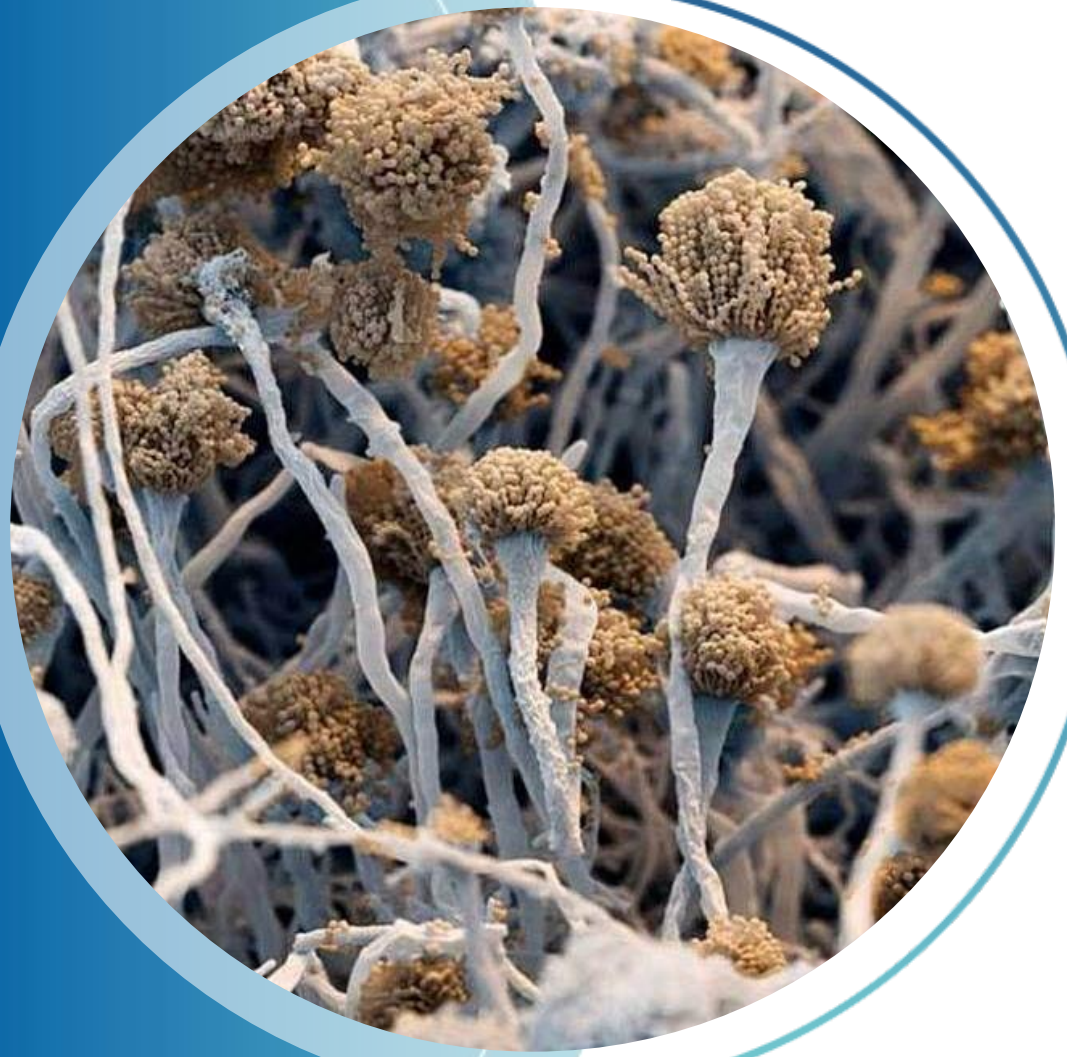

PROAGRO LECTORIUM

Mycotoxins in farm animal feed: Current status

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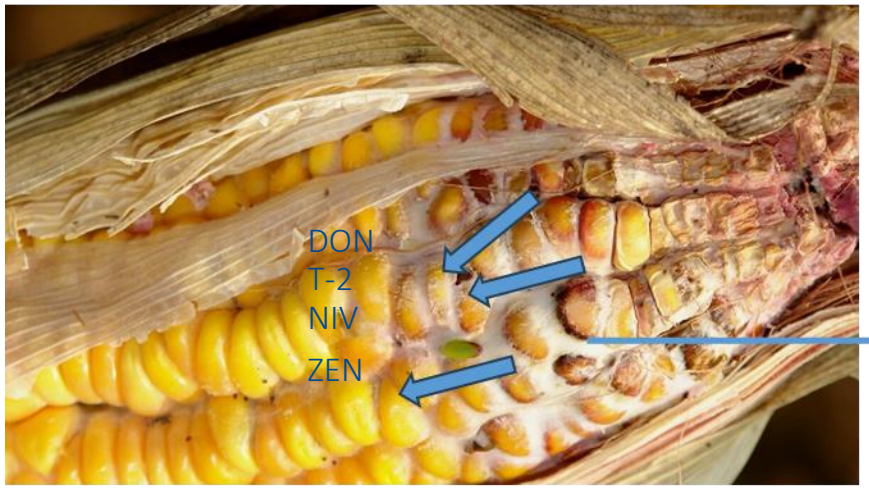
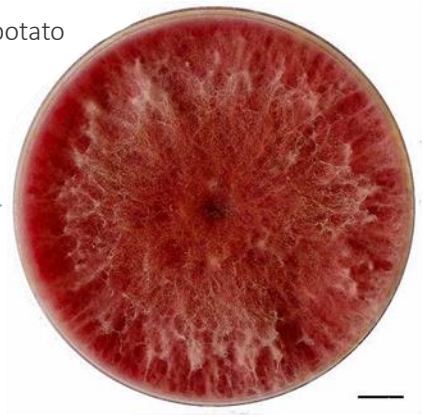
Mycotoxins: from Greek μύκης, mykes, mukos (“fungi”) and Latin τοξικόν

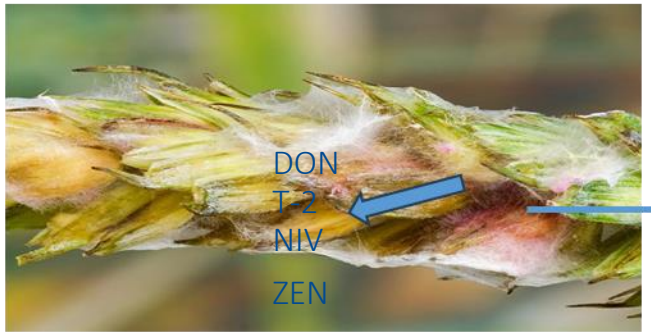
- Mycotoxins are low molecular weight, toxic secondary metabolites produced by microscopic fungi. Unlike primary metabolites (sugars, amino acids, and other substances), they are not essential for normal fungal metabolism.
- Toxigenicity is the ability of an organism to produce substances that have a toxic effect on other organisms.

