Molds and Mycotoxins in Grains During Storage, Distribution and Processing

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Food Safety

- Ensuring that the food supply is safe, is increasingly becoming a critical issue in every society.
- Despite many significant advances in science and technology, food-borne illnesses continue to be a major public health issue around the globe.
- Foods and crops can be a vehicle of many hazards, namely bacterial pathogens, viruses, parasites and natural toxins such as mycotoxins.

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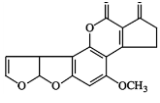
Mycotoxins - Definitions



- **"Mycotoxins** comprise a structurally diverse family of naturally occurring, fungal-elaborated toxins, many of which have been strongly implicated as chemical precursors of toxicity in humans and animals." (CAST, 2003)
- The term **mycotoxin** is derived from the Greek word "***mykes***" which means fungus and the Latin word "***toxicum,***" which means toxin, or poison.
- **Mycotoxigenic fungi** - Fungi capable of producing mycotoxins.
- **Mycotoxicoses** - Diseases caused by ingestion of foods containing mycotoxins.

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Mycotoxin-Producing Mold Genera

- *Aspergillus*
- *Penicillium*
- *Fusarium*
- *Claviceps*
- *Stachybotrys*
- *Alternaria…*

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Characteristics of Fungi

- Eukaryotic
- Heterotrophic
- Propagate by spores (sexual and/or asexual)
- Have cell walls of chitin, other polysaccharides, protein and lipids

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Important Mycotoxins / Mycotoxigenic Fungi Associated with Grains

Mycotoxin	Fungi
Aflatoxins	*Aspergillus flavus, A. parasiticus*
Fumonisins	*Fusarium verticillioides, F. proliferatum*
Deoxynivalenol	*F. graminearum, F. culmorum*
Zearalenone	*F. graminearum*
Ochratoxins	*A. ochraceus, A. carbonarius, Penicillium verrucosum*
Moniliformin	*F. proliferatum, and other Fusarium* species

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General Concerns about Fungi

- Food Spoilage
- Mycotoxins
- Fermentations
- Food Allergies
- Infections…

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Rating health risks from foods

High ↑ Low

Acute	Chronic
Microbiological	**Mycotoxins**
Phycotoxins	Anthropogenic contaminants
Some phytotoxins	Some phytotoxins
Mycotoxins	Unbalanced diet
Anthropogenic contaminants	Phycotoxins
Food additives	Microbiological
Pesticide residues	Food additives
	Pesticide residues

(Source: Kuiper-Goodman, 1998)

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Economic Effects of Mycotoxins in Grains

Mycotoxin contamination of grains affects all sectors, from production to consumption, economically:

- **Grain producers**: Reduction in yields, restricted markets and price discounts.
- **Grain handlers**: Restricted storage options, cost of testing and loss of end markets.
- **Grain processors**: Product losses, monitoring costs, and restricted end markets.
- **Consumers**: Health effects of mycotoxins and paying higher prices.
- **Society**: High cost due to regulations, research, low export costs, and high import costs.

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Mycotoxins - History

- Toxigenic molds have caused food safety problems for as long as foods and crops have been harvested and stored.
- Intensive scientific studies of toxigenic molds and mycotoxins have only been undertaken since 1960.

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Mycotoxins-History

Ergotism (St. Anthony's Fire) – France in 943A.D.
- Alkaloids produced by *Claviceps purpurea*

Alimentary Toxic Aleukia – Russia, WW II
- *Fusarium* toxins

Yellow Rice – Japan, 1940s
- *Penicillium* toxins

Turkey X Disease, England, 1960
- Discovery of aflatoxins

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Acute Aflatoxicosis in Kenya

"*Between **April and September 2004**, one of the largest known aflatoxicosis outbreaks occurred in Kenya, **with 317 reported cases and 125 deaths**. A less severe outbreak occurred in **2005, resulting in 16 deaths**. Food samples collected from households in the affected areas contained high levels of aflatoxin B_1 (20 to > 1,000 ng/g), suggesting that the outbreak was caused by acute aflatoxin poisoning. The outbreak resulted from aflatoxin contamination of locally grown maize that was stored under damp conditions*."

