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FUNGI AND MYCOTOXINS

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Mycotoxins represent nature at its most malevolent.

- They are toxic substances produced by fungi.
- Production is based on complex genetics (multiple genes involved).
- Production entails complex intermediary metabolism in the fungus.
- Mycotoxins are generally of no known use to the fungi that produce them.
- Metabolism of mycotoxins in the host is complex.
- Mycotoxins show multiple modes of pathogenesis (cause *mycotoxicoses*).
- Toxins tend to be processed by the liver for elimination via the kidneys. Some toxins are metabolically activated in the liver, rather than detoxified. Chronic exposures to mycotoxins often damage the liver, the kidneys, or both.

Toxigenic fungi

- Three genera (*Aspergillus*, *Fusarium*, and *Penicillium*) include many toxigenic species, but many other genera also include one or more toxigenic species.
- Not all members of a given species are toxigenic — even the most infamous species may include nontoxigenic strains.
- General classification is as *field fungi* vs *storage fungi*, depending on whether the fungus is most likely to enter the picture before or after harvest.
- Substrates are generally plant materials, especially grains.
- Molds must grow in the food or feed substrate in order to produce mycotoxins.
- Factors that govern mycotoxin production by a toxigenic mold include:
  - In the field — weather conditions, plant stress, invertebrate vectors, species and spore load of infective fungi, variations within plant and fungal species, and microbial competition
  - In stored grain and other products — water activity (a_w), temperature, crop damage, time, blending with moldy components, and a number of chemical factors, such as aeration (O_2, CO_2 levels), types of grains, pH, and presence or absence of specific nutrients and inhibitors

Genus *Aspergillus*

- The aspergilli are both field and storage fungi. They produce some very important toxins that threaten both human and animal health.
- The **aflatoxins** are produced primarily by *A. flavus* and *A. parasiticus*, but also by a few other *Aspergillus* species. The name, aflatoxin, is a contraction of *A. flavus* (+ "toxin").
  - They occur most often in peanuts, corn, cottonseed and other oilseeds, cereals, figs and other dried fruits, most tree nuts, milk, and sorghum. Optimal temperatures and a_w for the growth of *A. flavus* and *A. parasiticus* are around 35–37°C (range 6–54°C) and 0.95 (range 0.78–1.0), respectively; for aflatoxin production, they are 28–33°C and 0.90–0.95 (0.83–0.97), respectively.

- They are hepatotoxins, carcinogens, mutagens, and teratogens. Destruction of liver cells by these toxins can lead to death. In areas of the world where hepatitis B is endemic, the two agents combine to cause a high incidence of liver cancer.
- The most prevalent types are B1 & B2 (fluoresce *blue* on chromatograms), G1 & G2 (fluoresce *green* on chromatograms), and M1 (shed in *milk*). B1 is the most toxic. M1 is of special concern because it is shed in the milk of dairy animals fed aflatoxin-containing grains and is perceived as a threat to the health of children.
- Some aflatoxin levels (equilibrated to parts per billion of aflatoxin B1) and their effects are:

0.5	FDA action level in meat and milk.
1.0	Produces liver tumors in trout.
15	Produces liver tumors in rats.
20	Cutoff for interstate grain shipment
50	Maximum level in dairy feed to avoid residues in milk.
100	Produce illness in calves, chicks, piglets.
200	Adult pigs not affected.
300	Produce liver damage in young swine leading to impaired growth and productivity. Adolescent calves not affected.
450	Reproductive performance in adult swine not affected.
500	Liver damage in dogs.
600	Decreased productivity in dairy cows. Liver damage in feeder cattle.
1000	Acute poisoning in adult swine.

- LD_{50} in mg/kg body weight differs for various species — rabbits, 0.3; ducklings, 0.34; mink, 0.5–0.6; cats, 0.55; pigs (6–7 kg size), 0.6; trout, 0.8; dogs, 1.0; guinea pigs, 1.4–2.0; sheep, 2.0; monkeys, 2.2; chickens, 6.3; rats, 5.5 for male, 17.9 female; mice, 9.0; and hamsters 10.2.
- They are very heat resistant and are difficult to remove from foods and feeds. Alkali treatment (including ammoniation) is useful in some situations.