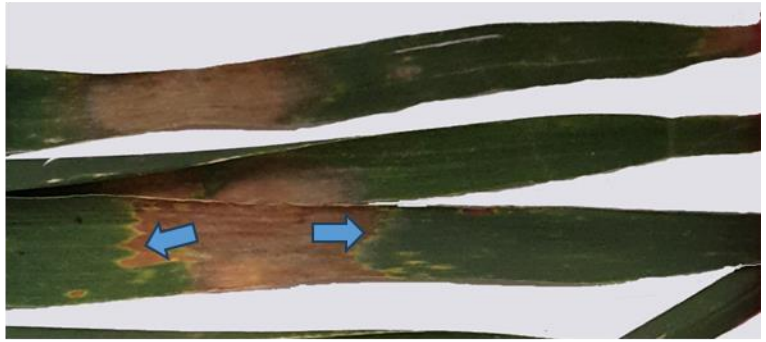
Chemical structure of compounds	Examples of mycotoxins
polyketides	aflatoxins, ochratoxin, patulin, zearalenone
terpenoids	trichothecenes
cyclopeptides	ergot alkaloids
sphingolipids	fumonisins



Fusarium culmorum on potato dextrose agar







Ergot alkaloids (clavines, ergopeptides); producers: Claviceps purpurea (C. fusiformis, C. paspali, C. africana)

12–15th centuries: mass poisonings in Europe
1951: poisonings in France
1977–1978: ergotism epidemic in Ethiopia

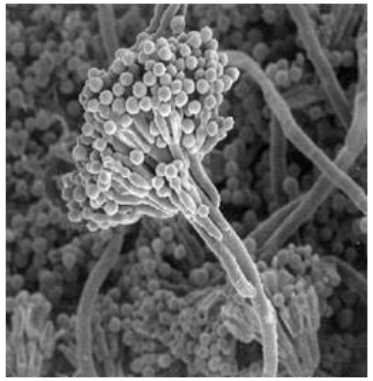
Matthias Grünewald. The Temptation of St. Anthony.

