ABSTRACTS OF
LECTURES AND POSTERS

November 6-8, 2006

THE World Mycotoxin Forum®

THE FOURTH CONFERENCE

The international networking conference
for the food and feed industry

Hilton Cincinnati Netherland Plaza
Cincinnati, Ohio, USA

FINAL PROGRAM
&
ABSTRACTS OF LECTURES AND POSTERS

THE
World Mycotoxin Forum®
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The international
networking conference for
the food and feed industry

November 6-8, 2006
Cincinnati, Ohio, USA

The World Mycotoxin Forum®

Advisory Committee

Dr. Daniel Barug	Ranks Meel, the Netherlands
Dr. Deepak Bhatnagar	U.S. Department of Agriculture, USA
Dr. Karl Dawson	Alltech, USA
Hans P. van Egmond, M.Sc.	National Institute of Public Health and the Environment, the Netherlands
Jan Willem van der Kamp, M.Sc.	International Association for Cereal Science and Technology, Austria
Dr. David F. Kendra	U.S. Department of Agriculture, USA
Dr. Xiumei Liu	Ministry of Health, China
Ton van Osenbruggen	TNO Quality of Life, the Netherlands
Dr. Joseph Shebuski	Cargill, USA
Dr. Gordon S. Shephard	Medical Research Council, South Africa
Prof.dr. Trevor K. Smith	University of Guelph, Canada
Dr. Angelo Visconti	Institute of Sciences of Food Production, Italy

Welcome at the fourth conference of The World Mycotoxin Forum®, Cincinatti, Ohio, USA, November 6-8, 2006!

Dear participant,

The World Mycotoxin Forum® – the international networking conference for the food and feed industry – is the leading international meeting series on mycotoxins of interest to the food and feed industry.

The main objectives of **The World Mycotoxin Forum®** are:

- to provide a unique platform for the food and feed industry, regulatory authorities and science;
- to exchange information and experiences on the various aspects of mycotoxins;
- to review current knowledge related to mycotoxins in food and feed; and
- to discuss strategies for prevention and control of mycotoxin contamination ensuring the safety of food and feed supply.

We are pleased to present you the lecture and poster abstracts of the fourth conference of **The World Mycotoxin Forum®**. As a comprehensive overview the fourth conference of **The World Mycotoxin Forum®** offers an excellent way to network and to share ideas, providing a reference source for anyone involved in this field. Invited speakers only will present their contributions to the conference in plenary meetings, parallel sessions and discussions. Poster sessions, spotlight presentations, workshops, and the concurrent instrument/ manufacturers exhibition are an integral part of this conference. They provide the opportunity for intensive informal discussions.

At the fourth conference of **The World Mycotoxin Forum®** key issues of mycotoxins will be discussed in relation to:

- regulatory issues and international developments;
- the food supply chain;
- animal health performance;
- sampling and analysis; and
- prevention and control solutions.

The World Mycotoxin Forum® invites you to take part in the discussions with participants from different disciplines and to meet business relations in your area. We wish you an active and fruitful meeting!

On behalf of the Advisory Committee,

Daniel Barug

The World Mycotoxin Forum®

Forum Secretariat

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Key to the abstracts of lectures and posters:

- abstracts of lectures, spotlight presentations and posters are grouped separately;
- the lectures and spotlight presentations are grouped according to the daily program;
- the posters are grouped according to theme and then in an alphabetical order according to the first author.

PROGRAM AT A GLANCE

Monday
November 6, 2006

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8:30 - 12:30	Workshop 2 *Mycotoxin detection and inactivation*
8:15 - 12:45	Workshop 3 (Invitation only) *Mycotoxins & biomarkers roundtable Think-Tank*

CONFERENCE PROGRAM

Monday
November 6, 2006

Plenary meeting
Mycotoxins – Setting the scene

Chair: Dr. Deepak Bhatnagar, U.S. Department of Agriculture, Agricultural Research Service (USDA/ARS/SRRC), USA

8:00 **Opening of The World Mycotoxin Forum® – the fourth conference**

Keynote address
Dr. Merle D. Pierson
U.S. Department of Agriculture, Deputy Under Secretary for Research, Education and Economics, Washington, USA

Keynote address
Frans Verstraete
European Commission, Health and Consumer Protection Directorate-General, Brussels, Belgium

8:45 *Mycotoxins – an unavoidable or avoidable risk?*
Dr. Monica Olsen
National Food Administration, Sweden

9:15 *Mycotoxins and worldwide regulations: recent developments*
Hans P. van Egmond, M.Sc.
National Institute for Public Health and the Environment, the Netherlands

9:45 *Mycotoxin regulations and food safety: the international trade dimension*
John S. Wilson
The World Bank, Development Economics Research Group, USA

10:15 *Mycotoxins: the ever-lower regulatory standards and toxicological significance*
Prof.dr. James J. Pestka
Michigan State University, Department of Food Science and Human Nutrition / Department of Microbiology and Molecular Genetics, USA

10:45 **Networking break & exhibition**

Monday
November 6, 2006

Parallel session 1
Mycotoxins – Food supply chain

Session chairs:
Dr. Joseph Shebuski, Cargill Corporate Food Safety, USA
Hans P. van Egmond, M.Sc., National Institute for Public Health and the Environment, the Netherlands

11:15 *Chairs' introduction*

11:30 *Probabilistic exposure assessment and risk analysis of mycotoxins for all age groups*
Dr. Tine Kuiper-Goodman
Health Canada, Food Directorate, Canada

12:00 *How to identify new mycotoxin risks?*
Prof.dr. Pierre Galtier
French National Institute for Agricultural Research, Pharmacology-Toxicology Unit, France

12:30 *Effects of processing on mycotoxin levels in food and food products*
Dr. Clare M. Hazel
RHM Technology, UK

13:00 **Lunch & exhibition**

Case studies

14:00 *A tale of two commodities: how EU mycotoxin regulations have hurt, or helped, food industries*
Dr. Felicia Wu
University of Pittsburgh, Graduate School of Public Health, USA

14:30 *Raisin and tree nut mycotoxin issues for export*
J. Michael Hurley
American Council for Food Safety and Quality (DFA of California), USA

15:00 *Mycotoxins in herbs and spices: a Trojan horse in the kitchen?*
Prof.dr. G. Devegowda
University of Agricultural Sciences, Bangalore, India

15:30 *Mycotoxin management in the dairy production chain*
Dr. Frank Driehuis
NIZO food research, the Netherlands

16:00 **Networking break & exhibition**

Monday
November 6, 2006

16:30 – 18:00 **Spotlight presentations theatre 1**
A special presentation facility for sponsors and exhibitors to promote their products in more detail.

16:30 *Development and application of multi-mycotoxin affinity columns*
Dr. Stephen P. Powers
VICAM group of Waters Technologies Corp., USA

16:45 *Qualitative and quantitative lateral flow methods for the determination of mycotoxins in grains*
Dr. Mark Tess
Charm Sciences, USA

17:00 *Development of a rapid and sensitive biosensor for the detection of aflatoxin M_1 in milk*
Dr. Steffen Rameil
R-Biopharm, Germany

17:15 *Rapid and quantitative determination of deoxynivalenol in raw grains using a FluoroQuant Don Plus test kit*
Dr. Zaneta Kubus
University of Natural Resources and Applied Life Sciences, Department for Agrobiotechnology (IFA-Tulln), Austria

17:30 *LFD (lateral flow diagnostics) for mycotoxin screening*
Frank Klein
Neogen, USA

17:45 *Mycotoxin reference materials*
Kraig K. Bond
Trilogy Analytical Laboratory, USA

18:00 *A new method for quantitative analysis of deoxynivalenol in grains*
Bob Robertson
Diagnostix, Canada

18:15 *Platforms for mycotoxin detection applications*
Dr. Liberty Sibanda
Euro-Diagnostica B.V., the Netherlands

18:30 **End of spotlight presentations theatre 1**

18:00 – 19:00 **Poster presentations at the Scientific Café**

Monday
November 6, 2006

Parallel session 2
Mycotoxins – Animal health performance

Session chairs:
Prof.dr. Trevor K. Smith, University of Guelph, Department of Animal and Poultry Science, Canada
Prof.dr. Johanna Fink-Gremmels, University Utrecht, Department of Veterinary Pharmacology, Pharmacy and Toxicology, the Netherlands

11:15 *Chairs' introduction*

11:30 *Mode of action of mycotoxins*
Dr. Ronald T. Riley
U.S. Department of Agriculture, Agricultural Research Service (USDA/ARS/SAA), USA

12:00 *Risk of mycotoxins to pig health: immunosuppressive effects*
Dr. Isabelle Oswald
French National Institute for Agricultural Research, Pharmacology-Toxicology Unit, France

12:30 *Interference of mycotoxins with poultry health and implementation of control programs*
Prof.dr. Elizabeth Santin
University of Paraná, Department of Veterinary Medicine, Brazil

13:00 **Lunch & exhibition**

14:00 *Effects of mycotoxins on ruminants*
Dr. Lon W. Whitlow
North Carolina State University, Department of Animal Science, USA

14:30 *Effects of mycotoxins in the equine – what we know and what we don't know*
Dr. Kyle E. Newman
Venture Laboratories, Inc., USA

15:00 *Mycotoxin concerns and the petfood industry*
Prof.dr. Herman J. Boermans
University of Guelph, Ontario Veterinary College, Canada

15:30 *Mycotoxins – a rising threat to aquaculture*
Prof.dr. Peter Spring
Swiss College of Agriculture, Switzerland

16:00 **Networking break & exhibition**

Monday
November 6, 2006

16:30 – 18:00 **Spotlight presentations theatre 2**
A special presentation facility for sponsors and exhibitors to promote their products in more detail.

16:30 *Counteracting mycotoxin contamination: the effectiveness of S. cerevisiae cell wall glucans for sequestering mycotoxins*
Dr. Alexandros Yiannikouris
Alltech, France

16:45 *In vivo trials in poultry with formulated clays against aflatoxin B_1, ochratoxin A and T-2 toxin*
Fernando Tamames III
Special Nutrients, Inc., USA

17:00 *A multifunctional wide-spectrum approach diminishing the impact on animal health and performance due to inevitable exposure to mycotoxins*
Koen Schwarzer
INVE Nutri-Ad, Belgium

17:15 *Efficacy of sequestrant/chelator Amadéite® in the binding of mycotoxins during transit through a dynamic gastrointestinal model (TIM) simulating the GI conditions of pigs*
Dr. Hervé Demais
Olmix, France

17:30 *A summary of a panel discussion on safety levels for mycotoxins*
Dr. Mamduh Sifri
ADM Alliance Nutrition, USA

17:45 **End of spotlight presentations theatre 2**

18:00 – 19:00 **Poster presentations at the Scientific Café**

Tuesday
November 7, 2006

Parallel session 3
Mycotoxins – Sampling and analysis

Chairs:
Dr. Gordon S. Shephard, Medical Research Council, PROMEC Unit, South Africa
Ton van Osenbruggen, TNO Quality of Life, the Netherlands

8:15 *Chairs' introduction*

8:30 *Theory and criteria for practical sampling*
Dr. Martien Spanjer
Food and Consumer Product Safety Authority, the Netherlands

9:00 *Mycotoxin analysis: an overview of classical, rapid and emerging technologies*
Dr. Mary W. Trucksess
U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition (FDA/CFSAN), USA

9:30 *Test kits and rapid methods for use in a non-laboratory environment*
Prof.dr. Sarah de Saeger
Ghent University, Department of Bio-analysis, Belgium

10:00 *The way to harmonize and validate analytical methods for mycotoxins*
Jan Willem van der Kamp, M.Sc.
International Association for Cereal Science and Technology (ICC), Austria

10:30 **Networking break & exhibition**

Subsession Analytical quality assurance

11:00 *Efforts of method validation*
Prof.dr. Tetsuhisa Goto
Shinsu University, Faculty of Agriculture, Japan

11:30 *Methodology required for proficiency testing*
Gina M. Clapper
American Oil Chemists' Society (AOCS), USA

12:00 *Use of certified reference materials to achieve reliable analytical results*
Prof.dr. Hendrik Emons
European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Belgium

12:30 **Lunch & exhibition**

Tuesday
November 7, 2006

Parallel session 4
Mycotoxins – Prevention and control solutions

Session chairs:
Dr. Angelo Visconti, Institute of Sciences of Food Production, Italy
Dr. Xiumei Liu, Ministry of Health, National Institute of Nutrition and Food Safety, China

8:15 *Chairs' introduction*

8:30 *Breeding new varieties to reduce pre-harvest mycotoxin contamination*
Dr. David F. Kendra
U.S. Department of Agriculture, Agricultural Research Service (USDA/ARS/NCAUR), USA

9:00 *Pre-harvest strategies for prevention of mycotoxin contamination*
Dr. Robert L. Brown
U.S. Department of Agriculture, Agricultural Research Service (USDA/ARS/SRRC), USA

9:30 *Managing the risk of mold and mycotoxin contamination in the supply chain: a global prospective*
Dr. Gerardo Morantes
Cargill Animal Nutrition, USA

10:00 *Development and use of biomarkers for mycotoxin exposure*
Dr. Karl Dawson
Alltech, USA

10:30 **Networking break & exhibition**

11:00 *Adsorbent compounds as feed additives to reduce mycotoxin bioavailability*
Prof.dr. Trevor K. Smith
University of Guelph, Department of Animal and Poultry Science, Canada

11:30 *Decontamination/detoxification of commodities containing mycotoxins: the microbiological approach*
Dr. Joshua (Jianhua) Gong
Agriculture and Agri-Food Canada / University of Guelph, Canada

12.00 *Mycotoxin contaminations along organic food chains: status and critical control points*
Dr. Gabriela S. Wyss
Research Institute of Organic Agriculture, Food Quality Unit, Switzerland

12:30 **Lunch & exhibition**

Tuesday
November 7, 2006

Plenary meeting
Mycotoxins – where do we go from here?

Chair:
Dr. David F. Kendra, U.S. Department of Agriculture, Agricultural Research Service (USDA/ARS/NCAUR), USA

14:00 – 15:15 **Wrapping up – stepping into tomorrow**

Mycotoxins – Food supply chain

Dr. Joseph Shebuski	Cargill Corporate Food Safety, USA
Hans P. van Egmond, M.Sc.	National Institute for Public Health and the Environment, the Netherlands

Mycotoxins – Animal health performance

Prof.dr. Trevor K. Smith	University of Guelph, Department of Animal and Poultry Science, Canada
Prof.dr. Johanna Fink-Gremmels	University Utrecht, Department of Veterinary Pharmacology, Pharmacy and Toxicology, the Netherlands

Mycotoxins – Sampling and analysis

Dr. Gordon S. Shephard	Medical Research Council, PROMEC Unit, South Africa
Ton van Osenbruggen	TNO Quality of Life, the Netherlands

Mycotoxins – Prevention and control solutions

Dr. Angelo Visconti	Institute of Sciences of Food Production, Italy
Dr. Xiumei Liu	Ministry of Health, National Institute of Nutrition and Food Safety, China

15:15 *Applying the 'omics' wrench: new tools (genomics, proteomics, metabolomics) for solving an age old mycotoxin contamination problem*
Dr. Deepak Bhatnagar
U.S. Department of Agriculture, Agricultural Research Service (USDA/ARS/SRRC), USA

16:00 **End of Plenary meeting**

WORKSHOP PROGRAM

Wednesday
November 8, 2006

8:30 – 12:30
Workshop 1
Structional and functional genomics of mycotoxigenic fungi
The workshop will provide basic information on various aspects of genomics; current information on the status of genomics of mycotoxigenic fungi; hypotheses on the origin and evolution of gene clusters for mycotoxin production; opportunity to discuss the use of available genomic information by researchers worldwide.

Workshop director:
Prof.dr. Gary A. Payne, North Carolina State University, Department of Plant Pathology, USA

Lead speakers:
Prof.dr. Joan W. Bennett, Rutgers, The State University of New Jersey, USA
Dr. Ignazio Carbone, North Carolina State University, USA
Dr. Nora Khaldi, Smurfit Institute of Genetics, Ireland
Dr. Frances Trail, Michigan State University, USA
Prof.dr. Charles P. Woloshuk, Purdue University, USA

Rapporteur:
Dr. Deepak Bhatnagar, U.S. Department of Agriculture, Agricultural Research Service (USDA/ARS/SRRC), USA

The availability of whole genome sequences of several mycotoxin-producing fungi has provided new tools to identify genes for pathogenicity and mycotoxin production. Genomic studies on several *Aspergillus* and *Fusarium* fungal species are underway in several laboratories in different parts of the world. Among these studies are structural and functional genomics of the toxin producing species *Fusarium graminearum* (trichothecene producer), *F. verticillioides* (fumonisin producer), and *Aspergillus flavus* (aflatoxin producer). Gene expression profiling experiments by DNA microarrays have successfully identified differentially expressed genes associated with mycotoxin production in the toxigenic fungi. This technology is also being employed to study environmental influences on the fungus, ecological/evolutionary significance of fungal propagation, fungal virulence, and global signal transduction within the fungus. Comparative genomics is being used to address the origin of mycotoxin gene clusters and their evolution in species of *Aspergillus* and *Fusarium*.

8:30 **Part I: Basics of genomics**
Definition and advent of genomics; genomic terminology; structural, functional and comparative genomics; DNA sequencing; analysis of DNA sequence (bioinformatics); microarray technology.
10:30 **Part II: Significance of genomics in mycotoxin research**
Structural and functional genomics of *F. verticillioides*; comparative genomics of *A. flavus*; genome wide functional analysis of polyketide synthases in *F. graminearum*; evolution and stability of the aflatoxin gene cluster; origin and evolution of the fumonisin gene cluster.
12:00 **Part III: General discussion**
Application of genomic information for control of mycotoxins; public access to genomic information; cooperative research using genomic data.
12:30 **End of workshop 1**

Wednesday
November 8, 2006

8:30 – 12:30
Workshop 2
Mycotoxin detection and inactivation

Sponsored and presented by Biomin and Romer Labs

8:30 **Part I: New ways to detect mycotoxins**
A hands-on workshop!
- Presentation of new products
- Hands-on training on the new products
- Discussion with the experts

10:30 **Part II: Mycotoxin deactivation**
What is the practical impact of a high-tech approach for the animal husbandry industry?
- Presentation of product news
- Presentation of new trial studies
- Discussion with the experts

12:30 **End of workshop 2**

Wednesday
November 8, 2006

08:15 – 12:45
Workshop 3 (Invitation only)
Mycotoxins & biomarkers roundtable Think-Tank

Sponsored by Alltech

Biomarkers play an increasingly important role when it comes to learning more about mycotoxins and their effects. The aim of this Think-Tank, therefore, is to bring together the leading experts in this area to discuss the calculation of expected exposure in consideration of the variations in the diet of different animal species and age, and production groups.

Special emphasis will be given to the opportunities and limitations to determine:
- biomarkers of exposure
- biomarkers of effects

08:15 **Registration**

08:30 **Introduction**
Prof.dr. Johanna Fink-Gremmels
University Utrecht, Department of Veterinary Pharmacology, Pharmacy and Toxicology, the Netherlands

Topics to be addressed
- Can neurological responses and behavioral changes be used as markers for mycotoxins?
- Immunological measures as response criteria for mycotoxin exposure?
- Can measures of oxidative stress be used as biomarkers of exposure?
- Biomarkers of effect - what can we learn from the recent understanding of the molecular mechanisms of toxin actions?

12:45 **End of workshop 3**

SEE YOU AT
THE WORLD MYCOTOXIN FORUM®
THE FIFTH CONFERENCE
AMSTERDAM, THE NETHERLANDS
NOVEMBER 2008

LECTURES

Mycotoxins – an avoidable or unavoidable risk?

Monica Olsen

National Food Administration, Sweden
monica.olsen@slv.se

Only a handful of risk assessments for mycotoxins have performed by international recognized expert groups such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA) due to the fact that there are still very limited data on the toxicological effects of most mycotoxins. For those that have sufficient data, such as aflatoxins, ochratoxin A, patulin and some to the *Fusarium* toxins, risk assessments have been performed. Several hundreds of mycotoxins have been identified being produced by moulds but still only a limited number have been surveyed in food.

Before proceeding we need to define the terms ‘hazard’ and ‘risk’. A hazard is something, which can have a negative effect on health, while risk is the probability of an adverse effect resulting from a hazard. In this specific case an avoidable 'risk' would then mean avoiding any adverse effect of mycotoxins resulting from the consumption of foodstuffs (i.e., an avoidable hazard). This cannot be completely achieved, but there are measures, which can be applied to reduce the risk significantly if the knowledge and resources are available.

In recent years of mycotoxins research, a lot of attention has been given to preventive and corrective actions leading to recommendations on how to avoid mould growth and mycotoxins formation in different crops. However, the knowledge of best practice is not sufficient to make the hazard avoidable. The socio-economic circumstances will affect the implementation of mycotoxins control measures in the production chain. Furthermore, when risk assessors like JECFA are asked to asses the health effect of certain risk management measures this normally includes a comparison of two different maximum levels (MLs) in food instead of measuring the effect of introducing preventive measures in the production chain. The latter would probably provide more favorable incentive for a successful adoption of preventive measures.

Aflatoxins, even though being the first described mycotoxins, are still among the most important mycotoxins both from a health point of view and as a trade obstacle. Whenever an European member of the Rapid Alert System for Food and Feed (RASFF) network[1] has any information relating to the existence of a serious direct or indirect risk to human health, this information is notified to the Commission under the RASFF. In 2005, the European RASFF received a total of 993 notifications on mycotoxins, of which 947 concerned aflatoxins. Most of these notifications concerned nuts (827) and especially pistachios (498) primarily originating in Iran (457). Specific safeguards measures are in place which, impose special conditions on the import of pistachios from Iran including control of every lot entering into the European union. Despite these control measures pistachios with aflatoxin levels above the ML and at levels, which may imply a health risk, are occasionally found on the market. Of the notifications concerning other mycotoxins than aflatoxins, a large majority of notifications concerned ochratoxin A (42) and to a much lesser extent patulin (6) and fumonisins (2).

[1] The RASFF is primarily a tool for exchange of information between food and feed central competent authorities in the European member states.

Aflatoxin contamination of pistachios has been well studied and the main preventive and corrective measures are described, but not adopted in all producing regions for different reasons. A recent report from Iran describes the main obstacles as the long-term establishment of the contaminating fungi, the number of varieties of pistachios and that the labor-consuming traditional methods still prevail. Combined with poor post-harvest handling makes staining of the nuts so abundant that the effectiveness of removing highly contaminated nuts by hand-sorting is decreased and other means of removing highly-contaminated are indispensable according to the report.

Brazil nut production is another area confronted with problems of contamination by aflatoxins. In this case the lack of infrastructure and huge distances between harvest areas in the rainforest and processing plants make a safe handling of the nuts very difficult. More knowledge is necessary to describe best practice in this kind of environment. However, in contrast to other nuts, Brazil nuts may be one of the few species of nuts that consumers may sort out highly contaminated nuts by the means of visual examination and thus protect themselves from consuming high levels of aflatoxins.

Ochratoxin A has been found as a contaminant in a number of cereals and cereal products and dried vine fruits (currants, raisins and sultanas). Other commodities such as coffee, cocoa, wine, grape juice, liquorice root and beer may also be affected. Ochratoxin A has also been found in food products from animals exposed to ochratoxin A in animal feedingstuffs. Through several different research initiatives, knowledge on how to prevent ochratoxin in most of these commodities are available and in regions where there are no socio-economic constraints to apply correct measures, exposure to hazardous levels of ochratoxin A are rare.

Fusarium toxins, such as trichothecenes, are produced on grain already in the field and even though tools for preventing and controlling these toxins are available, these contaminations are more difficult to avoid. Consequently, the exposures above the tolerable daily intakes of these toxins are likely to occur also in developed countries.

Some mycotoxins, such as patulin, can be important contaminations during preparation of food in the households. With the focus of decreasing the use of preservatives and sugar in the preparation of jams and other fruit products, mould growth are common to occur. In most cases the consumer don’t know whether the product is safe to eat or not. It is therefore important to give advice to consumers on how to avoid mould growth due to inadequate food preparation and how to act in cases where this occurs.

Africa is one of the regions where mycotoxins are recognized as being important constraints to improving human health and well being of people. Exposures of high levels of aflatoxins occur frequently and with lethal consequences in the worst case, such as in Kenya where more than 150 deaths due to aflatoxin poisoning occurred in 2004 and 2005. Consequently, our biggest future challenge is to make mycotoxins avoidable hazards for all consumers.

Mycotoxins and worldwide regulations: recent developments

Hans P. van Egmond and M.A. Jonker

National Institute for Public Health and the Environment (RIVM),
Laboratory for Food and Residue Analysis, the Netherlands
hp.van.egmond@rivm.nl

Regulations for mycotoxins have been established in food and animal feed in many countries since the late 1960's to protect the consumer from the harmful effects that mycotoxins may cause. Current regulations mostly concern the aflatoxins, but for other mycotoxins they are now rapidly developing. Various factors influence the decision-making process of setting limits for mycotoxins. These include scientific factors such as data about effects on man and animals, the levels of human and animal exposure, as well as the availability of suitable methods of sampling and analysis. Economical factors such as commercial interests and sufficiency of food supply, have their impact as well. Weighing the various factors that play a role in the decision-making process to establish mycotoxin tolerances is not an easy process, but it is of crucial importance. Despite the difficulties, mycotoxin regulations have been established in many countries during the past decades, and newer regulations are still being issued. In particular the European Union has been very active in this respect.

In the last decades various international inquiries on worldwide regulations and limits for mycotoxins were undertaken by RIVM for the Food and Agriculture Organization (FAO) of the United Nations. The latest completed inquiry resulted in the publication 'Worldwide regulations for mycotoxins in food and feed in 2003' (FAO, 2004). The FAO publication shows that there are approximately 100 countries worldwide with specific mycotoxin regulations for food and feed. The total population in these countries represents about 87% of the world's inhabitants. National and regional regulations have been established for a number of mycotoxins: the naturally occurring aflatoxins, aflatoxin M_1, agaric acid, deoxynivalenol, diacetoxyscirpenol, ergot alkaloids, fumonisins, ochratoxin A, patulin, phomopsins, sterigmatocystin, T-2 toxin and HT-2 toxin, and zearalenone.

In the presentation some insight will be provided in the way mycotoxin regulations and guidelines are achieved. An overview will be given on limits set for mycotoxins, in particular aflatoxins, ochratoxin A, patulin, deoxynivalenol, T-2 toxin, and fumonisins. The mycotoxin regulatory situation in various parts of the world will be reviewed, with a special focus on the European Union, where rapid developments take place. EU-harmonized limits now exist for 40 toxin/food combinations, and this number will expand steadily in the coming years. For almost 30 toxin/feed combinations harmonized legal and guideline limits are now in force. Various developments in Europe may have an impact on the further establishment of mycotoxin regulations. These include scientific conferences, large pan-European research projects, and activities driven by the European Commission (EC). Examples of the latter are the creation of the European Food Safety Authority (EFSA) and the Community Reference Laboratory (CRL) for Mycotoxins, as well as a mandate of the EC to the European Standardization Committee (CEN) in the field of methods of analysis for mycotoxins in food. It is expected that the scientific basis and transparency of the mycotoxin regulations will benefit from these developments.

References

FAO (Food and Agriculture Organization of the United Nations), 2004. Worldwide regulations for mycotoxins in food and feed in 2003. FAO Food and Nutrition Paper No. 81. Rome, Italy (ISBN 92-5-105162-3).

Mycotoxin regulations and food safety: the international trade dimension

John S. Wilson

The World Bank, Development Economics Research Group, USA
jswilson@worldbank.org

The relationship between food safety regulation and trade is at the forefront of policy debate. Moreover, how international standards are set for food safety, can have a profound effect on export prospects for the least developed countries.

The presentation will review empirical evidence on the impact of regulations on mycotoxin to achieve food safety goals on international trade. It will focus on analysis conducted at the World Bank on aflatoxin standards, and other studies related to food safety and trade (Otsuki et al., 2001; Wilson and Otsuki, 2003). The work on mycotoxin regulations examined the impact of adopting international food safety standards and the harmonization of standards on global food trade patterns.

In specific, research was conducted on the effect of aflatoxin standards in 15 importing (4 developing) countries on exports of selected commodities from 31 (21 developing) countries. Results from this work included estimates that world exports could increase by $38.8 billion if an international standard (Codex) were adopted, compared to the divergent national standards in place. The impact of the adoption of harmonized international standards vs. specific national regulation will be addressed in this session, as well as suggestions for further research on food safety regulations and trade.

References

Otsuki, T., Wilson, J.S. and Sewadeh, M., 2001. Saving two in a billion: quantifying the trade effect of European food safety standards and African exports. Food Policy 26:495-514.

Wilson, J.S. and Otsuki, T., 2003. Food safety and trade: winners and losers in a non-harmonized world. Journal of Economic Integration 18:266-287.

Mycotoxins: ever-lower regulatory standards and toxicological significance

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Since the discovery of aflatoxins in the early 1960s, there has been intensive investigation worldwide on the identification, detection, occurrence and toxicology of foodborne mycotoxins. Regulatory limits have been established based on existing toxicologic data, occurrence and prevalence in foods, and availability of analytical methods as balanced against the need for a sufficient and nutritious food supply. While analytical methods were an early limiting factor in the rigor of such standards, advances in instrumental and immunochemical methods have now enabled the detection and screening of extremely low concentrations of mycotoxins. These increased analytical capabilities have increased public awareness of contamination of agrifoods by very low levels of mycotoxins. This knowledge has driven new evaluations or reevaluations of the potential risks of human exposures to mycotoxin that have led to proposals for new or lower regulatory limits for a number of mycotoxins. Controversy has arisen in some cases as to whether these limits are overly conservative or might be insufficiently rigorous.

In recent years, there has been a remarkable increase in our understanding of the molecular basis of action for the major mycotoxin families including the aflatoxins, ochratoxins, fumonisins and trichothecenes. These findings have the potential to provide extraordinary insight into cellular targets, toxic thresholds, human sensitivity and reversibility and possibility for cumulative effects. Such information can complement the formal health risk assessment approach used by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and various regulatory agencies. This presentation will survey recent advances in understanding of molecular modes of action of mycotoxins and discuss how this knowledge might impact assumptions that underlie existing and proposed regulatory guidelines.

Probabilistic exposure assessment and risk analysis of mycotoxins for all age groups

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To gain a better understanding of the risk assessment of mycotoxins and their management in foods, Health Canada has launched a program involving probabilistic exposure assessments based on Canadian occurrence data for various finished foods or raw food commodities, gathered over the last decade. Such assessments tend to provide a realistic estimate of exposure for various age/sex groups of the population, since they utilize available information regarding variability and uncertainty in model variables. For food consumption, we used recent USDA food consumption data (two non-consecutive days), which included extensive information on infants and young children. The software package FARE® as well as SAS programs, developed in house, were used to assess exposure. Various approaches used to derive exposure will be discussed and the resulting exposure data will then be compared with various risk metrics, such as chronic or short-term tolerable daily intakes (TDIs). Recently completed results for patulin will be presented, as well as preliminary data for some other mycotoxins (ochratoxin and deoxynivalenol).

Patulin in apple juice, the major food of concern for this mycotoxin, was treated on a single commodity basis. Its mean occurrence in apple juice was 10.3 ng/g (imputed, no maximum residue limit or ML) using a parametric distribution for censored data. Older infants and young children (less than 5 years of age) had the highest frequency of consumption (up to 38%, with up to 44% repeat consumers). Because the highest 2-day average juice consumption was observed in one-year olds (mean 16.8 and 90th percentile 34.1 g/kg bw) we focused subsequent detailed analyses on this age group. Adjusting these data to usual (i.e. chronic) consumption reduced the mean consumption values by about 20%. Mean and 90th percentile usual exposure values were below the chronic TDI of 0.4 μg/kg bw. A short-term TDI was developed to consider the impact of occasional excursions above the chronic TDI. Applying a ML of 50 ng/g, decreased imputed mean occurrence to 8.1 ng/g by eliminating the highest occurrence values, thus decreasing variability in exposure. Based on current toxicity information, there appears to be no need to implement lower MLs, even for young children.

Because of their ubiquity, ochratoxin A and deoxynivalenol were analyzed on a multi-commodity basis. Most persons (age one year and up) consumed some of the 37 different food categories containing these mycotoxins on both days of the survey. As a result there was little difference between 'two-day average' exposure of 'all persons' or 'eaters only' and the day one of 'usual' exposure.

For ochratoxin A, our exposure data for the adult population were similar to recent results by Counil et al. (2005, 2006) for persons aged 15-74+ in France, the only age group investigated by that group. Our results show that ochratoxin exposure (ng/kg bw) for young children was higher than for older age groups, as expected. Mean adjusted exposure for all age groups tended to be below 5 ng/kg bw; generally, the 90th as well as 95th percentiles were below 15 ng/kg bw. The major contribution of ochratoxin in the diet came from wheat-based foods (up to 70 % in 7-18 yr-olds), followed by oats (hot oat cereal and other oat based products), rice, and breakfast cereals. Coffee, wine and beer also contribute to total ochratoxin exposure, but these foods are generally only of importance in adults. Of these, coffee was the greatest contributor, especially at ages >30 years. As expected, exposure values decreased when the

European MLs were applied to the occurrence data.

Preliminary results for deoxynivalenol show that mean adjusted exposure tended to be below the TDI of 1μg/kg bw, except for young children (ages1-4), where it reached a maximum value of 1.14 μg/kg bw. The major contribution (87-90% for age 1 and up) came from wheat-based foods. Although infant cereals were an important contributor, mean adjusted exposure from this source for infants was less than 0.17 μg/kg bw. Mean exposure from breakfast cereals was relatively minor, reaching a maximum at age 3 (0.06 μg/kg bw). All other foods provided only a minor contribution to deoxynivalenol exposure.

The use of exposure models, as described above, greatly help in selecting age/sex groups at higher risk, in developing appropriate scenarios to measure the risk, and to model the impact of various ways of reducing these risks. As risk analysis is an iterative process, using this methodology also helps to identify which foods or mycotoxins are of greatest concern. This may result in examining the need for further monitoring of specific foods, and whether current detection limits are appropriate; on the appropriateness of current analytical methodology (i.e. extraction methods for bound mycotoxins); and on toxicology (i.e. development of appropriate TDIs or other risk metrics that consider underlying mechanisms of carcinogenicity or other concerns, and that are appropriate for infants and young children or the elderly).

Acknowledgements
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How to identify new mycotoxin risks?

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New mycotoxin risk can be defined as the risk for consumers exposed to poorly investigated fungal metabolites with putative deleterious effects in humans or animals. Risk assessment is classically based on both the description of toxicological properties and the exposure to the considered toxins through natural occurrence and consumption data. Three major classes will be considered in this review: ergot alkaloids, tremorgenic mycotoxins and *Alternaria* mycotoxins. Due to both their vasoconstrictive and their tremorgenic properties, *Neotyphodium* mycotoxins will be also included in this review.

