Mycotoxins and plant toxins in food

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Summary

Mycotoxins (toxic fungal metabolites) have challenged human and animal health since early times; dating back to the 900’s. Some metabolites have pharmaceutical characteristics, but most are known for adverse effects on human and animal health.

Food and feed raw materials are seldom contaminated with one mycotoxin only, new mycotoxins are discovered and old ones re-emerge, while recent observations on masked mycotoxins may influence risk assessments.

This asks for wide range screening methods, new analytical approaches and low detection limits. Producers need fast methods for decisions on the use of raw materials. Effect based toxicity assays will improve the screening for unknown mycotoxins. Recent developments in analysis will be discussed.

Changing society and environment forces food safety officers and scientists to be aware of mycotoxin risks (occurrence and re-introduction). Trends in consumption may lead to (re-)introduction of risks from mycotoxins. Consumer demand for more healthy diets might increase the demand for oats (slow release of energy) and subsequently (re-)introduce or expand the area of cultivation in certain regions. The consumer perception of soy-GMO, and the demand for highly nutritious proteins for meat replacement will increase the demand for other legumes, such as lupine, with the risk for phomopsin contamination. Plant diseases and insects may spread due to alterations in climate and may force fungi to produce (more) mycotoxins. The use of food crops for biofuel production and the treatment of biofuel raw material (storage, use as animal feed) is a challenge for the agrichain and needs to be monitored closely.

The major challenge for food producers and food safety officers today is to incorporate the risk assessment for mycotoxins for every change in the agri-chain. This requires smart and reliable monitoring as well as close cooperation between governments, scientists, plant breeders and food and feed producers.
Risks of mycotoxins and plant toxins in food and feed

Monique de Nijs, CONFIDENCE presentation October 4, 2011

Program
1. RIKILT
2. Confidence
3. Mycotoxins and plant toxins (Mw. de Nijs)
   1. Introduction
   2. Mycotoxins
   3. Plant toxins
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   5. Challenges
4. Risk assessment and regulations for mycotoxins (Mr. van Egmond)

RIKILT Institute of Food Safety
- Detection, identification, functionality and effects of substances in food and feed.
- RIKILT:
  - Specific research, with CIVI and RIVM, for the Netherlands new food safety authorities (NVWA).
  - Laboratory for VWA feed and aid.
- Contract work:
  - According to guidance document.
  - Report duty, as any laboratory, when legal limits are exceeded.

RIKILT Institute of Food Safety
- Environment & process contaminants:
  - Dioxins in eggs in Germany.
  - Flu in Roerdijk (influenza insect);
- Radioactivity:
  - Imports from Japan;
- Pesticides;
- Natural toxins (mycotoxins, plant toxins, phycotoxins);
- Animal treatment medicines and residues;
- GMOs;
- Allergens;
- Nutrients / Quality:
  - Identify organically produced eggs;
  - Authenticity identification (is this the fruit juice I selected and ordered several months ago?)

RIKILT Institute of Food Safety
- Part of Wageningen UR since 1998
- About 200 employees

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**Introduction**

Natural toxins in food and feed

Compounds that are poisonous to humans and/or animals that naturally occur in food and feed can be produced by:

1. Microorganisms (fungi (mycotoxins) and bacteria);
2. Plants (plant toxins or phytotoxins);
3. Algae/shellfish
   (a) Zootoxins produced by snakes, bees, frogs)

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**Introduction**

Zootoxin

Liken van kop hallucinerende pad kan levensgevaarlijk zijn

Poison, licked from the head of a certain toad, can give strong hallucinations; can even kill a grown-up person

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**Introduction**

Secondary metabolites

All natural toxins secondary metabolites

Secondary metabolite ≠ toxin

Secondary metabolites: metabolites that are not directly related to growth of cells, or to development and reproduction of an organism;

Primary metabolites: amino acids and glucose and substances related to growth of cells, and to development or reproduction
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Mycotoxins Challenges Climate
- Expected changes in range of latitudes, where certain fungi are able to compete;
  - Example: F. graminearum growth: MY increase
- Drought, flooding and other consequences of climate change may result in more mycotoxins and changed toxin profiles;
  - Example: aflatoxins found in Italy since 2003.
  - and in other parts of Central Europe;
- Response of insects and plant diseases to climate change poorly understood, but increases expected