- The **ochratoxins** are produced by *A. ochraceus* (for which they were named), as well as several other species of aspergilli and penicillia (especially *Penicillium viridicatum*).
  - Most ochratoxin producers are storage fungi. They can grow in a range 4–37°C and at a_w as low as 0.78, but optimal conditions for toxin production are narrower, with temperature at 24–25°C and a_w values >0.97 (minimum a_w for ochratoxin A production is about 0.85).
  - Ochratoxins are most associated with cereal grains and seeds — barley, beans, cereals, coffee, feeds, maize, oats, rice, rye, wheat. They may also occur in products derived from animals that were fed ochratoxin-containing feeds.
  - Ochratoxins are potent nephrotoxins and teratogens. They have caused health problems in humans and swine in Europe, but have not been a major problem in US foods. The oral LD_{50} in rats is approximately 22 mg/kg for ochratoxin A which is the most toxic of the ochratoxins. Monogastric animals are most sensitive (horses, swine, poultry, young ruminants). Adult ruminants are less sensitive (rumen protozoa hydrolyze the peptide bond of ochratoxin). Levels of 2–40 ppm ochratoxin in feed were toxic to young ruminants. A lethal dose was 0.2 mg/kg body weight in dogs

over a 14-day period.

- **Sterigmatocystin** is a naturally occurring hepatotoxic and carcinogenic mycotoxin produced by fungi in the *Aspergillus*, *Bipolaris*, and *Chaetomium* genera and *Penicillium luteum*. It is a precursor in the metabolic pathway to aflatoxin production, but is toxic in its own right, though not as potent as aflatoxin B1. Several other minor toxins are produced by aspergilli.

Genus *Fusarium*

- The fusaria are often field fungi. Their most dangerous toxins are the fumonisins and trichothecenes.
  - **Fumonisins** are a group of toxic metabolites produced primarily by *Fusarium verticillioides* (previously *F. moniliforme)*, one of the most common fungi colonizing corn throughout the world. Fumonisins are most frequently found in corn, corn-based foods, and other grains (such as sorghum and rice). Toxigenesis usually begins in the field, but may continue in grains in storage. The fumonisins are carcinogens and hepatotoxins that may also cause cardiovascular, neurological, and respiratory problems. They are probably more injurious to farm animals than to humans. Guidelines for fumonisin levels in feeds to be used for various animals were established by the US FDA in 2000. Fumonisin B1 levels are limited to 5, 10, 20, 30, 60, and 100 ppm in corn and corn by-products to be used for horses and rabbits, "other" (dogs, cats, etc.), catfish and swine, breeding stock (ruminants, poultry and mink), ruminants and mink, and poultry, respectively. The fumonisin B1 levels are limited to 1, 5, 10, 15, 30, and 50 ppm in the total ration for the above animal species, respectively (e.g., 1 ppm for horse and rabbit).
  - A well-documented **trichothecene** incident involved the disease called alimentary toxic aleukia (ATA) that resulted in more than 5000 deaths in humans in the Orenberg district of the USSR during World War II. The cause of this outbreak was later determined to be trichothecenes produced by fungi growing on grain allowed to stand in the field during winter.
    The most important trichothecenes are deoxynivalenol, from *F. graminearum* and other species; T-2 toxin, from *F. sporotrichioides*, etc.; and zearalenone, from *F. graminearum*, etc.
    Deoxynivalenol is a neurotoxin most associated with corn.
    T-2 toxin is involved in ATA and affects many parts of the body; it is most associated with corn, feeds, and hay.
    Zearalenone is a genitotoxin that has estrogenic potency, as well as a teratogen; it is most associated with cereals, corn, feeds, and rice. It causes animal reproductive problems, particularly in swine.
- Many other toxins are produced by this genus, and some of the toxins discussed are also produced by members of other genera.

Genus *Penicillium*

- The penicillia are principally storage fungi.

- Cyclochlorotine, luteoskyrin, and rugulosin have long been considered to have been involved in the “yellow rice disease” during World War II. They are hepatotoxins and also produce hepatomas in test animals.
- Several other mycotoxins, including **patulin**, penicillic acid, citrinin, cyclopiazonic acid, citreoviridin, and xanthomegnin, which are produced primarily by several species of penicillia, have attracted some attention because of their frequent occurrence in foods.
- Patulin is often found in damaged apples, apple juice, apple cider and sometimes in other fruit juices and feed. Its highly reactive double bonds readily react with sulfhydryl groups in foods, so patulin is not very stable in foods containing these groups. Nevertheless, patulin is considered a health hazard to humans. At least 10 countries have regulatory limits, most commonly at a level of 50 μg/kg, for patulin in various foods and juices.
- Penicillic acid has been detected in “blue-eye corn” and meat.