Ergot is the common name of a fungus in the genus *Claviceps*. It is most prevalent on rye but occurs to some extent on wheat, triticale, barley, oats, forage seed grasses, and native grasses. The *Claviceps* sclerotia contain a large array of chemicals, which are also commonly found in the endophytic infected grasses: ergotamine, ergocristine, ergocryptine and ergocornine. These alkaloids possess to a greater or a lesser degree, biological direct peripheral effects on smooth muscle such as uterine contraction or vasoconstriction and indirect effects as serotonin antagonists or adrenergic blockers. They are known to cause problems to livestock and humans who have consumed grains contaminated with ergot sclerotia. Consumption of infected rye bread has been associated with human outbreaks, which date back some 2,000 years. Livestock are also often poisoned when fed contaminated grains. The symptoms may include one or more of the following: lameness, loss of body parts from gangrene, abortions in pregnant animals, seizures, and eventually death (Berde and Schild, 1978). Consumption of contaminated feeds with sub-lethal doses may lead to problems such as reduced growth performance or loss of milk production in lactating animals. Due to the easy identification of sclerotia in cereals, ergot alkaloids would not represent a current dietary risk for humans. In case of livestock, current recommendations generally indicate that ergot may be present at levels up to 0.1 per cent of the feed intake. Even though the fate of ergot alkaloids in breeding animals is not really investigated, these regulations guarantee safety of animal-derived food products such as meat and milk.

Ryegrass infected with the endophytic fungus *Neotyphodium* spp. contains several classes of toxic alkaloids, including ergovaline and lolitrem alkaloids (Porter, 1997). Ergovaline is a vasoconstrictor normally associated with endophyte-infected tall fescue. Lolitrem B, a potent tremorgen, is generally considered to be the predominant alkaloid in endophyte-infected perennial ryegrass. The symptoms of ergovaline poisoning in livestock include hyperthermia, lower feed intake, weight loss, lower pregnancy rates and decreased milk production. Horses are particularly sensitive to ergovaline and are prone to developing reproductive problems including abortions, difficult births and foal deaths. Fescue foot is also associated with ergovaline and is most apparent in winter months. Animals with this condition develop swelling in the legs and restricted blood flow, causing possibly gangrene, tremors, severe uncoordination and falling down. This condition requires a 7 to 14 day exposure period and clinical signs usually disappear in 2 to 3 days if animals are removed from toxin containing feed. The threshold levels above which clinical signs typically occur are 2,000 µg/kg lolitrem B and 300-400 µg/kg ergovaline. Due to the very low concentrations of lolitrem B detected in milk or tissues of livestock, human exposure to the neurotoxic effect of this toxin through animal-derived food products is unlikely.

Among others, *Aspergillus clavatus, A. fumigatus, Penicillium crustosum, P. cyclopium, P. roqueforti* and *P. verruculosum* produced tremorgenic mycotoxins such as fumitremorgins, penitrem A, roquefortine C, tryptoquivalines or verruculogen (Abramson, 1997). These toxins have been detected in maize, silages or forages and some of them would contaminate beer, cheeses and nuts. These tremorgens have been implicated in a number of neurologic diseases of cattle collectively known as staggers syndromes, and pose significant agricultural and health problems for both animals and humans. They induce neurologic symptoms ranging from mental confusion to tremors, seizures and death, and are apparently the only class of mycotoxins with significant central nervous system activity. In mammals, verruculogen and penitrem A are highly toxic; however, pigs are less sensitive than sheep to these toxins. Tremorgens are lipophilic molecules that may cross the blood brain barrier and gain access to the central nervous system (Cole, 1997). Clinical investigation and *in vitro* studies in synaptosomes indicate that penitrem A and verruculogen affect neurotransmission by increasing spontaneous glutamate and aspartate release and by decreasing γ-aminobutyric acid (GABA) release. They increase also the presynaptic neurotransmitter release at the neuromuscular junction. By using different *in vitro* systems (Ames test, lymphocyte), fumitremorgin and verruculogen exhibited a certain degree of genotoxicity, but not penitrem A or roquefortine. Concerning roquefortine C, this toxin occurs naturally in silages and could be involved in dog outbreaks. Like for other tremorgens, there is a lack of documented cases of human toxicity. In terms of human food safety, the low levels of roquefortine C detected in cheeses and its relatively low toxicity make the consumption of blue cheese safe for the consumer.

Fungi of the genus *Alternaria* are parasitic on plants and other organic materials. *A. alternata* is a frequently occurring species of particular interest because it produces a number of mycotoxins, including alternariol (AOH), alternariol methyl ether (AME), altenuene (ALT), altertoxins (ATX), tenuazonic acid (TeA) and AAL toxins (Panigrahi, 1997). Natural occurrences of AOH, AME, and in some cases other *Alternaria* toxins have been reported in various fruits, including tomatoes, olives, mandarins, melons, peppers, apples, and raspberries. They have been found also in processed fruit products such as apple juice, other fruit beverages and tomato products, wheat and other grains, sunflower seeds, oilseed rape meal, and pecans. Concerning the biological activity of these toxins, some of them have been described to be cytotoxic or mutagenic by using *in vitro* tests. Due to these properties, *Alternaria* toxins have been proposed to play etiological role in human oesophageal cancer in China (Liu et al. 1991). In order to assess this hypothesis, the possible toxic effects of alternariol methyl ether (AME) and tenuazonic Acid (TeA) on oesophagus were investigated in mice fed daily with oral AME (50 or 100 mg/kg/d) or TeA (25 mg/kg/d) for 10 months (Yekeler et al., 2001). Moderate and severe dysplasia was only observed in TeA-treated animals. These results suggested that a high dose of TeA has higher toxicity as evidenced by dysplastic transformation. Conversely, this toxin has been found to protect mice against dimethyl-benzantracen induced skin carcinogenesis. Due to their fumonisin-related structures, AAL toxins would develop similar effects. So, in rat primary hepatocytes exposed for 40 h, both fumonisins and AAL toxins (TA and TB) disrupt sphingolipid biosynthesis by inhibiting ceramide synthase. AAL toxins TA and TB increased the concentration significantly above that of fumonisin B_1 (FB_1) while the sphinganine:sphingosine ratio was altered to the same extent. However, TA and TB were significantly less toxic to primary hepatocytes than FB_1 at all the concentrations tested (Van der Westhuizen et al., 1998).

In conclusion, it remains difficult to assess the risk for human consumers of poorly investigated mycotoxins. Mainly, there is a lack of *in vivo* studies in animal models identifying hazards through dose-response experiments for harmful “new mycotoxins” like ergot alkaloids, verruculogen and AAL toxins. In this perspective, mutagenic, immunotoxic and endocrine disruptor properties of these toxins should be particularly checked in future. Concerning the risk for animals, outbreaks have been clearly described in case of ergotism, fescue foot disease and staggers syndromes. However, regarding these toxins, only little

information is available on their natural occurrence in cereals, fruits or in animal-derived food products such as meat or milk. In consequence, exposure assessment and risk characterization remain also difficult to establish.

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Effects of processing on mycotoxin levels in food and food products

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Mycotoxins frequently occur in raw materials that are intended for food production. Consumer exposure to mycotoxins is the result of contamination of finished food products. The process of converting these materials into food and drinks for consumers has significant effects on the levels of toxins in the final food.

To identify the extent of exposure to mycotoxins it is critical to understand both:

- their occurrence in primary agricultural crops; and
- the effect of processing and food manufacturing on the mycotoxin levels in the foods.

Current existing and proposed mycotoxin regulatory limits in the European Union apply to both the raw cereals and to the final food products manufactured from these commodities. These reflect some of the current understanding of processing effects.

Food processing covers any physical, chemical or biological processes undergone by raw materials in the formation of food products, for example grain milling, baking, extrusion, fermentation. This presentation will focus on the most frequently occurring mycotoxins and recently published information concentrating on commercial scale processing of naturally contaminated raw materials.

The UK is currently undertaking a major collaborative project (funded by Government Agencies) to study the fate of *Fusarium* mycotoxins such as deoxynivalenol and related trichothecenes, zearalenone and fumonisins in the food chain. Data will be presented on the levels of the key *Fusarium* mycotoxins in wheat, maize and oats at intake to mills in the UK, the distribution of mycotoxins in the cereal various milling streams and the fate of *Fusarium* mycotoxins in the representative food processes included in the project; bread baking, breakfast cereal, cakes, biscuits, snacks and oat flakes production.

From initial studies it is clear that mycotoxin contamination of cereals changes from season to season and from region to region. Changes in mycotoxin levels due to milling and processing arise from cleaning regimes, concentration or reduction in the different milling streams and the conditions required in each process. Loss of mycotoxins can be by thermal breakdown, hydrolysis, binding to or release from, cereal constituents and solution in steep liquors.

The significance of these findings for food manufacturers and regulators will be discussed.

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A tale of two commodities: how EU mycotoxin regulations have hurt, or helped, food industries

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The European Union's (EU) regulation on aflatoxin is among the strictest in the world, at 4 µg/kg total aflatoxin in food. Several studies have indicated that this standard causes severe economic losses to food exporters in the United States, China, Argentina, and Africa (Otsuki et al., 2001; Vardon et al., 2003; Wu, 2004), without any noticeable gain in health benefits to European consumers. However, these studies are all based on partial-equilibrium economic models that do not take into account the multiple stakeholders and price fluctuations that are inherent in adjusting to the EU standard. This research corrects these problems by using general equilibrium principles, to show how the EU aflatoxin regulations can in some cases hurt and in some cases help export markets. It explores who really benefits and who suffers as a result of strict food standards.

We start with the question: does it ultimately help or hurt to have one market (EU) that imposes a strict aflatoxin standard? On a global scale, this creates a more tiered market, similar to the automobile industry. Those consumers who do not have such high standards can buy lower-quality food at a lower price, whereas those who do have high standards (EU) can buy higher-quality food at a higher price.

Such an arrangement certainly benefits high-quality producers (e.g., California pistachio producers), because their high-quality goods are recognized in the market, whereas before they may not have resulted in a price premium. Does it benefit *consumers*? This depends on whether consumers see value in the higher-quality good. We would assert that most Europeans, who may not be aware of aflatoxin at all, are *losing* as a result of the strict EU standards; because they must pay higher prices for a level of quality that is unimportant to them. From a purely health-related perspective, it does not benefit consumers significantly to have a 4 µg/kg aflatoxin standard as opposed to a more relaxed standard (Henry et al., 1999). (An analogy, to use the automobile industry again, would be a government that forced every citizen to buy a Porsche, regardless of whether everyone wanted such a high-quality car.)

However, EU consumers may not be the ones bearing the brunt of the cost. Rather, the cost is likely to be born primarily by EU food processing industries. This is because of three economic principles: supply shortage, price elasticity of demand, and substitutability of goods:

- The global supply of foods that can meet the 4 µg/kg standard is very small, so EU food processors must pay higher prices to find quality products.
- Food processors cannot pass all of this cost onto the consumers, because the price elasticity of demand P_d for luxury foods (e.g., tree nuts and their products) is high; meaning that a unit increase in price will result in drastically reduced demand for the good.
- Moreover, luxury goods are highly substitutable. If the price of marzipan, made from almonds, increases, consumers are likely to forego buying it and may buy another kind of confection instead, such as chocolate.

What is the situation for the producers? We examine two case studies: U.S. pistachios and almonds. The U.S. pistachio industry provides an example of a market that has benefited

from strict EU standards, for two reasons: (i) the consistently high quality of the product, and (ii) the ability to shift markets on a global scale. According to data from the U.S. Department of Agriculture Foreign Agricultural Service (USDA FAS, 2004a), ever since the EU aflatoxin standard was enforced beginning in 1998, U.S. pistachio exports to the EU – particularly to Belgium, Luxembourg, the Netherlands, France, and Italy – have increased relative to its exports to other nations. Moreover, the EU has paid a higher price for pistachios than other nations, so the U.S. pistachio industry has gained per unit product over the last decade.

Even other pistachio-exporting nations have benefited. In the last decade, the stricter EU aflatoxin standard has encouraged the Iranian pistachio industry to substantially improve aflatoxin management, both pre-harvest and post-harvest (Ketabi, 2005). As a result, Iran's pistachio exports to the EU have remained much the same in spite of the aflatoxin standard, while its exports to less-strict markets such as Hong Kong have increased significantly since 1998 (USDA FAS, 2004a).

The case for U.S. almonds is different. Unlike the case with pistachios, the U.S. is by far the largest exporter of almonds in the world, with Spain as its main competitor as well as a major importer. Both nations produce generally high-quality almonds. Therefore, there is very little room for global market shifting. Unlike pistachios, U.S. almond exports to the EU have not increased significantly since 1998 (UDSA FAS, 2004b). In fact, in recent years U.S. almonds have been subject to a number of EU rapid and information alerts (ultimately a rejection of the shipment); not surprisingly, almost entirely from Spain. In the latter half of 2005, for example, U.S. goods were subject to 23 EU rapid and information alerts for aflatoxin. Of these, 10 were leveled by Spain against U.S. almonds; the rest were from various other nations for a variety of U.S. commodities (EC, 2005). Each rejected shipment poses a significant cost to the food industry in terms of transportation, demurrage, storage, time, and labor. Therefore, on the whole, the U.S. almond industry has suffered as a result of the strict EU aflatoxin standard.

This analysis allows us to arrive at several conclusions.

- The EU, primarily food processing industries, and secondarily consumers, may in fact suffer the greatest economic loss from its own strict aflatoxin standards. This is because the strict standard results in a drastically reduced supply, which increases market price; and food processors cannot completely pass on this cost to consumers due to the luxury nature of the products. Moreover, health benefits from such a standard are insignificant. We believe that EU policymakers must consider these more nuanced economic impacts, to understand the potential adverse effects of their own regulations to the EU food processing industry.
- Under certain conditions, an export market (e.g., U.S. pistachios) may actually *benefit* from the strict EU aflatoxin standard. These conditions are: (i) a consistently high-quality good, (ii) a global scene that allows market shifts (i.e., high differentiation in quality among multiple exporters), and (iii) a lack of a competing export market from the EU.
- Export markets may also benefit from a strict aflatoxin standard abroad, because it forces them to adopt technologies and methods to control food quality.
- If the conditions above are not all in place, export markets are more likely to suffer as a result of the strict EU aflatoxin standard. In particular, rejections of shipments pose increasingly significant costs.

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Raisin and tree nut mycotoxin issues for export

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The American Council for Food Safety and Quality also known as DFA of California has been in existence since 1908. The non-profit agricultural association represents 75% of the dried fruit and tree nut processed in California. Approximately 50% of California's tree nut and dried fruit commodities are exported. A brief discussion will describe the company and our many programs including the sanitation audit programs: HACCP, BRC and EurepGAP. A pictorial tour of the laboratory will be will be followed by a discussing of a few of the export issues that the California industry faces. The internationally recognized laboratory has close ties with U.S. Food and Drug Administration (FDA), U.S. Department of Agriculture (USDA), California Department of Health Services (CDHS), and the Japanese Department of Health and Welfare. The laboratory specializes in analytical testing, fumigation research and recently received a grant from the USDA. The challenges of mycotoxin sampling, testing and certification will be addressed. It is imperative to create a smooth flow of the high quality products grown in California to our export markets. Future considerations like Country of Origin Testing will also be discussed.

Mycotoxins in herbs and spices: a Trojan horse in the kitchen?

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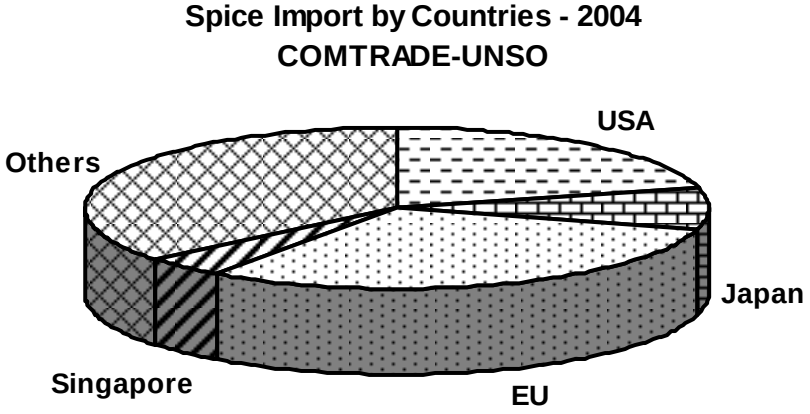
Mycotoxins are a continuous threat in the food and feed chains. By their presence in almost every ingredient of food, the risk of exposure to mycotoxins is of great significance at the point of consumption. This presentation focuses on various aspects of mycotoxin contamination in some herbs and spices of culinary importance. Spices are nature's own preservatives, contributing to the color, flavor and taste of food, spicing up diets as well as consumer's health. Various spices have several health benefits such as antioxidant activity, cholesterol reducing effect, potentiation of insulin activity, antiseptic properties, cardiovascular benefits, etc. The use of these natural nutraceuticals in human food and animal feed has been continuously on the raise in the past years. Though spices are being generously used to improve taste and health, they may also contain potentially harmful agents like toxigenic fungi, mycotoxins, etc., forming a dreaded Trojan horse.

Globally, the production of spices has shown a continuous positive trend. Though China is the undoubted leader in the production and trade of herbs, India is the major producer of spices and culinary herbs with around 86% of the total production (FAO).

World spice production in tons, 2003-2004, data from FAOSTAT		
India	1,600,000	86%
China	66,000	4%
Bangladesh	48,000	3%
Pakistan	45,300	2%
Turkey	33,000	2%
Nepal	15,500	1%
Other countries	60,900	3%
Total	**1,868,700**	100%



Spices like cinnamon, clove, turmeric, thyme, etc., exhibit substantial antifungal activity. Several spices have been found, which prevent the growth of fungi like *Aspergillus flavus, A. parasiticus, A. ochraceous* and also reduce mycotoxin production under testing conditions. Surprisingly, however, there have been reports of these very spices being contaminated with molds, i.e., factors like humidity, temperature, handling, hygiene, etc., can modulate fungal growth. In addition, the concentration at which spices are used in food and feed may not always be high enough to prevent mold growth. *A. flavus*, *A. parasiticus*, and *A. ochraceous* are the fungal species, which are reported widely. The genera *Penicillium*, *Fusarium*, *Alternaria*, *Mucor* have also been reported, but only in a sporadic manner.

Aflatoxin is the major mycotoxin reported in spices worldwide. Most countries have specified upper limits for aflatoxin contamination levels in spices. For example, the European Union (EU) has notified its upper limits at 5 µg/kg for aflatoxin B_1 and 10 µg/kg for total aflatoxins (B_1+B_2+G_1+G_2). Natural contamination with aflatoxins has been reported in almost all the spices. In chillies, pepper, fennel, cumin, coriander, nutmeg, ginger, turmeric, cardamom, etc., various researchers have reported levels of aflatoxins varying generally between 0.5 µg/kg to 25 µg/kg (Giridhar and Reddy, 1999; Heperkan and Ermis, 2005).

Ochratoxin contamination is also a commonly reported phenomenon in spices. The EU has yet to specify a restriction on the level of ochratoxins, which can be found in spices, although it has one already for cereals, beer and wine. Ochratoxin A, produced by *A. ochraceus* or *Penicillium verrucosum*, has been reported in chillies, pepper, coriander, turmeric, ginger (Tirumala Devi et al., 2001; Scheuer and Gareis, 2001), usually occurring in the range of 2.5 to 30 µg/kg.

Other mycotoxins like zearalenone, sterigmatocystin, cyclopiazonic acid, penicillinic acid, citrinin, deoxynivalenol, fumonisins, patulin, etc., have been reported only sporadically. At present time the information available on other mycotoxins in spices, is too less to get a clear picture. In a long term study, ten different spices (45 samples each) were sampled for a period of 3 years from different geographical regions in India during different seasons Of the samples analyzed 48.07% of black pepper, 31% of caraway, 30% of dry chilli, 28% of fennel, 60% of ginger, and 35% of turmeric samples contained one or more of the other mycotoxins. Along with aflatoxins sterigmatocystin, citrinin and cyclopiazonic acid were found (Giridhar and Reddy, 1997).

There is no technology available, which can fully prevent the mycotoxin contamination of food at pre- or post-harvest. However, in food and feed several methods of mycotoxin decontamination have been studied and proven to be effective at varying levels. Among these, application of a glucomannan-containing yeast product (GYP) has been the most promising. As a biotechnological approach, which is also organic, GYP has been shown to decontaminate several mycotoxins co-occurring in animal feeds. After careful validation this concept may be helpful in improving the safety levels of herbs and spices.

In conclusion, herbs and spices are highly beneficial in improving the taste of food as well as consumer's health. However, the presence of mycotoxins is an unavoidable associated risk. Newer concepts in reducing preformed mycotoxins could be a reality in the near future. However, as of now, strict monitoring is the only way by which food and feed operators can ensure that the spices are in accordance with the levels of the mycotoxin regulations in their respective countries.

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Mycotoxin management in the dairy production chain

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Control of the dairy production chain with respect to mycotoxins is required to secure safe milk and milk products. The dairy production chain can be divided in three parts: dairy cattle farming and milk production, milk processing and production of dairy products, and storage and handling by retail and consumers. Control of mycotoxins focuses on the first part of the production chain, in particular on the occurrence of mycotoxins in dairy cattle feeds. The most important mycotoxin for the dairy industry is aflatoxin M_1. Aflatoxin M_1 is formed in lactating dairy cattle from aflatoxin B_1 occurring in contaminated feedstuffs and has a relatively high carry-over rate to milk. In many countries there are stringent regulations for aflatoxin M_1 in milk and dairy products and for aflatoxin B_1 in feedstuffs for dairy cattle. Increasing attention in the dairy sector is given other mycotoxins in feed, in particular to deoxynivalenol (DON), zearalenone and fumonisins. These mycotoxins are of concern to the dairy sector primarily because of their potential negative effects on animal health. Due to their very low carry-over rate to milk they are not of significant concern with respect to the safety of dairy products. The other two parts of the production chain are of less concern. With current production processes in the dairy industry the risk of introducing mycotoxins during manufacturing or storage of dairy products is very low. Mould strains present in commercial starter cultures used in the production of mould-ripened cheeses are generally tested to be non-mycotoxigenic. Mycotoxins may occur in cheeses or other dairy products that become molded due to spoilage, but there is no evidence that consumption of molded foods is a relevant food safety issue.

Control of aflatoxin B_1

Strict control of the aflatoxin B_1 level in feed stuffs for lactating dairy cattle is required in order to be able to control the level of aflatoxin M_1 in milk and milk products. In a number of countries aflatoxin B_1 in feedstuffs and aflatoxin M_1 are monitored routinely. As the risk of aflatoxin B_1 contamination is high in geographical regions with a tropical or sub-tropical climate monitoring of feedstuffs generally focuses on ingredients imported from these regions, such as palm kernel, sunflower cake, maize gluten, cotton seed and soy beans. However, formation of aflatoxin B_1 in maize has also been observed in regions with a more temperate climate in periods with high temperature and drought. In the Netherlands the different players participating in dairy production chain (feed industry, farmers associations and dairy industry) collaborate since 1990 to ensure the lowest possible level of aflatoxin M_1 in milk. As a result of this collaboration the average level of aflatoxin M1 in farm milk has decreased significantly.

Control of *Fusarium* mycotoxins

The *Fusarium* mycotoxins DON, zearalenone and fumonisins in feed are of concern because of their potential impact on animal health and productivity. These mycotoxins are common in cereals and grains and their by-products. In particular maize and maize-based products, including locally grown maize, which is usually fed as maize silage, are important sources of DON, zearalenone and fumonisins. In contrast to feed ingredients used in concentrates there is little knowledge about the levels of these mycotoxins in locally grown feeds. A recent study in The Netherlands showed that maize silage accounts for a significant contribution to the total intake of DON and zearalenone of dairy cattle. Maximum tolerance levels in feed were not exceeded.

Silage-associated mycotoxins

Forages such as grass, whole crop maize and lucerne, generally represent 50 to 80% of the diet of dairy cows. In many countries silage making is used to preserve these forages after harvesting. High levels of moulds are frequently detected in silages, particularly in surface layers. Mold growth in silage is associated with air infiltration during storage, which jeopardizes the conservation. A number of mycotoxigenic mould species have been detected in silages. The most frequently detected species is *Penicillium roqueforti*, a micro-aerophilic, acid-tolerant species capable of producing roquefortin, mycophenolic acid and other mycotoxins. However, there is little knowledge about level of these mycotoxins in silages fed to dairy cattle and their potential impact on animal health. Based on available literature data about toxicity of and metabolism in animals the occurrence of roquefortin and mycophenolic acid in silages is presumably not a significant food safety issue.

In conclusion, aflatoxin M_1 contamination of milk and milk products can effectively controlled by monitoring programs of feed suppliers and the dairy industry. DON and zearalenone are frequently occurring in concentrate feeds as well as forage feeds. Control of these mycotoxins in dairy cattle feeds is primarily an animal health issue. Finally, the occurrence of silage-associated mycotoxins and their relevance for animal health requires more research.

Mode of action of mycotoxins

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Mycotoxins are a structurally disparate group of toxic chemicals. There only common link is that they are all produced by fungi. It is therefore not surprising that their biochemical mechanisms of action are as diverse as their taxonomic origins. The study of biochemical and cellular mechanisms of toxicity is founded on the fact that the action of a toxic chemical is distinct from its effects. Mechanism of action is not a precisely defined concept. The easiest way to envision mechanism of action is as a series of events initiated by an interaction between a specific toxic chemical and some biochemical entity (the proximate cause) and from which there occurs secondary events at the subcellular and cellular level that cascade into a complex array of additional downstream events leading ultimately to observable effects at the organismal level. Secondary effects are often misidentified as the proximate cause (initiating event). For the more toxic mycotoxins, the ultimate observable effect is often cell death; however, adverse physiological effects, which can be expressed as normal cellular responses but at inappropriate times, are also common. The simplest mechanism of action is when a toxic chemical mimics a physiologically relevant agonist (first messenger) for a classical receptor. This can occur by binding to a specific receptor and either initiating or inhibiting the normal function of the receptor-mediated response. For mycotoxins, this is most easily seen with zearalenone, which is known to bind to cytosolic estrogen receptors and initiate the estrogenic response in target tissues. Unfortunately, most of the mechanisms of action are not as simple as those that involve specific binding to classical receptors and the observed effects are also not easily connected to the initial biochemical action (the proximate cause). For example, patulin binds with high affinity to protein- and non-protein-sulfhydryls. The downstream effects can range from inhibition of membrane transporters to lipid peroxidation depending on the proteins/peptides that are most affected. Other mycotoxins such as the fumonisins, cyclopiazonic acid and ochratoxin mimic substrates for specific enzymes/proteins and thus interfere with specific protein functions. The ultimate downstream effects are difficult to predict and are often influenced by subtle differences that are cell or tissue specific and can be modulated by other environmental and physiological factors. For example, cyclopiazonic acid is a specific inhibitor of the sarcoplasmic and endoplasmic reticulum calcium transport ATPase (SERCA). The consequences of inhibition of calcium transport in muscle are quite different than the effects of cyclopiazonic acid in epithelial cells. Likewise, fumonisins inhibit *de novo* sphingolipid metabolism resulting in changes in several sphingolipid intermediates that are ligands for receptors in signaling pathways that control both cell death and cell survival. Examples of environmental factors that can modulate the downstream response to fumonisin inhibition of ceramide synthase include folate sufficiency and antioxidant status. Another example of specific binding is deoxynivalenol (DON) (and other trichothecenes) which binds to the eukaryotic ribosome (28s rRNA) and, thus, inhibits translation of mRNA into protein and initiates the ribotoxic stress response in affected cells. The observed toxic effects of DON inhibition of protein synthesis will be quite different in tissues comprised of quiescent cells compared to tissues in which cells are rapidly proliferating. Finally, some mycotoxins must be metabolized in order to be highly toxic. For example, aflatoxin B_1 (AFB_1) when metabolized to its epoxide readily reacts with DNA and if metabolized to the dihydrodiol reacts readily with proteins. Thus, the ability of the tissue to metabolize AFB_1 is a critical

determinant for toxicity.

In conclusion, the modes of action of mycotoxins are as diverse as their chemical structures (for additional reading see references). While proximate causes have been identified for many mycotoxins, the cascade of downstream events that eventually lead to observed effects are not as easily elucidated since there are many factors that can modulate the expression of toxicity.

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Risk of mycotoxins to pig health: immunosuppressive effects

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The clinical toxicological syndromes caused by ingestion of moderate to high amounts of mycotoxins are well characterized. They range from acute mortality, to slow growth and reduced reproductive efficiency. Consumption of lesser amounts of fungal toxins may result in impaired immunity and decreased resistance to infectious diseases. Mycotoxin-induced immunomodulation is significant for several reasons. First of all, from an agricultural standpoint, it is conceivable that altered immune function may contribute mechanistically to the symptoms of some animal mycotoxicoses. Mycotoxins could also predispose livestock to infectious diseases and reduce productivity. Secondly, from a public health perspective, increased infections in animals may well result in increased animal-to-human transmission of pathogens and/or increased antibiotic concentrations in animal products, as a consequence of animal treatment (Oswald et al., 2005). The sensitivity of the immune system to mycotoxin-induced immunosuppression arises from the vulnerability of the continually proliferating and differentiating cells that participate in immune mediated activities and regulate the complex communication network between cellular and humoral components. Mycotoxin may act on both innate and acquired immune responses. In terms of pig health, mycotoxin intoxication may eventually decreases resistance to infectious diseases, reactivates chronic infection or reduces vaccine and therapeutic efficacy.

Mycotoxins and innate immune response

Mycotoxins can affect the innate immune response by altering barrier function of the epithelial monolayer (Bouhet and Oswald, 2005) and through their action on phagocytes (macrophages and neutrophils). They may directly affect phagocytes viability or impair their functional capacities or their secretory functions. In pigs, an alteration of the inflammatory response by aflatoxin (AF) has been reported. *In utero* exposition of piglets to AF (through exposition of sows) alters the functional capacities of both macrophages and neutrophils. We demonstrated that weanling piglets feed for 4 weeks with low doses of AF have a reduced synthesis of pro-inflammatory cytokines and an increased of anti-inflammatory one (Marin et al., 2002). Recent studies have also provided *in vitro* evidence that fumonisin B_1 (FB_1) influence the inflammatory response. The exposure of chicken peritoneal macrophages to FB_1 reduced cell viability to 80% of the control level. Similarly, incubation of swine alveolar macrophages with FB_1 led to a significant reduction of the number of viable cells and cell death by apoptosis (Liu et al., 2002).

Mycotoxins and humoral immune response

Mycotoxins also affect humoral immunity. Of particular interest is the effect of deoxynivalenol (DON), also called vomitoxin, on antibody synthesis. In mice, one of the most dramatic effects of this toxin is a pronounced elevation in serum immunoglobulin A (IgA) and concurrent depression in IgM and IgG. The associated immunopathology, which includes glomerular IgA accumulation and hematuria, is very similar to human IgA nephropathy. These effects can persist a long time after the withdrawal of DON from the mouse diet but intermittent exposure is less effective at increasing IgA levels than continuous exposure. DON-induced increased IgA production may be mediated by T lymphocytes and macrophages and especially through the superinduction of cytokine genes such as IL-2, IL-5 and IL-6 (Pestka et al., 2004). In pigs, an increase of IgA in the serum of animals receiving DON contaminated feed. However, in these experiments the levels of IgG and IgM in the

serum as well as the levels of expression of several cytokines were not influenced by the diet (Accensi et al., 2006; Drochner et al., 2004; Swamy et al., 2002).

Mycotoxins and cellular immune response

AFs alter cell mediated immunity. Their effects on humoral immunity require higher toxin concentration and are inconsistent across different species (Meissonnier et al., 2006). In pigs, attempts to evaluate the effects of AF on the cellular immune response have lead to conflicting results. Several papers have demonstrated a reduction in lymphocyte stimulation in animals receiving contaminated feed. By contrast, other investigators have not observed any suppression of the lymphoproliferative response. Developing piglets might be especially susceptible to this toxin. Indeed, after exposure of sows to AFB_1 or AFG_1, the lymphoproliferative response of piglets was reduced as well as their monocytic functions (Silvotti et al., 1997). In human as well as in chicken, a genetic component has involved in AFB_1-related cell-mediated immune suppression. The molecular-cellular basis and general mechanism responsible for the broad immunosuppressive effects of AFB_1 appears to be directly related to impaired protein synthesis. AF alters cytokine synthesis by macrophages and/or T cells. Ultrastructural studies show that AFB_1 causes selective mitochondrial damages in murine lymphocytes and does not affect other cellular organelles and external structures of the lymphocytes.

Significance to pig health

Susceptibility to infectious diseases

The broad immunosuppressive effect of mycotoxins may decrease host resistance to infectious diseases. This has also been shown in mice and in domestic animals. In pigs, consumption of feed contaminated with AF increased the severity of the *Erysipelothrix rhusiopathiae* infection. Ingestion of ochratoxin A (OTA) contaminated feed also increases susceptibility to natural infectious disease. Indeed, salmonellosis arose spontaneously in all piglets receiving an OTA contaminated diet and when the animals were vaccinated against salmonellosis, the consumption of contaminated feed lead to spontaneous *Serpulina hyodysenteriae* and *Campylobacter coli* infections (Stoev et al., 2000). In our laboratory we demonstrated that FB_1 constitutes a predisposing factor to *Escherichia coli* infection in piglets (Oswald et al., 2003). Additional *in vitro* and *in vivo* experiments indicate that FB_1 decrease the synthesis of IL-8, a cytokine involved in the recruitment of inflammatory cells during an infection. FB_1 also alters the integrity of the epithelial cell monolayer and increases the translocation of bacteria across the epithelium. Both phenomenons may participate in the increased susceptibility of the animals to intestinal infections (Bouhet al al., 2004, 2006).

Reactivation of chronic infection

The effect of mycotoxin intoxication on the reactivation of chronic infection was also investigated, however the experiment was not performed with pigs but with rodents. In the immunocompetent host, *Toxoplasma gondii* infection progresses to a chronic phase characterized by the presence of encysted parasites. Cyst rupture may occur, but infection remains latent and reactivation is prevented. In immunosupressed animals and human subjects, such as HIV infected patients, rupture is associated with the formation of new cysts and disease. Low and repeated doses of either AFB_1 or T-2 toxin are able to accelerate *Toxoplasma* cyst rupture in previously infected mice (Venturini et al., 1996).