Pieter Bruegel. The Beggars (The Cripples).

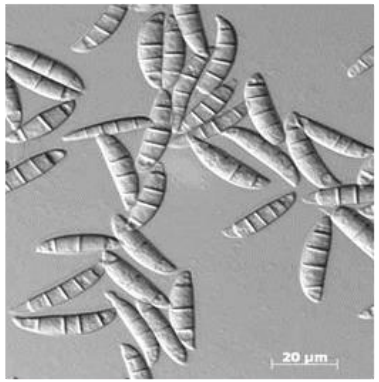
The most dangerous and widespread toxigenic species of fungi

Aspergillus

Penicillium



Fusarium



“storage molds” = “barn fungi”

“field fungi”

Fungi are the main producers of mycotoxins

Fungi genus	Main mycotoxins produced
Aspergillus	Aflatoxins B1, B2, G1, G2, M1 Ochratoxin A Sterigmatocystin Cyclopiazonic acid
Penicillium	Ochratoxin A Citrinin Patulin
Fusarium	Type A trichothecenes: T-2 and HT-2 toxins, diacetoxyscyrpenol, neosolaniol Type B trichothecenes: nivalenol, deoxynivalenol, fusarenone X Zearalenone Fumonisins (B1, B2, B3, B4) Moniliformin

Toxic effect of mycotoxins

Mycotoxins	Main toxic effect
aflatoxins B1, B2, G1, M1; **ochratoxin** A, sterigmatocystin, cyclopiazonic acid, fumonisin B1	carcinogens
aflatoxin B1, luteoskyrin, fumonisin B1	hepatotoxins
ochratoxin A, citrinin	nephrotoxins, teratogens
aflatoxin B1, **ochratoxin** A, trichothecenes, moniliformin	immunosuppressants
ergot alkaloids, cyclopiazonic acid, fumonisin B1	neurotoxins
zearalenone	estrogen
moniliformin	cardiotoxin
aflatoxins, patulin, luteoskyrin	mutagens

More about the toxic effects of Fusarium toxins

Mycotoxins	Mechanisms of action	Toxic effect
Trichothecenes	Inhibit ribosomal protein synthesis; inhibit DNA and RNA biosynthesis; cause cellular oxidative stress, apoptosis, and cell membrane dysfunction	Immunosuppressants. Growth retardation, reproductive disorders, feed refusal (anorexia), vomiting, hemorrhage, diarrhea.
Zearalenone	Binding to estrogen receptors	Estrogen, anabolic. Reproductive system disorders.
Fumonisins	Inhibition of sphingolipid biosynthesis; inhibition of ceramide synthase; cell membrane structure and function impairment	Carcinogens. Neurotoxins adversely affect embryos and cause liver and kidney disorders.
Moniliformin	Inhibition of protein synthesis, cytotoxicity, and chromosome damage. Damage to myocardial mitochondria.	Cardiotoxin. Acute heart failure. Immunosuppressant. Weight loss, hemorrhages.

Examples of intoxications caused by mycotoxins

Aflatoxicoses
Cause: A. flavus; mycotoxins: aflatoxins
1960: mass death of 100,000 turkeys in the UK (turkey X disease)
1974: liver cancer in ~400 people in India

Patulin toxicosis
Cause: Penicillium spp.; mycotoxin: patulin
1954: mass deaths of cows in Japan

Cereal scab
Cause: F. graminearum; mycotoxin: DON
1880–1890: Primorsky Territory, Russian Far East

Alimentary toxic aleukia (ATA) = septic angina
Cause: F. sporotrichioides; mycotoxin: T-2 toxin
1932–1946: Ural region, Kazakhstan, Siberia, central and northwestern regions of the USSR
1989: India
1993: China

Impact of mycotoxins on animal health

Mycotoxicoses are diseases caused by the consumption of low-quality food contaminated with toxic fungal metabolites.

Mycotoxicoses are diseases caused by the consumption of low-quality food contaminated with toxic fungal metabolites.