(Mwancha N. Okioma, 2008)

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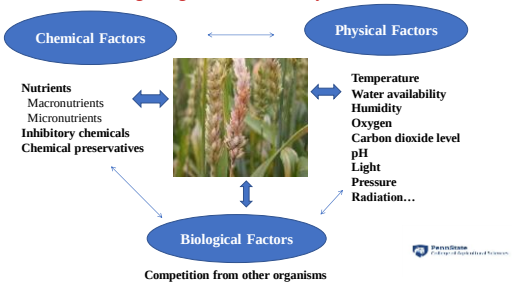
Factors Affecting Fungal Growth and Mycotoxin Production



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Factors Affecting Fungal Growth and Mycotoxin Production
Pre-Harvest Conditions

- Soil fertility, seed quality, plant variety
- Plant density in the field
- Presence of other plant diseases
- Geographical regions
- Crop rotation
- Planting time, temperature, rain, humidity
- Drought stress
- Insect infestation…

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Factors Affecting Fungal Growth and Mycotoxin Production
Harvest and Post-Harvest Conditions

- Climate (temperature, moisture…)
- Types of grains (Content in carbohydrates, proteins, fat…)
- Crop damage (insects, mechanical)
- Timeliness (Grains should be harvested at their optimum maturity)
- Cleanup
- Drying (Not storing grains with more than 12% moisture)
- Use of antifungal agents

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General Conditions for Germination of Spores and Mold Growth

- Relative humidity (RH) over 70%
- High moisture content of the crops (20% or higher in corn)
- Temperatures over 30°C (86°F) are generally needed for several days
- Stress to the crops: drought, flooding, or insect infestation

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Water Activity (a_w)

$a_w = P/P_0 = ERH/100$

P = Partial pressure of water vapor in food

P_0 = Vapor pressure of pure water at same temperature

ERH = Equilibrium Relative Humidity (%)

Range: 0-1

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Minimal Water Activities for Growth of Selected Fungi

Species	Minimal Aw for growth
Aspergillus flavus	0.78 – 0.8
Aspergillus ochraceus	0.76 – 0.83
Byssochlamys nivea	0.84 – 0.92
Cladosporium herbarum	0.85 – 0.88
Fusarium graminarium	0.89
Penicillium expansum	0.82 – 0.85
Xeromyces bisporus	0.61

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Physiological Characteristics of Fungi

- **Water activity and pH**

Foods with a_w <0.95 will mostly be spoiled by fungi. Foods with a_w< 0.85 will be spoiled predominately by xerophilic fungi.

Fungi grow over wide range of pH, commonly 2-8. Below pH 4.0, yeasts and molds are generally the dominant contaminants.

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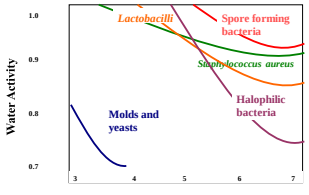

A schematic diagram of the combined influence of pH and a_w on microbial growth

(Pitt and Hocking, 1999)

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Field Fungi

- Field fungi invade grain kernels while they are developing on the plant.
- Principal field fungi:
  - *Alternaria*
  - *Fusarium*
  - *Cladosporium*
  - *Botrytis…*
- Field fungi require ERH of 90-100%

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Storage Fungi

- **Storage fungi** grow at lower Water Activity (a_w).
- **Xerophilic fungi** are capable of growth at a_w below 0.85.
- **Storage Fungi**
  - *Aspergillus* species (*A. flavus*, *A. ochraceus*, *A. candidus*, *A. restrictus*…)
  - *Penicillium* species

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Major Mycotoxin-Producing Mold Genera in Cereal Grains

- *Aspergillus*
- *Penicillium*
- *Fusarium*

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Negative Aspects of Aspergilli

- Degradation of agricultural products before and after harvest
- Production of mycotoxins such as aflatoxins, ochratoxins, citrinin, sterigmatocystin…
- Some of the Aspergilli species are allergenic
- Some cause infections known as aspergilloses in humans and animals. *Aspergillus fumigatus* causes most of these infections, followed by *A. flavus*, *A. terreus*, and *A. ochraceus*.