Vaccination efficacy

Immunity acquired through vaccination is also impaired by mycotoxin ingestion. For example, AFB_1 interferes with the development of acquired immunity in swine following erysipelas vaccination with bacterin preparation of *Erysipelothrix rhusiopathiae*. We have demonstrated that ingestion of low doses of another mycotoxin, FB_1, decreases the specific antibody response mounted during vaccination in pigs. Indeed, a prolonged exposure to feed contaminated with 8 mg/kg of FB_1 does not modify the serum concentration of total

immunoglobulin but significantly decreases specific anti-body response towards a model antigen. *In vitro* analysis on pig lymphocytes reveals that this toxin inhibits cell proliferation and alters cytokine production. FB_1 increases the synthesis of IFN-γ, a Th1 cytokine involved in the cell mediated immune response and decreases IL-4 synthesis, a Th2 cytokine involved in the humoral response. This alteration of both lymphocyte proliferation and cytokine production might explain the failure in vaccination that we observed *in vivo* (Taranu et al., 2005; Marin et al., 2006). The effect of DON on pig vaccinal immune response is more controversial. Indeed some reports indicate that DON decreases specific antibody response (Overnes et al., 1997; Rotter et al., 1994), whereas other suggest that this toxin increases both total and specific antibody response (Pinton et al., 2006). The presence of low levels of mycotoxins in the feed can lead to a breakdown in vaccinal immunity and may lead to the occurrence of disease even in properly vaccinated flocks. These reactions are of considerable consequence in animals for which we rely on an effective vaccination program for disease prevention.

In conclusion, several mycotoxins alter immune-mediated activities in pigs. Furthermore, mycotoxin-induced immunosuppression may result in decreased host resistance to infectious disease and decrease vaccine efficacy. However, some considerations have not been taken into account. First, mycotoxin mixtures are likely to occur naturally and these may alter immunity in an additive or synergistic manner as it has been described for aflatoxin and T-2-toxin or for deoxynivalenol and fusaric acid. Second, nutritional effects associated with feed refusal may also contribute to observed alterations. Finally, while systemic immunity is the focus of most investigations, it is probable that mycotoxins have their greatest effect on mucosal lymphoid tissue (particularly gut) before they are absorbed and subsequently metabolized.

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Interference of mycotoxins with poultry health and implementation of control programs

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Mycotoxicoses are diseases caused by fungal toxins. Poultry mycotoxicoses due to factors such as fungal growth and mycotoxin production in the field, during storage and processing of animal feed, are very common. Reduction in metabolism and performance of animals happen long before macroscopic lesions can be seen. Macroscopic lesions in birds affected by mycotoxicoses are not pathognomonic, and can be similar to a lot of different diseases. Actually, mycotoxicosis is a metabolic disease and many different lesions can be associated with it. In addition, there are more than 300 different compounds classified as mycotoxins and each one has a specific mode of action and target organ. The interaction of different types of mycotoxins – a very common situation in the field – can produce different clinical signs, normally associated with enteric syndromes. Some mycotoxins can cause contact lesions, especially T-2 toxin, which causes in oral lesions and enteritis. Fumonisins are also associated with enteritis in poultry. Other mycotoxins, such as aflatoxins and ochratoxins, are potent hepatotoxins, reducing digestive activity and animal metabolism. Aflatoxins have been reported to reduce the activity of digestive enzymes (pancreatic amylase, lipase and trypsin), resulting in non-digested feed in the animal intestine. This can also increase the activity of the intestinal microbiota resulting in enteritis. Affected animals show non-digested feed in the litter, low uniformity in the flock, despigmentation and, at necroscopy, severe duodenitis, non-digested feed and gas in the other part of the intestine, with or without mucosal inflammation; the liver can show different scores of esteatosis. On the other hand, some nefrotoxins such as aflatoxins and ochratoxins, can cause severe kidney lesions resulting in urate deposition in the feces; and at necropsy kidney hypertrophy and urate deposition on ureteres, muscles or intestines can be seen. Although all these lesions can be very important to animal health, probably the most important damage caused by mycotoxins in poultry production is immunessupression, which can happen before the development of macroscopic lesions. Most mycotoxins interact in a way with the immune system of poultry and can affect the sensitivity of these animals to other pathogenic agents. For example, mycotoxins reduce the response to various vaccinations (infectious bursal disease, Newcastle disease and coccidiosis) and so these pathogenic agents can cause reduction in animal production and even mortality. Field experiments have shown that most of the mycotoxicoses are subclinic, while it is very difficult to determine exactly which mycotoxins are actually involved. The reduction in vaccination response, the increase in sensitivity to pathogenic agents and the reduction in animal digestive and absorption capacity reduce strongly the economic viability of poultry production.

Table 1. Nutritional losses in corn stored for 60 days at different moisture contents.

Moisture at storage (%)	CO_2 production	Fat content (%)	Crude protein (%)	Specific weight (kg/m^3)
12	3,380	4,6[a]	8,7[a]	689[a]
15	10,750	4,0[a]	8,9[a]	622[b]
18	24,266	2,2[b]	9,5[b]	538[c]

[a,b,c] Different letters in the same column mean statistical differences($P<0,05$) in the Tukey test (adapted from Krabbe, 1995).

As fungi produce mycotoxins, it is always mentioned it as the worse problem promoted by fungi growth in cereal to animal production. However, fungi can also cause losses in nutritional values of for example cereals, especially in fat content (energy level) and specific weight (Table 1). This will result in losses in animal performance and in the cost of feed production, as nutritional corrections of diet formulation are needed.

It is very important to implement measures, which reduce the impact of mycotoxins in animals. The ideal method is to avoid fungal growth and to prevent contamination with mycotoxins. The method of choice is the implementation of a complete HACCP program, or at least the HACCP principles:

- Conduct a hazard analysis.
- Determine the critical control points (CCPs). CCPs could be, for example, the quality of cereals used, storage conditions, the accumulation of dust and organic material inside the equipment for feed production, transport conditions, the presence of 'old' organic material in animal feeders.
- Establish critical limits.
- Establish monitoring procedures. Monitoring procedures should be fast and easy, for example visual inspection of grains at intake, routine analysis of animal health (vaccinal litter, hematocrit values, seric protein, activity of seric enzymes such as aspartate aminotransferase).
- Establish corrective actions. At pre-harvest, hybrids less susceptible to molds should be cultivated in areas with perennially fungal growth and/or mycotoxin problems. Identifying the prevalent molds will be necessary prior to selecting the appropriate hybrids. Although hybrids vary in susceptibility, no hybrids are completely resistant to grain moulds. For example, hybrids with very tight husks are more susceptible to *Gibberella* ear mold, and less *Fusarium* and subsequent mycotoxin contamination has been reported in corn-borer resistant Bt-hybrids. An effective insect management plan during the growing season as well as during post-harvest shipping and storage of the grain can alsoreduce mould and mycotoxin contamination. Grain handling should be designed to minimize mechanical damage. At post-harvest grain moisture content should be reduced to less than 15% within 48 h after harvesting to minimize the potential for fungal growth and mycotoxin production. Storage bins and feed production equipment should be cleaned thoroughly each time. The use of fungicides is a very common management practice during storage being very efficient to reduce fungal growth and its consequence for losses and mycotoxin production. In addition, adsorbents to control mycotoxins in animal feed can be applied: organic (yeast cell wall components) and inorganic adsorbents (clay derivatives such as aluminosilicates, bentonites, zeolites). These adsorbent differ in composition and mode of action (Watts et al., 2003; Aravind et al., 2003).
- Establish verification procedures.
- Establish record-keeping and documentation procedures.

The interference of mycotoxins with poultry health and implementation of control programs will be discussed in more detail during the presentation.

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Effects of mycotoxins on ruminants

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Ruminants may be exposed to greater mycotoxin risks than monogastric animals. Ruminants consume a diverse milieu of feedstuffs, including grains, by-product feeds, and various forms of forages, which increases the potential exposure to a broad array of mycotoxins, some having received little research attention. Contaminated or damaged grains diverted from use for monogastrics may be included in ruminant diets. By-product feeds such as grain screenings, spent grains, and hulls have potentially greater risk for mycotoxin contamination than do pure grains. Forages in the form of pasture, hay or silage may harbor mycotoxins, which are different from those found in grains. If not carefully handled, wet feeds present their own inherent mycotoxin risks.

Ruminants are somewhat protected against high concentrations of mycotoxins because of partial degradation of mycotoxins by microbial action in the rumen. Prior to the development of a functional rumen, young ruminants are more susceptible to mycotoxins. Ruminal degradation of mycotoxins may reduce the incidence of acute mycotoxicosis in ruminants, but may mask the effects of mycotoxins and result in a greater incidence of chronic toxicities as a result of long term exposure to lower levels of mycotoxins. Degradation of mycotoxins in the rumen also results in formation of some toxic derivatives that may produce symptoms that are different and may be unexpected in relation to the known mycotoxin exposure. Unexpected symptoms may also result from mycotoxin interactions resulting from the broad array of possible mycotoxins in ruminant feeds. Chronic exposure to low levels of mycotoxin may result in reduced performance and increased disease and yet in practice be perceived as simple digestive upsets, faulty diet formulation, poor forage quality, and various stresses associated with weather, production or management. The idea of chronic mycotoxicoses in ruminants may have received greater attention in recent years due to increases in production stresses and greater attention to the details of production management.

While it has long been known that ergots affect ruminants, toxicities caused by other mycotoxins have been uncovered in the last 45 years, and yet with only a modest amount of research directed at the ruminant animal. In particular, the discovery of aflatoxin secretion in milk resulted in a period of research with dairy ruminants that characterized this process and led to the establishment of aflatoxin guidelines worldwide. In addition, some forage mycotoxins that can produce acute toxicities, have received research attention, but most of the research has been influenced by the mycotoxins occurring in grains.

Some of the better recognized forage mycotoxin problems include fescue toxicosis, ryegrass staggers, paspalum staggers, slobber syndrome, locoism, photosensitization and sweet clover disease. Fescue toxicity results from the production of ergot alkaloids that can produce symptoms of vasoconstriction, necrosis of the extremities to include foot disorders, fat necrosis syndrome, reduced performance, and lower prolactin levels reducing immune function and causing agalactia. Ryegrass staggers results from lolitrems (indole terpenoids) that produce a staggers syndrome with unsteady gait, muscle twitching and weakness, which can progress to convulsions but rarely death. Slobber syndrome, most commonly associated with red clover, is caused primarily by slaframine but perhaps in combination with swainsonine, both alkaloids. Swainsonine, produced by an endophyte in certain legumes generally referred to collectively as locoweeds, is responsible for locoism resulting in neurological symptoms, fetal developmental disorders, heart damage and death.

Photosensitization disease has been observed in some countries and is the result of hepatotoxicity associated with sporidesmin. One way the disease is being managed is by use of genetically resistant animals. Sweet clover disease causes a hemorrhagic condition and results from the mold induced production of dicoumarol in sweet clover or sweet vernal grass.

The major classes of mycotoxins include the ergot alkaloids, aflatoxins, trichothecenes, fumonisins, zearalenone, and ochratoxin. Ruminants are affected by ergots regardless of source (forage or grain). In ruminants, aflatoxin not only appears in milk, but also affects rumen fermentation and damages the liver and intestine, resulting in anemia, jaundice and hemorrhage. Aflatoxin also reduces immunity, reproduction and performance. Trichothecenes are known to affect ruminants causing anorexia, weight loss, digestive tract irritation, altered rumen fermentation, immune suppression, reproductive disorders and death. Fumonisin affects ruminants by appetite reduction and liver damage. Zearalenone is associated with estrogenic effects in ruminants. Estimates of ochratoxin degradation in the rumen have been variable, but data suggest that mature ruminants are not very sensitive to ochratoxin, however, tissue residues can occur, and pre-ruminant calves can be affected.

Beyond the major mycotoxins, some other mycotoxins may be of greater importance to ruminants than monogastrics because they occur in ruminant feeds such as silage or other wet feeds and include PR toxin, mycophenolic acid, roquefortines, patulin, penitrem A, gliotoxin, fumigaclavine A and C, fumitremorgens A and B, sterigmatocystin, satratoxins and others. Of these other mycotoxins, perhaps those produced by *Penicillium* should receive more rigorous investigation because of the prominence of *Penicillium* in silage and because *Penicillium* produced mycotoxins have been associated with ruminant toxicity.

Ruminants may be affected more routinely and by a broader range of mycotoxins than are monogastrics because of the mycotoxins that occur in forages, particularly silages, in wet feeds and in by-product feeds. Research is needed to better define the interaction of mycotoxins and rumen fermentation. Research is needed on the economic cost of chronic exposure to low levels of mycotoxins on performance and health in ruminants. Research efforts should pursue the antibiotic effects of mycotoxins on digestive microorganisms and tissue residues. Also needed, are improved methods to monitor animal exposure to mycotoxins and to better diagnose toxicities.

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Effects of mycotoxins in the equine – what we know and what we don't know

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Mycotoxins present a unique problem in the equine to other farm animals. These other species are bred for growth, and meat yield and have a relatively short lifespan while in most cases the horse is bred for athletic performance, confirmation, temperament, beauty and/or durability. Limiting values or safe concentrations of specific mycotoxins are, for the most part, unknown for the horse. However, recent studies have help to expand our knowledge of these toxins on the equine. In addition to the threat of mycotoxins in grains, the equine may also be exposed to forage toxins and mycotoxins that may be present in bedding material. For these reasons, probably more so than other species, the safe amount of specific mycotoxins is unknown for the horse. In addition to the degree of exposure, incidence of disease can also be influenced by the presence of multiple mycotoxins. Factors influencing susceptibility to mycotoxins include; disease, stress, poor nutrition, drug interactions, presence of multiple toxins, crowding, age and reproductive status. A summary of equine aflatoxicosis cases indicates a very large range from approximately 55 to 6,500 µg/kg to be associated with clinical symptoms with duration of exposure and previous history of exposure playing a role in aflatoxicosis.

Fusarium mycotoxins have also been demonstrated to have deleterious effects on the equine. Horses could come in contact with deoxynivalenol (DON) and/or fusaric acid from the consumption of corn, wheat, wheat bran, wheat by-products in grain concentrates and/or from the consumption of wheat-straw bedding. Since the studies examining DON effects on horses represent a large range in potential toxic levels, it is suggested that, until more precise research is completed, the maximum tolerable level for DON in the total feed for horses be comparable to levels allowed for humans. Zearalenone is an estrogenic *Fusarium*, non-tricothecene mycotoxin. It was found that the corn screenings fed to the horses contained on average 2.7 mg/kg of zearalenone. These screenings fed to a herd of horses on a commercial farm produced strong estrogenic symptoms after a feeding period of 30 days. Fumonisin in horses can cause equine leukoencephalomalacia (ELEM), which is typified by staggers, stupor, lameness, seizure (due to brain necrosis) and death. In the equine, the consumption of fumonisins produces a brain neurological disease identified by multi-focal liquefactive necrosis of the white matter affecting multiple horses in a herd. Once clinical signs appear, the majority of affected horses die. Horses that survive typically have some degree of permanent neurological disorder. Corn screenings can be heavily contaminated with fumonisins and should never be fed to horses. Concentrations of fumonisin B_1, B_2 and B_3 in equine feed should not exceed 5 mg/kg and should not exceed 20% of the diet on a dry-matter basis. Studies on the effects of T-2 toxin on the horse are sparse, with only one report involving the feeding of T-2 toxin. The feeding of 7 mg of purified T-2 toxin to equines *per os* daily, to mimic a 1 mg/kg concentration in feed, had no effect on the ovarian activity of mares. In other species, oral and intestinal lesions are frequently observed in animals consuming T-2 or diacetoxyscirpenol (DAS) at levels as low as 100 µg/kg for a period of 25 days.

Mycotoxins in forages have been documented to cause illness and death in the horse. One of the earliest recognized mycotoxin diseases was stachybotryotoxicosis. The fungus causing this illness produces black, 'soot-like' spores in poorly prepared hay and straw and has been found to grow extensively on moist drywall in houses. Traditionally, the first cases

of this disease appear in the autumn when animals are stabled and inhale or consume fodder containing the toxin. There is a subsequent increase in affected animals as the winter months continue into April when the toxicosis begins to decline as horses are returned to pasture and stall confinement is diminished. The presence of a fungus in fescue grasses has been associated with reduced weight gain, rough hair coats, higher body temperatures, reproductive problems and necrosis of the foot, tail and ears of horses and cattle. Increases in the number of cycles for conception and early embryonic death have also been associated with endophyte-infected fescue. In addition, increased length of gestation, retained and so-called 'red bag' placentas, higher numbers of weak or stillborn foals, and agalactic mares were associated with endophyte infected fescue consumption compared to mares consuming endophyte-free fescue. In addition, toxins associated with ryegrass and clover have been documented in the horse. It can be argued that given the complex interactions of mycotoxins with feed ingredients, varying exposure situations and the state of the individual horse, safe levels cannot be identified and that no levels of mycotoxins can be demonstrated to be safe in a field situation. Levels can be reduced with the testing of feeds and forages, increased awareness, proper management and possible the use of compounds that have been shown to have binding capabilities on mycotoxins.

Mycotoxin concerns in the pet food industry

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The human-animal bond has resulted in the pet being considered a companion and now over 64 million households in the USA have one or more pets. The general pet owning public considers pet food to be safe and nutritious while unaware of the potential presence and risk of mycotoxins in pet food. While the pet owners provide the pet food industry a large market for their product, emotional concern of owners for their pet's health creates a major responsibility for the pet food industry to provide a safe product.

Pet foods have been shown to contain a number of mycotoxins. Mycotoxins such as aflatoxins, trichothecenes, zearalenone, ochratoxins, fumonisins, and tremorgens are found at varied levels, from different sources, and known to produce different physiological effects. Outbreaks of mycotoxicosis have occurred in pets and the potential of adverse effects of mycotoxins in pet food is a real but poorly recognized fact. The pet owner relies on the pet food industry to address the mycotoxin problem. Cereal grains and nuts can be contaminated with mycotoxins at pre-harvest, or during transportation and storage; efforts are being made to reduce contamination at these levels. The existence of these natural toxins produced by fungi in grains, however, cannot be totally avoided. The producers of pet foods must address the mycotoxin threat to maintain public confidence. The approach to controlling the mycotoxin risk begins with rigorous analysis. Analytical capabilities have vastly improved but at increasing costs. The uneven distribution of mycotoxins in crops creates sampling difficulties and mycotoxins can potentially escape detection. Screening techniques with confirmation of suspect samples must be used with stringent sampling protocols. Plants may bind glucose to toxins and produce 'conjugated mycotoxins' that can escape detection in standard analytical protocols. Sampling and analytical issues must be addressed from the raw ingredients up to the final product.

At present regulatory policy, which determines acceptable levels of mycotoxins in pet food mostly reflect the analytical detection limits, regional prevalence and trade relationship amongst different countries. Toxicological evidence, however, is not given adequate consideration by the regulatory agencies. To determine the significance of the mycotoxins and the levels detected in raw ingredients and pet foods, producers must consider 'risk' in estimating safety. Risk is the probability of an adverse effect and is determined using the inherent toxicity and the exposure level of the mycotoxin in the feed. In estimating risk, pet specific toxicity data and the most sensitive adverse effect should be used if possible. Unfortunately little pet specific toxicity data is available and risk requires extrapolation of alternative species data. While genomics and proteomics may provide useful information on mechanism of toxicity, it is questionable whether toxicological findings at the molecular level should be considered clinically significant as those at the organ or cellular level. Thus ever more sensitive methods of detecting toxicity may not result in ever-lower concentrations in regulatory standards.

The safe level of mycotoxin in pet food would best be determined using the No-Observed-Adverse-Effect-Level (NOAEL) of that mycotoxin**.** Unfortunately the NOAEL is difficult to determine as it requires the detection of a dose at which there is no effect. Toxicity data commonly generates the Lowest Observed Adverse Effect Level (LOAEL), generally the lowest dose used in the study, but there are levels that would cause effects below this value.

Therefore to determine a safe level of mycotoxin in pet food, Safety Factor numbers (SFn) should be used similar in principle to those used in human exposure limits. These numbers consider such uncertainties as species-to-species variability, conversion from LOAEL to NOAEL, and severity of response. Pet food regulations, however, should not attempt to be equivalent to human safety limits. Consequence of such strict regulations would be increased competition with the human food chain, increased pet food costs, decreased profits and the possibility of promoting mycotoxin outbreaks. With the application of safety limits, further pet food safety can be achieved through processing techniques such as abrasive pearling and dilution, or application of mycotoxin adsorbents. These solutions, however, should never be used alone to avoid safety responsibilities.

The potential of mycotoxicosis from pet food is therefore a controllable risk. Future research should consider real world or field conditions of multiple mycotoxin exposure. The additive or synergistic effects of mycotoxins are beginning to appear in the literature. The public perception that 'organic foods' are safer must also be examined. With the avoidance of fungicides and insecticides there is the real threat of increased fungal growth and mycotoxin contamination. The pet food industry must support pet specific research to increase pet food safety and boost public confidence.

Mycotoxins – a rising threat to aquaculture

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Over the last 10 years plant based ingredients have been increasingly used in fish diets. The main reasons for replacement of fish meal and oil with plant based ingredients is due to increasing economic/market pressure placed upon the fish meal and oil manufacturing industry and animal feed compounder; coupled with the consequent search, development and use of lower cost and more sustainable alternatives by the aqua feed manufacturing sector (Tacon, 2004). The aquaculture sector will have no choice but to further reduce its dependency on fishmeal and oil in the future, in order to sustain its growth and competitiveness. As plant ingredients pose a high risk for mycotoxin contamination, moving to such protein sources in the aqua feed industry demands careful risk assessment regarding mycotoxins as well, as the development of appropriate protection strategies for fish fed contaminated feeds. In aquaculture, mycotoxin contamination of feeds and their ingestion affects growth, feed utilization, livability, reproduction, immunity and product quality. There are three primary mechanisms by which mycotoxins exert their effects. Firstly, there is an alteration in the content, absorption and metabolism of nutrients. Secondly, there are changes in the endocrine and neuroendocrine function. Thirdly, and most importantly, there is a suppression of the animal's immune system. Although several hundred mycotoxins are known, the mycotoxins of most concern in aquaculture, based on their toxicity and occurrence are aflatoxin, ochratoxin A, trichothecenes (deoxynivalenol, T-2 toxin), zearalenone, fumonisin, and moniliformin.

Effect of single toxins and toxin combinations

Aflatoxin and ochratoxin A

As the fungi which are producing alfatoxin and ochratoxin A require higher temperatures for mycotoxin production, those toxins are more often found in warmer, humid climates.

Alfatoxin can be transferred at low rates into edible tissues. Therefore, it is not only of concern to animal health, but also to humans consuming food from animal origin. Aqua diets have been reported to be contaminated with aflatoxin up to 1,000 µg/kg. However, much lower levels can have detrimental effects in both shrimp and fish. As in other animal species, aflatoxin exerts carcinogenic effects in fish. Different research groups have reported that long-term exposure of less than 1 µg/kg of dietary aflatoxin B_1 can be sufficient to cause hepatomes (Lee et al., 1968; Sinnhuber et al., 1965).The carcinogenic or toxic effects of aflatoxin for fish seem to be species specific, with rainbow trout being particularly sensitive to the toxin. Studies with tilapia also show an age dependent effect, with fingerlings being affected at lower inclusion levels than older animals. Aflatoxin can lead to irreversible organ damage. Thus, it has been observed that growth rate remained at a lower level, when switching back to uncontaminated feed, indicating that the fish were not able to fully overcome a previous exposure. As aflatoxin can impair immune function (Ottinger and Kaarrari, 2000), exposure will render the fish more susceptible to disease. Reduced immune function has been reported in Indian major carps (*Labeo rohita*) following exposure to aflatoxin B_1 in the feed at doses as low as 1.25 mg/kg body weight (Sahoo and Mukherjee, 2001). Aflatoxin has also been shown to significantly reduce shrimp performance. Bautista et al. (1994) have reported a significant reduction in performance of pre-adult shrimp (*Penaeus monodon*) at aflatoxin B_1 concentrations of 75 µg/kg in a 60-day study. At the same level of challenge, higher susceptibility to shell diseases was also noted. Histopathological changes in the hepatopancreas of shrimp were observed at the lowest inclusion level of 25 µg/kg, and became more pronounced with increasing dietary toxin concentrations.

Ochratoxin A in aquaculture has not been studied to the same extent as aflatoxin. In catfish, ochratoxin A has been shown to reduce weight gain when fed at 1,000 µg/kg for 8 weeks (Manning et al, 2003a), although 500 µg/kg did not affect weight gain. Higher inclusion levels (4-8 mg/kg) significantly reduced feed conversion as well as hematocrit values. Interestingly, in contrast to observations in mammalian and avian species, the toxin did not lead to any necrotic changes in the renal tubules. Necrosis was only reported in hepato-pancreatic tissue at toxin concentrations as low as 1,000 µg/kg. More recently, Manning et al. (1995) showed an increase in susceptibility towards *Edwardsiella ictaluri* in catfish fed diets containing 4.0 mg/kg of ochratoxin A. Mortality in challenged fish was 68.28% compared to 80.49% in control fish.

Trichothecenes and zearalenone

Trichothecenes and zearalenone are produced by *Fusarium* molds in temperate climates, and represent typical field toxins. Arukwe et al. (1999) reported that zearalenone could affect reproductive success and the development of fish eggs. Alpha-zearalenone, one of its metabolites, has been shown to reduce the number and quality of sperm in carp (Sándor and Ványi, 1990). Trichothecenes have been intensively researched and are known to affect aquatic species. In rainbow trout, Woodward et al. (1983) reported that diets containing levels of 1.0 to 12.9 mg/kg of deoxynivalenol (DON) caused progressively greater reductions in 4-week live weight gain on juveniles. The depression in weight gain ranged from -12 to -92% compared to the control, and resulted from adverse effects on both feed intake and feed conversion. Complete feed refusal was observed when dietary DON concentrations reached 20 mg/kg or more. Catfish seem to be more able to tolerate dietary DON (Manning, 2004). Despite their tolerance towards DON, catfish seem quite susceptible towards T-2 toxin. Levels as low as 625 µg/kg have been shown to reduce weight gain in catfish (Manning et al., 2003), and higher concentrations (5,000 µg/kg) significantly reduced feed conversion, survival rate and hematocrit concentrations. Histological inspections revealed an increased incidence of gastritis, a finding that is in agreement with intestinal lesions and gastritis reported in other species. As in other animal species trichothecenes have been shown to be immune suppressive and thus to enhance disease susceptibility in fish. T-2 toxin at concentrations of 2.0 and 4.0 mg/kg has been shown to significantly increase mortality catfish challenged with *Edwardsiella ictaluri* (Manning et al., 1995). In shrimp, Trigo-Stockli et al. (2000) reported that DON-concentrations as low as 0.2 mg/kg led to significant reductions in growth rate in the last phase of a 16-week performance trial. Overall body weight was affected at a level of 0.5 mg/kg (11.22 g vs. 10.43 g). Toxic levels reported in both trout and shrimp seem comparable to concentrations reported in swine, where concentrations of 1 mg/kg or more are considered problematic. Lower concentrations have been shown to reduce feed intake in young piglets (Spring and Strickler, 2004).

Fumonisin and moniliformin

Fumonisin and monoliformin are of concern to the aquaculture industry as it is considered a major problem in corn, as it commonly contaminates corn and its by-products. A survey of catfish feed ingredients in Alabama and Mississippi revealed that 80% of the corn samples contained detectable levels of fumonisin. Concentrations ranged from 1.3 to 10 mg/kg (Lumlertdacha and Lovell, 1995). As with other mycotoxins, younger animals also seem more susceptible towards fumonisin than older ones. Twenty mg/kg of fumonisin has been shown to reduce growth rate in fish with an average weight of 1.5 g (Yildirim et al., 2000). Only two-year old catfish had reduced weight gain when exposed to 80 mg/kg fumonisin. Toxic effects of fumonisin have been reported in Nile tilapia at concentrations similar to catfish (Nguyen et al., 2003). Immune suppression has been reported at lower dietary concentrations, which have not been shown to affect growth performance (Lumlertdacha and Lovell, 1995). Beside its effects on the immune system, fumonisin has also been shown to

act as a hepato-toxic agent, and inhibits sphinganine biosynthesis (Wang et al., 1991).

Channel catfish have been shown to tolerate moderate concentrations of moniliformin, but levels of 20 mg/kg or more significantly reduced weight gain compared to non-contaminated control diets. When feeding moniliformin in combination with fumonisin, a synergistic negative interaction between the two toxins on weight gain was observed. This is in agreement with research data from mammalian and avian species (Smith et al., 1997). As toxins are often present as a cocktail in a single ingredient or a final diet, toxic effects due to synergistic action occur on a daily bases in the field. Therefore, one has to be careful when interpreting mycotoxin concentrations and potential risks. A concentration that does not adversely affect animal performance in a semi-purified diet, can lead to problems in a natural diet in the presence of a mycotoxin cocktail.

Risk assessment and prevention strategies

Research comparing mycotoxins in different aqua species has demonstrated that they pose a risk to fish and shrimp performance and health. However, it is impossible to determine a safe level for mycotoxin exposure, as the effects depend not only on the dietary concentration, but also on the presence of other mycotoxins, the length of exposure, the fish species, the age of the fish and their nutritional and health status. As the exposure of mycotoxins increases with heavier reliance on plant-based raw materials, mycotoxin risk assessment plans, as well as the appropriate prevention strategies, should be put in place in any aqua production system. Prevention strategies must target the production chain from cropping systems to animal feeding. As the removal of mycotoxins from contaminated diets is not feasible, adsorbents that bind mycotoxins and decrease their bioavailability show a great deal of promise as tools for use in strategies that control mycotoxin-induced toxicosis. Due to their high affinity and high adsorption capacity, yeast-derived glucomannan preparations have shown effective in alleviating negative effects of mycotoxins in mammalian and avian species. Some basic research is required to determine the degree of efficacy in aquaculture.

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Theory and criteria for practical sampling

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Worldwide consumer's health is protected by enforcing legislation that sets maximum levels for specific mycotoxins in food. This implies that food inspectors, mainly at points of import and factories, as well as quality controllers in industrial companies that are subject to the requirements of HACCP, have to perform regular checks. To prevent contaminants from entering the food chain, this control is preferably made on the raw material before any processing starts. The crucial point is that the analytical result of the sample determinates the judgement of the lot from which it originates. For this reason a sampling scheme is based on extensive scientific mathematical statistical calculations, based on analysis of a large amount of subsamples of a lot, as to investigate chances on false positive (producers risk) or negative (consumers risk) results (Whitaker, 2006). For the European Union (EU) a number of sampling plans has been developed on different contaminant-commodity combinations for bulk food, containers, or individual packing such as sacks, bags, bottles, vacuum packs and retail packages. The method of sampling may be applied to different forms in which the commodities are put on the market (EC, 2006).

So far theory, but how to handle this in practice? To judge this let us consider for example the EU sampling directive of import control on nuts packed in sacks. The container of an incoming ship is put ashore. Before inspection this container must be completely unloaded. A 20-ton container may hold 333 sacks of 60 kg each. All sacks must be removed from the container. Food inspectors must take 100 incremental samples of 300 g each, requiring them to open 3 out of every 10 sacks. These 100 sacks then must be closed and the container must be reloaded again to create a proper truckload. Two food inspectors need half a working day to sample this one container, because they have to move all these heavy bags; and they must do it together because the weight exceeds the maximum one person can lift. The owner of the lot loses time waiting for sampling and analysis. Sometimes the result must be delivered within a certain period, as regulated by directives (i.e., customs regulations), which puts time constraint on all interested parties and also on preparing the affected cargo for further trading. And all this work is required to sample only one container of just one ship!

In the above example it is more ore less possible to carry out the proposed sampling plan, because the ship will be unloaded in a warehouse and the commodity is packed in bags. In many other cases it turns out in practice that it is rather impossible to expect sampling to be performed according to the rules. This can be due to the fact that the commodity is loaded in bulk, or just the opposite, when it consists of consumer or vacuum packages. Consider sampling of grain as an example of bulk, which can be necessary in silos, ships or truckloads. Taking 100 increments in this type of bulk is more likely a challenge then understanding one's job. In practice this means that mostly less increments are taken as to collect some 1-3 kg wheat. How does this influence the analytical result, e.g., for deoxynivalenol (DON) (Champeil et al., 2004; Whitaker et al., 2000)? Another feature that can be discussed is whether the distribution for all mycotoxins is the same. This has been investigated at truck load level for DON and ochratoxin A in wheat (Biselli et al., 2005; Hart and Schabenberger, 1998). From these data it can be estimated what the influence will be on the analytical result.

Vacuum and consumer packages present other problems. When for example hazelnuts are vacuum packed per 5 or 10 kg, it is most unlikely that 100 packs will be opened to collect 100

increments. The EU sampling directive states: *"If it is not possible to carry out the method of sampling described above because of the unacceptable commercial consequences resulting from damage to the lot (because of packaging forms, means of transport, etc.) an alternative method of sampling may be applied provided that it is as representative as possible and is fully described and documented"*. A logical remark in this respect will be: what is as representative as possible? When just 2 packages of 5 kg are taken, the sampling uncertainty will raise enormously (Ozay et al., 2006).

Sampling raisins has similar problems. And when they contain ochratoxin A as well as aflatoxins, they can be subject to different heterogeneity patterns (Möller and Nyberg, 2003). Collecting incremental samples is enlarging this challenge, since they are mostly handled in consumer packages. Regarding retail sampling there is an interesting study of Central Science Laboratory, which handles raisins and pistachios (MacArthur et al., 2006). In this respect it has to be kept in mind that whatever the sampling plans, an individual consumer can eat just that unit. For that reason there is also an interest in the costs of sampling and analysis versus the costs of a possible misclassification, i.e., a recall (Ramsey et al., 2001).

These cases are explained more in detail during the presentation. They are given as an example of the practical problems one has to face when applying regulated sampling schemes. The consequences of deviations from the sampling plans, as performed in practice when compared with the original schemes, are estimated. Possible adaptations and/or improvements are discussed.

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Mycotoxin analysis: an overview of classical, rapid and emerging technologies

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Numerous analytical methods have been published for detecting and quantifying mycotoxins in food and feeds. Some of these have been validated by collaborative studies. Methods are used for survey of incidence and occurrence of mycotoxins, and to establish or enforce regulatory limits. The improvement and advancement of methods are keeping pace with the development of instruments and computer technology. Analytical procedures consist of three inter-related steps, extraction (where necessary), purification or isolation (cleanup), and determination. Extraction solvents such as chlorinated hydrocarbons have been largely replaced with aqueous methanol or acetonitrile and large open columns have been replaced with small solid phase extraction columns and multifunctional cleanup columns. Many manual procedures have been automated. The major revolution in mycotoxin methodology has been the development of antibodies for the toxins, used in immunoaffinity cleanup columns and enzyme-linked immunosorbent assays (ELISAs), and the advancement of the liquid chromatography/mass spectrometry (LC/MS) technology that has allowed simultaneous multitoxin testing.