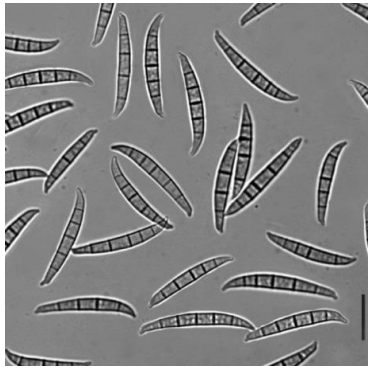
Manifestations of mycotoxicosis:

- Feed refusal
- Reduced productivity
- Immunosuppression
- Damage to internal organs (liver, kidneys, reproductive organs, etc.)
- Ulcerative stomatitis
- Epidermal necroses

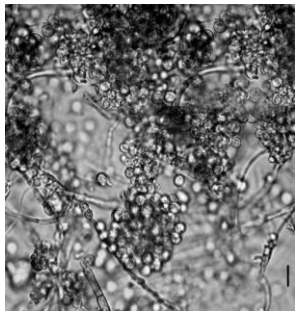
Thermal stability of mycotoxins

Mycotoxin	Molecular mass, g/mol	Melting point, °C
Ergot alkaloids	>70 compounds	162–249
Aflatoxin B1	312	268–269
Aflatoxin M1	328	299
Ochratoxin A	403	169
Patulin	153	105–108
Deoxynivalenol	296	151–153
Nivalenol	312	222–223
T-2 toxin	466	150–151
Diacetoxyscirpenol	366	162–164
Zearalenone	318	164–165
Moniliformin	120	345–355

Fusarium fungi



F. graminearum



Fusarium fungi producing mycotoxins

Fungi type	Trichothecene mycotoxins				Zearalenone (ZEN)	Fumonisins (FUM)	Moniliformin (MON)
	Deoxynivalenol (DON)	T-2/HT-2 Toxins	Nivalenol (NIV)	Diacetoxyscirpenol (DAS)			
F. graminearum	+++		+		+++		
F. culmorum	++		+		++		
F. sporotrichioides		+++		+	+		
F. langsethiae		+++		++			
F. poae			++	++			
F. cerealis			++		+		
F. avenaceum							+++
F. tricinctum							+++
F. equiseti				++	++		
F. verticillioides						+++	+
F. proliferatum						++	++
F. subglutinans					+	+	++
F. oxysporum						+	+

A particular fungal species can produce a specific set of mycotoxins
One fungal species can produce several mycotoxins
The same mycotoxin can be produced by several fungal species

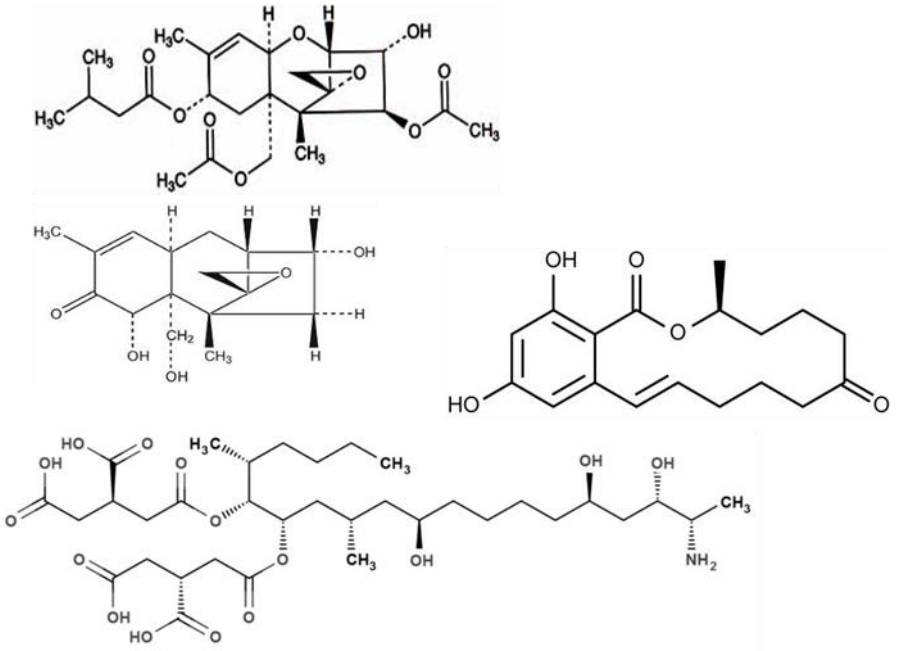
Standardization of Fusarium toxins in grain and grain products

T-2 toxin 100 µg/kg

Deoxynivalenol 700–1,000 µg/kg

Zearalenone 200–1,000 µg/kg

Fumonisins 4,000 µg/kg
200 µg/kg in corn meal for baby food



SanPiN 2.3.2.1078-01, 2012, as amended;
CU TR 015/2011 On Grain Safety; CU TR 021/2011 On Food Safety;

Recommended safe maximum allowable concentrations (MACs) of mycotoxins in grain and grain products

The MAC of mycotoxins varies depending on the type of grain crop, the type of product, and its consumer.

