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FUNGI AND MYCOTOXINS

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Introduction:

Mycotoxin is a toxin which is formed during fungus growth. "Myco" means mold and "toxin" represents poison. These toxins are mainly low molecular weight proteins produced by mushrooms and molds. Under proper environmental conditions (temperature, moisture, oxygen …), mycotoxin levels become high. Mycotoxins are generally of no known use to the fungi that produce them. Some mycotoxins have lethaleffects; some cause specific diseases or health problems; some of them affect on the immune system; some act as allergens or irritants; and some have no known effect on human and animal health. Some mycotoxins act on other micro-organisms, such as penicillin's antibiotic action.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.

History:

Mycotoxin problems such as ergotism and mushrom poisoning have been recognized for centuries. An outbreak occurred in the UK in 1960, in which 100,000 turkeys died of an unknown disease. The cause of death was identified as aflatoxin-contaminated peanut meal. Alimentary toxic aleukia (ATA), with more than 5000 deaths caused by trichothecene mycotoxins in grain in the USSR late in World War II, was one of the well-documented reports of mycotoxicosis.

In last four decades, several mycotoxins and their sources have been discovered. Some of these are effective in animal feed or food, and sometimes both, and the effects of some of them on human and animal health has been proven.

Mycotoxin production and occurrence:

Mycotoxins can occur in the field pre-harvest and later, during processing, transportation and storage. *Aspergillus flavus* and some *Fusarium* species are frequently found at the field level; some *Fusarium* species and *Trichothecium* are also introduced at harvest time; and *Penicillium* are mainly found in storage, in addition to *A. parasiticus ,A. flavus* and several other *Fusarium* species.
Mycotoxin production at the field level needs proper weather conditions, plant stress, vectors, fungal species and microbial competition; and during storage and transportation, factors such as water activity (a_w), temperature, crop damage, time, blending with moldy components, and chemical properties such as oxygen availability are influential.

Mycotoxins contamination could be prevented or minimized by:

- Plant breeding
- Good agronomic practices
- Detoxification

Aflatoxin

Aflatoxins can be produced by three species of *Aspergillus*—*A. flavus*, *A. parasiticus*, and the rare *A. nomius*—which contaminate plants and plant products. *A. flavus* produces only B aflatoxins, while the other two species produce both B and G aflatoxins. Aflatoxin B1 is a potent liver carcinogen and DNA-damaging agent. 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.
Aflatoxins sometimes cause serious problems, such as outbreaks of jaundice with high case-fatality rates. Preliminary laboratory testing of food collected from the affected area revealed high levels of aflatoxin, suggesting that the outbreak was caused by aflatoxin poisoning. Aflatoxins M_1 and M_2 are the hydroxylated metabolites of aflatoxins B_1 and B_2 and can be found in milk or milk products from livestock that have ingested contaminated feed. The main feed sources of aflatoxins are peanut meal, maize and cottonseed meal; but it is suggested that in Iran sorghum, as a prevalent animal feed, is another source of aflatoxin. Investigations concerning the biological fate of aflatoxin B_1 (AFB_1) in lactating dairy cattle have demonstrated the transmission of residues into milk, occurring as the metabolite aflatoxin M_1 (AFM_1). Although AFM_1 is considered to be less carcinogenic than AFB_1 by at least an order of magnitude, its presence in dairy products should be limited to the lowest level practicable. The amount of daily ingested AFB_1 that is transferred into milk as AFM_1 is in the range of 0.17 to 3.3%.
Aflatoxins are very heat resistant and are difficult to remove from foods and feeds. Alkali treatment (including ammoniation) is useful in some situations.

Ochratoxins

Ochratoxins comprise groups of mycotoxins produced by *Aspergillus* and *Penicillium.* Ochratoxin A (OA) can be produced in a range of 4–37ºC and an a_w as low as 0.78; it occurs primarily in cereal grains and mixed feeds, but it can also be found in beans, coffee, fruit juices, nuts, olives, cheese, fish, pork, milk powder, pepper, wine and beer. The role of OA in human pathogenesis is still speculative; some reports considered the symptoms associated with nephropathy in Tunisia. They may also occur in products derived from animals that were fed ochratoxin-containing feeds. A recent study in South America showed that *Aspergillus niger* aggregate and *A. ochraceus* species would be the main sources of OA.