In addition to method performance (specificity, sensitivity, accuracy and precision), many other factors should be considered before selecting a method of analysis: the skill of the analyst, availability of instruments, cost of reagents, time available, number of analyses, and environment. In a laboratory setting traditional methods such as liquid chromatography (LC), LC-MS, LC-MS/MS, instrumental thin-layer chromatography (TLC), gas chromatography (GC), GC/MS and microwell-format ELISA are commonly used. Most rapid methods are antibody-based methods such as flow through membrane-based immunoassays, lateral flow dipstick tests and hand held biosensors. All rapid methods are field-portable and are used in food processing plants or at grain elevators. Minimal training and skill are needed to perform these tests.

Emerging analytical technologies for mycotoxins include the developments of solid phase micro-extraction (of liquid foods), surface plasmon resonance, fiber optic sensors, electrochemical immunosensors, fluorescence-based immunoassays, and the use of molecularly imprinted polymers for binding the mycotoxins. Recently, non-invasive techniques such as near infrared reflectance spectroscopy, electronic noses and acoustic analysis have been used successfully for detecting the effects of *Fusarium* spp. and correlating the effects to the *Fusarium* toxin levels.

The challenges for all of these technologies remain appropriate sampling plans and sample preparation of different commodities, efficient extraction of multitoxins, and method validations.

Test kits and rapid methods for use in a non-laboratory environment

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In the last decade there has been a continuous growth in development of rapid methods for mycotoxin analysis. Moreover, rapid screening techniques which could be used outside the laboratory environment, at the place of sampling, are becoming more and more important. Indeed, results are expected immediately, so that commodities can be further processed without delay. Food and feed industry are forced to reduce costs, employ cheaper labor, but deliver in time safe goods which comply with U.S. and/or European Union (EU) regulations. Ideally, such rapid tests should be used at different points in the pre- and post- harvest supply chain. So, they could represent key tools in a HACCP type approach to mycotoxin control. In general there are two basic requirements for rapid testing in a non-laboratory environment. Firstly, test kits should be simple to use, and secondly, results should be easy to interpret. It means consisting of a simple sample extraction, minimal manipulations, little assay steps, short assay time, and no or minimal use of toxic solvents. Also, it should be easy to see the difference between positive and negative results. Ideal non-laboratory rapid tests are non-instrumental, making use of visual evaluations. In some cases, however, simple, low-cost, handheld instruments are used. Time for analysis is in terms of minutes. Test kits should be portable, containing stable prepacked reagents, and delivered with easy to use instructions. Low levels (µg/kg and ng/kg range) of mycotoxins should be detected and results should be accurate and reproducible. Therefore, to provide confidence to the end-users a well-performed validation of these tests is of outmost importance.

In a first part of the presentation an overview will be given of commercially available test kits for mycotoxin detection. These are immunochemistry-based and include ELISA, fluorometric assays, and membrane-based tests (flow-through, lateral flow). Advantages and limitations of the different techniques will be compared. The second part of the presentation will deal with emerging technologies of potential application in the rapid analysis of mycotoxins. Examples are column-based tests, immunosensors, molecular imprinted polymers, aptamers, and multiplexed assays. Currently there are two major tendencies, one being improvement of speed and user-friendliness, and the other being the simultaneous determination of multiple mycotoxins. Until now, only few attempts have been made in multi-mycotoxin rapid testing.

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The way to harmonize and validate analytical methods for mycotoxins

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There is a need worldwide for standard and validated methods for the detection of mycotoxins. There is also a need for protocols for screening methods, sampling and sample preparation. Currently individual countries and bodies are developing such methods on an *ad hoc* basis. A co-ordinated approach to the issues would be beneficial to all countries so that harmonized methods might be developed which would enable standards to be set globally and be approved by each country's approval bodies. With the objective to address these needs, The ICC Task Force on Mycotoxins and Sampling was established in autumn 2005. This was done in close agreement with organizations such as AOAC International, AOCS (American Oil Chemists' Society), AACC International, IUFoST (International Union of Food Science & Technology).

A number of steps are envisaged in order to achieve the goals:

- Investigation of the *status quo* – what methods currently exist or are under development. Collect all the methods that are currently used and readily applied by manufacturers.
- Identify any gaps – where methods are still needed, requirements which are unique to particular countries.
- Validation criteria – gather data on these.
- Publication of the information gathered in trade journals and scientific press – it is hoped that this would bring the initiative to the attention of all possible interested parties.

Once the information had been gathered then relevant, efficient, cost effective and widely acknowledged methods could be developed. It was suggested that deliverables for the Task Force would be:

- 'new standards' or rather a 'global agreement' of existing standards;
- guidelines;
- network of interested parties;
- validation studies.

At present, experts of over 100 organizations have joined in this Task Force. Activities on harmonization and validation of methods, also those of this Task Force, face the problem of a lack of resources. With this in mind ICC submitted – successfully – a proposal for a Network of Excellence to the European Commission: 'MONIQA - Towards harmonization of analytical methods for monitoring food quality and safety in the food supply chain'. The general objective of European Union (EU) Networks of Excellence is to integrate research efforts of participating organizations. In MONIQA, the core consortium (= partners / members) seek to establish mechanisms for coordinating and finally merging research activities, personnel and infrastructure. The industry and SME (small and medium sized enterprises) sector will benefit through application of the harmonized detection method and technologies, as will the consumers of high quality and safe food. One of the 10 MONIQA Work Packages – WP4 – is titled 'Harmonization and standardization of analytical methods'. Most objectives of the ICC Task Force on Mycotoxin and Sampling will be incorporated in this WP. The WP will also go beyond the objectives of the Task Force, by producing guidelines for harmonized validation procedures and by evaluation of the socio-economic impact of implementation of these harmonized methods. Although MONIQA is funded by the

European Commission, it is a global network. The core consortium comprises a network of 34 members. The geographic diversity covers 11 EU member states (22 partners), 4 associate countries (5 partners), and 5 other countries (7 partners, most of Asia). From the 34 members, 12 are research institutions, 11 are centres of higher education, 2 are industry partners and 5 are other organizations (NGOs (non-governmental organizations), small companies). Other interested parties can apply for associate membership via the ICC website (www.icc.or.at). MONICA will start early in 2007 and will be financed for 5 years. After this period this will result in an efficient and quality assured network management scheme that can be sustained without the EU funding. In the first year the focus will be on setting up the infrastructure of the Network, and on making a start with the integration of research activities. After this year for structuring results of this integration efforts can be expected. Since MONIQA will focus more on analytical methods than on sampling protocols, ICC, together with other organizations, will address sampling by organizing, endorsed by ISO (International Organization for Standardization), in the first half of 2007 a workshop on sampling. The workshop will, most probably be held in one of the large ports of Canada. This will enable government regulators to see the practice of sampling and the potential impact of regulations.

Method validation efforts

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Mycotoxin contamination of various agricultural products is a worldwide economic and health problem for both producers and consumers, respectively. As a result, significant efforts to develop analytical methods to measure mycotoxins in various commodities continue to be expended. The key to this development and method validation is characterized by the term 'method' which refers to three different parts, the analyte, the matrix and the 'how to do' (method). However, the many matrixes that can contain the specific analyte (mycotoxin) and the available techniques continue to grow and to evolve. With this evolution, the demand for validated methods continues to increase for both regulatory and commercial purposes. To satisfy these demands, considerable effort is expended on method validation in addition to method development for mycotoxin analysis.

For method validation, the organization AOAC International has led the world in this field for over a century. For mycotoxin method validation, their method committee D – Natural Toxins and Food Allergens – has actively validated mycotoxin related methods. Also, a more recent European organization – CEN (European Committee for Standardization) – has also been actively validating methods. Validation by these organizations is based on a so-called international harmonized protocol. Under this protocol, a collaborative study with 10 or more laboratories is carried out and the performance of the methods is characterized. For method performance, traditionally several characteristics are studied including accuracy, linearity, LOD (limit of detection), LOQ (limit of quantitation), and specificity. In addition, recovery and uncertainty are also determined and evaluated for method validation. The use of recovery and uncertainty for regulatory purpose is still not totally agreed, but they are important characteristics of these methods.

Some on-going issues are the expansion or modification of methods to accommodate the demand for analysis of new matrixes, the development of new techniques, and environmental considerations. The AOAC International official method board have discussed and agreed to chemical quantitative method, with a goal towards simple analyte expansion without any substantial changes in the method itself. The board agreed to use a single laboratory for validation purposes, instead of a full collaborative validation. However, the method modification issue is still under discussion.

In this presentation the current method validation issues will be discussed.

Methodology required for proficiency testing

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Why do we need consistent methods? Too many methods lead to inconsistent results and missed specifications (under or over). Agreement on test methods allows for meaningful comparison of products, variation control, and quality standards for regulatory issues and health claims. How do you know if you are correctly performing a method? Proficiency testing (PT) programs, such as the one administered by AOCS, are designed to provide an ongoing means for laboratories and chemists to check their adeptness in analytical procedures and techniques through inter-laboratory test comparisons.

Chemists use specified methods to analyze samples and then report the analysis results back to the PT provider for tabulation. A statistical report, including mean, standard deviation, individual deviation, z-score, etc., is sent to each participant. This allows each chemist the opportunity to judge their performance and their laboratory's capabilities against other chemists in their industry.

There are several benefits to participating in PT schemes in addition to the regular, independent assessment of data quality. Comparison of performance with peers is invaluable, especially when used in conjunction with an internal quality assurance system. Participation in external quality assurance programs supports accreditation or certification standards such as ISO 17025 as well as offers the opportunity to train new staff and identify measurement problems. The commercial benefits include using their participation in PT as a marketing tool for their dedication to quality analytics.

Data from several AOCS Mycotoxin series will be presented, including information regarding sample preparation, representative sample size, method variability, and participant performance.

Use of certified reference materials to achieve reliable analytical results

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Reliability and consequently also comparability of analytical data can only be assured if adequate metrological traceability and measurement uncertainty have been established for the analytical results. But many of the decisions on the assessment and management of real-world problems, such as food and feed safety and quality, are still often based on data which are either obtained without appropriate analytical quality assurance (QA) at all or which are not accompanied by sufficient QA information in the report of analysis.

Therefore, the further enhancement of the understanding and application of problem-adequate analytical quality assurance and quality control (QA/QC) is of crucial importance for allowing the international and longer-term comparability of analytical data, also in the field of mycotoxins. This presentation will explain the role of reference materials (RM) in such QA/QC systems. The interdependence between material and information characteristics of reference materials and their applicability in the various steps of the total analytical process will be outlined. It will be stressed and explained that so-called 'calibration standards' are also belonging to the RM family. Such substances have to possess the basic characteristics of reference materials, namely material homogeneity and stability fit for their intended use. Various types of reference materials with different minimum quality characteristics and accompanied information are used for the development of new methods and lab-internal statistical quality control of routinely applied methods. But the crucial steps of method validation as well as the establishment of metrologically traceable results for a particular analysis require the use of adequate certified reference materials (CRMs). Properly selected CRMs provide information about the quantity of the analyte of interest together with a statement about the uncertainty and metrological traceability of this value. Recommendations for the various stakeholders which are producing, evaluating, assessing and using analytical data will be derived. Those include in particular consequences for the proper selection of RMs and accreditation efforts.

The main application areas of reference materials are method development and validation, calibration, proof of method performance and proficiency testing of laboratories. In this context modern approaches to compare own analytical data with certified values on CRM certificates and the establishment of control and warning limits for control charts will be explained.

The European Commission's Institute for Reference Materials and Measurements (IRMM) is already offering the largest range of reference materials for QA/QC in mycotoxin analysis. But the demands from analytical laboratories, stipulated further by accreditation requirements, and from the recently established Community Reference Laboratory (CRL) for mycotoxins in food and feed at IRMM can only be met by additional RM developments. Therefore, an update on related RM projects such as new CRMs for calibration of aflatoxin measurements and for ochratoxin A will be provided. The general IRMM strategy for providing mycotoxin RMs as a systematic set of material presentations, from pure substances or their solutions for calibration up to matrix RMs matching as close as possible the real-world samples to be controlled for food and feed will be outlined.

Breeding new varieties to reduce pre-harvest mycotoxin contamination

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Plant breeding has played a major role in the advancement of human civilization. The domestication and continued improvement of plant species allowed more people to be fed by a significantly smaller portion of the population thereby allowing other individuals to focus on improving other facets of civilization. Even with the advent of new breeding strategies and methodologies, maize breeders traditionally discard moldy ears and use visually healthy seed for future breeding. Although humans have been consuming grains for thousands of years, the importance of mold and mycotoxin contamination has only recently been recognized as a significant health risk. As more countries implement regulations to limit mold and mycotoxin contamination in their food and feed, breeders face increasing pressure to successfully identify, develop, and commercialize resistant germplasm. To date, most mycotoxin resistance breeding strategies have focused on germplasm selection as well as development and validation of screening methods. The integration of genetic and molecular methods to elucidate gene-to-phenotype relationships has significantly improved germplasm breeding; unfortunately disease and mycotoxin screening methods have not advanced as rapidly due to inherent variation in the screening systems thereby impeding the development of commercially acceptable resistant germplasm. The impact of abiotic stresses is a major contributor to this variation due to their effect on both plant and pathogen growth and development. Additionally, the stable performance of commercial products from modern plant breeding programs depends on a decreasing number of elite genetic combinations, and any undetected stress sensitivity may lead to yield reductions and mycotoxin contamination across a significant area.

Recent plant breeding strategies to develop germplasm that is resistant to multiple stresses, represent a unique opportunity to also characterize fungal and mycotoxin resistance. Historically, selection for abiotic stress resistance relied on performance evaluations averaged over multiple locations each with similar environments. Due to the stochastic nature of weather-associated stresses and the limited number of similar environments, plant breeders are relying on managed stress environments which allow stringent control of the environment, timing, and intensity of imposed stresses.

This paper will discuss the utility of managed stress environments to breed new varieties resistant to pre-harvest mycotoxin contamination.

Pre-harvest strategies for prevention of mycotoxin contamination

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Aflatoxins are carcinogens produced by *Aspergillus flavus* and *A. parasiticus* when these fungi infect crops before and after harvest. Contamination of foods and feeds with aflatoxins, therefore, threatens both human and animal health. Traditional control methods (e.g. cultural practices, pesticides) have not been effective in controlling aflatoxin-producing fungi. Our research, therefore, focuses on acquiring knowledge of: (1) the interruption of aflatoxin biosynthesis, (2) fungal populations and biocontrol using atoxigenic *A. flavus* strains, and (3) enhancement of host plant resistance to aflatoxin contamination through (a) gene insertion, and (b) breeding along with the identification of resistance markers in newly-developed lines. Strategies for interrupting aflatoxin biosynthesis could be significantly benefited by previous studies conducted in our lab that identified the cluster of pathway genes, corresponding biosynthetic enzymes, and precursor intermediates involved in aflatoxin B_1 and B_2 synthesis. Our current study of *A. flavus* genomics provides an innovative strategy and rapid investigative tool for simultaneous analysis of the biochemical function and genetic regulation of fungal genes to understand the molecular regulation of aflatoxin biosynthesis. A project is currently underway to identify, through EST (expressed sequence tag) technology, the complex gene array involved in fungal virulence, aflatoxin formation, signaling pathways between the fungus and the environment and fungal reproduction/survival. Microorganisms have often been suggested as agents for control of aflatoxin contamination. The best biocompetitive agent to control *A. flavus* would be atoxigenic strains of *A. flavus*, because these strains are expected to be adaptable to environmental conditions identical to the toxigenic strains and would be biologically active. Research demonstrating success in the competitive exclusion of toxigenic strains by atoxigenic strains and subsequent drastic reductions of aflatoxin levels in cottonseed have led to an industry-ARS partnership in support of extensive field trials in Arizona and the large-scale development of atoxigenic strains. Recently, this technology and the assessment of its impact has been extended to areas of Texas and California and now include rotation crops, particularly corn. Plant breeding for resistance is practicable for corn due to the existence of a large germplasm pool with differential resistance. However, genetic engineering may be essential for cotton since it contains little resistance to aflatoxin contamination in its seed. Genes encoding antifungal proteins have been identified and cotton has been transformed with these genes and is being tested for enhanced resistance to aflatoxigenic fungi. A rapid and simple laboratory-based kernel screening assay (KSA) was developed that enhances resistance-screening for corn breeders and facilitates the study of kernel resistance mechanisms. The strategy of comparing kernel proteomes of resistant with susceptible genotypes has facilitated the discovery of antifungal and stress-related proteins associated with resistance (RAPs). This strategy is enhanced by the discovery of closely-related corn lines differing in resistance among lines produced in a West African breeding program combining resistant germplasm from the U.S. with that discovered in Central and West Africa. Confirmation of a role for resistance-associated proteins/corresponding genes in resistance would yield markers useful to breeders to develop agronomically superior resistant corn lines.

Managing the risk of mold and mycotoxin contamination in the supply chain: a global prospective

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The grains global supply chain handles and stores a significant amount of the world's food and feed industries flows. Cereal grains, in general, can be stored for long periods of time. When that occurs the primary objective is to maintain the quality of an important volume of *nutrients*, that later can be utilized by the primary users, the food and the animal feed industry. If storage conditions are inadequate and pertinent measures are not implemented, the losses due to poor quality therefore, economic losses, can become significantly important for the animal production industries because of poor performance as well as increased live production costs. There are many variables that affect grain quality during storage. When these variables are not controlled, molds will develop (field and storage molds). Mycotoxins are a group of structurally diverse secondary metabolites of molds that occur as contaminants of grains worldwide. Many of these secondary metabolites can cause serious health problems in animals and their presence in agricultural commodities may result in serious economic losses.

The following table is an illustration of the financial impact of aflatoxicosis (Shane, 1994). The simulation study indicates that aflatoxin-induced growth depression, poor feed efficiency and increased mortality could cost a poultry producer as much as $110,000 per week or an increase of 10.2% in production cost.

Table 1. Example of economic impact of aflatoxicosis in broilers[1].

	Normal feed	Aflatoxin contaminated feed	Difference
Live weight, kg	1.8	1.7	
Feed:gain ratio	2.00	2.15	
Mortality, %	7.0	10.0	
Downgrades, %	1.0	3.0	
Meat produced[2], kg	1,292,663	1,157,598	
Revenue[3], US$	1,163,397	1,041,838	
Cost of goods sold[4], US$	956,571	944,871	
Weekly income, US$	206,826	96,967	109,859
Unit cost, US$/kg	0.74	0.816	0.076

[1] Adapted from Shane (1994).
[2] Assuming 1M chickens are processed per week and processing yield is 78%.
[3] Assuming broiler meat sells at $0.9/kg.
[4] Assuming the feed cost is $200/ton and the ratio of feed:nonfeed cost is 7:3.

The Council of Agricultural Science and Technology (CAST) report (2003) includes a chapter by the FDA's Peter Vardon and coworkers, which analyzes the potential economic cost of mycotoxins in the US. Verdon estimated an annual range of losses from $0.5 billion to $ 1.5 billion from aflatoxins (corn and peanuts), fumonisin (corn), and deoxynivalenol (wheat). The main costs considered were: (i) the value of the food/feed crop losses, (ii) the costs incurred to mitigate the contamination, and (iii) the value of the livestock losses. Cost of testing for the toxins, either to commodity producers or to the public through the FDA budget, and cost of

handling affected crops was not included. They are estimated at about $ 500 million on average.

It is the objective of this presentation, to discuss the most important factors affecting grain quality and storage, during its transformation by the animal feed industry, as well as the use of the MYCON™ tool for risk assessment of mold contamination and mycotoxin production in the grains and feeds throughout the supply chain in the grain marketing system and feed production systems relevant to the grains global supply chain.

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Development and use of biomarkers for mycotoxin exposure

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Mycotoxins are known to induce a variety of detrimental physiological and toxicological effects on animal tissue. However, many of these responses may not be readily apparent, since their clinical symptoms are often not easily observed or described. While the toxins may have major long-term effects on performance and animal health, the most striking effects of fungal intoxications can often only be seen after the most critical damage has occurred. Many different approaches have been used to evaluate or describe the effects of mycotoxins in animal systems. Changes in immunological characteristics, in neurological and hormonal responses, in growth and performance, and in reproductive efficiency have all been used to describe mycotoxin effects. Most require long periods of observations, and few of them provide quantitative data that would allow for easy assessment of remediation strategies. Additionally, definitive identification of specific toxicity problems is extremely difficult. There is a significant need for new tools and biomarkers that can be used to rapidly and quantitatively evaluate the effects of the wide variety of mycotoxins in human and animal feeds.

Recent advances in our understanding of animal genomes and their construction has allowed for the development of many new techniques that will undoubtedly change the way we examine the toxic effects of mycotoxins. The construction of specific oligonucleotide microarrays or gene chips allows for a detailed study of differential gene expression patterns and for an improved understanding of the toxic effects of mycotoxins at the most basic regulatory level. The results of these analyses allow for a global view of toxicological effects. Microarray evaluations of gene expression can provide tremendous amounts of quantitative data and be used to rapidly describe the basic physiological changes that are associated with specific types of mycotoxin exposure. The term toxigenomic has been used to describe the application of genomics in basic toxicological studies. Innovative uses of these sciences will be useful in identifying key biomarkers and diagnostic tools. The power of the toxigenomic approaches to understanding mycotoxins and strategies for addressing mycotoxicoses has been illustrated in a recent study of the effects of the fugal toxins associated with endophyte-infected fescue using an interspecies microarray analysis (Jones et al., 2004). This study demonstrated that exposure to the fungal toxins resulted in differential expression of genes associated with neural functions, with transport mechanisms, and with cell cycling and programmed cell death in luteal tissue from heifers. While these changes can be related to the known physiological changes observed in animals grazing on endophyte infected pastures, it was also striking that the gene expression patterns could be used to demonstrate the effectiveness of a domperidone treatment that has the potential of reversing the toxic effects associated with these infected pastures. This clearly shows how such technologies will be useful in evaluating strategies for reducing the detrimental effects of mycotoxins.

Applications of modern microarray and other molecular techniques promises to change the way we view the effects of fugal toxins, and will give us tools for rapidly evaluating toxicity control strategies.

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Adsorbent compounds as feed additives to reduce mycotoxin availability

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Mycotoxin adsorbents are inert, non-nutritive materials that can be added to livestock and poultry feeds to reduce the absorption of mycotoxins from the digestive tract. Such materials are usually non-digestible and non-fermentable high-molecular weight polymers which sequester smaller molecules while moving through the digestive tract thereby preventing uptake into the blood stream and transport to target tissues. Mycotoxin adsorbents are most effective when used as preventative agents for mycotoxicoses. If animals have already been exposed to mycotoxins and adsorbents are used therapeutically to treat tissue damage, their application is limited to preventing further tissue damage and increasing biliary excretion of metabolites and a small fraction of parent compound. Contaminated feeds typically contain multiple mycotoxins. It is desirable, therefore, that mycotoxin adsorbents have broad substrate binding capabilities. This will ensure that at least part of a given challenge of mycotoxins will remain toxicologically unavailable thereby minimizing the chances of toxicological synergy between different mycotoxins which could increase the severity of mycotoxicoses. It is desirable, therefore, that the chemical composition of mycotoxin adsorbents be heterogeneous and derived from semi-purified, naturally-occurring materials. The use of synthetic, homogeneous adsorbent polymers reduces the chances of simultaneous adsorbence of combinations of mycotoxins with widely varying polarity and molecular weights. It is also desirable that mycotoxin adsorbents have a high mycotoxin binding capacity thereby ensuring a minimal level of dietary inclusion.

The main advantage of the use of mycotoxin adsorbents as agents to prevent mycotoxicoses is that potentially irreversible multiple tissue damage is prevented through the use of a single feed additive. To produce this result, however, the level of inclusion of the adsorbent must be appropriate for the degree of mycotoxin challenge. If a given adsorbent has a relatively low mycotoxin binding capability, the level of inclusion may be unacceptable due to a reduction in dietary nutrient density. High levels of adsorbent addition may also adversely affect physical characteristics of the feed including palatability and pellet forming properties.

Mycotoxin adsorbents can be categorized as being either inorganic or organic. The common inorganic adsorbents used commercially are silica polymers which can include clays, zeolites, bentonites and diatomaceous earth. It is important that such naturally-occurring materials be relatively free of contaminants such as heavy metals and dioxins. Organic adsorbents are carbon polymers. Examples include activated charcoal, yeast cell wall extracts and plant fibres such as those from alfalfa. Mycotoxin adsorbents present an attractive short term approach to minimizing the harmful effects of mycotoxicoses on livestock and poultry production efficiency until longer term strategies eliminate mycotoxins from animal feeding systems.

Detoxification of mycotoxins: a microbiological approach

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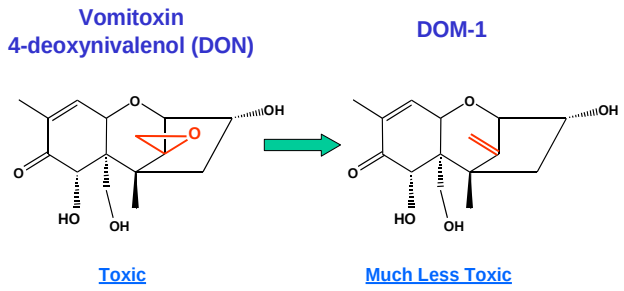
Mycotoxin contamination of food and feed has been an ongoing and serious threat to human health and food and livestock industries due to high toxicity to humans and animals and widespread occurrence in agricultural commodities. Prevention through plant breeding and pre-harvest use of fungicides has been one of major strategies to protect cereal grains from mycotoxin contamination. Physical and chemical techniques have mainly been used for post-harvest treatment to decontaminate mycotoxins. These post-harvest treatment techniques are, however, generally limited by their insufficient efficiency, high cost, or side effects on the grain quality. Thus biological approaches, particularly the use of enzymes and microbes, have become an attractive choice of technology for post-harvest decontamination because of their high specificity and efficiency as well as environmental soundness. A number of mycotoxins, such as aflatoxins, fumonisins, fusaric acid, ochratoxin A, and trichothecenes, have been inactivated by microorganisms of both pure and mixed cultures. Trichothecene mycotoxins, including 4-deoxynivalenol (DON, vomitoxin) and T-2 toxin, are primarily produced by *Fusarium* spp. fungi on a variety of cereal grains and are known to be associated with several diseases in animals and humans. The 12,13-epoxide ring in trichothecenes has been identified as the active toxic site, since removal of the epoxide group (i.e., deepoxy) results in a significant loss of toxicity (Figure 1). This epoxy reduction could be achieved through an enzyme reaction by intestinal microorganisms from chickens, pigs, bovine, and sheep and by soil microorganisms.

Figure 1. Chemistry of bio-detoxification



DON has a high incidence in Canada, which continues to pose toxicological hazards to Canadians and causes significant losses to Canadian cereal and livestock industries, particularly to the swine economy. To address this issue, we initiated a study aiming at the development of a microbial agent that can effectively detoxify DON in feed. We examined intestinal microbiota from different types of chickens and from different regions of the chicken gastrointestinal (GI) tracts for its ability to convert DON to its less toxic form (DON-dE, alsocalled DOM-1). While the microbiota from broilers showed no activity in converting DON to DOM-1, the microbiota from both White Leghorn and Isa Brown hens exhibited a

significant level of the conversion. The activity of deepoxidation (100% conversion) was found in the large intestines of 11 out of 12 hens. However, only 50% of the hens had the same level of activity in their small intestines. A low level of deepoxidation (26% conversion) was detected in 25% of the hens. Through a selective procedure, we were able to identify and isolate several bacterial strains from the chicken GI tracts with the capacity for detoxifying DON. Two of the strains, LS100 and SS3, were investigated by liquid chromatography-mass spectrometry for their activity to degrade twelve different trichothecene mycotoxins (Young et al., 2006). Two pathways were observed: deacylation and deepoxidation (Table 1). Essentially complete conversions to the deepoxy metabolites were observed for the non-acylated trichothecenes DON, nivalenol, and verrucarol. However, deacetylation was the predominant pathway for the monoacetyl trichothecenes 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol (15ADON), and fusarenon X. Small amounts of the deepoxy metabolites were observed from 15ADON and large amounts from 15-monoacetoxyscirpenol where steric hindrance protected the C-15 acetyl groups from enzymatic attack. Diacetylated trichothecenes diacetoxyscirpenol and neosolaniol exhibited only deacetylation. The larger isovaleryl functionality was resistant to removal and deepoxidation was the prevalent reaction in HT-2 toxin and T-2 triol, whereas T-2 toxin showed only deacetylation. These observations were in agreement with the report by Fuchs et al. (2002) with a pure culture of *Eubacterium* strain isolated from rumen fluid. Further studies to determine the potential application of the bacterial isolates in feed are underway.

Table 1. Product ratios for treatment of selected trichothecene mycotoxins with pure cultures of chicken intestinal bacteria.

Mycotoxin	UnRx[a]	dE[b]	dA[c]	dEdA[d]	dA_2[e]	diV[f]	dEdV	dAdEdV	Unk[g]1	Unk2
Alcohol										
DON	0[h],0[i]	100,100	na[k]	na	na	na	na	na		
NIV	-[j],0	-,100	na	na	na	na	na	na		
VER	0,-	100,100	na	na	na	na	na	na	4,-	
MonoAcetyl										
15ADON	0,0	0,7	7,4	93,89	na	na	na	na		
3ADON	2,6	0,0	94,70	6,4	na	na	na	na		
FUS	10,0	0,0	85,56	5,16	na	na	na	na	0,19	0,10
15MAS	0,0	88,84	na	na	na	na	na	na	12,16	
DiAcetyl										
DAS	38,51	0,0	42,33	1,0	12,19	na	na	na	7,7	
NEO	5,20	0,0	86,45	0,0	5,34	na	na	na	5,6	
iValeryl										
T2T	0,0	23,30	na	na	na	14,0	33,32	na	30,37	0,1
iValeryl Acetyl										
HT2	0,0	81,67	0,0	4,11	na	4,7		1,0	10,14	
T2	-,27	-,0	-,61	-,0	-,10	-,0		-,2		

[a] Unreacted material; [b] deepoxidation; [c] deacetylation; [d] deepoxidation deacetylation; [e] dideacetylation;[f] deiso-valerylation; [g] unknown; [h] observed from the treatment with a bacterial isolate, SS3, from the chicken small intestines; [i] observed from the treatment with a bacterial isolate, LS100, from the chicken large intestines; [j] not measured; [k] not applicable. Abbreviations: 3ADON, 3-acetyldeoxynivalenol; 15ADON, 15-acetyldeoxynivalenol; amu, atomic mass units; APCI, atmospheric pressure chemical ionization; -dA, deacetyl; -dE, deepoxy; -dV, deisovaleryl; DAS, diacetoxyscirpenol; DON, 4-deoxynivalenol; FUS, fusarenon X; HOAc, acetic acid; HOiV, iso-valeric acid; HT2, HT-2 toxin; LC, Liquid Chromatography; 15MAS, 15-monoacetoxyscirpenol; MS, Mass Spectrometry; NEO, neosolaniol; SCP, scirpentriol; T2, T2 toxin; T2T, T-2 triol; UV, Ultraviolet; VER, verrucarol.

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Mycotoxin contaminations along organic food chains: status and critical control points

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Within the 5th EU-framework project 'Recommendations for improved procedures for securing consumer oriented food safety and quality of certified organic foods from plough to plate' (QLRT-2002-02245; 'Organic HACCP'), a systematic analysis was carried out among selected certified organic food production chains, such as wheat bread, tomatoes, apples, milk and several more, to investigate current procedures of production management and quality assurance. Among others (there were six more), the quality and safety criteria 'microbial toxins' was analyzed to identify Critical Control Points (CCPs) and to suggest ways how the control of quality and safety can be further improved. CCPs were defined as the steps in supply chains where the quantities of the final product can be controlled most efficiently. The new aspect within the Organic HACCP project was thus to improve how consumer concerns are addressed (Torjusen et al., 2004), through the use of the CCP concept for a wide range of criteria including social and ethical aspects of farm management or freshness and taste of organic produce, not only safety as with the topic microbial toxins.

Mycotoxins have been reported in organic produce. Contamination of organic produce must be taken seriously and weak points of the production system need to be identified and eliminated. One theory is that organically-grown products are likely to contain higher concentrations of mycotoxins than conventionally-grown products. However, there is little evidence to support this theory (Benbrook, 2005; Tamm and Thürig, 2002). Higher or lower mycotoxin contents in feed and food made in different production systems may be caused by (i) systematic differences in the production systems during pre-harvest (e.g., use of agrochemicals), (ii) differences in post-harvest handling (e.g., storage, transport), and (iii) differences during the transformation of raw products into processed foods. Also differences that are in fact due to improper handling procedures during harvest or post-harvest tend to occur systematically if there are systematic differences in the type of equipment used or in the technical qualifications of those who handle the products. Obvious omissions regarding quality assurance lead to poor quality but this phenomenon is not linked to organic agriculture in particular (Tamm, 2001).

A questionnaire was set up for the assessment of the awareness for problem situations and management steps regarding the prevention of mycotoxins along food chains such as tomatoes, apple, milk and wheat bread. An internal database was established to carry out the analysis of collected data, representing several regions typical for the selected commodity and to overview the current management steps and their critical control points. The database contains information on (i) the background of the CCPs, (ii) the quantitative risk related to other chains in the analysis or, if relevant, compared with data from other studies, (iii) how and why the step is controlled in the chain or suggestions of means for improved control, and (iv) discussions in relation to the differences between the chains. Possibilities which may alleviate the problem at a later stage, if relevant, were also included. Based on the internal database, an external, publicly accessible database has also been established which is accessible on the public homepage (www.organichaccp.org). The objective of the Organic HACCP project was to utilize some from the Hazard Analysis and Critical Control Points (HACCP), a standard system which identifies, evaluates and controls hazards that are significant for food safety (NACMCF, 1997) in order to improve the control

of quality and safety of organic food in a broader context. The project concentrated on the aspects of procedures for analysis of risks, working with representative examples, in order to provide a systematic framework for the formulation of recommendations for improving existing procedures, rather than with a view to their commercial use or as a means of obtaining HACCP certification. The definition of a CCP is a step at which control can be applied and is essential to prevent or eliminate a risk (instead of a food safety hazard) or reduce it to an acceptable level.

Risk of mycotoxins in selected commodities

Tomatoes

In the investigated retail units, tomatoes were sold fresh and the sales managers were aware of the risks to sell rotten fruits. The selling turnover in the shops was either very fast (fruit delivery daily with temperature control) or fruits were stored at lower temperatures such as 14 ˚C prior to display and marketing. It is recommended to check tomatoes in retail storage daily and to discharge spoiled tomatoes.

