

The threat of mycotoxins to man

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Mycotoxins are harmful metabolites of moulds (fungi) that can be synthesised on plant materials pre-harvest. Mycotoxins can also accumulate when plant products are stored under suboptimal conditions.

Formation of mycotoxins in the field will depend on climatic conditions at the time of harvest and during critical stages of plant development.

High moisture content is often a key promoter of fungal growth and mycotoxin production, but drought conditions can also promote mycotoxin contamination of grains as a result of environmental stressors on the fungi. Foods of plant origin present the greatest chance of human exposure to mycotoxins. Lower quality grains and nuts are typically diverted to the animal feed chain, while higher quality plant materials, which have lower levels of contamination, are normally used as human foods.

Numerous processing techniques can also be employed to further reduce mycotoxin concentrations in foods of plant origin. Foods of animal origin usually present a lesser threat to human health.

Ruminant animals including cattle, sheep and goats are protected to some degree

from the harmful effects of feedborne mycotoxins due to the detoxifying actions of rumen micro-organisms. This usually results in vanishingly low residues of mycotoxins and metabolites in meat products.

Milk, however, is monitored for the carcinogenic mycotoxin metabolite aflatoxin M1. Monogastric animals such as pigs and avian species, including broiler chickens, laying hens and turkeys, are more sensitive than ruminants to the effects of feedborne mycotoxins but there is still considerable potential for mycotoxin degradation which minimises harmful residues in pork, poultry meat and eggs.

Residues of the carcinogenic mycotoxin ochratoxin A, however, are monitored in certain pork products.

Carcinogenic mycotoxins

Aflatoxin B1 is likely the carcinogenic mycotoxin of greatest concern to human health. This compound and related metabolites are produced mainly by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* (Table 1).

These fungi are usually considered to be tropical or semi-tropical moulds which

thrive under conditions of high temperature and humidity.

Exposure to foodborne aflatoxin can result in liver cancer and this has been estimated to be the cause of approximately 500,000 deaths annually. Edible nuts are particularly prone to aflatoxin contamination and aflatoxin contamination of peanuts and peanut products and fluid milk are commonly monitored.

Human nephropathies, including Balkan endemic nephropathy and tumours of the urinary tract have been attributed to foodborne ochratoxin A. This mycotoxin is produced by *Penicillium verrucosum* and *Aspergillus ochraceus* which commonly infest cereal grains in storage including barley.

Whole pig carcasses can be condemned in Denmark if ochratoxin A residues in kidney tissue exceed a legal maximum.

Foodborne fumonisins, produced in corn by *Fusarium verticillioides*, has been linked to outbreaks of oesophageal cancer in South Africa and China.

Pharmacologically active

Foodborne mycotoxins also have the potential to alter brain neurochemistry causing physiological and behavioural changes.

Fusaric acid, a tryptophan analogue produced by many different strains of *Fusarium* fungi has the potential to reduce brain concentrations of norepinephrine by inhibiting the activity of the synthetic enzyme dopamine-beta-hydroxylase.

The physiological consequence of this property is a decline in blood pressure and fusaric acid has been studied as a possible drug to control human hypertension. Orally administered fusaric acid has also been shown to result in sequential increases in brain concentrations of tryptophan, serotonin and 5-hydroxyindoleacetic acid. This can trigger vomiting, reduced appetite, loss of muscle co-ordination and lethargy.

Fusarium trichothecene mycotoxins have been shown to have similar effects including T-2 toxin. The feeding of a blend of wheat and corn naturally contaminated with *Fusarium* mycotoxins including deoxy-

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Table 1. Human diseases suspected to be related to mycotoxin contaminated foods (Galvano et al, 2005).

Disease	Food	Aetiological agent	Mycotoxin
Alimentary toxic aleukia	Cereal grains	<i>Fusarium</i> spp.	Trichothecenes
Balkan nephropathy	Cereal grains	<i>Aspergillus</i> and <i>Penicillium</i>	Ochratoxin A
Cardiac beriberi	Rice	<i>Aspergillus</i> and <i>Penicillium</i> spp.	Citreoviridin
Ergotism	Rye, cereal grains	<i>Claviceps purpurea</i>	Ergot alkaloids
Oesophageal tumours	Corn	<i>Fusarium verticillioides</i>	Fumonisin B1
Hepatocarcinoma	Cereal grains, nuts	<i>Aspergillus flavus</i> , <i>A. parasiticus</i>	Aflatoxin B1
Human neural tube defects	Corn	<i>Fusarium verticillioides</i>	Fumonisin B1
Kashin Beck disease	Cereal grains	<i>Fusarium</i> spp.	Trichothecenes
Kwashiorkor	Cereal grains	<i>Aspergillus flavus</i> <i>A. parasiticus</i>	Aflatoxin B1
Reye's syndrome	Cereal grains	<i>Aspergillus</i> spp.	Aflatoxin B1
Testicular cancer	Various	<i>Penicillium</i> spp, <i>Aspergillus</i> spp.	Ochratoxin A

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valenol (DON, vomitoxin) also caused elevated brain serotonin concentrations. Ergot alkaloids produced by *Claviceps purpurea* in rye and related grains are also pharmacologically active.

Reproduction and immunity

The most significant foodborne mycotoxin with effects on the endocrine system is zearalenone. This mycotoxin is a resorcylic acid beta-lactone which has powerful oestrogenic activity in mammals.

Studies with pigs have shown that zearalenone can cause infertility, abortions, ovarian atrophy and uterine enlargement in females. A feminising effect is seen in males.

The implications for human health are clear. Zearalenone is produced by *Fusarium* spp. and is commonly found in cereal grains together with deoxynivalenol and other mycotoxins.