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Penicillium Species

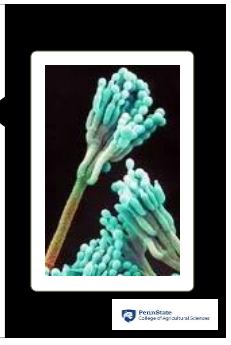
Penicillium species are the most diverse fungal group, in terms of the number of species and range of habitats.

Ubiquitous

Some species are more specialized (e.g., *P. expansum*)

Many are psychrotophic

More difficult taxonomy by comparison with the other mold groups



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Fusarium species

- *Fusarium species* are characterized by the production of septate, fusiform to crescent shaped cells – Macroconidia
- Some *Fusarium* species also produce smaller 1-2 celled conidia called microconidia.
- *Fusarium* colonies are characterized by intense colors: white, cream-colored, yellow, brown, pink, red or violet.

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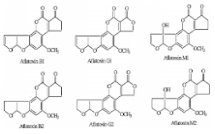
Aflatoxins

Aflatoxins: B_1, B_2, G_1, G_2, M_1, M_2

Occurrence:

Occur in a wide range of foods including peanuts, corn, pistachios, spices, figs…



Produced by:

Aspergillus flavus and *Aspergillus parasiticus.*

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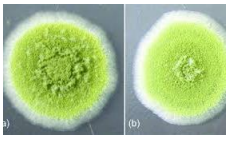
Aflatoxins



- Turkey-X Disease. Occurred in England in 1960.
- Large number of Turkey poults and duckling died after the consumption of peanut meal feed contaminated with the mold, *Aspergillus flavus*.
- Analysis of the feed revealed the presence of fluorescent compounds named a**fla**toxins for ***Aspergillus flavus*** **toxins.**

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Toxicological Characteristics of Aflatoxins

- Potent human hepatocarcinogen
- Dose effects
  - **High doses:** lethal if consumed - lung, myocardial and kidney tissues
  - **Sub-lethal doses** cause chronic toxicity, e.g., liver cirrhosis
  - **Low level exposure:** human hepatocellular carcinoma (liver cancer)
- Teratogenicity (Embryonic abnormalities)
- Impaired growth in children
- Immunosuppression

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Ochratoxin A

- Produced by: *A. ochraceus*, *A. carbonarius*, and *P. verrucosum*…
- Toxicology: Necrosis of the cells in the kidneys and liver, teratogen, carcinogen
- Detected in: beans, peanuts, barley, coffee beans, grapes…

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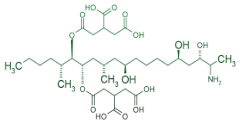
Fumonisins

- Produced by: *Fusarium* spp (*F. moniliforme*, *F. proliferatum*…)
- Toxicology: Equine leucoencephalomalacia, liver cancer, oesophageal cancer
- Detected in corn



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Deoxynivalenol (DON) (Commonly known as Vomitoxin)

- Produced by: *F. graminarium*, *F. culmorum*
- Toxicology: Haematotoxic, immunosuppressive, in animals vomiting, feed refusal and diarrhea, immunological issues and other symptoms. Swine are aften the most susceptible animals.
- Commonly affect wheat (Fusarium Head Blight)

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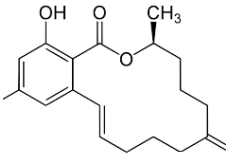
Zearalenone

- Produced by *Fusarium graminearum*.
- Found in corn, moldy hay and feed.
- Affect swine, dairy cattle, chicken, turkey, guinea pigs and other animals.
- Toxicity: Estrogenic effects, atrophy of testicles, atrophy of ovaries, enlargement of mammary glands and abortion.
- Production of zearalenone is favored by low temperatures coupled with a high humidity.