Apple

The risk for mycotoxin formation during production and trade was generally controlled well. Farmers and wholesalers stored apples under CA (controlled atmosphere) storage. In retail situations, the storage period was kept low by periodical orders for new apples. Sometimes, apples were kept at room temperature and controlled on a regular basis. Fungal growth and formation of the mycotoxins patulin and citrinin on apples with small rotten areas during storage can be controlled by early detection, before and after storage, and discarding of rotting apples as well as optimal storage conditions including CA (controlled atmosphere) storage or storage at about 2˚C and 96% rH.

Milk

In the investigated milk chains, the risk of mycotoxin contaminated milk was variable, even within single chains. All farmers produced their own feed, such as fresh and dry roughage and silage. Some produced also grass pellets, rape and corn. This reflects the typical practice in organic livestock of feeding large proportions of hay, grass and silage, which reduced opportunity for mycotoxin-contaminated feed. All investigated farmers also bought concentrates from feed companies. Most of the farmers did not check the fodder upon delivery for obvious mould growth or smell. They trusted the feed companies and the analysis certificate that accompanied the feed. None of the farmers mentioned the option that mycotoxins may evolve under storage in his/her own storage facilities. This represents a serious risk: spoiled fodder is not detected and then fed to lactating cows. Therefore, it is highly advisable that all farmers establish a quality assurance system, including written checklists for inspecting newly delivered feed and own storage practice. As organically raised livestock are fed greater proportions of hay, grass and silage, rather than corn, there is reduced opportunity for mycotoxin-contaminated feed to lead to contaminated milk (FAO, 2000).

Wheat production

Measures of good agricultural practice as applied by most of the investigated farmers can help to reduce the risk. All farmers maintained a well balanced crop rotation with potatoes, peas, alfalfa, lucerne or vegetables to break the chain of infection. Only a few farmers grew maize, which is susceptible to *Fusarium* and could transfer the fungus onto wheat through diseased crop residues. Most of the farmers claimed to have chosen their wheat cultivars because of their storage quality, their disease resistance and early ripeness. To some producers the long regional tradition of growing a particular cultivar was an important factor for their choice which indicates the use of locally adapted and therefore possibly more disease and stress resistant cultivars. The risk can be reduced by maintenance of good crop husbandry practices such as crop rotation, timed sowing and harvesting.

Wheat harvest
The farmers in the investigated chains were very well aware of this risk and most producers did not harvest during wet periods or dried their grains after harvest.

Wheat storage
In each of the investigated chains the grain was handled through several facilities and was stored by at least two different actors such as the producer, the wholesaler, the mill or the bakery. These different actors seem to use not only a variety of facilities but to have a different understanding of this risk; not all of the operators considered storage facilities with humidity and temperature control as an important part of their quality assurance concept. The grain was stored with or without humidity and temperature control and over periods between one and twelve months. Thus the risk of mycotoxin production in grains ranges from high with one producer who stores up to 180 days without a temperature and humidity control to low, in the mill where flour is stored for 60 days under controlled conditions.

Conclusions
The project allowed the built up of knowledge on management steps and quality assurance activities throughout selected organic food chains in various European countries. For various safety and quality criteria such as microbial toxins a set of recommendations were developed in leaflets with advice to producers, processors, retailers or consumers, respectively. Overviews of the analyzed supply chains as well as the identified CCPs can be accessed without password from the website (www.organichaccp.org) together with instructions on how to use the databases and how to establish simple quality control procedures within enterprises. In general, there was a big awareness on the critical control points regarding the prevention of microbial toxins among the actors in the selected food chains.

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Applying the 'omics' wrench: new tools (genomics, proteomics, metabolomics) for solving an age old mycotoxin contamination problem

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Mycotoxin contamination of food and feed is an age old problem lasting over four decades. For the first time it appears that control measures for this problem are within reach. To hasten this process, additional information is needed rather rapidly, particularly for understanding the specific molecular factors (both in the plant and the fungus) during host plant-fungus interaction. The information derived from the use of tools such as genomics, proteomics and metabolomics provides us with the best and the quickest opportunity to achieve a clear understanding of the survival of toxigenic fungi in the field, the fungal invasion of crops, and toxin contamination process under various environmental conditions. Significant progress has been made by many researchers around the world in understanding the genomic make-up of some of the most significant toxin producing fungi namely *Aspergillus flavus*, *Fusarium graminearum* and *F. verticillioides.* Progress has also been made in the study of fungal-host crop interactions for these and other fungi. For this presentation, the information available on aflatoxin production by *A. flavus* will be used as a model system in the use of genomics, proteomics and metabolomics in deriving the requisite information required for developing effective strategies to interrupt the machinery in fungus required to produce these toxins, as well as to assist in the development of host-resistance against fungal invasion and aflatoxin contamination of crops.

Functional genomics is needed to speed up our understanding of the field biology of the fungus in relationship to its interaction with the host plant. But this insight into the behavior of these biological systems first requires the sequence information (structural genomics) of the mycotoxigenic fungi of interest. Subsequently, the fundamental strategy in a functional genomics, metabolomics and proteomics approach is to expand the scope of biological investigation from studying single genes, individual compounds (fungal secondary metabolites) or plant proteins to studying all genes, metabolites and proteins at once in a systematic fashion.

Understanding the complex interrelationships of plant and fungal gene products during the host plant-*A. flavus* interaction is key in developing strategies to interrupt the aflatoxin contamination process. Plant factors have been discovered through the use of proteomics and natural product chemistry that may influence fungal processes involved in invasion and aflatoxin contamination. These factors can also be divided into three categories: (i) seed proteins/inhibitors of fungal cell wall degrading enzymes, (ii) seed/kernel natural products which may influence fungal growth and/or aflatoxin synthesis, and (iii) plant stress responsive proteins.

A. flavus genomics and proteomics of seed-based resistance provide the best investigative tools for simultaneous discovery and analysis of the biochemical function and genetic regulation of the critical genes governing fungal development, plant fungal interaction and aflatoxin biosynthesis. Sequencing and annotation of *A. flavus* expressed sequence tags (EST) identified 7,218 unique EST sequences. Genes that are involved in or potentially

involved in aflatoxin formation were identified from these ESTs. Gene profiling using micro-array has thus far identified hundreds of genes that are highly expressed under aflatoxin-production conditions. Annotation of the *A. flavus* whole genome sequence data (36.3 Mb) has been completed. Further investigations on the functional involvement of these genes in aflatoxin biosynthesis are underway. Environmental influences on the fungus, ecological/ evolutionary significance of *A. flavus* propagation, fungal virulence, and aflatoxin formation as manifested by changes in gene expression profiles and global signal transduction within the fungus are now being rapidly analyzed using this genomics information.

The new genomics resources that are becoming available for *A. flavus* will greatly aid our understanding of the ecology and metabolism of this fungus as well. Results from the whole genome sequencing project show that the genome of *A. flavus* (36.3 Mb) is larger than that of *A. nidulans* (30.1 Mb) or *A. fumigatus* (29.4 Mb) and thus capable of a more complex pattern of secondary metabolites. The expanded genome of *A. flavus* over other *Aspergillus* species suggests that *A. flavus* is adapted to growing in complex environments. An analysis of the function of these extra genes may reveal those genes that make this fungus a successful saprophyte as well and a pathogen of plants and animals. Interestingly, Comparative genomic studies show that *A. flavus* is highly similar to *A. oryzae* (37.6 Mb) with respect to genome size and number of genes for secondary metabolism. These results support the conclusions of others that *A. oryzae* is not a separate species, but rather is a domesticated ecotype of *A. flavus*.

In metabolomic studies with *A. flavus*, comparisons of the chromatographic fingerprints of the wild type and a specific atoxigenic mutant revealed large differences in the small molecule compositions of the two strains. Although the mutant strain appeared to have some unique components when compared to the wild-type, chromatograms of the mutant samples lacked many of the prominent peaks present in wild type. Further experiments are in progress, using mass spectrometry and pulse/pulse-chase technique, to identify specific compounds and pathways impacted by the mutation. Additionally, this metabolic profile, when compared with a transcriptional profile (using an EST microarray) to determine the changes in gene expression resulting from the mutation will provide insights into the signaling pathways in the fungus that control not only toxin formation but also secondary metabolism as a whole.

The results from genomics are expected to provide information for developing novel strategies to control aflatoxin contamination by identifying targets for inhibiting fungal growth or toxin production, as well as developing ‘designer’ biological control agents. With the large volume of information being reported with respect to proteomics of host and genomics and field ecology of the fungus, novel strategies will also emerge based on a clear understanding of the aflatoxin contamination process, especially at the molecular level. If the interaction between the plant and the fungus can be better understood through use of proteomics, genomics and natural product chemistry, particularly in how plant factors may influence fungal processes contributing to virulence and aflatoxin contamination, this information could accelerate development of breeding through marker selection and/or gene insertion technologies for enhancing host plant resistance.

SPOTLIGHT PRESENTATIONS

Development and application of multi-mycotoxin affinity columns

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With regulations being established for an increasing number of mycotoxins it has become advantageous to determine the levels of multiple mycotoxins from the same sample. The method of choice for this is HPLC or HPLC/MS. In order to provide adequate sample clean-up for these methods, we have developed several immunoaffinity columns capable of isolating multiple families of mycotoxins. Mycotoxins of interest are aflatoxins (A), ochratoxin (O), zearalenone (Z), fumonisins (F), deoxynivalenol (D) and T-2 toxin and HT-2 toxin (T2). To date columns for isolating AO (Can et al., 2004), AOZ (Göbel and Lusky, 2004), and AOFZD (Benvenuti et al., 2004) have been described. Recoveries from alcoholic beverages using both the AOFZD column and the AOZ column ranged from 70-100% for all toxins. In addition to these, several other combinations of mycotoxins including AOFZDT2 are in development. Application of these columns to multiple mycotoxin analysis will be discussed.

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Qualitative and quantitative lateral flow methods for the determination of mycotoxins in grains

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A method to detect mycotoxins has been developed using lateral flow test strips and a reflectance reader to detect analyte concentrations with ng/kg (ppt), µg/kg (ppb), or mg/kg (ppm) results. The test procedure consists of an extraction, extract dilution, and sample addition with incubation in a packaged lateral flow strip. The reflectance reader compares the binding of the test zone and control zone to determine the concentration of the mycotoxin.

Charm ROSA P/N qualitative tests (3 min incubation) utilize a test line and a control line to determine if a sample is positive/negative at a defined screening level. The qualitative assays can be interpreted either visually or with a reader. This method has received USDA-GIPSA (U.S. Department of Agriculture-Grain Inspection, Packers & Stockyards Administration) approval for the detection of aflatoxin at 10 and 20 µg/kg using a 70% methanol extraction, and 20 µg/kg using a 50% ethanol extraction of the sample. Deoxynivalenol (DON, vomitoxin) testing also has been GIPSA approved with detection at 0.5 mg/kg in wheat, and 1.0 mg/kg in wheat and barley using a water extraction.

Charm ROSA Quantitative tests (10 min incubation) utilizes two test lines and a control line to increase the detection range. The Charm ROSA Aflatoxin (Quantitative) assay has received GIPSA approval for aflatoxin detection from 5-100 µg/kg in 16 commodities using a 70% methanol extraction. Tests kits also have been developed for aflatoxin testing from 0-25 µg/kg using a 50% ethanol extraction, and zearalenone testing from 0-1.0 mg/kg using a 70% methanol extraction.

Development of a rapid and sensitive biosensor for the detection of aflatoxin M_1 in milk

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In a collaboration between R-Biopharm and Universal Sensors, potentiometric sensors have been developed, coated with specific antibodies against aflatoxin and tested for milk analysis. The UTS™ sensor technology provides potentiometric sensors using polypyrrole on a carbon electrode as the transducer. On this surface immunoassays can be constructed by coating bioreagents very similar to established ELISA procedures.

Proprietary technology ensures that the transduction layer is applied to the electrodes in a very controlled and reproducible way. This enables immunoassay performances with 10% CV or better. The interaction of conjugated polymers with electron acceptors or donors in particular causes changes in both carrier density and mobility, leading to significant changes in the conductivity and the over potential of the electrode which enhances the measured change in potential. The change of the potential is measured against a silver/silver chloride reference electrode. A purpose built 12 channel reader and software is available for R&D purposes.

The sensitivity of the current protocols is similar or better than established ELISA tests. Results of a competitive 2 step immunoassay will be presented using the following protocol:

- Add the milk sample to the tracer (aflatoxin-peroxidase conjugate), and incubate for 10 min with the biosensor.
- Remove the sensor, rinse with buffer and wipe dry. Place the sensors into the reader, and dip the sensors into the substrate solution. Measure the potential for up to 60 s. Read the results.

Data will be shown from spiked samples, which include raw and skimmed milk.

The protocol can be altered according to needs. In this competitive test format, the signal (mV) is inversely proportional to the concentration of aflatoxin M_1 in the sample. Reliable quantitative measurements are obtained when the sample signal is subtracted from that of a zero-standard and read against a predetermined standard curve.

This test format takes less than 15 min and gives clear discrimination between contaminated samples above 500 ng/kg (ppt) aflatoxin M_1 and samples, which are acceptable according to U.S. legislation. This test format can also be tailored to the European regulatory limit of 50 ng/kg. In the future this technology will allow simultaneous measurements of different, independent parameters, such as mycotoxins and antibiotics. Potentiometric biosensors like this could significantly contribute to food safety and quality control.

Rapid and quantitative determination of deoxynivalenol in raw grains using a FluoroQuant DON Plus test kit

Zaneta Kubus

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Romer Labs® offers the best choice for a rapid and quantitative analysis of deoxynivalenol in raw cereals using a fluorometric test system. FluoroQuant® DON Plus detects deoxynivalenol (DON, vomitoxin) in wheat, maize, barley and malted barley. The test is designed for single-sample testing and can be applied at the early stages of post-harvest grain control. Possible users are grain elevators, grain import and export terminals, mills and breweries.

The analytical procedure of the test takes approximately 10 min for extraction, purification, derivatization and end measurement. The test requires calibration only once per day, with ready to use and reusable calibrators. Each test kit comes complete with all accessory materials such as columns, filter paper, pipette tips, cuvettes and reagents. FluoroQuant® DON Plus is available in kits of 25 tests.

The advantage of this system is its accuracy, speed and easy procedure, which can be performed by a person with little chemical training. The test system does not require much equipment. All the reagents required are stored at room temperature. The developed quantitative method has been pre-validated for a working range 0.25 mg/kg to 5 mg/kg DON in wheat. The limit of detection (LOD) obtained for wheat, barley and maize is 0.1 mg/kg. The limit of quantification (LOQ) obtained during pre-validation study was 0.2 mg/kg for wheat, 0.3 mg/kg barley and 0.2 mg/kg maize. The reproducibility of the method at a level of 1.1 mg DON per kg, which is close to the regulatory limit of the European Commission for wheat (EC, 2006), is 12%, involving three operators with five measurements each. The obtained recoveries and RSD (relative standard deviation) values fulfil European Union (EC, 2006) and GIPSA criteria (USDA/GIPSA). It is planned to adapt FluoroQuant® DON Plus to enable to use this test to test more matrices.

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LFD (lateral flow diagnostics) for mycotoxin screening

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As immunochromatographic devices have increased in popularity due to ease of use and rapid results, the demand for these tests have increased. The use of these devices for incoming materials has been increasingly adopted, and the need for archiving and semi-quantitation is also a desired output. Neogen has continued to deliver high quality diagnostic devices to the industry.

The newest additions to the mycotoxin line of products will be reviewed. This will include a look at the Reveal LFD devices and AccuScan technology.

Mycotoxin reference materials

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Trilogy Analytical Laboratory provides reference materials for mycotoxins in a wide variety of different matrices. These materials can be utilized in many capacities such as technician training, technician certification, method validation, check samples, and day to day quality assurance. The samples are naturally contaminated with commonly found toxins including, aflatoxins, fumonsins, deoxynivalenol, zearalenone, ochratoxin A, and T-2 Toxin. Matrices available include corn, wheat, barley, sorghum, coffee, peanut paste, wine, and animal feeds. Materials are ground to a powder consistency and mixed to achieve a homogeneous product. Samples are then tested by AOAC International reference methods repeatedly to determine the average concentration and standard deviation. Each reference sample must be extracted and analyzed 10 times on a minimum of 3 separate runs for a total of 30 individual results before it is considered certified. Samples are available in a variety of different sizes. These reference materials can be a valuable tool in adding validity to mycotoxin results.

A new method for quantitative analysis of deoxynivalenol in grains

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Diagnostix Ltd. has recently released a new product (the EZ-Tox DON Test Kit) designed for rapid, quantitative on-site determination of deoxynivalenol (DON) in grain samples. The kit is a homogeneous enzyme immunoassay incorporating ready-to-use liquid reagents in a simple 3-step procedure. Unlike a conventional ELISA (enzyme-linked immunosorbent assay) kit, the EZ-Tox DON Test Kit does not require wash steps and timed incubations, resulting in a considerably faster and simpler process (results for five samples can be obtained in about five minutes with less than two minutes of hands-on time). The quantitative range of the test is 0.25-6.00 mg/kg (ppm) DON, with accuracy and precision identical to ELISA. A high-quality microplate reader and customized software are supplied standard with the kits and offer several added features including the ability to store and graph sample results. The EZ-Tox DON Test Kit is certified for use by USDA/GIPSA (U.S. Department of Agriculture-Grain Inspection, Packers & Stockyards Administration) and the test reagents are manufactured in a FDA (U.S. Food and Drug Administration) compliant ISO 9001 certified facility.

Platforms for mycotoxin detection applications

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Euro-Diagnostica is committed to achieving the highest level in the supply of a qualitatively superior package of diagnostic products tailored to food safety control. We service customers all over the world with a team of experienced product managers with an in-depth scientific background. We make use of a worldwide network of distribution partners who are dedicated to the goals of our organization, providing the best possible solutions for our customers.

We have two platforms for mycotoxin detection in various matrices. These comprise the Flow-Through Rapid Test and the Enzyme-Immunoassay (EIA) which is a purely screening test. This platform is optimized to suit end-user applications under non-laboratory conditions. Signal differentiation between positive and negative samples has been enhanced for visual interpretation (new device). The test is simple to the extent; it can be carried out without any laboratory training. It is also rapid with results obtained within 10 min. It is far less costly as it does not require equipment. The Flow-Through Rapid Test is currently available for aflatoxin B_1 (AFB1), ochratoxin A (OTA), and zearalenone (ZEA). These have been validated for matrices ranging from simple cereals to the more difficult spice matrices with at least 95% accuracy.

Euro-Diagnostica B.V. also developed and validated sensitive ELISA methods for the detection of various mycotoxins. The ELISAs for detecting mycotoxins are competitive assays based on the competition for binding sites on the specific antiserum between the mycotoxin in question and the peroxidase (enzyme) labeled mycotoxin. The EIA products we have are for deoxynivalenol (DON), OTA, fumonisins, ZEA, T-2 toxin, AFB_1 and total aflatoxins. For the AFB1 mycotoxin two different kits are available. One of these kits is ultra-sensitive, suitable for detecting AFB1 in baby food and food intended for children due to its very low sensitivity (standard curve ranging from 2.5 to 40 ppt), and shows minimum effects for the different matrices down to 30 ppt (ng/kg).

Both the Flow-Through Rapid test for the qualitative screening of mycotoxins and the EIA for quantitative analysis of mycotoxins are cost effective and reliable aids for your screening needs.

Counteracting mycotoxin contamination: the effectiveness of *S. cerevisiae* cell wall glucans for sequestering mycotoxins

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Several natural strategies for controlling the disease processes associated with mycotoxins by sequestration the mycotoxin within the gastrointestinal tract of an animal are being investigated. Some of these strategies target yeast cell wall as an efficient organic sequestering agent that decreases the toxicological properties of mycotoxins. It is important to understand the fundamental chemistry of the interaction to clarify the adsorption process involved in toxin clearance. More than a simple 'binding assays', interaction kinetic models based on overall capacity, standardized affinity rate and stereochemical views are becoming available. These models are required to complete and support in a meaningful way the advances in applied nutrition needed to define in the beneficial role of organic adsorbents prepared from yeast cell wall. The chemical mechanisms involved in the sequestering activity of *Saccharomyces cerevisiae* cell wall components with several major mycotoxins were investigated. The *in vitro* methodology was based on the comparison of several sources of yeast cell wall differing in their relative glucan/mannan/chitin content. ß-D-glucans, composed of linear chains of ß-(1,3)-D-glucans branched with ß-(1,6)-D-glucan side chains, were clearly important in defining the efficacy of cell wall materials against aflatoxin B_1 (AFB_1), deoxynivalenol (DON), zearalenone (ZEA), patulin (PAT) and ochratoxin A (OTA). The evaluation of the affinity rate using Hill's equation and resultant biological parameters proved a strong efficacy of ß-D-glucans for AFB_1, ZEA, DON and PAT structures. Complementary work showed a significant efficacy for sequestering T-2 toxin, as well as endophytes associated toxins. The affirmation of the chemical interaction between mycotoxins and adsorbent was demonstrated using molecular mechanics investigation because the affinity was not only depending on the quantities of ß-D-glucans present in the cell wall but also on their structure and the network organization defined in their single and/or triple helix structure. This powerful physical/chemical modeling technique together with complementary NMR, X-ray structural data analysis, assessed the overall stability of the modeled molecules in all their most stable possible conformations and allowed the calculation of the statistical probability of the existence of each conformation. Furthermore, the site-specific interactions between a mycotoxin and the adsorbent was investigated so that translations, rotations, and up and down positioning were carefully explored. It was concluded that the defining chemical interactions involved weak chemical linkages such as hydrogen and Van der Waals bonds occurring between ß-D-glucans and the hydroxyl and cyclic groups of mycotoxins, if available. The consequence was the production of several *in silico* models showing mycotoxin molecules caged inside the helix-shaped ß-(1,3)-D-glucans, which were firmly stabilized by ß-(1,6)-D-glucan branched side chains. X-ray defraction showed that a key feature of the glucan molecule was the geometric similarities between the spatial organization of mycotoxins molecules and the active site on the single-helix conformation containing six ß-D-glucopyranose residues per turn of the ß-(1,3)-D-glucan chain. Basic science has helped confirm the sequestering properties of organic adsorbents and extending our understanding on the observed effectiveness of *S. cerevisiae* cell wall at limiting the toxicological activities of mycotoxin. The plasticity of the structure of ß-D-glucans exhibiting diverse stereochemistry was undoubtedly responsible for the affinity on a large range of mycotoxins. Affinity rates varied widely between toxins due to their structural and physico-chemical disparities. Nevertheless, we concluded that ß-D-glucans may have strong affinities for mycotoxins exhibiting 'aflatoxin-like', 'deoxynivalenol-like' or 'zearalenone-like' structures.

In vivo trials in poultry with formulated clays against aflatoxin B_1, ochratoxin A and T-2 toxin

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Clays as mycotoxin binders have been widely used in the feed industry. There has been the erroneous presumption that all clays such as hydrated sodium calcium aluminosilicate (HSCAS), bentonites and zeolites are equal and that they can only adsorb aflatoxin. Most clays, due to their common mineralogical characteristics, can only absorb aflatoxin, but a few of them, due to their different mineralogical characteristics are capable not only to absorb aflatoxin, but also other mycotoxins such as ochratoxin A and T-2 toxin.

Hydrated sodium calcium aluminosilicate

Aflatoxin and ochratoxin A

Two experiments were conducted to study the efficacy of a low inclusion commercial HSCAS (Myco-Ad®) in preventing the deleterious effects of aflatoxin B_1 (AFB_1) and ochratoxin A (OTA) in broiler chicks (Casarin et al., 2005a). Arbor Acres broiler males individually caged were used in both experiments. The feed was experimentally contaminated with synthetic AFB_1 or OTA from Sigma Labs, USA. In Experiment 1, 96 four-day old chicks were randomly assigned four dietary treatments with 24 replications each. T I was a sorghum-soybean meal control diet, T II control + 2.5 kg/t Myco-Ad, T III control + 7.5 mg/kg AFB_1, and T IV control + 7.5 mg/kg AFB_1 + 2.5 kg/t Myco-Ad. At 24 days of age, birds fed 7.5 mg/kg AFB_1-contaminated diet showed severe macroscopic liver lesions, higher mortality, lower body weight, poorer feed conversion, and higher liver weight than chicks fed the control diet. The addition of Myco-Ad significantly prevented the impaired performance and the gross liver lesions observed in chicks fed AFB_1. In Experiment 2, 64 seven-day old chicks were randomly divided into four dietary treatments with 16 replications each. T I was a sorghum-soybean meal control diet, T II control + 2.5 kg/t Myco-Ad, T III control + 2 mg/kg OTA, and T IV control + 2 mg/kg OTA + 2.5 kg/t Myco-Ad. Feeding OTA-contaminated diet plus Myco-Ad resulted in statistically significant heavier and more efficient broilers, with markedly reduced macroscopic kidney lesions than those fed 2 mg/kg OTA at 28 days of age. In both experiments, the addition of 2.5 kg/t of Myco-Ad to chick diets did not show any statistical difference in performance compared to the control diet, demonstrating its lack of interference with the absorption of nutrients. These results indicated that Myco-Ad at 2.5 kg/t was effective in preventing the toxic effects of AFB_1 and OTA in broiler chicks.

T-2 toxin

Two experiments were conducted to study the efficacy of HSCAS (Myco-Ad) in preventing the deleterious effects of T-2 toxin (T-2) in broiler chicks (Casarin et al., 2005b). Feed in both experiments was experimentally contaminated with synthetic T-2 from Sigma Labs, USA. In Experiment 1, 75 one-day old Arbor Acres straight-run broilers individually caged were randomly distributed into three dietary treatments with 25 replications each. T I was a corn-wheat-soybean meal control diet, T II control + 1 mg/kg T-2, and T III control + 1 mg/kg T-2 + 2.5 kg/t Myco-Ad. At 40 days of age, birds fed 1 mg/kg T-2 contaminated diet showed significant lower body weight (bw), poorer feed conversion (FC), smaller bursa and severe macroscopic oral lesions than chicks fed the control diet. The addition of Myco-Ad significantly prevented the impaired performance (bw 1,840 v.s 1,381 g; FC 2.02 v.s. 2.12), bursa damage and the severe oral lesions observed in chicks fed T-2. In Experiment 2, 32 five-day old Ross male chicks were randomly divided into four dietary treatments with 8 replications each. T I was a sorghum-soybean meal control diet, T II control + 2.5 kg/t Myco-

Ad, T III control + 1.25 mg/kg T-2, and T IV control + 1.25 mg/kg T-2 + 2.5 kg/t Myco-Ad. Feeding T-2 contaminated diet plus Myco-Ad resulted in statistically significant heavier (bw 1,837 v.s. 1,563 g) and more efficient (FC 1.89 v.s. 2.19) broilers, with substantially reduced gross oral lesions and microscopic organs lesions (tongue, gizzard, thymus, bursa, spleen, liver, kidney) than those fed 1.25 mg/kg T-2 at 38 days of age. The addition of 2.5 kg/t of Myco-Ad to chick diets did not show any statistical difference in performance and bone ash compared to the control diet, demonstrating its lack of nutrients absorption. These results indicated that Myco-Ad at 2.5 kg/t was effective in preventing the toxic effects of T-2 in broiler chicks.

Phylosilicate

T-2 toxin

An experiment was conducted to study the efficacy of a very low inclusion commercial purified phylosilicate (Myco-Ad A-Z) in preventing the deleterious effects of T-2 in broiler chicks (Casarin et al., 2006). Thirty-two five-day old Ross male broiler chicks individually caged were randomly distributed into four dietary treatments with 8 replications each. Birds were fed a basal sorghum-soybean meal mash diet containing or exceeding NRC (National Research Council) recommended nutrients levels. The feed was experimentally contaminated with synthetic T-2 from Sigma Chemical Company, USA. Treatments were: (i) control diet; (ii) control + 1.0 kg/t Myco-Ad A-Z; (iii) control + 1.25 mg/kg T-2; and (iv) control + 1.25 mg/kg T-2 + 1.0 kg/t Myco-Ad A-Z. Results at 38 days of age indicated that broilers fed 1.25 mg/kg T-2 contaminated diet presented significant lower body weight, poorer feed conversion, and severe macroscopic oral lesions than chicks fed the control diet. The addition of Myco-Ad A-Z to the contaminated diet resulted in statistically significant heavier (bw 1,772 v.s. 1,563 g) and more efficient (FC 1.97 v.s. 2.19) broilers, with statistically reduced gross oral lesions and substantial reduction in microscopic organs lesions (tongue, gizzard, thymus, and bursa) than those fed 1.25 mg/kg T-2. The addition of 1.0 kg/t of Myco-Ad A-Z to chick diets did not show any statistical difference in performance and bone mineralization compared to the control diet, demonstrating its lack of interference with nutrients absorption. These results indicated that Myco-Ad A-Z at 1.0 kg/t was effective in preventing the toxic effects of T-2 in broiler chicks.

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A multifunctional wide-spectrum approach diminishing the impact on animal health and performance due to inevitable exposure to mycotoxins

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Mycotoxins are a group of structurally diverse secondary fungal metabolites that occur as contaminant of grains worldwide. *Aspergillus, Fusarium, Penicillium,* and *Claviceps* species of fungi are ubiquitous in nature and under ideal conditions often infect crops and forages already in the field, during storage, transportation and processing. The effects of mycotoxins in animal rearing are well recognized. The clinical response is both dose- and time-dependent and can vary from acute to chronic. Many of these mycotoxins can cause severe health problems in livestock and their presence in agricultural products may result in serious economic losses. Most often used approach by the livestock industry to protect animals from the toxic effects of mycotoxins is the inclusion of clay minerals in the diets. The ability of binding fungal metabolites and decrease their absorption in the digestive tract is constrained to mainly aflatoxins. However, raw materials and inevitably feeds are contaminated with different mycotoxins such as ochratoxin A, zearalenone, fumonisins, trichothecenes, etc. The true impact of moulds and mycotoxins is difficult to measure; the risk associated with their presence even at low level is well established. Reduced liver function is due to overload of the liver in its different functions. Overload will saturate the different pathways in the liver. Detoxification will lead to high amounts of free radicals. All this will cause cell degeneration:

- affecting negatively transformation of carbohydrates, proteins and fats;
- risk of fatty liver;
- reduced detoxification of elements;
- higher risk for toxins, dead cells, microorganism in the bloodstream;
- dysfunction of immune system; and
- reduced feed intake.

A multifunctional approach with a wide-spectrum of activity is required to neutralize the impact on the health and performance of animals by these chemically diverse toxic molecules. The adsorption of the more polar mycotoxins with specific high adsorbent clay minerals, silicates is one mode of action. Complex forming of other types of mycotoxins by parts of yeast cell walls is another action. Stimulating biotransformation in the digestive tract and in specific organs modifying the molecular structure of mycotoxins, is acting at another level. Reducing immune-suppression caused by mycotoxins and stimulating the functionality of specific organs by ferments and botanicals is a further step in reducing the harmfulness of mycotoxins. Avoiding lipid peroxidation, initiated by certain mycotoxins, with antioxidants is critical. Protecting essential vitamins from being damaged already in the digestive tract and limiting tissue damage covers the final issues.

Mycotoxins in animal diets have a severe impact on animal health and performance. The presence of various types of mycotoxins and their diversity in molecular structure requires a multifunctional approach. The functionality of certain organs is vital to cope not only with mycotoxins, but also with other harmful chemicals present in feed raw materials, feed and or digestive tract.

Efficacy of sequestrant/chelator Amadéite®, in the binding of mycotoxins during transit through a dynamic gastrointestinal model (TIM) simulating the GI conditions of pigs

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The objective of this study was to investigate the efficacy of a pillared interlayered clay Amadéite® on the binding of various mycotoxins and consequently to inhibit the availability for absorption of mycotoxins during gastro-intestinal transit of the TNO *in vitro* dynamic gastrointestinal model, the TIM-1 system. The experiments in TIM-1 were performed under the average physiological conditions of the gastrointestinal tract of young adult pigs after the intake of a solid pig meal artificially contaminated with the mycotoxins deoxynivalenol (DON) (approx. 1 mg/kg), and fumonisin (approx. 2 mg/kg). The feed and pooled dialysate samples were analyzed on the concentrations of the two mycotoxins (DON, fumonisin). The difference in absorbed amounts between the control experiment (0% level of adsorbent) and the experiments with 0.01% and 0.1% adsorbent added to the pig feed, determines the efficacy of the mycotoxin-binding in inhibiting mycotoxin absorption. The feed and dialysate samples of each TIM-run are analyzed on some specific nutrients: nitrogen Kjehldahl analysis (to determine the protein digestibility); free glucose (to determine the carbohydrate digestibility); and vitamin B1 (thiamin) and B2 (flavine dinucleotide) (as example-vitamins to determine the bioaccessibility of water-soluble vitamins). The difference in absorbed amounts of nutrients between the control experiment (without adsorbent) and the experiments with two levels of adsorbent/chelator, determines the potential binding effect of the sequestrant on the feed nutrients.

The results showed that Amadéite has a binding capacity for fumonisin, even at the low dose of 0.01% in the pig feed contaminated with two different mycotoxins at the levels of 0.8 to 2 mg/kg. It inhibits the bioaccessibility of fumonisin with approx. 50% (0.01% level of Amadéite) to 60% (0.1% level). Besides fumonisin, Amadéite also inhibits the bioaccessibility of DON. Added to the contaminated pig feed at the level of 0.1%, the inhibition was approx. 40% in comparison to the control without Amadéite. Previous studies in the TIM system with activated carbon demonstrated a reduction in the bioaccessibility of DON of 30-40% in comparison to the control experiment (Avantaggiato et al., 2004). However, the level of activated carbon in the feed ranged from 0.5% up to 2%. The adsorbance capacity of Amadéite did not inhibit the digestibility of proteins and carbohydrates as shown by an unchanged bioaccessibility of nitrogen and glucose, respectively. The addition of Amadéite to the pig feed at the level of 0.1% showed a 30% increase in the bioaccessibility of vitamin B1 in comparison to the control, but did not change the bioaccessibility of vitamin B2.

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A summary of a panel discussion on safety levels for mycotoxins

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In 1984, Prof. Hamilton wrote in his paper 'Determining safe levels of mycotoxins' (Hamilton, 1984): *"What are safe levels of mycotoxins?' It is a very simple, obvious and intensely practical question, which deserves a simple, straightforward answer. Unfortunately, this question has been poorly investigated. The main reasons for this inactivity have been that we have taken refuge in regulatory guidelines promulgated to satisfy legal requirements rather than scientific requirements and that in consequence, we have abdicated our responsibility for the orderly and logical development of a new area of science.…."*

Hamilton summarized the current knowledge at this time about safe levels of mycotoxins by plotting risk incurred against concentration of mycotoxins consumed. This idealized illustration assumes proportionality between risk and mycotoxins. There is a zero intercept without a threshold. The prudent person will probably assume that any level carries a risk. The higher the concentration and frequency of mycotoxin exposure, the higher will be the risk. However, the presence of accessory interactants increases this risk. It should be noticed that the y-axis is labeled risk rather than response. The importance of interacting factors to the manifestations of mycotoxins means that a given amount of toxin will not ensure a given response under field conditions.