Other, **miscellaneous fungi**

- The mycotoxins named above, and many others, can also be produced by members of other fungal genera.
- Conditions for toxigenesis, and the foods and feeds targeted, vary widely.

Ergot, a historic threat

- Ergotism is a human disease that results from ingestion of the ergot body, the sclerotium of the fungus, in rye or other grains infected by members of the genus *Claviceps*. Two types of ergotism have been documented.
  - In the convulsive type, the affected persons have general convulsions, tingling sensation of muscles, and sometimes the entire body is racked by spasms. Epidemics occurred between 1581 to 1928 in European and other countries. Although it has been suggested that the consumption of ergots that cause convulsive ergotism played a role in "Salem Witchcraft" incidents, this is still a controversial issue.
  - In the gangrenous type of ergotism, the affected parts became swollen and inflamed with violent, burning pains, hence, the "Fire of St. Anthony." In general, the affected area became numb first, turned black, then shrank, and finally became mummified and dry. Outbreaks occurred in the Middle Ages and into the 19th century. In some areas of France, grain contained as much as 25% ergots, and patients died as a result of ingestion of ~100 g of ergots over a few days. Between 1770–1771, about 8000 people died in one district alone in France.
- *Claviceps purpurea* is the principal agent of ergotism. Other potential producers include *C. paspali*, *C. cinerea* and *C. fusiformis*. The fungus invades cereal grains and pasture grasses under high-moisture conditions. The developing hyphae produce sclerotia which fill the florets and protrude being 2–20 mm long with a distinct purple-black color. The sclerotia contain many ergot alkaloids. Some of these are derivatives of lysergic acid.
- As little as 0.2% by weight of ergot sclerotia in grain can cause mild symptoms in humans. Usually, 2% ergots in grain is sufficient to cause an epidemic. European and most other countries have a regulatory limit of 0.1–0.2% ergots in flour. The suggested maximum level for safety is 0.05%. In the US, wheat or rye with 0.3% sclerotia is considered unsafe and oats, triticale, or barley having more than 0.1% sclerotia are

classified as ergoty and unfit for consumption by man. Ergots can be mechanically separated from grain with reasonable effectiveness, if the needed equipment is available.

- Typical gangrenous ergotism occurred in an Ethiopian epidemic in 1978 from ergot-infested oats. Approximately 50% of the affected persons died. Similar problems occurred in India in the 1970's due to infestation with *Claviceps fusiformis*.

Mushrooms — food or poison?

- Mushrooms have been a very special category of food for a very long time. The National Museum of the Czech Republic, in Prague, has an entire department devoted to mushrooms, principally for their gastronomic and toxicological significance.
- Although several species of mushrooms are cultivated for human consumption and others are harvested in the wild and eaten, a significant number of mushroom species are highly toxic.
- Important toxic mushrooms in the US are ***Amanita phalloides*** **(Death Cap)**, *A. ocreata* (Death Angel), as well as *A. virosa* (Destroying Angel), *A. verna* (Destroying Angel), and *A. bisporigera* (Destroying Angel). The latter three species are found in the Eastern US in hardwood forests.
  - *Amanita phalloides* contains 2–3 mg of amatoxins per gram of dry tissue. A single mushroom can kill an adult human. The amatoxins are cyclic octapeptides, while the related phallotoxins are cyclic heptapeptides. The phallotoxins are much less toxic than the amatoxins, perhaps due to poor absorption from the GI tract.
  - The mechanism of action of amatoxins is inhibition of **RNA-polymerase**. This is a key component for the transfer of DNA information to transfer RNA. The result of inhibition halts protein synthesis in the cell that leads to cell death of the intestinal epithelium and liver tissue. Liver failure follows.
  - Ingestion of the amatoxin-containing mushroom results in vomiting, nausea, abdominal pain, and bloody diarrhea that develops within 6–24 hours. These signs may lessen for a short period of 12–24 hours followed by confusion, delirium, seizures, and coma. Within 3–4 days, patients develop icterus (yellow skin), low blood sugar (hypoglycemia), bleeding (coagulopathy), changes in urine flow (renal failure), and sepsis (bacterial infections). The mortality rate in humans is 10–40%, due to the variation in the amount of mushrooms eaten. In patients who become comatose (unconscious), death usually follows.
- ***Amanita muscarina*** is called the fly agaric and is characterized by muscarine, which affects the autonomic nervous system. Within a few minutes to a few hours of consumption of mushrooms containing these toxins, the patient will experience perspiration, salivation, and lacrimation (PSL) syndrome, blurred vision, abdominal cramps, watery diarrhea, constriction of the pupils, hypotension, and a slowed pulse. Death does not usually occur when these are the only toxins in the poisonous mushrooms, but fly agaric also contains amatoxins and phallotoxins, so a fatal combination of symptoms may occur.
- ***Clitocybe dealbata*** typically contains muscarine but not amatoxins or phallotoxins, so is less likely to kill. Other mushrooms whose principal toxicant is muscarine are *Inocybe*