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Control and Management of Mycotoxins Processing and Manufacturing

Good Manufacturing Practices (GMPs) in processing of cereal grains
- Control of temperature and humidity
- Removal of damaged kernels

Strict Quality Control
- Frequent analysis of mycotoxins (Aflatoxin) is performed

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Control and Management of Mycotoxins Detoxification Methods

Physical methods of separation
- Mechanical separation
- Density segregation (Corn, Peanuts…)
- Dry Milling
  - Distribution of aflatoxins low in grits and high in the germ and hull
  - Removal of hulls of corn kernels reduced fumonisins significantly
- Wet Milling
  - Wet milling of corn resulted in aflatoxin mainly in steep water and fiber, and less in gluten and germ

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Control and Management of Mycotoxins Detoxification Methods

Physical methods of detoxification

- Thermal inactivation: Mycotoxins are heat-stable. Degradation of aflatoxins is function of temperature, heating time and moisture content.
  - Cooking and boiling can reduce aflatoxin: boiling (by 28%), Frying (by 34-53%) and alkaline cooking (by 20-90%).
  - Roasting resulted in the reduction of aflatoxin content in nuts, oilseeds and corn.
  - Baking temperatures did not reduce aflatoxin level in the dough.
- Irradiation
  - Gamma irradiation did not degrade aflatoxin.
  - Exposure of peanut oil to UV light resulted in significant reduction in the levels of aflatoxin.

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Control and Management of Mycotoxins Detoxification Methods

Biological methods

- Microbial inactivation
  - *Flavobacterium aurantiacum*
  - Lactic acid bacteria (*Lactobacillus* spp.)
- Fermentation
  - Fermentation of contaminated grains resulted in degradation of aflatoxins and Ochratoxin A.

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Control and Management of Mycotoxins Detoxification Methods

Chemical methods

- Ammoniation
- Ozonation
- Nixtamalization
- Addition of hydrogen peroxide
- Addition of sodium bicarbonate

Adsorption techniques

- Addition of Chemisorbents to animal feed: Zeolite, Hydrated Sodium Calcium Aluminosilicate (HSCAS)

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Preventing Fungal Spoilage

- Good manufacturing practices (GMPs)
- Monitoring and reduction of the level of fungi in raw materials
- Reduction of water activity
- Reduction of oxygen level
- Lowering pH
- Addition of preservatives
- Heat processing with aseptic packaging

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Control of Mold Growth in Cereal Grains

- Dry crop to a_w below 0.70
- Cereals should be harvested at maturity
- Cereals should be dried as quickly as possible
- Avoid damaging grains during harvesting and handling
- Maintain uniform low temperature during storage by using artificial aeration
- Use controlled atmosphere storage (20% carbon dioxide or higher)
- Use preservatives (e.g., propionic acid, acetic acid) especially for animal feed

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Effect of Modified Atmosphere on Fungi

- Some storage fungi are capable of growth at low partial pressure of oxygen.
- High carbon dioxide levels are more inhibitory to mold growth.
- The degree of inhibition is affected by temperature and moisture content.
- Atmospheres high in carbon dioxide are more effective in controlling mold growth than those which exclude oxygen by replacement with nitrogen.
- Many spoilage molds are capable of growth at oxygen levels < 1%.
- Atmospheres with about 20% carbon dioxide generally inhibit mold growth.
- To prevent fungal deterioration of high moisture commodities more than 80 % carbon dioxide is needed.

(A.D. Hocking, 1990)

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Effect of Modified Atmosphere Storage on:

Corn

- MA storage could be used for temporary holding of high moisture corn before drying
- Most fungi are not killed by low oxygen atmospheres
- Safe storage of high moisture corn is limited

Wheat

- Wheat is usually drier than corn
- *A. flavus* and *Fusarium* spp. cause fewer problems in wheat
- Higher levels of carbon dioxide (>50%) are more effective. At 80% CO_2 there is almost complete inhibition of molds
- Tolerant species: *Eurotium* species and *A. candidus*

Rice

- Most storage fungi are inhibited at atmospheres of < 1% O_2 at Aw of 0.87 or lower
- Under 100% CO_2 or N_2 there is no fungal growth
- At Aw of 0.90 or higher bacteria like lactic acid bacteria proliferate

(A.D. Hocking, 1990)