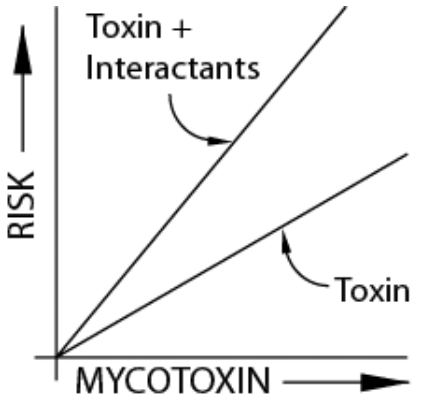

Illustration of prudent view about safe levels of mycotoxins. (Hamilton *et al.*, 1984)

Can we really assess the hazard of mycotoxins? Mycotoxins show a great diversity of chemical structures, therefore they have a wide range of toxicological effects (Coulombe, 1993). Mycotoxicosis, the disease resulting from exposure to a mycotoxin, may be manifested as acute to chronic and ranges form rapid death to tumor formation in animals and humans. The diagnosis of mycotoxicoses is difficult because the effects observed are not necessarily unique to a given mycotoxin, but may be shared by other toxins or interactions with other mycotoxins. Many fungal species also are capable of simultaneously producing several mycotoxins (Bottalico et al., 1998). Therefore, an individual grain may be naturally contaminated with more than one mycotoxin (Trenholm et al., 1989), or the incorporation of numerous grain sources, which are each contaminated with a different mycotoxin, into a single feed may result in a diet that contains a number of different mycotoxins. We are already aware that different mycotoxins can synergistically react with each other. Synergistic effects can be one reason, why mycotoxicoses occur under the detection limit of mycotoxins, but there might be another threat: the presence of mycotoxin conjugates (Berthiller et al., 2004). In the early eighties of the last century, so called 'masked' mycotoxins became a topic. Fungi of the genus *Fusarium* are also plant pathogenic and responsible for agricultural diseases. Plants protect themselves by detoxifying deoxynivalenol (DON) and its derivative 15-acetyl-deoxynivalenol with the UDP-glycosyltransferase, which transfers glucose to these toxins (Berthiller et al., 2004; Poppenberger et al., 2003). The resulting mycotoxin conjugates are not detectable with common analytical methods. Gareis et al. (1980) described another conjugate (zearalenone-4-ß-D-glucopyranoside) and demonstrated that it is decomposed during digestion, releasing the parent mycotoxin zearalenone. These uncertainties are conveyed by the upper line in the figure of Hamilton. Do these considerations suggest that there is no magic level above which mycotoxins are unsafe and below which they are safe?

The participants of the panel discussion at Biomin's World Nutrition Forum, 7-8 September 2006, Vienna, Austria, were international experts in the field of mycotoxins: Dr. Mamduh Sifri, ADM Animal Health & Nutrition, USA; Prof.dr. Johanna Fink-Gremmels, University Utrecht, Department of Veterinary Pharmacology, Pharmacy and Toxicology, the Netherlands; Prof.dr. Vincent Cheng, National I-Lan University, Department of Animal Science, Taiwan; Prof.dr. Carlos Mallmann, Universidade Federal de Santa Maria, Brasil; Prof.dr. Wayne Bryden, University of Queensland, Australia; Prof.dr. Gonzalo Diaz, College of Veterinary Medicine, Toxicology Laboratory, Colombia

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POSTERS

Food supply chain
P1 – P8

P1 *Survey of aflatoxins and ochratoxin A in Brazilian paprika*
A.P. de Almeida, J. Alaburda, L.C.A. Lamardo, L. Shundo, S.A. Navas, V. Ruvier and M. Sabino
Instituto Adolfo Lutz, Seção de Química Biológica, Brazil

P2 *Effect of industrial processing on the distribution of fumonisin B_1, aflatoxins and zearalenone in corn milling fractions*
C. Brera, F. Debegnach, C. Catano, B. De Santis, V. Minardi and M. Miraglia
Istituto Superiore di Sanità, Italy

P3 *Exposure assessment to ochratoxin A from the consumption of Italian wines*
C. Brera, V. Minardi, B. De Santis, F. Debegnach, E. Pannunzi and M. Miraglia
Istituto Superiore di Sanità, Italy

P4 *Fumonisins in traditional Xhosa maize beer in South Africa*
P.M. Gatyeni, G.S. Shephard, L. van der Westhuizen, N.I.M. Somdyala, H. Burger and W.F.O. Marasas
Medical Research Council, PROMEC Unit, South Africa

P5 *Determination of aflatoxin producing Aspergillus flavus - A. parasiticus in dried figs*
F.K. Güler and D. Heperkan
Istanbul Technical University, Faculty of Chemical and Metallurgical Engineering, Department of Food Engineering, Turkey

P6 *Mycotoxins: a risk assessment of Rhenosterkop, a rural farming community in Mpumalanga, South Africa*
D. Naicker[1,4], G. Marais[2,5], H. van den Berg[1], M.G. Masango[1], D. Moodley[3] and A.A. Chuturgoon[3]
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P1

Survey of aflatoxins and ochratoxin A in Brazilian paprika

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A survey to determine the levels of aflatoxins and ochratoxin A (OTA) in paprika samples was carried out in our country after alerts concerning the contamination of Brazilian paprika with these mycotoxins. According to the Hungarian food safety authorities, a number of paprika samples marketed in Hungary were contaminated with aflatoxins above EU (European Union) permitted limits. It is thought that the paprika originated in Brazil and entered the EU via Spain. This incidence led the Hungarian authorities to issue a ban on the use of ground paprika products. Aflatoxins have been shown to cause cancer of the liver in laboratory animals and to directly damage DNA. They are also considered to cause liver cancer in humans. The EU established a maximum level of 5.0 μg/kg for AFB_1 and 10.0 μg/kg for total aflatoxins in spices, including paprika. OTA has been shown to damage, and cause cancer of, the kidneys in laboratory animals. Because the uncertainties about the mechanism of carcinogenicity of OTA and concerns over the potential genotoxicity of OTA, a tolerable daily intake (TDI) have been proposed for OTA by scientific committees (5 ng/kg bw per day). A total of 70 samples of paprika were collected from the market in the city of São Paulo-Brazil from January to April 2006. Aflatoxins were determined by immunoaffinity column (IC) and bi-directional thin layer chromatography (bi-TLC). OTA was determined by IC and high performance liquid chromatography (HPLC). For aflatoxins B_1, B_2, G_1 and G_2, the limit of quantification (LOQ) was 1.0 µg/kg, with mean recovery values of 66.8, 64.4, 66.2 and 61.2% and relative standard deviation (RSD) of 14.4, 15.0, 14.3 and 19.7%, respectively. The mean recovery values for 2 µg/kg were 62.4, 60.0, 62.4 and 60.6% with RSD of 8.6, 5.6, 8.6 and 7.7%, and for 5.0 µg/kg 71.7, 70.1, 71.0 and 82.0% with RSD of 6.3, 10.4, 8.8 and 6.5% for aflatoxins B_1, B_2, G_1 and G_2, respectively. For OTA, the limit of detection (LOD) and limit of quantitation (LOQ) were 0.24 and 0.80 µg/kg, respectively and the mean recovery values were 108.0% (2 µg/kg) and 92.5% (10 µg/kg), with RSD of 4.6 and 7.8%, respectively. Of a total of 70 paprika samples, 56 (80.0%) contained aflatoxins with levels ranged from 1.17 to 11.2 µg/kg and 60 (85.7 %) contained OTA with levels ranged from LOD to 97.23 µg/kg. According to our results many paprika samples exceeded the TDI for OTA and some paprika samples were above of the maximum levels established by the EU. The high incidence of mycotoxin contamination in paprika samples represents a health risk especially in traditional paprika-consuming communities. The high extent of the aflatoxins and OTA contamination in paprika in Brazil could be explained by the tropical and subtropical climatic conditions under which the peppers are grown. High temperature and humidity offer a favorable environment for mould growth and mycotoxin development. Controlling the levels of aflatoxins and OTA in the paprika produced in our country are important in order to guarantee a product without contamination for decreasing the health risks and improve the quality of the products to export.

P2

Effect of industrial processing on the distribution of fumonisin B_1, aflatoxins and zearalenone in corn milling fractions

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The aim of this study was to investigate the distribution of fumonisin B_1 (FB1), aflatoxins (AFs) and zearalenone (ZEA) levels in various corn-milling fractions processed by an Italian industrial plant. Corn kernels and six derived milling fractions (germ, bran, large and small grits, flour, and animal feed flour) were sampled during a real processing cycle. The industrial plant worked continuously at a rate of 60 tons per day. Two sublots of 5 tons each were investigated with samples of derived products taken at regular time intervals. The sampling scheme was derived from the European Directive 98/53/EC for Afs. For both lots, germ, bran, and animal feed flour showed a marked concentration factor from 239 to 911% for Afs and ZEA, and from 157% to 192% for FB_1. These marked concentration factors could be related to both the low yields of the derived products and the distribution of mycotoxin contamination in the outer parts of the kernels. Conversely, a reduction factor of four times (AFs and ZEA) and of ten times (FB_1) from raw material to finished products was observed. To evaluate the effect of cooking, samples of polenta were prepared starting from naturally contaminated flour obtained from the same industrial processing cycle. Polenta samples resulted unaffected by the cooking process, with contamination levels of all mycotoxins similar to those of starting flour.

P3

Exposure assessment to ochratoxin A from the consumption of Italian wines

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Ochratoxin A (OTA) is mainly produced by the secondary metabolism of some ubiquitous molds of genera *Aspergillus* (south regions) and *Penicillium (*north regions). In grapes, the predominant species is *A. carbonarius,* that grows very early and even if it is not able to punch the peel, it penetrates the grape through the hurts, and when in contact with the juice, it starts to produce the toxin. The development of the fungi occurs at moisture levels of 72-90% and temperatures ranging from 12°C to 39°C. The International Agency for Research on Cancer (IARC) classified ochratoxin A as possible carcinogenic agent for humans (group 2B). In this study, a total of 1,166 wine samples including 773 red, 290 white, 75 rosé and 28 dessert, produced in the years 1971-2005 in Italian regions, were analyzed for ochratoxin A using inmunoaffinity column (IAC) clean-up and HPLC with fluorimetric detection. For red wines, 64% of the samples were positive for OTA ranged from 0.01 to 7.50 ng/mL. Furthermore, OTA was detected in 61% of dessert in the range from 0.01 to 1.90 ng/mL, in 92% of rosé from 0.01 to 4.01 and in 45% of white wine samples in the range from 0.01 to 1.95 ng/mL. As for the correlation between OTA concentration and geographical area of production, the south of Italy presented a cluster of higher contamination levels. On the basis of the obtained results, a study of OTA daily exposure assessment by the Italian consumer was also carried out outlining a quite low contribution to the overall intake in comparison with the Tolerable Weekly Intake (TWI) (120 ng/kg bw per week) as stated by the European Food Safety Authority (EFSA) in April 2006.

P4

Fumonisins in traditional Xhosa maize beer in South Africa

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Fumonisins are a group of fungal toxins occurring worldwide in naturally contaminated maize. They are mainly produced by the fungus *Fusarium verticillioides*. The most prevalent naturally occurring analogues are fumonisin B_1 (FB_1), B_2 (FB_2) and B_3 (FB_3). The production and consumption of home-brewed Xhosa maize beer is a widespread traditional practice in the former Transkei region of South Africa. HPLC determination of FB_1, FB_2 and FB_3 in maize beer samples collected in two magisterial areas, Centane and Bizana, showed a wide range of levels. During the period from 2001 to 2004 when the samples were being collected, a survey was conducted to ascertain the extent to which moldy maize is used for various purposes in households in these two districts. In Bizana, 50% of households reportedly used moldy maize in beer brewing, whereas in Centane, only 25% admitted to this practice. Apart from its widespread use in maize beer, householders reported that they either discard the moldy maize or give it to animals. All samples were positive for FB_1, with a mean level of 281 ± 262 ng/mL and a range from 38 to 1,066 ng/mL. Total fumonisins (FB_1 + FB_2 + FB_3) ranged from 43 to 1,329 ng/mL, with a mean of 369 ± 345 ng/mL. However, the range of levels in beer observed in both districts was large, and statistical analysis revealed that there was no significant difference between the areas ($p > 0.05$). The results of this research indicated that the consumption of maize beer in rural areas of the former Transkei, South Africa, can significantly enhance fumonisin exposure, and that among beer consumers it was found to be well above the provisional maximum tolerable daily intake of 2 µg/kg of bw per day set by the joint FAO/WHO Expert Committee on Food Additives (JECFA).

P5

Determination of aflatoxin producing *Aspergillus flavus - A. parasiticus* in dried figs

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Figs are among the most widely produced second group fruits in the world. Mathot (1989) reported that the amount of aflatoxin found in figs could be high: 1,150 µg/kg (non-fluorescent) and 4,170 µg/kg (fluorescent); the amount of aflatoxin found in a single fig could be 180 and 360 µg/kg, respectively. Such amounts could be risky for the consumer. Akerstrand and Möller (1989) and Rasmussen and Pedersen (1989) also found samples containing aflatoxin over 100µg/kg. In this study 115 fig samples from 7 different orchards in the Aegean region have been collected during drying to establish *Aspergillus flavus - A. parasiticus* contamination and to determine morphological and mycotoxigenic properties. Aflatoxin analyses were carried out by thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC). The procedure was as follows: identified *A. flavus - A. parasiticus* isolates were inoculated on Yeast Extract Agar (YES) and Czapek Yeast Extract Agar (CYA), and incubated at 25ºC. After 7 days of incubation the content of the petri dishes were extracted with 50 ml methanol (70%):water in a stomacher for 5 min. After filtration 0.45µm extracts were injected into the HPLC. For TLC three agar plugs were cut out of the colony centre. The plugs were placed into eppendorf tubes and mixed with 1 mL chloroform for 30 s in a vortex. TLC plates were dipped in 8% oxalic acid in water and dried in air before spotting the extract. Toluene:ethyl acetate:formic acid (98%)(5:4:1) was used as developing solvent. In the two-year study 59 *A. flavus - A. parasiticus* were isolated from a total of 115 dried fig samples. Seventy-five percent of the isolates produced aflatoxins on synthetic media. Consequently, aflatoxin-producing *A. flavus* and *A. parasiticus* appeared to be very high in dried figs.

P6

Mycotoxins: a risk assessment of Rhenosterkop, a rural farming community in Mpumalanga, South Africa

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Mycotoxins may be present in feeds without any visible sign of mold contamination. There is therefore a need for rapid and accurate measurement of mycotoxins for purposes of continual monitoring and identification of risk commodities. Extracts from chicken feed, grass, lucern and hay samples, and milk from cows were analyzed for the presence of mycotoxins and fungi. Results of mold profiles conducted show that most samples are contaminated by molds belonging to the genera *Alternaria, Aspergillus, Cladosporium, Fusarium, Penicillium*, *Mucor* spp., *Phoma sorghina* and *Rhizopus oryzae.* The chicken feed samples tested contained mycotoxins below the safe intake levels for poultry. Grass contained mycotoxins below the safe intake levels for dairy and beef cattle. However, the lucern sample contained levels of zearalenone greater than the safe intake level in dairy cattle (0.3 mg/kg). All the grass samples collected revealed levels of zearalenone greater than the safe intake levels for beef and dairy cattle (250 µg/kg). Milk obtained from the cows contained aflatoxin M_1 below the safe intake levels of 0.5 µg/kg. A dye reduction assay (MTT) in conjunction with cell culture was used to evaluate the cytotoxicity of the chicken feed extracts and grass extracts in human peripheral blood lymphocytes. Extracts from chicken feed display immunotoxicity, with some extracts displaying < 30% cell viability. A positive control consisting of dog feed containing aflatoxin B_1 (320 µg/kg) was extremely immunotoxic (87% cell mortality). Extract 5 from cattle feed induced a 64% cell viability, while extract 6 stimulated cell proliferation. It appeared that cell death due to exposure to extract 2 was predominantly via apoptosis with 65% of cells undergoing programmed cell death. The dog feed positive control exhibited a 50% programmed cell death. The other extracts displayed < 30% apoptosis with more cells being viable than necrotic.

P7

Survey of aflatoxin M_1 in raw and pasteurized milk in Mexico

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Aflatoxin M_1 (AFM1) means a public health risk. AFM1 is a mycotoxin that can occur in milk from dairy cows that consume aflatoxin B1-contaminated feeds. Some countries have established regulatory limits for AFM1 in milk and dairy products. Mexico issued an official norm to regulate the maximum level of AFM1 in milk (NOM-091-SSA1-1994); the permitted concentration of AFM1 is 0.05 µg/kg (ppb). The aim of this work was to survey the contamination of both raw and pasteurized fluid milk marketed in Mexico to know the compliance with the norm. 265 Samples of raw milk of 1 L each were obtained from 11 small dairy farmers (6 samples/week/four weeks) in the Tehuacan region. The small dairy farmers sell daily, house to house, their current milk production. In addition, 90 samples of pasteurized milk were obtained from groceries of some cities in central Mexico. The samples include all national accepted brands of milk. A portion of 50 mL of each sample was tested by HPLC, using a commercial immunoaffinity column cleanup according to previous work (Dragacci et al., 2001). The results demonstrated that a very high percentage of samples of raw milk complied with the norm of 0.05 ppb. Some samples of pasteurized milk were contaminated at levels of 0.5 ppb. The complete results of this study are summarized in this poster.

P8

Sphingoid base levels in humans consuming fumonisin-contaminated maize from high and low esophageal cancer incidence areas

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High esophageal cancer (OC) incidence rates have been associated with the consumption of fumonisin contaminated maize in certain areas around the world. In the former Transkei region of the Eastern Cape Province, Centane magisterial area had high and the Bizana area low OC incidence rates, which corresponded with high and low levels of fumonisins in the home-grown maize from these areas. In this cross sectional study we investigated sphingoid base levels as a possible biomarker for fumonisin exposure in plasma and urine of male and female volunteers from Centane in 1997 and Bizana in 2000, sampling healthy home-grown maize from the same areas where the volunteers resided. Maize was analyzed for the major naturally occurring fumonisin analogues (FB_1, FB_2 and FB_3) and the plasma and urine were analyzed for the sphingoid bases, sphinganine (Sa) and sphingosine (So), by reversed-phase HPLC utilizing fluorescence detection. The mean male ($p < 0.05$) and female ($p < 0.0001$) plasma Sa/So ratios, as well as the mean female plasma sphinganine ($p = 0.01$) level, were significantly higher in Centane than in Bizana. In urine the male sphinganine and sphingosine levels were significantly lower ($p < 0.0001$) than the female levels in both Centane and in Bizana. Furthermore the male and female urinary sphingosine levels were significantly higher ($p < 0.0001$) in Centane than in Bizana. The male urinary Sa/So ratios were very similar between the areas, whereas the female urinary Sa/So ratio was significantly lower ($p < 0.05$) in Centane than in Bizana due to increased So levels. Mean total fumonisin ($FB_1 + FB_2 + FB_3$) levels in healthy homegrown maize collected in Centane during 1997 was 0.58 mg/kg, whereas in Bizana during 2000, the mean fumonisin level was 0.92 mg/kg. Given that elevated Sa levels or Sa/So ratios have been postulated as potential biomarkers for fumonisin exposure in humans, the situation in Centane of higher plasma Sa levels and Sa/So ratios in a year of lower fumonisin levels indicates that alternative biomarkers should be sought. The fumonisin levels in maize fluctuate seasonally, complicating investigation into the effect of fumonisin consumption on sphinganine and sphingosine and their role as a biomarker in humans.

P9

Effects of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins on performance, metabolism, hematology and immunology of turkeys

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An experiment was conducted to investigate the effects of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins on performance, metabolism, hematology and immunological parameters of turkeys. The efficacy of polymeric glucomannan mycotoxin adsorbent (GMA, Mycosorb®, Alltech, Inc., Nicholasville, KY) in preventing the adverse effects of *Fusarium* mycotoxins was also evaluated. Three hundred one-day old male turkey poults were fed wheat, corn and soybean meal-based starter (0-3 wk), grower (4-6 wk), developer (7-9 wk), and finisher (10-12 wk) diets formulated with uncontaminated grains, contaminated grains and contaminated grains + 0.2% GMA. Feeding contaminated grains to turkeys significantly decreased body weight gains during the grower and developer phases. Feeding contaminated grains did not, however, alter feed intake or feed efficiency. Supplementation of the contaminated diet with GMA significantly improved body weight gains during the grower and developer phases. The feeding of contaminated grains reduced ($p<0.05$) the total lymphocytes counts at wk 3 and increased percent $CD4^+$ lymphocytes population during wk 6, however there was no change in the percent $CD8^+$ and B-lymphocytes during experiment period. Contact hypersensitivity to dinitrochlorobenzene, which is a $CD8^+$ T-cell-mediated delayed type hypersensitivity response, was significantly decreased after 24 h and 72 h by feed-borne mycotoxins compared to controls. Supplementation of the contaminated diet with GMA prevented the decrease in response after 24 h. Mitogenic responsiveness of peripheral lymphocytes to concanavalin A and pokeweed was not affected by the feeding of contaminated diet. Supplementation of GM polymer to the contaminated diet increased total plasma protein levels compared to control and contaminated diet. Plasma uric acid concentrations were increased at the end of the experiment compared with controls when birds were fed contaminated grains and the feeding of GMA prevented this. It was concluded that turkey performance, metabolism, hematology and some immunological parameters were adversely affected by chronic feeding of a combination of *Fusarium* mycotoxins, and that GMA prevented many of these effects.

P10

The effect of feed-borne *Fusarium* mycotoxins on performance, metabolism and immunity of dairy cows

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There is limited information regarding the sensitivity of dairy cattle to *Fusarium* mycotoxicoses. Two experiments were conducted, therefore, to determine the effect of feed-borne *Fusarium* mycotoxins on performance, metabolism and immunity of dairy cattle. The efficacy of polymeric glucomannan mycotoxin adsorbent (GMA) (Mycosorb^{TM}, Alltech Inc., Nicholasville, KY) in preventing of *Fusarium* mycotoxicoses was also determined. In each experiment a blend of naturally contaminated feedstuffs was fed to 18 mid-lactation Holstein cows with milk production ranging from 33-36 kg/day. Diets included: (1) control TMR (2) contaminated TMR, and (3) contaminated TMR + 0.2% GMA, and were fed for 56 days. Wheat, corn and hay were the contaminated feedstuffs. Deoxynivalenol (DON), the major contaminant was found in the TMR at up to 3.6 mg/kg dry matter. Body weight, body condition score, DMI, $NE_{L,}$ milk production, milk composition, SCC, blood serum chemistry, hematology, total Ig count and coagulation profile were measured. Rumen ammonia concentration, neutrophil phagocytosis activity and antibody response to ovalbumin immunization were also evaluated. Data were analyzed by analysis of covariance using the mixed model of SAS as a completely randomized design with repeated measures. Pre-treatment measurements were used as a covariate. Multiple comparisons were performed. Milk production, milk composition, body weight, DMI and NE_L were not changed under current experimental conditions. Serum globulin and total protein levels increased significantly in cows fed contaminated TMR compared to controls after 42 days, while albumin:globulin ratio decreased. Serum urea concentrations were significantly elevated throughout the experiment in the same group. Serum IgA concentrations decreased significantly in cows fed the contaminated diet after 36 days. Phagocytosis in neutrophils was depressed throughout the experiment in cows fed the contaminated diet. The feeding of GMA prevented these effects. Serum sodium concentrations and osmolality were high throughout the experiment in both groups fed contaminated TMR. Primary antibody response to OVA immunization was higher in both groups fed contaminated diets compared to controls. It was concluded that feed naturally contaminated with *Fusarium* mycotoxins, even in low concentrations, can affect metabolic parameters and immunity of dairy cows and GMA can prevent many of these effects.

P11

Mycotoxin evaluation in pet food by liquid chromatography - tandem mass spectrometry

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Mycotoxins in food for pet nutrition have not been quite evaluated in Brazil. On the other hand, feed for animals to be used as raw material in the meat industry has been widely monitored. Pet food industries, in parallel to the agribusiness in the country, have increased quite widely. Due to its demand, the quality of that type of feed became essential. Among the main mycotoxins responsible for feed contamination and that can cause serious harm to animal health are: aflatoxins (AFLs: AFB_1, AFB_2, AFG_1 and AFG_2), ochratoxin A (OTA), zearalenone (ZON), and fumonisins (FBs: FB_1, FB_2, FB_3). Their toxicological effects in animals can cause damages to liver, kidneys, brain and/or gastrointestinal tract. They can also interfere with animal reproduction and even be lethal. Considering the importance of feed safety for pets, a study was carried out aiming to evaluate the extent of mycotoxin contamination in feed for birds, cats, dogs, hamsters, horses, rabbits, pet-fish and turtle. A total of 123 samples of different animals and brand pet feeds were collected in pet shops, veterinary clinics and supermarkets in Florianopolis city, Santa Catarina State, southern Brazil from February to July, 2006, and also from horse stables. The total number of samples for each animal was: 19, 19, 46, 6, 26, 3, 3 and 1 for birds, cats, dogs, hamsters, horses, rabbits, pet-fish and turtle, respectively. The methodology used was liquid chromatography-tandem mass spectrometry (LC-MS/MS) utilizing as ionization source eletrospray and APCI modes. All the samples presented some contamination of one or more than one mycotoxin surveyed, except for the turtle sample. As far as the types of animal feed are concerned, the ones that presented contamination were more often for dogs (100% AFB_1), rabbits (100% AFB_1), hamsters (100% ZON), pet-fish (100% ZON), birds (94.7% AFB_1) and cats (84.2% AFB_1). Total AFLs levels varied from 0.879 to 209 µg/kg. The highest AFB_1 contamination was detected in hamster feed with 185 µg/kg and the lowest in horse feed (1.14 µg/kg). Horse feed presented low levels of FB_1 ranging from 4.76 to 91.6 µg/kg (MRL: 5000 µg/kg). Despite of the levels detected, only AFL total and ZON, corresponding to 6 (4.9%) and 19 (15.5%) of the total samples surveyed, presented levels above international regulations (50 and 50/100 µg/kg for AFLs and ZON, respectively).

P12

Effects of feedborne *Fusarium* mycotoxins on performance of broiler breeder chickens

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A study was conducted in order to investigate the effects of feeding grains naturally contaminated with *Fusarium* mycotoxins on performance of broiler breeder chickens. Forty-two 26-week old broiler breeder hens and nine roosters were fed diets including: (1) control (2) contaminated grains, and (3) contaminated grains + 0.2% polymeric glucomannan mycotoxin adsorbent (GMA, Mycosorb, Alltech, Inc., Nicholasville, KY) for 12 weeks. The major contaminant was deoxynivalenol (12.6 mg/ kg feed) with lesser amounts of zearalenone and 15-acetyl deoxynivalenol. Feed consumption and body weights were not affected by diet. The feeding of contaminated grains did not significantly affect egg production. Decreased eggshell thickness was seen, however, at the end of week 4 and dietary supplementation with GMA prevented this effect. There was no effect of diet on other egg parameters measured. There was a significant increase in early (1-7 d) embryonic mortality in eggs from birds fed contaminated grains at week 4 but mid (8-14 d) and late (15-21 d) embryonic mortalities were not affected by diet. There were no differences in newly hatched chick weights or viability. The ratio of chick weight to egg weight was not affected by the feeding of contaminated grains. Weight gains of chicks fed a standard broiler starter diet at 7, 14 and 21 days of age were not significantly affected by previous dietary treatments for the dam. There were also no differences in weekly or cumulative feed efficiency of the progeny. There was no effect of diet on the relative weights of liver, spleen, kidney, and testes. Blood biochemistry and hematological parameters were not affected by the feeding of contaminated grains. Rooster semen volume and sperm concentration, viability and motility were not affected by the feeding of contaminated diets. The feeding of contaminated grains decreased antibody titers against infectious bronchitis virus at the end of week 12 and this was prevented by dietary supplementation with GMA. There was no effect of the diet on serum antibody titers against Newcastle disease virus. It was concluded that the feeding of diets containing *Fusarium* mycotoxins to broiler breeders could possibly affect performance and immune status.

P13

Investigation of imprinted polymers for the selective solid phase extraction of moniliformin

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Moniliformin is a toxic metabolite produced by several *Fusarium* species, such as *proliferatum* and *subglutinans*. This low molecular weight mycotoxin is generally isolated as the sodium or potassium salt of semisquaric acid. Studies have found concentrations of moniliformin to be associated with adverse effects in a variety of animals, including rats and poultry. A reported mode of action of moniliformin is the inhibition of pyruvate dehydrogenase, resulting in decreased mitochondrial respiration through the disruption of the Krebs cycle. Current methods for moniliformin detection include the use of conventional analytical techniques such as HPLC-UV and mass spectroscopy. In addition, a method has been developed using capillary zone electrophoresis diode array detection. With simple derivatization of moniliformin, fluorescence and gas chromatography can be accomplished. Several procedures have been developed for moniliformin extraction and clean-up. Extraction has been reported with various concentrations of acetonitrile/water and with ion pair reagents. Extracts are typically put through clean-up steps, which include the use of C18 or SAX columns. A moniliformin selective binding material can assist in the clean-up and the determination of moniliformin levels. Molecularly imprinted polymers (MIPs) are materials that can be designed and optimized for selectivity. Imprinted polymers that exhibit selective moniliformin binding compared to non-imprinted polymers have been characterized by binding assays using HPLC-UV to detect moniliformin levels. Moniliformin binding MIPs were prepared with moniliformin analogs as templates and optimal polymer compositions were dependent on the imprint molecule. Evaluation of these materials in molecularly imprinted solid phase extraction (MISPE) columns identified several imprinted polymers possessing selective moniliformin binding compared to non-imprinted polymers in reported extraction solvents. In addition, bound moniliformin was eluted from the MISPE columns with the LC-mobile phase. The imprinted polymer was capable of the preconcentration and clean-up of moniliformin from moniliformin spiked corn extracts. These materials show potential to assist in the determination of moniliformin levels in corn.

P14

Fluorescence polarization, a rapid and reliable technique to quantify mycotoxin contamination: study for zearalenone

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Mycotoxins pose a threat to human and animal health through the ingestion of contaminated food. The Food and Agriculture Organization (FAO) of the United Nations has estimated that 25% of the world's food crops are contaminated by mycotoxins each year. Zearalenone (ZON) is produced by the fungus *Fusarium* spp. (Diekman and Green, 1992). It possesses estrogenic activity and is able to bind to tissue receptors that normally bind estrogens. Symptoms of consumption of ZON-contaminated feed are often observed in swine, i.e., prepubertal gilts including enlarged mammae, swelling of uterus and vulva, and atrophy of the ovaries. The fluorescence polarization immunoassay technique is a practical and appropriate solution to rapid and reliable mycotoxin detection in food and feed, allowing quantitative determination in the µg/kg (ppb) range within a few minutes. When a small fluorescent tracer is illuminated by plane polarized light those molecules within their electric transition moment parallel to the electric vector of the excitation light are excited. The subsequent fluorescent emission however will be largely unpolarized since the molecule is free to rotate during the time taken for the electronic transition of the fluorescence to occur. The rotational relaxation time is much shorter than the fluorescence decay time and the molecular orientation effectively becomes randomized before fluorescence occurs. In the fluorescence polarization assays a fluorescent labeled ZON reacts with anti-ZON, this results in a slowing down of the rotation of the molecule. In an immunoassay application, the changes in fluorescence polarization between free and bound tracers can be rapidly and accurately measured and related to the concentration of unlabeled ZON present in a sample. Results will be presented for detection of ZON in wheat, triticale and corn and compared to HPLC results.

P15

BioCop, new technologies to screen multiple chemical contaminants in foods: towards an enhanced rapid detection technique for deoxynivalenol, nivalenol, T-2 toxin and HT-2 toxin

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The BioCop project is an EC funded FP6 Integrated Project aimed at developing new technologies for the screening of multiple chemical contaminants in foods. Part of this work focuses on developing a rapid (< 60 min) analysis technique for trichothecenes in cereal based food products. Trichothecenes are an important class of mycotoxins, formed widely in cereals, which are stable during most food processing operations. Currently analytical methods for identifying trichothecenes are mostly based on chromatographic or immunochemical procedures, but these methodologies are prone to variability. While several LC- and GC- methods exist, low-cost methods with wide applicability for rapid screening at an early stage in the food chain are needed to supplement these reference methods. The poster details the first results obtained in this work. A range of pure (>97%) trichothecenes (deoxynivalenol [DON], nivalenol [NIV], T-2 toxin [T-2] and HT-2 toxin [HT-2]) have been produced and characterized. Monoclonal antibodies (mAbs) for some of these trichothecenes have also been produced by immunization of mice. Three types of test materials have been produced: baby food, maize and breakfast cereals, both blank materials and materials with naturally incurred trichothecenes. The purified trichothecenes will be used within the project to develop screening assays based on gene expression fingerprints with the aim of developing a high-throughput low-cost DNA-microchip platform for the detection of trichothecenes as well as an electrochemical biosensor. The monoclonal antibodies will be used in the development of a universal antibody library to produce high quality binders for assay development. The test materials will be further characterized and then used in prototype testing and validation of the newly developed techniques.