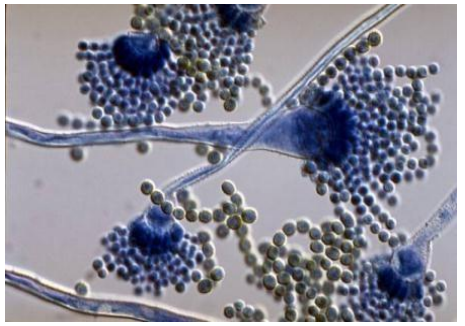
Mycotoxins	Content in grain, µg/kg (ppb)			
	food-grade		feed-grade	
	Russia[a]	EU[b]	Russia	EU
DON	700–1,000	200–1,750	1,000	900–12,000
T-2 toxin	100	15–200	100	250–2,000
Zearalenone	200–1,000	20–350	1,000	100–3,000
Fumonisins	200–4,000	200–4,000	5,000	5,000–60,000

aSanPiN 2.3.2.1078-01, 2012, as amended;
CU TR 015/2011 On Grain Safety;
CU TR 021/2011 On Food Safety;

bCommission Regulation (EC) No 1881/2006; Commission Recommendation 2006/576/EC и 2013/165/EU

Aspergillus fungi producing mycotoxins

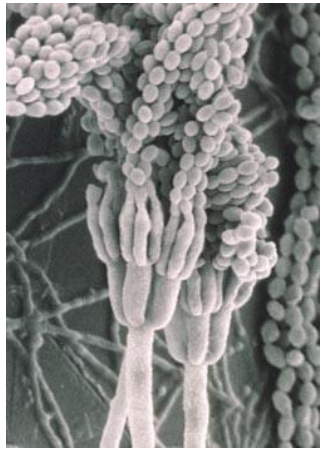
Producer	Mycotoxin
A. flavus, A. parasiticus, A. nomius	Aflatoxin B1
A. ochraceus, A. westerdijkiae, A. steynii, A. clavatus	Ochratoxin A
A. nidulans, A. versicolor	Sterigmatocystin
A. niveus, A. terreus, A. clavatus	Patulin
A. niveus, A. oryzae, A. terreus	Citrinin
A. flavus	Cyclopiazonic acid

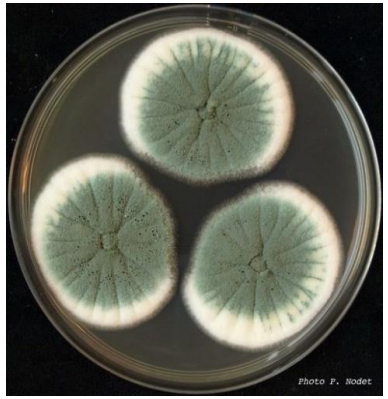


A. ochraceus

Penicillium fungi producing mycotoxins

Producer	Mycotoxin
P. verrucosum	Ochratoxin A
P. citrinum, P. verrucosum, P. expansum	Citrinin
P. expansum, P. carneum, P. paneum	Patulin
P. commume, P. camamberti, P. palitans	Cyclopiazonic acid

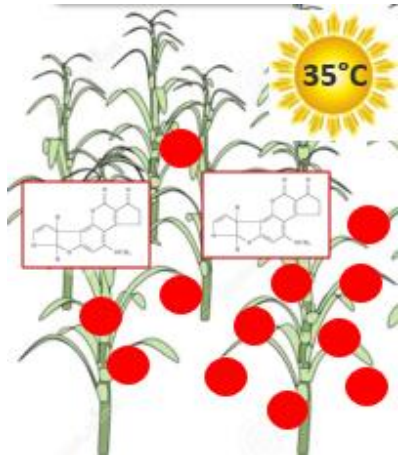




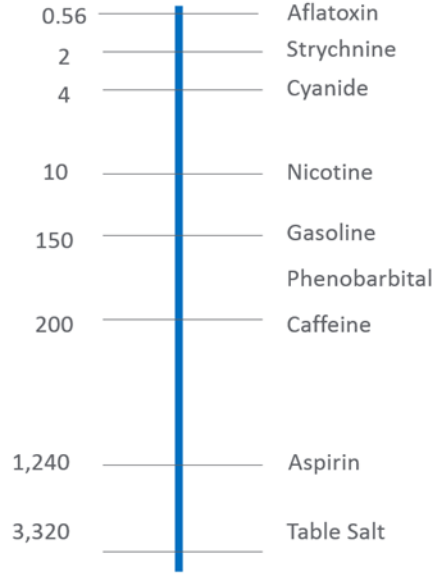
Briefly about aflatoxins

Aspergillus flavus Aflatoxin (AFB1)