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Effect of Modified Atmosphere Storage on Mycotoxin Production

Aflatoxins

- Increase in the concentration of CO_2 causes a decrease in the aflatoxin formation

Ochratoxin

- Ochratoxin production was found to be completely inhibited at 30% or more CO_2, regardless of oxygen level

Patulin

- Some studies have shown that patulin was inhibited at 20% CO_2

"Mycotoxin production is more sensitive than fungal growth to MA conditions, but may still occur if other conditions (Temperature, Aw) are favorable." (A.D. Hocking, 1990)

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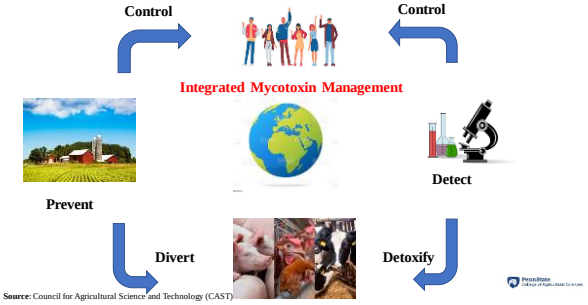

Source: Council for Agricultural Science and Technology (CAST)

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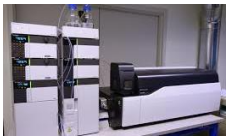
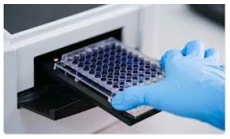
Detection Methods of Mycotoxins

- Sampling
- Extraction
- Cleanup
- Detection
  - Enzyme-Linked Immunosorbent Assay (ELISA)
  - High-Performance Liquid Chromatography (HPLC)
  - Liquid Chromatography-Mass Spectrometry (LC/MS)
  - Gas Chromatography
  - Thin Layer Chromatography (TLC)





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Advantages and Disadvantages of Methods for Mycotoxin Analysis

Method	Advantages	Disadvantages
TLC	• Simple, inexpensive and rapid • Can be used for screening • Simultaneous analysis of multiple mycotoxins	• Poor sensitivity • Poor precision • Adequate separation may require 2-dimensional analysis • Quantification only when used with a densitometer
GC	• Simultaneous analysis of multiple mycotoxins • Good sensitivity • May be automated • Provides confirmation	• Expensive equipment • Derivatization is required • Matrix interference problems
HPLC	• Good sensitivity • Good selectivity • Good repeatability • May be automated • Short analysis time • Official methods available	• Expensive equipment • Specialist expertise required • May require derivatization

(Pascale & Visconti, 2008)

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Advantages and Disadvantages of Methods for Mycotoxin Analysis

Method	Advantages	Disadvantages
LC/MS	• Simultaneous analysis of multiple mycotoxins • Good sensitivity • Provide confirmation • No derivatization required	• Very expensive • Specialist expertise is needed • Calibration curve
ELISA	• Simple sample preparation • Inexpensive equipment • High sensitivity • Simultaneous analysis of multiple samples • Suitable for screening • Limited use of organic solvents • Visual assessment	• Cross-reactivity with related mycotoxins • Matrix interference problems • Possible false positive/negative results • Confirmatory analysis required • Semi-quantitative

(Pascale & Visconti, 2008)

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FDA Action Levels for Aflatoxin in Human Food, Animal Feed and Animal Feed Ingredients

Intended Use	Grain, Grain By-Products, Feed or other Products	Aflatoxin Level [parts per billion (p.p.b.)]
Human consumption	Milk	0.05 (Aflatoxin M_1)
Human Consumption	Foods, peanuts and peanut products, brazil and pistachio nuts	20
Immature animals	Corn, peanut products, and other animal feeds and ingredients, excluding cottonseed meal	20
Dairy animals, animals not listed above, or unknown use	Corn, peanut products, cotton seeds, and other animal feeds and ingredients	20
Breeding cattle, breeding swine, and mature poultry	Corn and peanut products	100
Finishing swine 100 pounds or greater in weight	Corn and peanut products	200
Beef, cattle, swine or poultry regardless of age or breeding status	Corn, peanut products, cotton seed meal	300