P16

New rapid and cost-effective SPE procedure for the clean-up of *Fusarium* toxin contaminated cereals and cereal-based foods

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Fusarium fungi are probably the most prevalent toxin-producing fungi of the northern temperate regions and are widely distributed in the food chain in America, Europe and Asia. The main sources of intake are products from cereals, in particular wheat and corn. *Fusarium* toxins can produce a wide range of mycotoxins including several type A and type B trichothecenes, which can be harmful to humans and animals. Suitable sensitive and reliable multitoxin methods for the analysis of trichothecenes and other *Fusarium* toxins are therefore required. Traditional sample preparation for trichothecene analysis typically involves extraction with acetonitrile/water and clean-up on polar columns. Analytical interfering substances are retained while trichothecenes are not adsorbed on the packing material. As the trichothecenes differ a lot in polarity and solubility, recoveries of the more polar analytes are often compromised with this approach. Another approach is the use of immunoaffinity columns (IAC). These provide highly selective extractions with high recoveries, but separate IAC columns are needed for each toxin. To overcome the limitations of these methods there was a need to develop an extraction and clean-up method for the simultaneous determination of several trichothecenes with high recoveries for the polar toxins by minimizing the matrix effects. This poster shows the optimized method extraction and single column clean-up step of 12 type A- and B-trichothecenes (deoxynivalenol (DON), nivalenol (NIV), 3-acetyldeoxynivalenol (3ADON), 15-acetyldeoxynivalenol (15ADON), fusarenon-X, T-2 toxin, HT-2 toxin, neosolaniol, monoacetoxy-scirpenol, diacetoxyscirpenol, T-2 triol and T-2 tetraol) and zearalenone (ZEA) in cereals and cereal based food on Bond Elut® Mycotoxin, a newly developed solid phase extraction sorbent. Average recoveries and RSD obtained for 12 trichothecenes and ZEA from spiked wheat, corn, durum, oats, bread, muesli and cereal infant food samples after clean-up with Bond Elut Mycotoxin columns were calculated. The recoveries especially for the polar toxins DON, NIV, 3ADON and T-2 tetraol were increased up to 31% when compared to the extraction method on polar charcoal-aluminia cartridges. To calculate the amount of ZEA, the extracted matrices were spiked with a defined amount of zearalanone (ZAN) standard solution before the clean-up step on Bond Elut Mycotoxin. Using ZAN as internal standard, the recovery of ZEA was about 100%. The trichothecene content of six naturally contaminated samples after 3 different clean-up methods shows that up to 43% higher values were achieved in the analysis of naturally contaminated samples for the polar toxins DON, NIV, 3ADON, 15ADON and T-2 tetraol in comparison to the charcoal-alumina based method. If the determination of DON alone is of interest, then the highest content can be achieved with an extraction of 100% water and clean-up with IAC; however for the determination of 12 trichothecenes with different polarities the Bond Elut Mycotoxin provides comparable results. As the performance of the Bond Elut Mycotoxin cartridges is similar or even better and the columns are more cost-effective, the new clean-up procedure is a very good alternative to other commonly used standardized methods.

P17

Rapid fluorometric test for the quantitative determination of deoxynivalenol in cereals

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Human and animal health can be at risk due to toxins produced by moulds. In order to reduce the exposure to mycotoxins, regulations and laws are enforced by the respective authorities. According to the Regulation of the Commission of the European Communities 856/2005 that came into force on July 1, 2006, Member States have to adopt maximum levels for *Fusarium* toxins, such as deoxynivalenol (DON), in certain foodstuffs (EC, 2005). The availability of reliable testing techniques is a crucial issue to fulfil regulation and to prevent distribution of the mycotoxin in the food chain. The test system developed in our lab is designed for single-sample testing and can be applied at the early stages of post-harvest grain control, e.g. at grain elevators, grain import and export terminals, milling industry, and breweries. The analytical procedure of the developed fluorescence-based test for DON takes approximately 10 minutes for extraction, purification, derivatization and end determination. The advantage of this system is its speed and easy procedure, it can be performed by a person with no chemical training. The developed quantitative method has been validated for the working range 0.25 to 5 mg DON per kg in wheat. Using LC-MS/MS as a reference method, recoveries for naturally contaminated wheat samples vary between 86 to 105%. The limit of detection (LOD) obtained for wheat, barley and maize is 0.1 mg/kg. The reproducibility of the method at a level of 1.1 mg DON per kg, which is close to the regulatory limit of the European Commission for wheat (EC, 2005), is 12%, involving three operators with five measurements each. The obtained recoveries and RSD values fulfil EU (EC, 2006) and GIPSA criteria (USDA/GIPSA).

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P18

Efficiency of various solvents in the extraction of aflatoxin from naturally contaminated cottonseed meal, corn gluten meal, corn gluten feed, and dried distillers grain

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An evaluation was conducted to compare the extraction efficiency of aflatoxins from naturally contaminated cottonseed meal, corn gluten meal, corn gluten feed, and dried distillers grain using various solvents. The solvents compared were acetonitrile/water (84/16), acetonitrile/water (90/10), methanol/water (80/20), methanol/water (70/30), and chloroform. These solvents were chosen because they are commonly used for HPLC and test kit analysis of aflatoxins. Each naturally contaminated matrix was finely ground, well homogenized, and extracted using the various solvents in replicates of 10 and analyzed by HPLC using AOAC Official Method 994.08 substituting the Kobra cell for the TFA derivative. The data demonstrates that the extraction efficiency of various solvents is matrix dependent with acetonitrile/water (84/16) consistently exhibiting the best extraction of aflatoxin. Solvents containing methanol/water should not be used for the extraction of aflatoxin from cottonseed meal. These solvents containing methanol/water at ratios of 80/20 and 70/30 only extracted 17% of the aflatoxin from cottonseed meal compared to the acetonitrile/water (84/16) solvent. This comparison of extraction solvents demonstrates the importance of the evaluation of naturally contaminated matrices when validating new methodology.

P19

Determination of aflatoxins and ochratoxin A in various spices by liquid chromatography with fluorescence detection

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Liquid chromatography methods were developed and evaluated to determine aflatoxins and ochratoxin A in chili pepper, paprika, and red pepper. Aflatoxins were extracted with acetonitrile/water (84/16), purified using a solid phase cleanup column, and analyzed by liquid chromatography with fluorescence detection using the Kobra cell for post column derivatization. Ochratoxin A was extracted with methanol/water (70/30), purified using an immunoaffinity column, and analyzed by liquid chromatography with fluorescence detection. Accuracy and precision data for both methods will be presented. Samples of chili pepper, paprika, and red pepper from various suppliers were purchased from retail stores from several parts of the United States and analyzed for aflatoxins and ochratoxin A. All 12 samples of chili pepper were non detect for aflatoxins (detection limit 1 ppb [µg/kg]) with 6 positive for ochratoxin A (detection limit 1 ppb) ranging from 1.6 to 8.3 ppb. For the paprika, 1 out of 9 samples was positive for aflatoxin B_1 at 1.1 ppb with 5 samples positive for ochratoxin A ranging from 1.1 to 5.4 ppb. For the red pepper, 8 out of 9 samples were positive for aflatoxin B1 ranging from 2.9 to 27.7 ppb with all 9 samples positive for ochratoxin A ranging from 2.0 to 11.8 ppb.

P20

Development of a multi-residue method for mycotoxin analysis in feeds and grains: multi-residue separation and detection by HPLC and post-column derivatization

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Although *Aspergillus* (aflatoxins, ochratoxin A) is generally associated with peanuts and *Fusarium* (deoxynivalenol, zearalenone, fumonisins) with wheat, these fungi and those that produce other toxins are not host selective and so can cross plant species. This situation is complicated by the fact that the microscopic mold may not be visible to the naked eye. Also, when infected grains are processed, any visible mold is lost but the toxic metabolites carry over into the finished products. Thus, multi-residue analytical screens for toxins in grain and finished goods are a wiser choice than single-family protocols. We are presenting a single screen method to cover 5 families of toxins.

P21

Evaluation of analytical methods for the mycotoxin patulin in apple juice

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Patulin is a toxic, secondary metabolite produced by several fungal species, including *Penicillium expansum*, the most common 'blue mold rot' in apples. Patulin exhibits mutagenic, immunotoxic and neurotoxic effects in experimental animals. Current recommendations indicate that residual levels of patulin should not exceed 50 ppb in apple products intended for human consumption. The analytical determination of patulin generally involves extraction from apple juice, clean-up of the extract and subsequent HPLC determination with UV detection at 275 nm. The use of a photo-diode array (PDA) detector, which enables the simultaneous acquisition of spectral data, provides information for confirmation purposes. Four methods for the extraction and clean-up of patulin from processed apple juice were evaluated by HPLC-PDA. Samples were spiked with patulin at 10, 20, 50, 100 and 150 ng/mL (ppb) and extracted by one of four methods (three solid phase extraction and one liquid-liquid extraction). Two of the solid phase methods involved sorption cartridges (silica and hydrophilic-lipophilic balance (HLB) cartridges) and the third involved a proprietary method for impurity removal (Romer cartridge). The elution solvent mixture used for the HLB cartridge method was modified to reduce drying times and improve recoveries. Purified extracts were analyzed by a single isocratic HPLC method that could achieve baseline separation from hydroxymethylfurfural, a common contaminant in processed apple juice. The methods were validated for recovery and precision. All four methods showed good recoveries (>70%), although at low levels (10 ng/mL) the liquid-liquid extraction and the Romer method were markedly above 100%, implying the presence of co-eluting impurities. All methods showed within laboratory repeatabilities (relative standard deviation less than 20% at 10 ng/mL), which are considered acceptable. Based on all aspects, the method using silica sorption cartridges performed the best for routine analysis of processed apple juice.

P22

Recent survey of ochratoxin A in U.S. wines and an update on multi-mycotoxin analysis in alcoholic beverages using HPLC/ultraviolet/fluorescence/tandem mass spectrometry

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Multi-mycotoxin analytical methods to analyze substrates containing various mycotoxins have become increasingly important, but the predominant naturally occurring mycotoxin in grape wine is the ochratoxin A (OTA). Our previously published 84 U.S. wines surveyed consisted of wine vintages ranging from 1995 to 2001. Overall, there was about 31% incidence of detectable OTA ($\geq$ 0.01 μg/L) in the 84 domestic wines surveyed: 16.7% at 0.01 μg/L $\leq$ [C_{OTA}] $\leq$ 0.03 μg/L, 13.1% at 0.03 μg/L < [C_{OTA}] $\leq$ 0.20 μg/L and 1.2% at 0.20 μg/L < [C_{OTA}] $\leq$ 2.00 μg/L. In 2006, the sample preparations of 121 wine samples, including duplicates using immunoaffinity (IA) clean-up columns were performed using the AOAC Official Method 2001.01. Our previously reported study indicated acceptable recoveries ranging from 80.8% ± 1.7 (n = 3; RSD = 2.1%) to 95.8% ± 7.8 (n = 3; RSD = 8.1%) on 10 different wines spiked with 0.50 μg/L OTA. The HPLC/Fluorescence system was used to measure the OTA level in our domestic wines with various vintages, appellations and varietals. The OTA in U.S. wine survey prior to the 2004 harvest showed the presence of naturally occurring OTA at levels substantially below the 2 ppb set by the European Union (EU). EU had recently proposed the OTA limit of 2 ppb for wines obtained from the 2005 and subsequent harvests. The highest OTA level found in these 121 domestic wine samples surveyed was 0.08 ppb. In the last few years, the TTB-led collaborative multi-mycotoxin analysis project efforts had successfully optimized and developed novel multi-mycotoxin (aflatoxins, deoxynivalenol, fumonisins, ochratoxin A and zearalenone) IA sample preparations and analytical methods for alcoholic beverages using LC/UV/fluorescence/ tandem MS. The simultaneous analysis of several family groups of mycotoxins ('all-in-one'), including aflatoxins, deoxynivalenol, fumonisins, ochratoxin A, zearalenone, T-2 and HT-2 using prototype mixed-antibody resins IA and selected multi-functional SPE columns by HPLC/UV/fluorescence with post-column derivatization and multiple reaction monitoring mode MS/MS using electrospray ionization were re-visited. The multi-mycotoxin method detection limits were in the sub μg/L and μg/L range. The mixed-bed IA column method optimizations resulted in multi-mycotoxin recoveries, which were in acceptable range for the spiked alcoholic beverage samples. Our findings suggest the efficacy of both the customized IA and multi-functional SPE cartridges to bind with multiple mycotoxins and to sufficiently suppress alcoholic beverage sample-matrix interferences.

P23

Development of a rapid flow-through assay for the detection of deoxynivalenol in cereals

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A flow-through membrane-based immunological test was developed for the detection of deoxynivalenol (DON) in cereals. The DON monoclonal antibody (Mab) was used at a 1:6000 dilution in assay buffer (0.1% casein in PBS pH 7.4). The DON assay consisted of a simple extraction procedure: 5 g of sample was extracted by hand in 15 ml of extraction solution (80% Methanol/3% $NaHCO_3$). The extract (1 mL) was mixed with 1.4 ml of dilution solution (PBS) and assayed. The flow-through rapid test format is based on anti-species antibodies bound on the membrane. The membrane is superposed on an absorbent layer housed in a bottom member. These are retained at the top by means of a top member, which is in contact with the membrane. The analytical site of the membrane is exposed to reagents by means of an aperture. Firstly, 1 drop of DON Mab was added followed by 600 µl of diluted sample extract, and sequentially followed by DON-horseradish peroxidase (HRP) (1:1000). The membrane was washed with a washing solution and the results developed with 200 µl of tetramethylbenzidine (TMB) substrate. DON standard was prepared in 30% methanol/water (vol/vol), and was also spiked in wheat samples to represent the following concentrations: 0, 50, 100, 150, 200, 250 and 300 ppb (µg/kg). The results for 0 to 150 ppb were 2 blue lines. The first line that appears is the control line followed immediately by the test line. The concentrations 200 to 300 ppb resulted in 1 blue line. The cut-off level of this assay was 200 ppb, a sensitivity which is required for screening food destined for infants in the European Union (EC, 2005). The cut-off level is adjustable to the required limit depending on the prevailing regulation.

References

EC (European Commission), 2005. Commission Regulation (EC) No. 856/2005 of 6 June 2005 amending Regulation (EC) No. 466/2001 as regards *Fusarium* toxins. Official Journal of the European Union L143, pp. 3-8.

P24

Performance validation of the flow-through rapid test and microtitre plate enzyme immunoassay test for ochratoxin A in wine using a quantitative HPLC method

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The Flow Through Rapid Test (FTRT) kit and the microtitre plate Enzyme Immunoassay (EIA) from Euro-Diagnostica BV were successfully validated at the Laboratory of Food Analysis, Ghent University, in Belgium. The FTRT and EIA test kits were used for screening red wine for OTA and the results were validated by high performance liquid chromatography (HPLC) analysis. The samples, which consisted of red and rose wines, were from diverse origins. The FTRT test kits contained everything required for the test. The FTRT and EIA were performed according to the manufacturer's instructions. The EIA had an average recovery of 72%. According to the FTRT screening results 10 samples (26%) of the 38 red wine samples were positive for OTA. The HPLC confirmation showed that 21 samples (55%) were contaminated with OTA and 6 samples (16%) had levels above the 1 ppb (µg/kg) cut-off level. Four samples (10%) gave a false positive result and there were, however, no false negative results. The Flow Through Rapid Test, therefore, had a 90% correlation with the HPLC confirmatory analysis.

P25

Simultaneous detection immunochromatography using two colloidal gold-antibody probes for the detection of aflatoxin B_1 and ochratoxin A in grain and feed samples

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The objective of this study was the development of simultaneous detection immuno-chromatography (SD-ICG) for analysis of aflatoxin B_1 (AFB_1) and ochratoxin A (OTA). The SD-ICG was composed of three pads (sample pad, conjugate pad, absorbance pad) and a nitrocellulose membrane. Two kinds of monoclonal antibodies (MAbs) against AFB_1 and OTA were conjugated each with gold particles and sprayed in one conjugate pad; AFB_1-oxime-BSA, OTA-oxime-BSA conjugate and rabbit anti-mouse IgG were drawn on AFB_1 test line, OTA test line and control line in nitrocellulose membrane. The detection limit of simultaneous detection ICG for AFB_1 and OTA was 0.5 and 5 ppb (µg/kg), respectively. Feed samples spiked with various concentrations of AFB_1 (0, 20, 50, 100, 200 ppb) and OTA (0, 20, 50, 100, 200 ppb) were assayed by SD-ICG; the detection limit of SD-ICG for spiked samples was 20 ppb AFB_1 and 100 ppb OTA, respectively. Sixty-five feed samples were tested by SD-ICG. Five feed samples showed AFB_1 contamination, but OTA was not detected. All AFB_1 positive samples were analyzed by HPLC for confirmation of AFB_1 and OTA. AFB_1 contamination was shown for all positive samples by SD-ICG, but only 4 feed samples were confirmed for OTA contamination by HPLC. We verified that the SD-ICG was able to detect AFB_1 and OTA simultaneously in food, grain and feed.

P26

Development of immunoassays for the detection of zearalenone

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Zearalenone (ZEA) is a non-steroidal estrogenic mycotoxin produced by several *Fusarium* species. It is found worldwide in a number of cereal crops such as corn, barley, rice, wheat and oats. A direct competitive enzyme-linked immunosorbent assay (DC-ELISA) and a rapid immunochromatographic assay (ICA) based on the monoclonal antibody (MAb) were developed for determination of the ZEA. The cross-reactivities to other mycotoxins of MAbs against ZEA were α-zearalenol, β-zearalenol, α-zearalanol and β-zearalanol as 121%, 65%, 21% and 19%, respectively. The detection limit of ZEA was determined to be 0.35 ± 0.07 ng/mL and IC_{50} was equal to 2.34 ± 0.28 ng/mL analyzed by the DC-ELISA. The detection limit in the ICA was 1.00 ng/mL and required an assay time less than 15 min. The extraction method with methanol-water (80:20, v/v) gave higher extraction efficiency as percentages of recoveries ranging from 90.65 to 116.54% for ZEA determination in rice, barley and corn. Matrix effects can be avoided by a 1:9 dilution of rice, barley and corn samples in phosphate-buffered saline. The detection limit of the ZEA for rice, barley and corn was 0.42, 0.63 and 0.54 ng/mL, respectively by DC-ELISA and 2.5 ng/mL by ICA. All 80 rice and 50 barley samples were considered to be negative by using ELISA and ICA test strip. However, among 38 corn samples ZEA was detected in 9 and 5 samples by DC-ELISA and ICA, respectively. The positive samples of DC-ELISA and ICA were confirmed by HPLC, 7 samples were detected positive by HPLC. The data of ZEA determination in corn showed a very good agreement between DC-ELISA, ICA and HPLC. Development of DC-ELISA and ICA are therefore useful for screening of ZEA in rice, barley and corn samples.

P27

The TRI101 story: engineering wheat and barley to resist *Fusarium* head blight

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Fusarium head blight (FHB), caused primarily by *Fusarium graminearum*, is a major disease of wheat and barley in the United States and Canada. The disease process depends on high humidity and the presence of inoculum (fungal spores), and therefore, the amount of disease can vary from year to year with the amount and timing of rainfall. FHB epidemics have been on the increase since 1993 and have caused severe monetary damage to the growers and seed industry. Infection of wheat and barley by *F. graminearum* causes necrosis of the florets that gives the head a 'scabby' appearance, and results in moderate to severe reduction in grain yields. Along with the reduced yields, the presence of mycotoxins in the moldy grain constitutes a major problem for the grain industry. The fungus produces trichothecenes as it grows throughout the plant tissue. These mycotoxins pose health problems to humans and non-ruminant animals upon ingestion. As a result, governmental agencies, both in the U.S. and Europe, have imposed limits on the amounts of deoxynivalenol (DON), the primary trichothecene produced by *F. graminearum*, that are acceptable in grain. Cultivars of wheat and barley that are resistant to *F. graminearum* are needed. The fungus is able to protect itself from trichothecenes by modifying the core trichothecene as soon as it is biosynthetically formed. The gene responsible for this modification, TRI101, encodes a trichothecene acetyltransferase. The modified trichothecene has been shown to be less toxic to plants. DON is also involved with virulence in wheat, as lines of non-toxin-producing *F. graminearum* do not cause much disease. Utilizing this information, our laboratories investigated the novel strategy of introducing the fungal gene, FsTRI101, into wheat and barley. The extra FsTRI101 activity was expected to limit the accumulation of DON in the plant as well as limit the spread of disease within the plant. *Fusarium*-susceptible lines of wheat (Bobwhite) and barley (Conlon) were transformed with FsTRI101 constructs. Proof was obtained for the presence and expression of the gene in the transformants. Greenhouse tests showed that transgenic lines of both wheat and barley had a reduction in FHB symptoms. Barley transgenics showed a reduced accumulation of DON. These results demonstrated, for the first time, that FHB severity and DON accumulation can be reduced in wheat and barley by the introduction of a toxin-modification gene.

P28

Decision support systems, an aid in mycotoxin management

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Certain filamentous fungi, mainly belonging to *Aspergillus, Fusarium* and *Penicillium* genera, are capable of producing mycotoxins, which can be accumulated in infected plants, and in stored crops. The formation of mycotoxins in plant products is of great concern since their occurrence in feeds and foods is often associated with chronic or acute mycotoxicoses in farm animals and humans. Mycotoxins are a serious problem worldwide, because of the wide range of host crops for toxigenic fungi, and the key toxins differ from area to area and from year to year, depending on weather conditions. Existing knowledge on mycotoxins is far from exhaustive, but two main lines have been tracked to manage problems: (i) definition of legal limits for toxin content in raw and processed products, and (ii) development of guidelines for crop management to minimize toxin content in raw products and possible post-harvest decontamination. The first line is currently much more developed mainly because the second implies a considerable knowledge of fungi of interest, epidemiology, relations with the host and the role of farming system, as well as the interaction with other parasites; these data are difficult to acquire and arrange in an applicable way. Useful tools to manage complex systems dynamically are decision support systems (DSS). They include and manage relationships between pathogen, crop, environment and control measures to support strategic, tactical and operational decision-making in integrated production. Models simulating the pathosystem to be considered are the core of a DSS; their implementation follows the step-by-step approach, with four main steps: (i) problem definition, (ii) model development, (iii) validation, and (iv) improvement. In problem definition, information can be obtained by growers, technicians, advisors, consumers, researchers, or any other actor in the agrofood production who knows the problem to be modeled. The second step consists of model development, preferably following a mechanistic approach based on the system's analysis. A system is studied by distinguishing its major components, characterizing their changes, and the interconnecting elements. The system structure in plant pathology includes pathogen, host, environment, human actions and their relationships. Data collection from different sources (step 1) is the basis of a system-oriented approach, that starts with drawing a relational diagram and goes on by translating it into quantitative relationships, until the production of model outputs in the form of predicted fungal development and risk of mycotoxin presence. The simulation model is generally implemented in a computerized form.
The third step consists of model validation and evaluation. The objective of this step is to build up a final model, by testing the agreement between model outputs and the real system. During the last step, feedback from the application of the model in decision-making is collected. Final users are encouraged to refer back about model performances and to suggest new needs to the modeler. Unsatisfactory performances and/or new knowledge are used to update the model and to obtain a new version: model development is an ongoing process. In summary, the core of a DSS is a simulation model able to predict the behavior of a pathogen, in terms of effect on the host, in function of the factors identified as relevant, mainly ecological parameters and cropping practices. Outputs consist of predictions of the pathogen effect on yield, in relation to natural conditions, and possible yield improvement, in this case especially in terms of quality, obtainable with the optimization of the cropping system. A post-harvest part of the DSS would add relevance to this tool. The main advantages of DSS in managing mycotoxins can be summarized as follows: (i) manage all available data to define the effect of all relevant factors on product contamination, (ii) produce real time scenarios with actual or historical data, and (iii) simulate the effect of different cropping systems, meteorological conditions, storage conditions.

P29

Novel regulation of fumonisin biosynthesis by *Fusarium verticillioides* via a Zn(II)2Cys6 transcriptional factor

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Fungal toxins are natural products that can negatively affect animal and plant health. The fungal genes involved in toxin synthesis are co-regulated and are often clustered within the genome and encode structural enzymes, regulatory proteins, and/or proteins that provide self protection. Fumonisins are toxins synthesized by *Fusarium* species that may contaminate maize or maize products, are associated with several animal diseases and are linked with cancer in animals and humans. The fumonisin biosynthetic gene cluster includes 16 genes, none of which appear to be play a role in regulation. We identified a new gene (FUM21) located adjacent to the cluster and show that it plays a significant but not absolute role in fumonisin production. FUM21 mutants produce no fumonisin after 10 days and 70% less fumonisin on cracked corn and accumulate significantly less FUM1 and FUM8 transcripts compared to wild type in a liquid medium. The predicted FUM21 protein includes a Zn(II)2Cys6 DNA binding domain and another domain associated with fungal transcription factors. Transformation of a FUM21 mutant with an intact copy of FUM21 restored wild-type fumonisin production. The observation that FUM21 mutants are not completely blocked in fumonisin production suggests that genes may directly regulate fumonisin production.

P30

Efficacy of a modified montimorillonite to ameliorate the toxic effects of aflatoxin in broiler chicks

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Traditionally, clays have been incorporated in animal diets to improve feed manufacture. Montmorillonite (MONT), an aluminosilicate clay with a 2:1 layered structure, has been shown to improve growth performance, reduce bacterial contamination of the gut, and to protect the intestinal mucosa. It is well documented that pharmacological concentrations of copper (125 to 250 mg Cu/kg diet) can stimulate growth and feed efficiency in poultry. Selected adsorbents, including MONT, added to aflatoxin (AF) contaminated feeds, can sequester AF during the digestive process, allowing AF to pass harmlessly through the animal. In an attempt to combine the benefits of MONT and copper, a Cu-MONT was prepared at the Institute for Technology of Nuclear and Other Mineral Raw Materials, Serbia. Since it is possible that modifying MONT may affect its efficacy in binding AF, the objective of this study was to evaluate the efficacy of the Cu-MONT in ameliorating the toxic effects of AF. One hundred fifty-day old male broiler chicks were purchased from a commercial hatchery, and were fed NRC diets for 2 days. On day 3, chicks were weighed, wing-banded, and assigned to pens in stainless steel chick batteries. A completely randomized design was used with 5 pen replicate of 5 chicks assigned to each of the six dietary treatments. Dietary treatments evaluated included: (i) basal diet containing neither adsorbent nor mycotoxins (positive control); (ii) basal diet supplemented with 0.5% MONT; (iii) basal diet supplemented with 0.5% Cu-MONT; (iv) basal diet supplemented with 2 mg AF/kg diet; (v) basal diet supplemented with 0.5% MONT and 2 mg AF/kg diet; and (vi) basal diet supplemented with 0.5% Cu-MONT and 2 mg AF/kg diet. Culture material containing 690mg AFB_1/kg culture material was used to supply AF. Dietary AF concentrations were confirmed by analysis and diets were screened for the presence of known mycotoxins prior to the start of the experiment. Response variables evaluated included growth performance, serum chemistry, relative organ weights, and toe ash weight. Compared to controls, feed intake and body weight gain were reduced ($P< 0.05$) by 20% and 22%, respectively in birds fed AF ($P<0.05$). Addition of Cu-MONT to feed of chicks fed AF reduced the growth depressing effect of AF from 22% to 13%. MONT was not effective in ameliorating the growth depressing effect of AF. Compared to controls, relative liver and kidney weights increased ($P <0.05$) by 34% and 68%, respectively in birds fed AF. Both MONT and Cu-MONT were equally effective in ameliorating the effect of AF on relative liver and kidney weights. Birds fed AF had lower ($P<0 .05$) toe ash weight compared with controls (9.92 vs. 11.14 mg). The addition of Cu-MONT to the AF diet reduced the effects of AF resulting in toe ash weights that were not statistically different from controls. The addition of MONT to the AF diet was not effective in preventing the decrease in toe ash weight. MONT, but not Cu-MONT, reduced the effects of AF on serum total protein and prevented the reduction in serum P observed in birds fed AF. These results suggest that both MONT and Cu-MONT at 0.5% of the diet were partially effective in ameliorating the toxic effects of AF in broilers.

P31

The isoepoxydon dehydrogenase gene of the patulin metabolic pathway in *Penicillium* species and in *Byssochlamys nivea*

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Penicillium expansum is the fungus most often associated with production of the mycotoxin patulin in apples and apple juice. The sequence of the isoepoxydon dehydrogenase (idh) gene, which codes for the seventh enzyme in the patulin biosynthetic pathway, was determined in 12 different *Penicillium* species and in *Byssochlamys nivea*, all capable of producing patulin. Among patulin-producers, *B. nivea* is unique because it can produce ascospores that survive pasteurization and cause spoilage of heat-processed fruit products worldwide. Primer pairs for DNA amplification by PCR and sequencing of the idh gene of *Penicillium* species were designed based on the *P. griseofulvum* idh gene deposited in GenBank. It was necessary to construct GenomeWalker libraries for determination of the *B. nivea* idh gene because its second intron was two- to three-fold larger than the introns in *Penicillium* species. The identity of all of the isolates was confirmed by sequencing rDNA (ITS1, 5.8 S, ITS2 and partial sequences of 28S rDNA). Maximum parsimony analysis showed trees based on idh and rDNA sequences to be congruent, suggesting that lateral gene transfer between the species examined had not occurred. Numerous nucleotide substitutions in the idh gene of the *Penicillium* species and *B. nivea* were present, but because the nucleotide differences occurred in the third coding position, the amino acid sequence was usually not affected. One significant amino acid difference among the fungi examined was the substitution of a conserved lysine residue by threonine. *P. griseofulvum* and closely related *P. dipodomyicola* were the only species containing lysine, which is necessary for binding the cofactor $NADP^+$. It is anticipated that the genetic information presented will aid in understanding the biosynthesis of patulin and serve as the basis for developing oligonucleotide probes to identify these mycotoxigenic species.

P32

Modified glucomannans decrease negative effect of zearalenone on blood parameters, antioxidant system and reproductive performance of quails

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The aim of this study was to evaluate effects of modified glucomannans (Mycosorb™) on blood parameters and antioxidant profile of quails liver after inclusion of zearalenone in the diet. Fifty-four day-old Japanese quails were divided into three groups and were fed a corn-soy diet balanced in all nutrients *ad libitum* during 30 days. The diet of the experimental quail was supplemented with zearalenone at the level of 1.6 mg/kg (ppm) or zearalenone plus modified glucomannans at 1 g/kg feed. Samples of quail liver were collected after 30 days of experiment. The main carotenoids, α-tocopherol, γ-tocopherol, ascorbic acid were analyzed by HPLC-based methods. Inclusion of zearalenone in the diet was associated with changes in blood parameters (level of white blood cells, red blood cells, hemoglobin was significantly reduced due to zearalenone supplementation to the diet). Reproductive performance of quails was also compromised. We observed decrease of egg fertility (by 2.6%), hatchability (by 19%) and increase of embryonic death, by 3.0% during the first period of the embryonic development and by 15.5% during the last stages of the embryonic development. Inclusion of modified glucomannans in the toxin-contaminated diet provided a significant protective effect against changes in blood parameters, antioxidant composition quail liver and reproductive performance of quails. It is suggested that a combination of mycotoxin adsorbents and natural antioxidants could be the next step in counteracting mycotoxins in animal feed.

Table 1. Antioxidants in quail liver (mg/g) (M±m; n=10).

Antioxidants	Control	Zearalenone	Zearalenone + Mycosorb
α-tochopherol	18.4 ± 1.33	10.2 ± 0.96**	15.8 ± 0.99*
γ-tochopherol	1.9 ± 0.17	1.0 ± 0.09***	1.6 ± 0.06*
Carotenoids	4. 3 ± 0.13	2.7 ± 0.18**	3.8 ± 0.96*
Ascorbic acid	166.6 ± 8.6	101.1 ± 5.1***	150.8 ± 6.0*

*P<0.05; **P<0.01; ***P<0.001

Table 2. Blood parameters of quails (M±m; n=10).

Parameter	Control	Zearalenone	Zearalenone + Mycosorb
White blood cells (10^9/L)	98.97 ± 4.38	88.80 ± 5.57*	59.3 ± 4.63***
Red blood cells (10^{12}/L)	2.97 ± 0.45	3.13 ± 0.35	2.85 ± 0.39
Hemoglobin (g/L)	176 ± 4.73	161 ± 1.77*	175.3 ± 2.56
Thrombocyte (10^9/л)	14.67 ± 0.59	17.33 ± 1.58	14.36 ± 1.23

*P<0.05; **P<0.01; ***P<0.001

P33

Effect of modified glucomannans in layers fed low levels of naturally contaminated grain

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Mycotoxicological analysis of feed from 20 poultry farm in Ukraine during last 8 years showed that 214 from 399 samples were contaminated with mycotoxins. Every fifth sample contained T-2 toxin in concentrations: 20-100 µg/kg, 54 samples; 100-200 µg/kg, 21 samples and >200 µg/kg, 8 samples. When contaminated feed wan fed to layers it was observed the reducing of egg production and increasing of mortality rate, and very often the sign of necrotic stomatitis were found. The aim of our work was to elucidate the effect of Mycosorb on egg production and mortality rate in poultry farm in Ukraine. With this aim, two poultry houses on poultry farm "Gorlitsa", Sumy region, with 10,130 and 10,320 hen in each house were fed naturally contaminated grain (results of mycotoxicological analysis suggested low level, trace, of T-2 toxin). Egg production and morality rate during two months were measured. Hens of experimental group were fed ration with addition of Mycosorb (1kg/t). Mortality rate in hens fed Mycosorb was reduced by 5% in comparison with control group hens. Addition of Mycosorb to the ration of laying hens fed naturally contaminated feed resulted in obtaining of 8,355 eggs more in comparison with control group. Results of experiments suggested that even low level of mycotoxins presents in feed can reduce performance of poultry and modified glucomannans prevent their negative effect. These data, together with above mentioned other findings in relation to Mycosorb efficacy against various mycotoxicoses clearly suggest that glucomannans could be considered as an effective means to deal with mycotoxin-contaminated feeds. Indeed, it is likely that inclusion of Mycosorb into standard feeds for various farm animals and poultry could be used as a preventive measure for producers to decrease or even prevent detrimental effects of mycotoxins.

Table 1. Performance of layers fed naturally contaminated feed.

