feed materials: - Cereals and cereal products with the exception of maize by-products - Maize by-products	8 12
	Complementary and complete feedingstuffs with the exception of: - Complementary and complete feedingstuffs for pigs - Complementary and complete feedingstuffs for calves (< 4 months), lambs and kids	5 0,9 2
Zearalenone	Feed materials: - Cereals and cereal products with the exception of maize by-products - Maize by-products	2 3
	Complementary and complete feedingstuffs: - Complementary and complete feedingstuffs for piglets and gilts - Complementary and complete feedingstuffs for sows and fattening pigs - Complementary and complete feedingstuffs for calves, dairy cattle, sheep (including lambs) and goats (including kids)	0,1 0,25 0,5
Ochratoxin A	Feed materials: - Cereals and cereal products	0,25
	Complementary and complete feedingstuffs: - Complementary and complete feedingstuffs for pigs - Complementary and complete feedingstuffs for poultry	0,05 0,1
Fumonisin B1 + B2	Feed materials: - Maize by-products	60
	Complementary and complete feedingstuffs : - Pigs, horses (Equidae), rabbits and pet animals - Fish - Poultry, calves (< 4 months old), lambs and kids - Adult ruminants (> 4 months old) and mink	5 10 20 50

Table 21. EU recommendation regarding the maximum level of mycotoxins in feedingstuff. Remark: * indicates guidance value in mg/kg (ppb) relative to a feedingstuff with a moisture content of 12%. (Source: Commission Recommendation of 17 August 2006 on the presence

of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding (2006/576/EC). Official Journal of the European Union L 229/7, 23.8.2006 EN.)

Conclusion

Mycotoxins, the secondary metabolites of fungi of primarily toxic nature, are present in nature ubiquitously and, as such, have a remarkable contribution to human and animal lives. The understanding of the production, chemical and biological characteristics, metabolism in the body of humans and animals and circulation in the biological world of mycotoxins, with special regard to the contamination of food and feed products by them, is of high importance for veterinary practice, in general, and animal food hygiene, in particular. The present thesis surveyed the basic of this topic, primarily from perspective of veterinary sciences and animal nutrition and feed hygiene.

Acknowledgement

The author is expressing her gratitude to Dr. Zsuzsanna Szili for the choice of the topic and for the support and consultations on the topic. She also expresses her thanks to Dr. Balázs Gulyás for advice and support regarding the preparation of the present thesis.

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