Perhaps the greatest consequence of foodborne mycotoxins on human health is the potential for compromising immunity.

A wide variety of mycotoxins have been shown to be immunosuppressive. These include aflatoxin, ochratoxin, fumonisins, trichothecenes and fusaric acid.

Implications for human health include lingering disease conditions, lack of response to medications and failure of vaccinations.

With such a large number of different mycotoxins having immunosuppressive properties, the combined effects of even small amounts of individual mycotoxins could have profound effects on disease resistance. Such conditions are referred to as secondary mycotoxic diseases. Acute and chronic mycotoxicoses are easier to diagnose than diseases arising only indirectly from foodborne mycotoxins.

Identifying the root cause of such diseases is made even more difficult by environmental considerations. Environmental stress can also cause a degree of immunosuppression, arising from elevated circulating levels of glucocorticoid hormones.

This, coupled with mycotoxin induced immunosuppression may seriously compromise disease resistance.

Solutions to the hazards

One long term strategy that is being employed to reduce mycotoxin contamination of foodstuffs is the use of genetic engineering to improve the resistance of plants to fungal infestation.

One example of this is the development of Bt corn, which provides season long protection of plants against corn borer insect damage, through the introduction of insect control proteins derived from *Bacillus thuringiensis*. Field tests have shown that Bt corn is less susceptible to contamination with fumonisin and deoxynivalenol.

Future advances in plant genetics should

improve yield and further reduce corn plant susceptibility to environmental stress factors that contribute to mycotoxin contamination of grains pre-harvest.

Efforts have also been made in developing a mathematical model to predict contamination of wheat crops with deoxynivalenol.

The model (DONcast) takes into account coincidental weather around heading, varietal susceptibility to *Fusarium* infestation and the management of previous crop residue. Validations of the model have been successful in four countries.

Analytical advances

Another long term solution to the challenge of foodborne mycotoxins is improved quality control procedures based on more accurate analytical methodology. It has long been recognised that sampling error represents an important source of error in determining the degree of mycotoxin contamination of foodstuffs.

This is largely attributed to the heterogeneous nature of the distribution of mycotoxins in a given storage facility. The development of enzyme linked immunosorbent (ELISA) assays and commercial quick test kits has greatly increased the economic feasibility of mycotoxin analysis. The kits are rapid and inexpensive but are prone to false positives for certain types of samples.

More conventional HPLC methods and the more sensitive GC/MS and LC-MS/MS avoid this problem. An important development in analytical methodology is the increased understanding of the significance of 'masked mycotoxins'.

These are chemical forms of mycotoxins that are produced by plants following invasion by fungi. An example is a glucose conjugate of deoxynivalenol, glucose conjugates of zearalenone have also been identified.

Samples of naturally contaminated wheat and corn from Slovakia were analysed together with artificially contaminated grains and the fraction of DON found in conjugated form was up to 30%.

Such modified DON is not detectable by conventional analytical techniques but it is likely that the glucose conjugated DON can be hydrolysed by digestive enzymes to generate free DON in the lumen of the intestinal tract.

This means that the conjugated forms are toxic but non-detectable and this would lead to an underestimate of total DON in foodstuffs and an underestimate of the potential hazard posed by the consumption of contaminated grains.

More recently, samples of naturally contaminated barley grown in North Dakota, USA, were analysed for DON following hydrolysis with trifluoroacetic acid. The acid hydrolysis would potentially remove all conjugated molecules from DON including glucose. DON content increased up to 88% following acid hydrolysis.

This reinforces the concept that current

analytical techniques might seriously underestimate mycotoxin contamination of foodstuffs and the subsequent threat posed to human health.

Mycotoxin adsorbents

Mycotoxin adsorbents are non-nutritive, non-digestible high molecular weight polymers that can be included in animal feeds to minimise the potential for mycotoxin residues in foods of animal origin.

The adsorbents bind small molecules in the lumen of the small intestine to prevent intestinal absorption and transfer to target tissues. The adsorbent and adsorbed molecules are then excreted in the faeces.

Effective adsorbents have broad substrate binding capability which allows them to minimise the harmful effects of combinations of mycotoxins in a given diet.

Adsorbents can be inorganic silica polymers or organic carbon polymers, the equivalent of plant fibres. Examples of the inorganic adsorbents include zeolites, bentonites and diatomaceous earth and can be naturally occurring clays or synthetic polymers. Organic polymers include natural fibre sources such as activated charcoal, dehydrated alfalfa meal and glucomannan polymer extracted from the cell wall of yeast.

An example of the efficacy of adsorbents in reducing harmful residues of mycotoxin metabolites in foods is the study of Diaz et al. In this study, lactating dairy cows were fed aflatoxin contaminated feed and four adsorbents were compared for their ability to reduce the excretion of aflatoxin M1 into milk. The adsorbents tested included activated charcoal, calcium bentonite, sodium bentonite and yeast cell wall extract (Alltech Inc).

The feeding of activated charcoal reduced aflatoxin M1 content of milk by only about 5% and calcium bentonite achieved a 30% reduction. Both sodium bentonite and yeast cell wall extract reduced milk aflatoxin M1 concentrations by about 60% although the dietary level of inclusion of bentonite was 25 times higher than that for yeast cell wall extract. This study illustrates the use of mycotoxin adsorbents to improve the wholesomeness of foods of animal origin by minimising potentially harmful mycotoxin residues.

Conclusions

It can be concluded that mycotoxin contamination of foodstuffs can pose real threats to human health especially due to immunosuppression. Minimising the entry of mycotoxin contaminated materials into the food chain requires improved analytical methodology that will include detection of 'masked mycotoxins'. Mycotoxin residues in foods of animal origin can be minimised through the use of a suitable mycotoxin adsorbent. n

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