Mycotoxins Regulations EU Feed (1/2)
- Commission Recommendation 2006/576/EC: On the presence of deoxynivalenol, zearalenone, ochratoxin A, T 2 and HT 2 and fumonisins in products intended for animal feeding

Mycotoxins Regulations EU Food (1/2)
- Commission Regulation (EC) No 401/2006 (and its amendments): Laying down the methods of sampling and analysis for the official control of the levels of mycotoxins in food

Mycotoxins Regulations MS Food (2/2)
- The Netherlands: Warenwetbesluit bereiding en behandeling van levensmiddelen Artikel 12: Fungal and bacterial toxins in quantities that can be harmful to public health must be absent in food, drinks and raw materials

Regulations EU import controls
- Commission Regulation (EC) No 1152/2009: imposing special conditions governing the import of certain foodstuffs from certain third countries due to contamination risk by aflatoxins and repealing Decision 2006/504/EC
**Mycotoxins**

**Decontamination**

- EU regulatory limit for mycotoxins in feed: aflatoxin.
- EU recommended limit for mycotoxins in feed: deoxynivalenol, zearalenon, ochratoxin A, T2 and HT2 and fumonisins
  - Decontamination is not allowed in EU;
  - Dilution to lower contamination is not allowed in EU;

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**Mycotoxins**

**Decontamination**

- Feed: allowed are technological additives according to EU 1831/2003: substances for reduction of the contamination of feed by mycotoxins;
  - that can suppress or reduce the absorption;
  - promote the excretion of mycotoxins;
  - modify their mode of action.
  - need to be evaluated according to EU 2002/2008
  - Toxicity of the substance to animals;
  - efficacy;
  - Only when EU regulations are met!

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**Plant toxis**

**Contamination and exposure route**

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**Mycotoxins**

**Decontamination**

- Technological additives according to EU 1831/2003:
  - Biotransformation
    - E.g.: enzymes, enzyme producing microorganisms,
  - Organic binders
    - E.g.: wall cell wall components; synthetic polymers; humic substances; dietary fibers,
  - Nutritional feed additives:
    - E.g.: antioxidants, immunostimulatory agents,
  - Inorganic compounds (absorbents):
    - E.g.: clays, activated carbon

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**Plant toxis**

**Factors affecting contamination**

- Role: protect plant against being eaten (insects and animals) and infections;
- Plant species (star anise);
- Environmental circumstances:
  - Temperature;
  - Growing season;
  - Insects;
  - Etc. more research.
Plant toxins  Challenges  Carry over

Carry over from feed to animal products:
- Jacoline from Senecio jacobaea (Jacob’s milkermaid) to milk
- Mangiferin from Mangifera indica, a BCC plant (Mangifera indica L.)
- Incident in Afghanistan: possible PA's from other weeds carried over to goat milk/cheese;
- No reports on occurrence of plant toxins in meat.

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Plant toxins  Challenges  Climate

- Growing area of weeds can change;
- Drought, flooding and other causes;
- (Re) introduction of crops;
- New plants/weeds due to import/export.

Challenges  Analytical methods

- Single compound chemical analysis
- Multiple compound chemical analysis
- Single/multiple compound effect analysis

Plant toxins  Legislation

- Zn (1750 mg/kg) in oats; cotton (in cottonseed oil), all others, viz. soy, corn, sunflower, wheat, rice, flaxseed, barley, and Triticale (1500 kg/ha, 1000 kg/ha, respectively).
- Tolerance for soils, leafy vegetables, root vegetables, grains, and other food products.
- 2001 regulation on health regulations in food:
  - for antinutritional acids
    - Carrinex, aluminium = 1 μg/kg
- Cultivated plants not to be used in herbal preparations (even if frost the alones...)