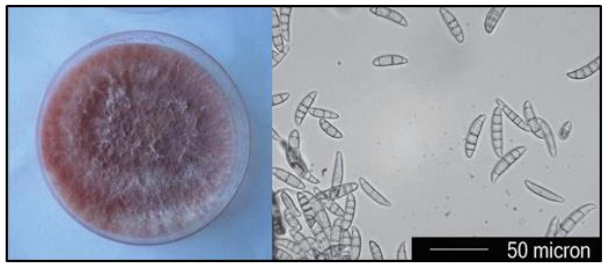
- A ubiquitous soil pathogen
- Spores germinate under hot (34–36 °C) and dry conditions
- MAC >5 µg/kg; makes grain unfit for human or animal consumption
- There are no conventional plant protection products on the market that can directly control aflatoxin levels

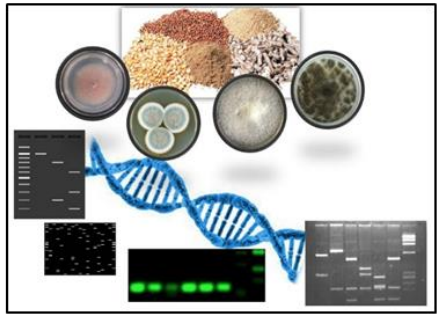


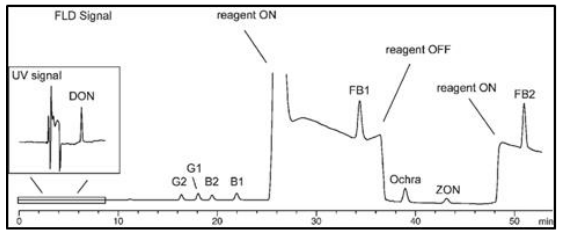
Acute oral toxicity LD_{50} mg/kg

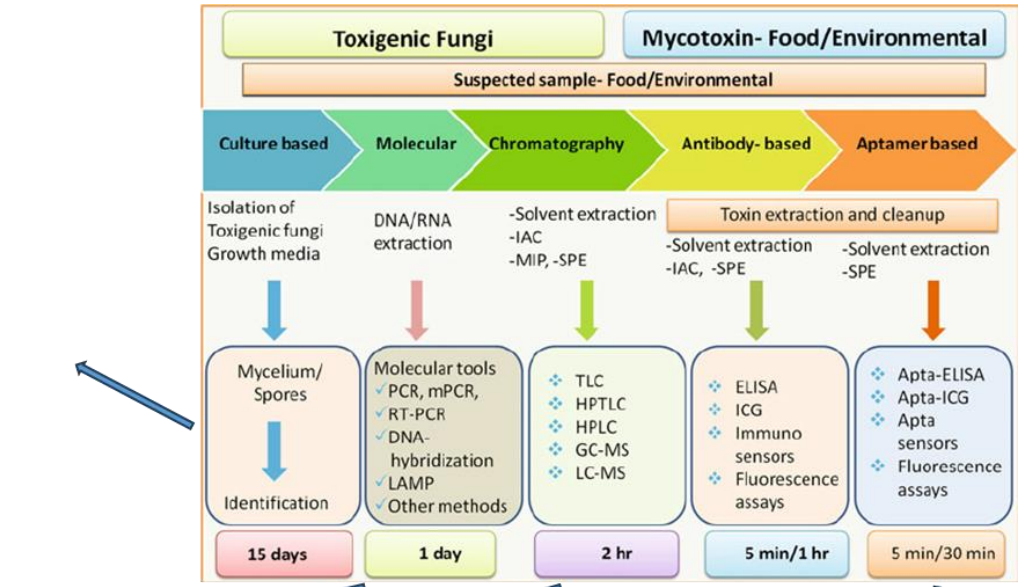


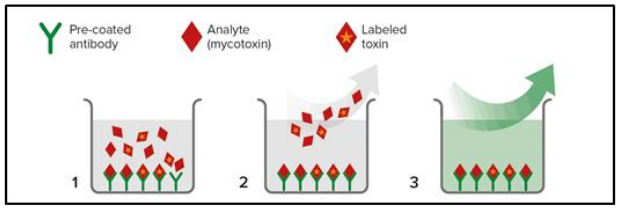
Diagnosis of mycotoxins. Instrumental methods