napipes (highest muscarine levels), *I. dulcamara*, *I. fastigiata*, *I. geophylla*, *I. patouillardii*, and *I. umbrina*,

- ***Coprinus atramentarius*** contains coprine, which causes symptoms only in conjunction with alcohol. Symptoms typically begin about 30 minutes after drinking alcohol and may occur for as long as 5 days after mushroom ingestion. The symptoms include flushing of the face and neck, distension of the veins in the neck, swelling and tingling of the hands, metallic taste, tachycardia, and hypotension, progressing to nausea and vomiting. These are the result of actions on the autonomic nervous system.
- ***Galerina* spp.** also contain **amatoxins**. They are small, brownish mushrooms commonly growing on wood. There are three toxic species of importance in the US: *G. autumnalis*, *G. marginata*, and *G. venenata*. These mushroom are easily confused with the edible two-toned Pholiota (*Kuehneromyces mutablis*) that also grows on wood in clumps.
- ***Gyromitra esculenta*** contains gyromitrin, which causes a bloated feeling, nausea, vomiting, watery or bloody diarrhea, abdominal pain, muscle cramps, faintness, and loss of motor coordination typically occur 6–12 hours after eating the mushrooms. In rare cases, the illness can progress to convulsions, coma, and death.
- ***Psilocybe mexicana*** are called "Mexican mushrooms, magic mushrooms, or shrooms." The toxins, including psilocybin and psilocin, are indoles. Symptoms begin about 30–60 minutes after ingestion and include pleasant or apprehensive mood, unmotivated laughter and hilarity, compulsive movements, muscle weakness, drowsiness, hallucinations, and finally sleep. Recovery is spontaneous. Mexican mushrooms have been used as recreational drugs for their hallucinogenic effects. However, the level of hallucinogens in these mushrooms is widely variable, so prolonged and severe side effects can be experienced. Death has occurred in small children who accidentally ate *Psilocybe* mushrooms. More commonly, patients experience persistent sequelae and are admitted to mental institutions.

Quorn™ — a special case

- Quorn™ is the brand name of a fungal protein that is sold as a meat substitute.
- The mycoprotein is derived from *Fusarium venenatum*, originally discovered growing in a field in Buckinghamshire in the UK. Research and development of the mycoprotein began in the late 1960s, based on its potential as a protein source.
- The manufacturer, Marlow Foods Ltd., states that Quorn™ foods have been available in Europe for over 17 years. To date, Quorn™ foods have been eaten by 20 million consumers, in nearly one billion servings.
- The product is new in the US, having been licensed by the FDA in 2002.
- Now, the Center for Science in the Public Interest (CSPI), a group that claims to represent consumers, says that hundreds of people are becoming violently ill with problems such as vomiting and diarrhea from eating Quorn™. They have a web site that solicits complaints.
- The company has acknowledged that some people do not tolerate the product well, just as some people have trouble digesting other proteins. They estimate that one in 146,000 people may have negative reactions.
- The FDA says it has contacted many of the people who have reported falling ill from Quorn™ products, but found no evidence of it being a serious threat to consumers.

- Inasmuch as the product is made by aerated fermentation followed by purification of the protein, this is probably not a mycotoxicosis issue, but who knows?

Summary

- Many fungi that grow on plants in the field and on feedstuffs and foodstuffs after harvest produce toxins that threaten animal and human health.
- Mycotoxins in animal feeds may eventually threaten humans via foods of animal origin.
- Once present in feeds or foods, mycotoxins are very difficult to eliminate.
- Several species of mushrooms also contain toxins that can cause serious illness and death if eaten.
- A new meat substitute made of fungal protein has attracted the attention of a consumer advocate organization, which wants it banned.

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