Challenges  Chemical analysis

- Single compound methods:
  - Optimized extraction,
  - SPE and/or IAC clean up (derivatization);
  - LC UV, LC Flu, GC ECD
- Multi compound methods:
  - Generic extraction;
  - (limited) no clean up;
  - LC MS/MS
  - T 2 and T 2/HTZ
  - Patulin alkaloids
  - Many other mycotoxins

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Challenges  Effect analysis (1/2)
In vitro testing: effects of mycotoxins on gene expression
- Cultured human Caco 2 cells
- Isolate RNA from Caco 2 cells
- Conversion to cDNA, amplify cDNA, labelling
- Hybridisation, detection and data equilibration
- Data analysis

Challenges  Effect analysis (2/2)
Affected genes whole array (predictions from 2000 genes)
Green: down-regulated
Further method development, e.g.:
- Caco 2 cell lines with luciferase expression when exposed to mycotoxins
- Multiplex qRT PCR on specific up- and down-regulated genes

Conclusions
- Similarities in occurrence and toxic effects of mycotoxins and plant toxins:
  - Route of exposure: mainly through plant materials
  - Carry over through animals (meat, milk) is known
  - Toxic profiles occur and toxicity differs between toxins
  - Animal species very in sensitivity
  - Climate will affect occurring profiles and concentration
  - Gape in knowledge about and toxicity of many myco and plant toxins
- Plant toxin intoxication often resulting from mistake. The results can be devastating.

Natural toxins
Past performance is no guarantee of future results!

Thank you for your attention
Questions?
Mycotoxins are small (low-molecular weight) secondary metabolites (products of metabolism not essential for survival), produced by some filamentous fungi and often found in food. Chemically-diverse, they can cause disease and death to man and livestock. Adverse effects include tissue damage, immuno-suppression and neurological disorders following short- (acute) or long-term (chronic) exposure. In many countries, mycotoxin contamination is addressed by legislation. Successful management of mycotoxins is, however, effected through thorough knowledge of their cause and implementation of appropriate control mechanisms, which are verified through use of reliable sampling and robust analytical methods. European Union Rapid Alert System for Food and Feed (RASFF) data suggest that harmonisation of mycotoxin detection methods would improve compliance at regulatory and commercial limits.

Aspergillus carbonarius: a) black rot of berries caused by A. carbonarius; b) A. carbonarius artificially inoculated on grape berry; c) A. carbonarius on PDA plate –
Dr Giancarlo Perrone, Institute of Sciences of Food Production, National Research Council, Italy

Aflatoxins are produced by many Aspergillus species, most notably A. flavus and A. parasiticus. There are four different types of aflatoxins: B₁, B₂, G₁ and G₂. Aflatoxin B₁, the most toxic, is a potent carcinogen linked to adverse health effects in humans including liver cancer. Aflatoxins are associated with commodities produced in the tropics and subtropics such as cotton, maize, spices, peanuts, pistachios and dried fruits. Aflatoxin M₁ is found in the milk of animals that are fed aflatoxin B₁-contaminated feed. Susceptibility to aflatoxin-related diseases is affected by species, age, nutrition, sex and exposure to other toxins.

Patulin is produced by Aspergillus, Penicillium, Byssoschlamys and Paecilomyces species, but mostly P. expansum, which is found in a range of moldy fruits and vegetables, especially rotting apples, but destroyed by fermentation. Patulin is not carcinogenic, but it can damage the immune system and intestine. In 2004, the EU set limits on patulin in food at 50 μg/ kg (fruit juice), 25 μg/ kg (solid apple products for direct consumption) and 10 μg/ kg (children’s apple products including juice), respectively.
Ochratoxins are produced mainly by A. carbonarius, A. ochraceus and P. verrucosum. Ochratoxin B is a non-chlorinated form of Ochratoxin A whilst Ochratoxin C is an ethyl ester form Ochratoxin A. They are one of the most abundant food-contaminating mycotoxins, largely arising from improper storage. Ochratoxin A is a carcinogen and nephrotoxin, affecting kidney function, and it has been linked with urinary tract tumours but research in humans is limited.

Citrinin was originally isolated from P. citrinum as an antibiotic but it is also produced by Penicillium and Aspergillus spp. including those used in food manufacturing (e.g. cheese – P. camemberti, sake, miso and soy sauce – A. oryzae). However, although associated with many human foods (wheat, rice, corn, barley, oats, rye, and food coloured with Monascus pigment), its full significance in human health is unknown.

Ergot Alkaloids: Ergot or ergot fungi refers to a group of fungi of the genus Claviceps. The most prominent member of this group is Claviceps purpurea. This fungus grows on rye and related plants, and produces alkaloids that can cause ergotism in humans and other mammals consuming grains contaminated with its fruiting structure (ergot sclerotium). Ergot alkaloids cause vasoconstriction leading to gangrene and loss of limbs and/or affect the central nervous system causing hallucinations and irrational behaviour and convulsions. However, ergot alkaloids have and continue to be used in medicine including control of post-partum haemorrhage.