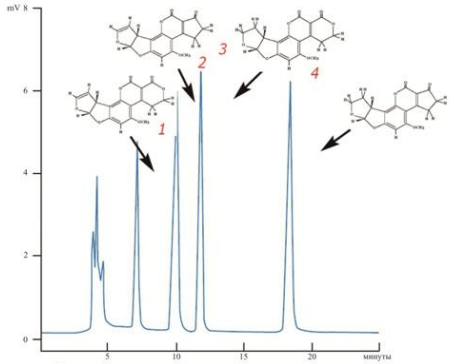


Chromatography

AGRASTRIP PRO
Ochratoxin WATEX
Test Kit

ELISA test strips

EXPRESS TEST AFLASENSOR QUANTI
KIT062: for aflatoxin



1: derivatized aflatoxin G1; 2: derivatized aflatoxin B1; 3: aflatoxin G2; 4: aflatoxin B2.
Figure 1. Typical chromatogram of a sample containing mycotoxins

Test systems for laboratory diagnosis of mycotoxins using ELISA

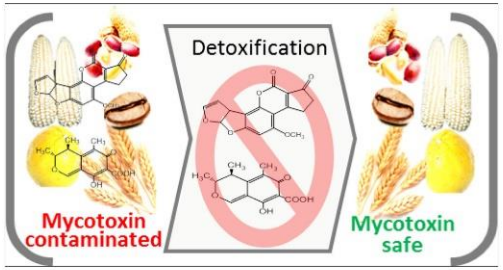
Mycotoxin control

Mycotoxin control is critical for public health and improving the economic situation in the country.

Therefore, several solutions for reducing and controlling mycotoxins have been considered in various parts of the world.

Mycotoxin control includes:

- Preventing the growth of mold or fungi on crops and other feed;
- Decontamination of feed/food contaminated with mycotoxins, as an additional strategy;
- Continuous monitoring of mycotoxin levels in crops, animal feed, and human food.



Preventing the growth of mold or fungi on crops and other feed

In the field

- Monitoring of primary manifestations of pathogens producing mycotoxins (ear and cob dark mildew, cereal scab, Alternaria spot)
- Optimal fertilizer system
- Optimal crop rotation
- Compliance with the rules of procurement and storage of feed raw materials
- Seed treatment with fungicides and seed dressers in the initial phases of disease development
- Optimal harvesting time

Example: Mycacid (propionic acid, sorbic acid, ammonium, polyglycerol polyricinoleate, brilliant blue dye, water) is a mold and pathogenic microflora inhibitor for feed and feed raw materials

Feed in storage

- Grain quality control at receipt into the storage facility
- Reducing storage temperature below 15–18 °C
- Feed moisture less than 11–12%
- Ventilation system in the storage area
- Humidity in storage facilities below 60%
- No violations of grain integrity
- Storage pest control

Grain in storage facilities

- Use of mold inhibitors
- Mechanical separation of spoiled grain

Mycotoxin adsorbents and neutralizers

- Binding and removal of mycotoxins occurs in the digestive tract of animals and poultry.
- Can remove up to 30–40% and even 70% of various toxins from the body of pigs, livestock, and poultry.
- Are recommended to be applied with feed continuously as a prophylactic agent, with the application rate of 0.2–0.5 to 2% of the diet.

More than 100 imported and domestic products are registered in Russia. About 30 products are recommended for neutralizing aflatoxin, and the rest are registered as effective against other mycotoxins, such as DON, zearalenone, T-2 toxin, fumonisin, etc.

They can be classified into two types based on their method of action: direct action against mycotoxins (adsorbing and biotransforming agents) and secondary action (reducing the negative effect of a mycotoxin on the animal's body).

Kormomix®SORB
CLEANFEED
SIMBITOX, etc.

Adsorbing effect of mineral components of products: binding and removal of a number of mycotoxins from the digestive tract.
Inorganic adsorbents include natural (zeolites, clinoptilolite, bentonites, diatomites, sepiolite, illite, kaolinite) and synthetic (potassium, sodium, aluminium silicates).

Inorganic adsorbents

- Low cost; can bind vitamins, amino acids, and enzymes in the animal body.
- The majority of inorganic adsorbents are only used against aflatoxins.

Adsorbing agents include β-glucans of cell walls of inactivated yeast and algae, as well as lignin and carbon.