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FDA Advisory Levels for Vomitoxin

Intended Use	Grain or Grain By-Products	Vomitoxin Levels in Grains or Grain By-Products and Complete Diet** (parts per million (p.p.m.)]
Human Consumption	Finished wheat products	1 p.p.m.
Swine	Grain and grain by-products not to exceed 20% of diet	5 p.p.m. (1 p.p.m.)
Chickens	Grains and grain by-products not to exceed 50% of diet	10 p.p.m. (1 p.p.m)**
Ruminating beef and beef lot cattle older than 4 months	Grain and grain by-products*	10 p.p.m (5 p.p.m.)**
Ruminating dairy cattle older than 4 months	Grain and grain by-products not to exceed 50% of diet*	10 p.p.m. (10 p.p.m.)**
Ruminating beef and feed lot cattle older than 4 months, and ruminating dairy cattle older than 4 months	Distillers grains, brewers grains, gluten feeds, and gluten meals	30 p.p.m. (10 p.p.m. beef/feedlot)** (5 p.p.m dairy)**
All other animals	Grain and grain by-products no to exceed 40% of diet	5 p.p.m. (2 p.p.m.)**

*88% dry matter basis **Complete diet figures shown within parentheses.

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FDA Guidance Levels for Fumonisin for Corn and Corn Products Intended for Human Food

Product	Total Fumonisins (FB1, FB2 and FB3) [parts per million (p.p.m.)]
Degermed dry milled corn products (e.g., flaking grits, corn grits, corn meal, corn flour with fat content of <2.25%, dry weight basis)	2
Cleaned corn intended for popcorn	3
Whole or partially degermed dry milled corn products (2.g., flaking grits, corn grits, corn meal, corn flour with fat content of equal or more than 2.25% dry weight basis)	4
Dry milled corn bran	4
Cleaned corn intended for masa production	4

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FDA Guidance Levels for Fumonisin in Animal Feed

Class of Animal	Grain or grain By-Product	Total Fumonisins (FB1, FB2 & FB3) Levels in Grain or Grain By-Products and (Complete Diet) [parts per million (p.p.m.)]
Equids and Rabbits	Corn and corn by-products not to exceed 20% of the diet	5 (1)
Swine and Catfish	Corn and corn by-products not to exceed 50% of the diet	20 (10)
Breeding Ruminants, Breeding Poultry and Breeding Mink	Corn and corn by-products not to exceed 50% of the diet	30 (15)
Ruminants > 3 Months Old being Raised for Slaughter and Mink being Raised for Pelt Production	Corn and corn by-products not to exceed 50% of the diet	60 (30)
Poultry being Raised for Slaughter	Corn and corn by-products not to exceed 50% of the diet	100 (50)
All Other Species or Classes of Livestock and Pet Animals	Corn and corn by-products not to exceed 50% of the diet	10 (5)

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Regulations of mycotoxins in grains in Ukraine and European Union (Maximum levels, ppb)

Crop	Mycotoxin	Ukraine	European Union
Wheat	Aflatoxin B_1	2	2
Barley	Ochratoxin A	5(25-100)*	5
	DON (Vomitoxin)	1250	1250
	Zearalenone	100	100
Soybean	Aflatoxin B_1	5(25-100)	4
	Zearalenone	1000 (1000)	2 (0.1-0.5)
	T-2 toxin	100 (200)	-
	DON (Vomitoxin)	500 (1000-2000)	8 (0.9-2)
	Ochratoxin A	5	0.25 (0.05-0.1)
Oat	DON (Vomitoxin)	1750	1750
	Zearalenone	100	100
Corn	Ochratoxin A	2	5
	T-2 toxin	100 (200)	3 (0.1-0.5)
	DON	750 (1250)	750 (1250)
	Fumonisins	1000 (4000)	1000 (4000)
	Zearalenone	350	350

*Animal feed figures are shown within parentheses. Information for soybean was obtained from Ukrainian standards;
(-) Not regulated.

Assembled by Dr. Nataliia Voloshchuk (NULES).

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Thank You

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