Fusarium is a large genus of filamentous fungi widely distributed in soil and in association with plants, which are generally harmless. However, in cereal crops such as wheat and maize, some produce a range of mycotoxins including fumonisins – affecting the nervous systems of horses and cause cancer in rodents – trichothecenes, which are associated with chronic and fatal toxic effects in animals and humans and zearalenone, which causes hyperestrogenism, particularly in swine. Other major Fusarium toxins include beauvercin and enniatins, butenolide, equisetin and fusarins.

MoniQA and mycotoxin analysis methods: In Europe, sampling and analysis for the control mycotoxins in foods must be in accordance with those set out in Commission Regulation 401/2006. For each mycotoxin, analytical method performance (recovery, repeatability and reproducibility) must fall within the range of acceptability. MoniQA undertook a survey on current laboratory practices for commercially-significant mycotoxins [aflatoxins B1, B2, G1, G2 and M1, fumonisins FB1 and FB2, ochratoxin A, deoxynivalenol, patulin, zearalenone, and T-2 and HT-2 toxins]. Nineteen control, commercial and research laboratories from 12 countries (UK, IT, BE, ES, DE, NL, BG, HU, GR, TR, NZ and CN) participated, 14 of which were accredited to ISO 17025:2005. Participants stated they receive laboratory samples weighing between 4g-1kg. The number and types of food matrices varies for the different mycotoxins with the highest number recorded for ochratoxin A. Most participants use HPLC coupled with either a fluorometer, ultraviolet or mass spectrometric (MS) detectors; one uses GC-MS for analysis of T-2 and HT-2 toxins and two use TLC-based methods for all mycotoxins except fumonisins. The use of LC-MS methods by eight (42%) laboratories is remarkable because these are not CEN- or AOAC-recognised method for mycotoxin analysis. ELISA kits are used by three (16%) laboratories for a range of mycotoxins whilst other test kits are used for the determination of ochratoxin A and deoxynivalenol, specifically. Six definitions of limit of detection (LOD) and nine definitions of limit of quantification (LOQ) were reported with signal-noise ratio being the most popular. In some cases, the values of LOD, LOQ and the measurement uncertainty varied from laboratory to laboratory for the same mycotoxin. This survey suggests laboratory accreditation, sample size, most appropriate and validated analytical methods, proficiency testing, reference/ certified materials/ standard solutions, and use of the same definition/ calculation for LOD, LOQ, recovery and measurement uncertainty need to be addressed as a priority. Solfrizzo et al. (2009) The use of mycotoxin methodology in practice: a need for harmonization QAS Crops & Foods 1(2): 121–132 DOI: 10.1111/j.1757-837X.2009.00021.x
Mycotoxins and rapid test methods: Rapid test methods are promoted as tools for food companies to validate and/or verify efficacy of food-safety management systems. Little is known about their uptake by the industry or current and future needs. In order to obtain more information, MoniQA conducted a survey in 17 countries (AL, BE, BG, CN, FI, FR, DE, GR, HU, IT, NZ, NO, PL, ES, TR, UK, VN) designed to explore routine analytical regimens operated by the food industries and the use of rapid test methods. More than 2600 questionnaires were sent to companies across the food chain (661 replies). At a strategic level, the survey revealed raw materials and final products are most commonly analysed, and the major analytes are microbiological contaminants, heavy metals, pesticides, foreign bodies, mycotoxins and allergens. Two-thirds of respondents use rapid test methods; almost all would be interested in extending the range of tests performed particularly for microbiological, food allergens and mycotoxins analyses. Lebesi et al. (2010) Rapid test methods: a versatile tool to assist food-safety management QAS Crops & Foods 2(4): 173–181, DOI: 10.1111/j.1757-837X.2010.00080.x

Formation of both field or storage mycotoxins in commodities and the interaction of a selection of external modifying factors on their formation – Dr Anton J. Alldrick, Campden BRI, United Kingdom

More about mycotoxins

- European Mycotoxins Awareness Network – www.mycotoxins.org
- HGCA – Agriculture and Horticulture Development Board (AHDB) – www.hgca.com (crop research)
- Food Standards Agency – http://www.food.gov.uk/safereating/chemsafe/mycotoxins

For further information please visit: www.moniqa.org/xxx or contact moniqa@moniqa.org.

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