- Mycosorb A+ is based on algae cells and glucans of yeast cells.
- Novasil Plus (BASF): aflatoxins.

Biotransformation: effects of specific enzymes or agents on mycotoxins. Toxin destruction occurs directly in the gastrointestinal tract.

Mycofix Plus 5.0 and Mycofix Select 5.0: ochratoxin A is deactivated by enzymatic cleavage of the amide bond in the toxin; fumonisin B molecules are deactivated by cleavage of diether bonds; trichothecenes are deactivated by cleavage of epoxy group bonds; and zearalenone is deactivated by cleavage of the lactone ring.

Novus uses a specific agent: modified lecithin.

The use of pectins as organic sorbents, a complex of microorganisms suppressing Clostridium bacteria and mold fungi, an enzyme complex responsible for toxin biotransformation, and the use of milk thistle as a hepatoprotector ensure the efficacy and high demand for BACITOKS-2.0 (RDC BIO LLC).

Lutein, known for its hepatoprotective properties, is used in the complex product Toxiban Max (Novus Int.) alongside adsorbing (bentonite) and biotransforming components (modified lecithin) to help animals and poultry resist mycotoxins and their harmful effects on the body.
Some manufacturers use essential oils (Zaslon by Biotroph) or a complex of whole plant components (Unike Plus by Nutriad, Escent by Innovad) in complex products.

Probiotics with antifungal properties are a promising area of environmentally friendly animal husbandry. For example, mycotoxin neutralizer Fungistat (Elest) contains spores of Bacillus subtilis bacteria, which inhibit the development of molds, while producing amino acids and vitamins in the gastrointestinal tract.
Mycotoxin neutralizers may contain organic acids and their salts, which disrupt the energy balance, protein synthesis, and enzyme synthesis in mold cells, resulting in their death (AtoxBio Plus by TeknoFeed, Excential Toxin Plus by ORFFA). Mycosoft (AgroSystem RPC) contains humic acids and fumaric acid.
Chitosan is a powerful organic sorbent. Chitolose (Elest) is based on chitosan.
Complex products of the Toxy-Nil family (Nutriad, Belgium) containing clay minerals (aluminum silicates, bentonites, sepiolites, etc.), yeast cell walls, organic acids, antioxidants, and phytobiotics are effective at deactivating mycotoxins.
The feed additive Apsabond (Andres Pintaluba) contains three types of purified natural clays and inactivated yeast cells. Excential Toxin Plus (ORFFA) has five activity levels and combines several mineral adsorbents (phyllosilicates and tectosilicates) for sorption of polar mycotoxins, MOS for sorption of nonpolar mycotoxins, ammonium propionate with fungistatic properties, and betaine. The experts of AgroBalt Trade have developed Amigo, a product containing two adsorbents with complementary specificity for binding specific groups of mycotoxins.
Foreign manufacturers of mycotoxin neutralizers: Alltech, BIOMIN, BASF, Nutriad, OLMIX, Daavision, GRASP, Kemin, CEVA, Impextraco, Daavision, Biochem, Liptosa, Andres Pintaluba, ORFFA, etc.
Domestic manufacturers of mycotoxin neutralizers: Agroakademiya LLC, AgroSystem RPC, Mustang Feeding Technologies, AgroBalt Trade, Biotroph, BIOROST, VitOMEK, RDC BIO, Sibbiopharm, TeknoFeed, Elest, Ekokremniy, etc.

Conclusions

Mycotoxins are naturally occurring toxic substances produced by certain species of mold fungi.

- Mold can appear both before and after harvesting, during storage, and/or on finished food products under favorable temperature conditions and high humidity.
- Most mycotoxins are chemically stable and are not destroyed by heat treatment.

Among the hundreds of known mycotoxins, the most common and dangerous to human and livestock health include aflatoxins, ochratoxin A, patulin, fumonisins, zearalenone, and nivalenol/deoxynivalenol.

The following is recommended to reduce the animal health risks associated with mycotoxins:

- Analyzing incoming grain and feed for mycotoxins
- Monitoring the primary manifestations of pathogens producing mycotoxins
- Preventing damage to grain before and during drying and storage, as damaged grain is more susceptible to mold infestation and thus to mycotoxin contamination
- Avoiding prolonged storage of feed prior to use
- Storing grain with low moisture content
- Creating optimal conditions for feed storage
- In the presence of mycotoxin-producing fungi and mycotoxins in the feed: treating grain with mold inhibitors and adding adsorbents and neutralizers to the diet of animals.

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Thank you!