Fumonisins

Fumonisins (Fms) are a group of toxic metabolites produced primarily by *Fusariun verticillioides*, which is one of the most common fungi colonizing corn and corn-based

foods as well as other grains (such as sorghum and rice) throughout the world. The level of contamination varies considerably ranging from 0 to >100 ppm.
The impact of low levels of Fms in humans is not clear; there are several reports of a possible role in the etiology of human esophageal cancer.

Trichothecenes

Trichothecenes (TCTC) are a group of toxic metabolites produced in the genera *Fusariun.* They have various toxic effects in animals and humans. TCTC mycotoxicoses affect many organs, such as the gastrointestinal tract and the hematopoietic, nervous, immune, hepatobiliary and cardiovascular systems.
Contaminated foods likely contain moldy corn or scabby wheat. One of the famous human diseases is hemorrhagic syndrome and alimentary toxic aleukia (ATA).

T-2 Toxin
T-2 toxin is a highly toxic type A TCTC

Deoxynivalenol

Deoxynivalenol (DON) mycotoxin is produced primarily by *F. graminearum*. Because DON causes feed refusal and emesis in swine, the name "vomitoxin" is also used. The level of contamination of this toxin in corn and wheat is often high; however it is considerably less toxic than most other mycotoxins.

Sterigmatocystin

Sterigmatocystin is a naturally occurring hepatotoxic and carcinogenic mycotoxin produced by fungi in the *Aspergillus*, *Bipolaris*, and *Chaetomium* genera and by *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 *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.

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.

Mycotoxin preventive measures

Toxin formation

The field and storage have the most important roles in regard to preventing mycotoxin production. Using resistant varieties, good agricultural practice, and drying grain to less than 10–13% are the most effective (but not all are successful) methods in this regard. Antifungal agents and phenolic antioxidants and antibiotics have some effects in post-harvest storage.

Monitoring programs to avoid human exposure

Effective monitoring and measures of mycotoxins in food to control human exposure by mycotoxins would be most effective. Monitoring of animal feed also would be another effective way.
Different methods of monitoring mycotoxins exist: TLC, HPLC and GC techniques, in addition to new chemical methods such as capillary electrophoresis, fluorescence polarization immunoassay and biosensors. . Immunoassay methods are now accepted as a simple approach to screening mycotoxins.

Remove mycotoxins from commodities

There are several methods to remove mycotoxins from commodities: however, these have some difficulties and in some cases add high costs to food products. Chemical detoxifiers, including acids, alkalis, aldehydes and oxidizing agents and gases such as chlorine, sulfur dioxide, $NaNO_2$, ozone and ammonia are used for aflatoxins, fumonisin and trichothecene.
Cooking and pasteurization treatments in processing do not destroy mycotoxins generally; however, extrusion cooking is effective for detoxifying DON, and roasting has some effect in reducing OA in coffee.

Other, miscellaneous fungi

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 and pasture infected by *Claviceps purpurea* as principal agent of ergotism. Other potential producers include *C. paspali*, *C. cinerea* and *C. fusiformis.* 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. Between 1770–1771, about 8000 people died in one district alone in France.

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.
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.
The corn smut fungus, *Ustilago zeae*, which occurs accidentally on corn ears in most of the world, has long been cultivated in Mexico as a special delicacy, called *huitlacoche* or *cuitlacoche*. It is used as an alternative to meat in many dishes, such as soup, tamales, quesadillas, etc.

***Amanita phalloides* (Death Cap)**

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.
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. The mortality rate in humans is 10–40%.

Amanita muscarina is called the fly agaric and is characterized by muscarine, which affects the autonomic nervous system.
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.

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.

***Galerina* spp.** also contain **amatoxins**. They are small, brownish mushrooms commonly growing on wood. 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. Death has occurred in small children who accidentally ate *Psilocybe* mushrooms.

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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