Parameters	Control	Mycosorb
Number of birds	10,320	10,130
Mortality (birds)	155	147
Egg production (eggs)	371,520	379,875

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Evaluation of the efficiency of an organoaluminosilicate to diminish the toxic effects of zearalenone in prepubertal sows

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Zearalenone (ZEA) is a mycotoxin, produced by *Fusarium graminearum*, which has estrogenic effects. It is found as a contaminant in sorghum and corn. Optimal production conditions are at low temperatures and high humidity percentages. ZEA is an autumn mycotoxin produced in the field before harvest. Most ZEA effects are estrogenic. Swines are the most sensitive animals, particularly prepubertal sows (gilts), which develop hyperestrogenism manifested as vulvovaginitis and mammalian gland enlargement. A minimum of 1 mg/kg (ppm) contamination levels in the diet have been reported to produce hyperestrogenism in sows (CAST, 2003). The addition of aluminosilicates has not proven to reduce this problem. An alternative route is the utilization of organoaluminosilicates, which are chemical compounds formed through the reaction of certain aluminosilicates and organic compounds. Their adsorption capacity is based on the capacity to adsorb low polarity compounds, such as ZEA. An additional characteristic of these adsorbents is their innocuity and their easy incorporation into finished feeds. The objective of this study was to determine the effectivity of a commercial organoaluminosilicate (ZEOTEK™) to decrease the toxic effects of ZEA in balanced gilt feeds and to prove that the incorporation of the organoaluminosilicate does not negatively affect animal development. 18 Recently weaned gilts were selected and placed in individual corrals with porcine floor, sucker water suppliers and weaning troughs. The first seven days were for adaptation. Thereafter, each animal was given one of the four experimental diets, which were identified as: (i) control diet, with no adsorbent nor ZEA (two gilts); (ii) innocuity diet, with ZEOTEK on a 3 kg/t dose (two gilts); (iii) ZEA diet, 2,000 µg/kg (ppb) contaminated (7 gilts); (iv) challenge diet, with ZEOTEK (3 kg/t dose) and ZEA (2,000 ppb) (7 gilts). The experimental period was 21 days. ZEA was prepared in NUTEK's laboratory (Fierro et al., 2002a,b). The feeds used were commercial (Lactomax), in which contamination with other mycotoxins was proven, such as aflatoxins, fumonisins, ochratoxin A, deoxynivalenol (vomitoxin) and T-2 toxin. The ZEA concentration in contaminated feeds was confirmed by HPLC quantification. Gilts were weighed at the beginning of the experiment (25 days of age) and individual weight was recorded every week, until the end of the experiment. Feed conversion was calculated weekly. No animal mortality happened during the trial. A daily observation of environmental conditions and animal health was performed. Starting the first week of experimentation, vulva measurements were taken (width and length) using calibrators. The result of width x length is an indicator of the effect of ZEA. Gilts were killed on day 21 of the experiment; the reproductive apparatus was removed and weighed. Samples for histopathological assays were also taken. The information obtained was analyzed as a factorial system 2 x 2 for variance analysis using the Fisher assay (minimum significant differences). The statistical significance value was based on a 0.10% probability. Due to the fact that the estrogenic effect of ZEA is manifested as a swelling, irritation and enlargement of the reproductive apparatus, the most adequate parameter for adsorbent efficiency measurement considered is the weight percentage of the reproductive apparatus in proportion to the full animal's weight, and the vulva size (width x length). Based on this, the required calculations were performed. The results proved that ZEOTEK reduced this problem in a statistically significant way ($p < 0.10$). In conclusion, ZEOTEK on a 3 kg/ton of feed dose is capable of reducing estrogenic effects in recently weaned gilts fed with diets containing 2,000 ppb ZEA during a period of 21 days.

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Dynamic in vitro gastrointestinal model (TIM): rapid and reliable studies on the effect of adsorbents on the bioaccessibility of mycotoxins and nutrients

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Improvement of mycotoxin binding adsorbents is an important issue for the feed industry. Different types of products are coming on the market. Claims on the efficacy of these binders to inhibit the bio-accessibility of mycotoxins in the gut has to be supported by reliable studies. One of the options is to study the efficacy in animal experiments, preferably in the target animals such as pigs and poultry. These *in vivo* experiments, however, are very time and money consuming. In fact, animal experiments are not a realistic option for testing the broad variety of (new) mycotoxin adsorbents in different formulations and at various concentrations. As unintended negative side effect these adsorbents might also bind essential nutrients. If fact this should be investigated as well before introduction of new products onto the market. These types of studies are even more difficult to perform in animal experiments. This means that there is a strong need for laboratory models with a high predictive quality for the *in vivo* situation. At our institute, a multicompartment, dynamic, computer-controlled model (TIM) has been developed, which closely simulates the *in vivo* conditions of the stomach and small intestine. The peristaltic movements in the GI tract are mixing the contents with secretion compounds and are transporting the contents gradually through the stomach and small intestines. During the digestion process, digested compounds and water are absorbed from the intestinal compartments via hollow-fiber semi-permeable membranes. To ensure that the results obtained in TIM are relevant for the human or animal situation, extensive validation studies, related to several areas of food/feed research, have been performed. A list of publications in peer-reviewed journals is available. We focus here on the results of studies on the availability for absorption (bioaccessibility) of mycotoxins in pig feed and the effect of binders on inhibiting the bioaccessibility of mycotoxins and there (lack of) side-effects on nutrients. It has been demonstrated with different studies that the results obtained with this dynamic model in relation to bioaccessibility of various mycotoxins (e.g., zearalenone, deoxynivalenol, nivalenol, aflatoxin B_1, fumonisin, ochratoxin A) and the efficacy of binding by various adsorbents (e.g., activated carbon, cholestyramine, expanded clays) are highly correlated with the results of animal studies. Based on the broad validation of the TIM-1 system for the digestibility of food/feed (e.g., proteins and carbohydrates) and the bioaccessibility of macronutrients (e.g., nitrogen and amino acids) and micronutrients (e.g., minerals, trace elements and vitamins) we performed studies on potential nutrient-inhibiting (or even -stimulating) effects of mycotoxin-binding products. The experiments performed so far, demonstrated that most of the binders have no (negative) effect on the bioaccessibility of nutrients. For one commercial product we showed a slight beneficial effect on the digestion of protein and the bioaccessibility of a single vitamin. In conclusion, the TIM system is a reproducible, reliable and relatively rapid and cost-effective laboratory model. In combination with sensitive analysis techniques recently developed at TNO (LC-MS method to analyze TIM samples on mycotoxins directly without cleanup), the system makes it possible to perform a broad variety of experiments on feed and functional additives, such as mycotoxin binders. The high '*in vitro – in vivo* correlation' showed the quality of the TIM system for extrapolation of results to the animal situation, such as the bioaccessibility of mycotoxins from contaminated feed, and the efficacy and safety of mycotoxin binders.

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A novel concept for simultaneous deactivation of various mycotoxins in piglets

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Mycotoxins are secondary metabolites produced by many fungi under various conditions. In animal husbandry mycotoxins impair productivity by decreasing weight gain and feed efficiency and increase disease incidence due to their immune-suppressive effects. In spite of all efforts to prevent formation of mycotoxins in feeds, significant contaminations still occur. Therefore strategies for detoxification have become very important. It has been known for years that clay minerals can be used for detoxification of aflatoxins but also that these enterosorbents do not work for other mycotoxins (Huwig et al., 2001). It has been reported that an anaerobic rumen bacterium *(Eubacterium* BBSH 797*)* is able to deactivate trichothecenes by biotransformation of their epoxide ring (Binder et al., 2001). Further a novel yeast strain (*T. mycotoxinivorans*) with the capability to deactivate ochratoxin A (OTA) and zearalenone (ZON) was recently isolated and characterized (Molnar et al., 2004). The objective of the presented study was to test a new feed additive based on the combination of clay minerals, *Eubacterium* BBSH 797 and *T. mycotoxinivorans* (MTV) in a feeding trial with weaning piglets for detoxification of 500 µg/kg OTA and 200 µg/kg ZON. A total number of 96 hybrid (white female x dark male) 21 day-old piglets were divided into four experimental groups. A negative control group (A) neither receiving mycotoxins nor the deactivator, a toxin group (B) receiving the mycotoxins and 2 test groups (C,D) receiving mycotoxins and the deactivator at 2 different concentrations (0.5 kg/to feed; 1kg/to feed). Duration of the trial was 42 days until the piglets reached an age of 63 days. The piglets were fed daily with mash feed *ad libitum*. Weight gain, feed consumption and feed conversion were evaluated on a weekly basis. At the end of the trial 10% of the population was selected randomly and slaughtered for histological assays of liver and kidney. After 42 days the piglets in the control group were heavier than the piglets in the toxin group (B). Piglets in group C (0.5 kg of additive) weighed 23.08 and in group B (1 kg of additive) weighed 24.39 kg. The average daily weight gain data showed a significant improvement in the test groups in comparison to the toxin group. Performance data were confirmed by clinical results like reduction of swollen vulvas, rectum prolapses and diarrhea in the test groups. Mycotoxins are well known for their adverse effects on animal performance. The results of the present trial confirmed these effects since 500 µg/kg OTA and 200 µg/kg ZON significantly decreased daily weight gain and thus body weight. The presence of OTA and ZON caused swollen vulva and prepuce, rectum prolapse, increased urination, vomiting and diarrhea in young piglets. This trial revealed that a combination of clay minerals, *Eubacterium* BBSH 797 and *T. mycotoxinovorans* is able to abolish negative effects of the mycotoxins OTA and ZON.

Table 1. Trial results and number of clinical findings (day 1-42).

	Final weight (kg)	Daily feed intake (g)	Daily weight gain (g)	FCR	Swollen vulva	Swollen prepuce	Rectum prolapse	Vomiting & diarrhea	Frequent urination
A	24.002[a]	648	434[a]	1.49	0	0	0	0	0
B	21.718[b]	598	380[b]	1.57	5	6	6	2	12
C	23.080[ab]	625	412[a]	1.52	2	2	2	0	0
D	24.393[a]	650	443[a]	1.47	0	2	1	0	0

a, b … different superscripts on the same column mean significant statistical difference (P<0.05).

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Effect of climate change on mycotoxin production in plants and crops

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In the future decades many regions of our planet are foreseen to be implicated in relevant climate changes that can influence in many respect the world's supply system. In this respect it is expected that climate changes may have significant influence not only on food security, but also on food safety through the influence both on chemical and biological contamination. Agriculture has always been susceptible to climate change and consequently in the next future it is expected that altered atmospheric conditions will influence the occurrence and gravity both of biotic and abiotic plant diseases. In particular fungi and mycotoxin contamination can be heavily influenced by climate change in many respects. This should be evaluated on a case by case basis since every fungal species has its own optimum conditions of temperature and water activity for growth and toxin formation. A worrying sign of the influence of climatic change on mycotoxin contamination has already been verified in 2003 in Italy: favourable climatic conditions induced heavy aflatoxin contamination in maize. The impact on human and animal health was minimized thanks to the rapid alert system of the control authorities, but tons of maize and milk had to be destroyed due to unacceptable levels of aflatoxins. Also indirect effects of climate and consequent environmental changes can influence mycotoxin contamination, such as insect attack and misuse of pesticides. Insect attack represents a main issue in fungal formation. Climate change can greatly affect this factor by influencing the insects' capacity of overwintering, their distribution over cultivated lands and the variety of attacking insects. Other environmental factors involved in climate change that can affect fungi infestation include the characteristic of soils and the kind of agricultural practices. In conclusion, climate change has to be considered as a relevant cofactor of emerging risk for mycotoxin contamination. It deserves focused studies on preventing and managing the issue.

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Field evaluation of a glucomannan polymer in commercial broilers fed natural mycotoxin-contaminated feed

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An experiment was conducted in commercial broilers (Ross) to determine the effects of supplementation of a glucomannan polymer (Mycosorb®) to feed containing multiple mycotoxins. Broiler chicks were fed corn and soybean based diets for 6 weeks. The diets included: (i) control diet (a regular commercial broiler diet) without any added mycotoxin adsorbent and (ii) diet with 500 g Mycosorb per ton feed. Diets were analyzed for aflatoxin B_1, ochratoxin A and T-2 toxin only. Both the diets on an average contained 35, 20 and 30 (µg/kg) ppb of aflatoxin B_1, ochratoxin A and T-2 toxin, respectively. Though these are the most common mycotoxins found in the Indian finished poultry feed, the presence of other mycotoxins can not be ruled out. Four replicated pens of 55 birds were fed each diet. Level of significance was set at $P \leq 0.05$. There was no significant effect of treatment on cumulative feed intake of birds, except in the first week. In the first week the birds fed Mycosorb consumed significantly less feed as compared control diet ($P = 0.025$). Until the age of 3 weeks, birds on Mycosorb weighed significantly higher than the birds fed control diet ($P \leq 0.05$). At later stages though there was no statistical difference, birds fed Mycosorb weighed 114 g more than control birds on day 42. Feed conversion ratio of birds fed Mycosorb was significantly lower than control birds on week 1 ($P=0.002$). There was no significant difference in FCR on week 2, 3, 4 and 5. However, at the end of the trial FCR again tended to be lower in Mycosorb group at the P value of 0.06. India, being a tropical country, has always faced mycotoxin challenges. Until recently only aflatoxin was targeted as the causative agent of mycotoxicoses. Now the awareness on multiple mycotoxins has increased and the poultry producers are looking for an effective multiple mycotoxin adsorbent. In this regard Mycosorb through the current trial has proved again that it can prevent the toxicity of multiple mycotoxins. Even though the individual mycotoxin levels are low, when present together they can cause significant reduction in body weight and increase in FCR as seen in the current trial. The feed intake was the same in birds fed the two diets, but feed efficiency was much better in Mycosorb fed birds. This can be attributed to Mycosorb preventing mycotoxin-induced liver and intestinal damage and, therefore, the better nutrient absorption. Economics calculation indicated 1:5.7 returns on investment. It can be concluded that Mycosorb can overcome the toxicity of diets containing blends of feed ingredients contaminated with multiple mycotoxins when fed to broilers. Return on investment is very encouraging, which allows the poultry producer to use it with greater confidence.

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Physcomitrella patens – a rapid assay system for transgenes conferring resistance to mycotoxins

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There is considerable interest in using transgenic approaches to enhance resistance to *Fusarium* head blight (FHB). Production of the tricothecene deoxnivalenol (DON) is thought to be a virulence factor for FHB during certain stages of infection, and it has been proposed that engineering resistance to DON might make plants less susceptible to infection with FHB. Testing transgenes for efficacy against DON in wheat or barley germplasm is time consuming and labor intensive. It would be extremely useful to be able to assay transgenes for efficacy against fungal mycotoxins and FHB, before they are introduced into wheat or barley. We have developed a rapid and efficient whole-plant system based on the recombinogenic plant *Physcomitrella patens*, an emerging model system for functional genomics. *Physcomitrella* is sensitive to physiological levels of DON and diacetoxyscirpenol (DAS) whose target is ribosomal protein L3. DON toxicity is substantially attenuated in transgenic *Physcomitrella* plants that express a modified ribosomal L3 gene (the L3Δ mutant) or in *Physcomitrella* plants in which other endogenous pathogen-induced transcripts have been deleted through gene targeting. We also tested the estrogenic mycotoxin zearalenone (ZON), which binds to the estrogen receptor in mammalian cells. Even though there is no estrogen receptor in *Physcomitrella*, ZON induced cell death in WT and attenuated cell death in transgenics. This suggests that the mechanisms of action of ZON may differ in plants and animals. We are extending these screens to explore the mechanisms of action of a range of different fungal mycotoxins. These results confirm that the sensitivity to mycotoxins is under genetic control in plant cells and demonstrate that sensitivity can be affected at a number of genetic control points. Current efforts are focused on establishing whether genes effective against DON, DAS and ZON act through similar or independent pathways. Our results form the basis for future functional studies designed to understand the mechanism of action of mycotoxins. This rapid assay system also allows transgenes to be assessed for efficacy, prior to their introduction into wheat or barley, and can be adapted for high-throughput screens for novel sources of mycotoxin resistance.

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Effect of a glucomannan polymer on performance and Gumboro vaccination response in broilers fed corn naturally contaminated with deoxynivalenol

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Three groups of 362 Ross 308 broilers were kept separately in one broiler house. The first group was fed a corn/soy based feed while the other two groups will be fed the same feed but with 30% of the corn replaced by 30% of corn contaminated naturally with deoxynivalenol (DON). Levels of DON in the feed were 2.03 mg/kg (ppm). One of these two groups received a polymeric glucomannan mycotoxin adsorbent (Mycosorb®) at a level of 2 kg/t from 7 days onwards. During the first 7 days, a standard starter feed was fed to all of the treatments. Body weight was measured at 10, 21 and 42 days of age by sampling at random 50-75 animals per group, while feed intake was also assessed at the same times. Mortality was measured daily and the dead birds were weighted to be able to make corrections for the feed conversion. At 14 days of age, animals were vaccinated against IBD (VMG91 strain) and at 21 days against NCD (La Sota strain) via the drinking water. Blood titers against IBD of 20 animals per treatment were taken at 14, 21, 28, 35 and 42 days of age. Statistical analysis on the blood titers was performed by Anova, followed by Duncan post hoc test. Results were considered significant when $P < 0.05$. No statistical analysis could be conducted on the performance data, as there were no repetitions present in the trial. Technical results demonstrate that due to the feeding of DON-contaminated corn reduced the end weight at 42 days with 76 g from 2,166 g for the control to 2,090 g for the mycotoxin-fed group. Adding Mycosorb at a dose of 2 kg/t on top of the mycotoxin-contaminated feed improved the end weight to 2,198 g, and thus counteracted the negative effect of mycotoxin on growth performance. No effect on FCR was noted (2.02 for control and mycotoxin-fed group and 2.05 for the Mycosorb fed group). IBD antibody titer was significantly reduced at 21 days (7 days post vaccination) when animals were fed mycotoxin-contaminated feed to which no Mycosorb was added. Adding Mycosorb resulted in titers, which were comparable with the titers of the control group. This negative results of mycotoxin on titers became smaller and insignificant later in time, however the coefficient of variation (=STD/Average*100) of the titers of the mycotoxin-fed group at 28 days, as on 21 days, was about twice the coefficient of variation of the titers of the control or the Mycosorb fed group. This higher coefficient was mainly due to the higher amount of broilers showing no or very poor response to the vaccination. It can be concluded from this trial that feeding 2.03 ppm DON to broilers had a significant negative effect on the vaccination success of IBD vaccination shortly after the vaccination (at least first 7 days), but anymore in the period of 14 days or more after vaccination. During this time, the animal is thus more sensitive to IBD infection.

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Investigations concerning the specificity of anorganic mycotoxin binders on the adsorption of various mycotoxins

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It is widely known and accepted in the scientific community that specific clay minerals like hydrated sodium calcium aluminosilicates (HSCAS) are able to bind aflatoxins very specifically (Phillips, 1999). Several *in vivo* studies supported *in vitro* findings that HSCAS do not significantly prevent the toxicity of other chemically diverse mycotoxins such as zearalenone, deoxynivalenol (DON), T-2 toxin and ochratoxin A (OTA). During the past years products based on chemically modified clays (e.g., organoclays) appeared on the market claiming to bind other mycotoxins than aflatoxins to a very high extent. The aim of our study was to investigate such products *in vitro* for the capability to bind DON. In addition to that one product was further investigated for its specificity to bind OTA and fumonisin B_1 (FB1). The mycotoxin concentrations for the binding assays were: 1mg/L DON; 0.2mg/L OTA; 1.0mg/L FB1. Products (MA, ZT, TS, DT) were added in a concentration of 0.2% either to a citrate buffer (pH 3.0, simulating acidic conditions) or to a phosphate buffer (pH 6.5, representing the neutral environment in the intestine). For the determination of the specificity binding tests were carried out in complex medium consisting of 10g/L glucose, 20g/L malt extract, 10g/L yeast extract and 5g/L peptone. Analysis was performed with HPLC and UV-detection for DON and fluorescence detection for OTA, respectively. For analysis of FB1 LC-MS detection was chosen. None of the processed binders showed good adsorption of DON, the main representative of trichothecenes (phosphate buffer: MA= 0.7%; ZT=2.86%; TS=1.00% and DT=1.45%; citrate buffer: MA=0; ZT=13.0%, TS=2.15% and DT=7.55%). Generally the adsorption was better in citrate buffer. However, binding rates did not exceed 13%, which is too low to ameliorate negative impacts of DON on animals consuming contaminated feeds.
In contrast to experiments with trichothecenes all products showed good adsorption concerning OTA and FB1 in a simple buffer system. One of these materials (TS) was subjected to a binding assay in a complex environment simulating conditions in the GI-tract. In comparison to the test in buffer solution binding of OTA was reduced by 15.8% (pH 3) and 34.2% (pH6.5) in the complex environment indicating that these material are not specific for OTA. Similar results were obtained with FB1. However, binding result was even more impaired in the acidic environment (-79.9%). The amount of FB1 adsorbed in the complex matrix decreased by 38% in comparison to the buffer solution. It can be concluded that products based on processed clays like organoclays are not capable of binding trichothecenes to an acceptable degree. This is in accordance with scientific publications (e.g., Döll et al., 2005). In case of OTA and FB1 those materials lack on specificity, because binding rates dropped very drastically in complex media in contrast to clear buffer solutions.

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P42

Biotransformation and microbial degradation – strategies for detoxifying mycotoxins in animal feeds

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The application of microorganisms to detoxify mycotoxins is an innovative approach to alleviate toxic effects on animals. This approach is very promising especially for non-adsorbable mycotoxins. However, for a successful in-feed application several requirements have to be fulfilled: metabolites of the biotransformation and biodegradation process have to be non-toxic; detoxification process has to be fast; microorganisms applied have to be safe; mode of action must not be influenced by the environment in the gastro-intestinal tract; and microbes have to be stable. A bacterial rumen strain (BBSH 797) has been shown to detoxify trichothecenes. This strain is able to biotransform deoxynivalenol (DON) to de-epoxydeoxynivalenol (DOM-1) (Figure 1). DOM-1 is known to be non-toxic or at least 500 times less toxic than its parent compound. In a comprehensive screening process our research group was able to isolate *T. mycotoxinivorans*, a yeast species able to degrade ochratoxin A (OTA) by cleavage of the amide bond (Figure 2). In further experiments this yeast strain was also able to transform zearalenone to a non-estrogenic metabolite. A screening for fumonisin (FB1) -degrading microorganisms led to an aerobic bacterium belonging to *Sphingomonadacae*. This isolate is able to detoxify fumonisins by a two step process. First the two tricarballylic side chains have to be cleaved followed by a deamination process (Figure 3). Biotransformation and microbial degradation comprise several advantages. They can be considered as very gentle processes, which are irreversible. However, for practical application above mentioned requirements have to be fulfilled.

Figure 1. Mode of action of BBSH 797.

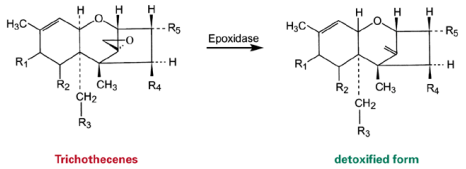


Figure 2. Detoxification of OTA by *T. mycotoxinivorans*.

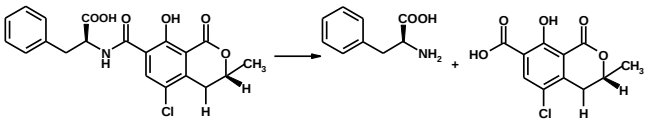
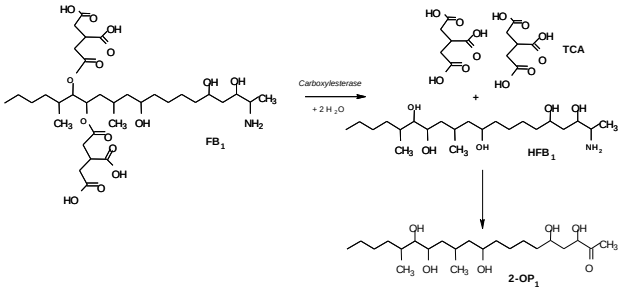


Figure 3. Detoxification of FB1 by bacterial isolate.



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The impact of Toxy-Nil Plus Unike Dry in diminishing the effect of aflatoxins in poultry

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Aflatoxins cause a variety of effects in poultry, including retarded growth rate, impaired feed conversion, increased liver weights, immunosuppression, negative effects on serum chemistry and hematological parameters, and histopathological lesions. The effect of aflatoxin on turkeys has been well documented. Extensive mortality was produced in young domestic turkeys that were given 400 µg aflatoxin/kg feed or more of dietary aflatoxin (Giambrone et al., 1985). Low levels of aflatoxin cause blood-clotting abnormalities, immune dysfunction, and poorer feed conversion (Witlock et al., 1981; Quist et al., 2000). Aflatoxins are very liposoluble compounds and are readily absorbed from the gastrointestinal tract into the bloodstream. Aflatoxins tend to infiltrate most of the soft tissues and fat deposits of the chicken; however, the majority of the accumulation occurs in organs such as liver and kidney (Lesson et al., 1995). The aim of the two trials was to investigate the efficacy of Toxy-Nil Plus Unike to counteract the toxic effects of aflatoxin in broilers and turkeys. In a first trial Toxy-Nil Plus Unike was included at 1.5 and 3.0 kg/t of feed which has been contaminated with aflatoxin at 2.8 mg/kg. Weight gain at 21 days of age was significant less reduced in birds fed diet containing 0.30% Toxy-Nil Plus Unike (18.8 %) compared to birds receiving control diet (24.9 %). The amount of feed intake of the birds fed aflatoxin-contaminated feed was significantly reduced by 21.6 %. The feed intake of birds fed aflatoxin-contaminated feed supplemented with Toxy-Nil Plus Unike was induced by 9.2%. Birds receiving Toxy-Nil Plus Unike showed the lowest relative liver weight among the intoxicated birds. Higher liver weight is an indication of increased liver activity, which is observed in acute aflatoxicosis. In a second trial turkeys were split in groups of 48 birds. One group received a non-contaminated trial whereas three groups received diets contaminated with 500 µg/kg (ppb) aflatoxins. Toxy-Nil Plus Unike was added at 0 %, 0.3 % and 0.5 % respectively. At 22 days the weighed gain with the treated groups was improved by 10.6 % over the control group receiving the contaminated feed. The feed intake reduction was diminished in the birds receiving Toxy-Nil Plus Unike supplemented to the contaminated feed by 9.8 % over the birds fed the control diet. The administered aflatoxin had a negative impact on performance parameters of broilers and turkeys. The addition of toxin in the feed significantly decreased feed intake, a parameter that interferes with the other aspects evaluated in the experiments. Several parameters evaluated in the trials indicate a significant protecting effect of Toxy-Nil Plus Unike Dry when broilers and turkeys were challenged with respectively 2.8 or 0.5 mg/kg (ppm) aflatoxins.

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Aflatoxin-phytoalexin interrelationship in peanut

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Most of the pathogens that attack peanut (*Arachis hypogaea* L.) are of fungal origin. *Aspergillus flavus* and *A. parasiticus* invade peanuts and subsequently lead to their contamination with carcinogenic aflatoxins. Pre-harvest aflatoxin contamination makes peanuts unsafe for consumption and is a major economic problem for peanut industries worldwide. Peanuts can naturally resist fungal growth when provided with adequate moisture throughout the growing season. The mechanism of resistance to fungal infection has been reported as the capacity to synthesize stilbene phytoalexins, which are antibiotic, low molecular weight metabolites. Research on peanut resistance to aflatoxin contamination under natural field conditions, such as drought stress and pest damage, may help outline measurable chemical factors that could be used in new programs for breeding resistant peanut cultivars. The objective of this research was to study the relationship between phytoalexin production and aflatoxin contamination in commercial peanut cultivars as influenced by insect damage and drought stress in the field. A two-year field study of the aflatoxin-phytoalexin interrelationship in disease-resistant and susceptible peanut genotypes was performed. Five cultivars (Georgia Green, Tifton 8, C-99R, GK-7 high oleic, and Mark I) differing in resistance to pre-harvest aflatoxin contamination and major peanut diseases were investigated for their ability to produce phytoalexins under normal and drought field conditions; drought is the major factor leading to pre-harvest aflatoxin contamination. Known major phytoalexins were quantitated in peanuts of different pod maturity (yellow, orange, brown, and black) without or with insect pod damage (externally scarified and penetrated). Comparison of Georgia Green (with small root system) and Tifton 8 (with large root system) showed that pods of the Tifton 8 genotype had higher water activity under drought stress. Damaged pods of Tifton 8 produced significantly higher levels of phytoalexins compared with Georgia Green. Insect damage of pods increased total aflatoxin contamination in both cultivars. However, Tifton 8 had significantly lower aflatoxin contamination in penetrated pods, which was associated with its ability to synthesize phytoalexins. Insect-damaged pods of C-99R and Tifton 8 cultivars had significantly higher concentrations of phytoalexins than other cultivars. The same cultivars were the most resistant to Tomato Spotted Wilt Virus (TSWV) and Late Leafspot (LLS), while Mark I, which is highly susceptible to these diseases, produced very low concentrations of phytoalexins. There was no significant difference in phytoalexin production by undamaged peanut pods of all tested cultivars. Trans-arachidin-3 and transresveratrol were the major phytoalexins produced by insect-damaged peanuts. There was a direct correlation between total phytoalexin production and published cultivar resistance to TSWV and LLS. Stilbene phytoalexins may be considered potential chemical markers in breeding programs for disease-resistant peanuts, including resistance to pre-harvest aflatoxin contamination.

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Determination of emerging mycotoxins in stored wheat grains: mycotoxigenicity potential of grain dusts and selected *Aspergillus* and *Penicillium* strains

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A bioassay tool for studying the mycotoxigenic potential of grain dusts was developed by incorporating (1% w/w) the grain dusts collected in the main agricultural region in Belgium (loamy region in central Belgium) in a triplicate sample (60 g/erlenmeyer) of wheat grain (*Triticum aestivum, Decan* cultivar) stored at 25°C for 4 weeks under 14, 17 and 20% moisture content (m.c.). Dusts act both as contaminant and as inoculum allowing the increases of the concentrations of ochratoxin A (OTA) and other mycotoxins in stored grains. OTA can be found at levels up to several hundreds µg/kg in dusts and can thus be considered as a threat to exceed the authorized maximum level of 5 µg OTA per kg in cereals. Some emerging mycotoxins like citrinin (CIT), gliotoxin, penicillic acid (PEA), secalonic acid D, helvolic acid, and zearalenol were detected in dusts and in stored grain under improper storage conditions. Patterns of mycotoxin production after grain inoculation with dusts vary according to the dust material and moisture content of wheat. Ergosterol content in stored grains was found to be a good and robust marker for forecasting the sum of OTA and CIT in stored grains, at reasonable moisture contents used as selected experimental conditions. Meanwhile, the potential of fourteen selected *Aspergillus* and *Penicillium* strains located at the 'Mycothèque de l'Université catholique de Louvain' were aseptically cultivated on wheat grains incubated at 25°C and 50% m.c. during 28 days. All the strains produced OTA (1.3-4,733.8 µg/g) whereas CIT (<0.01-56.6 µg/g) and PEA (<2.0-18.4 µg/g) were produced by 12 and 5 strains, respectively. Citreoviridin, cyclopiazonic acid, gliotoxin, helvolic acid, mycophenolic acid, patulin, secalonic acid D and sterigmatocystin were not detected. A significant positive correlation coefficient ($r = +0.976$, $n=14$, $p<0.0001$) has been derived between OTA and CIT concentrations with, however, high variations in CIT/OTA ratio, depending on strain ability. CIT production does not systematically represent a fixed fraction of OTA. OTA and CIT concentrations were higher in *Aspergillus* species than in *Penicillium* species. But, the reverse trend was somewhat obtained in the case of PEA production. By reinforcing our knowledge of the links existing between presence of grain dusts, fungal strains and mycotoxin production, mycotoxin hazards could be better predicted and the appropriate control measures undertaken, such as controlling and maintaining moisture at a reasonable level during storage of the raw material, paying more attention to the fungal infestation and to the cleaning of the stores before loading in the new harvests as well as the implementation of Hazard Analysis Critical Control Point systems. The question of the multi-contamination on cereals and the subsequent exposure of consumers to the mixture of toxins should be assessed in depth and therefore systematic monitoring of OTA and CIT as well as PEA is recommended in all foodstuffs under storage when they are susceptible to be contaminated by dusts and other *Aspergillus* and *Penicillium* inoculum sources.

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Multi-tiered approach to determine the *in vitro* ability of clay minerals to adsorb aflatoxin B_1, followed by *in vivo* performance evaluation

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Aflatoxin B_1 (AfB_1) is mainly produced by *Aspergillus parasiticus* and *A. flavus* primarily in agricultural products from tropical and subtropical regions (CAST, 2003). The utilization of adsorbents added to AfB_1-contaminated feed in order to bind the toxin in the gastro-intestinal tract before its resorption is a common way to counteract aflatoxicosis. *In vitro,* a broad range of mycotoxins including AfB_1 can be adsorbed by charcoal. However, charcoal is a relatively unspecific adsorbent, because it also adsorbs essential nutrients (CAST, 2003). A commercially available and intensively investigated HSCAS (hydrated sodium calcium aluminosilicate) was shown to have high affinity to AfB_1, and to prevent from aflatoxicosis (Huwig et al., 2001). Numerous *in vitro* tests deployed in this study were used for screening of 61 bentonites for their ability to adsorb AfB_1. The commercially available HSCAS product and a charcoal sample were used as a reference. Obtained results were compared to results of the reference materials. The evaluated chemisorption index (C_α) showed that bentonites can bind AfB_1 very strongly, a fact that indicates an adsorption process due to chemisorption. From the tested bentonites only a few reached or exceeded the C_α value as well the maximal adsorption capacity (q_{max}) of the reference binders. To verify the adsorption selectivity of the binders, tests were performed in real gastric juice and in vitamin mix solution. Charcoal proved to be a very unselective binder that definitely binds vitamins to a greater extent than bentonites do. AfB_1 adsorption of the reference materials was clearly decreased in real gastric juice. Only few investigated binders performed better than the reference materials. The different impact of the binders on vitamin adsorption, as well as the significant reduction of AfB_1 adsorption of some binders, when adsorption tests were performed in real gastric juice instead e.g. of buffer solution, highlighted the differences in the selectivity of adsorption and the need to perform a multi-tiered approach, when evaluating *in vitro* binders' performance. Mineralogical investigation (XRD, STA, FTIR and CEC) of selected bentonites could not indicate a property that influences AfB1 adsorption. One of the most promising tested material was part of a feeding trial with dairy cows aiming to determine carry over of aflatoxin into milk (3 groups: control diet; control diet + material at two concentration levels). The addition of the material to the dairy cow diet significantly reduced the milk aflatoxin M_1 content, while it apparently did not influence the feed intake and the milk production.

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Comparison of clays and yeast cell wall products, for their ability to adsorb aflatoxin B_1 and zearalenone

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One of the methods to control the negative impact of mycotoxins on animals is their adsorption in the gastrointestinal tract by an appropriate adsorbent material resulting in reduced bioavailability and excretion of the adsorbent-toxin complex. For evaluation of an adequate mycotoxin binder, high adsorption capacity, and affinity at different pH values are the most important criteria. Numerous adsorbent materials, such as bentonites and yeast cell wall products, have been examined *in vitro* and/or *in vivo* for their ability to bind, among other mycotoxins, aflatoxin B_1 (AfB1) and zearalenone (ZON). Some hydrated sodium calcium aluminosilicate (HSCAS) and bentonites have shown great adsorption capacity for AfB1 (Vekiru et al., 2006), but not for ZON. Other adsorbents based on yeast cell wall ingredients, like glucomannans, were reported to bind 95% AfB1 and 77% ZON among other toxins (Yiannikouris et al., 2002). In this *in vitro* study different adsorbents were tested in buffer solutions and in real gastric juice for their ability to adsorb AfB1 and ZON; ash content in the adsorbents was additionally determined. The following products were compared (Table 1): (A) Captex Fusa from Dox-Al Italia Spa, a product containing a modified hydrated sodium calcium aluminosilicate, resindox, esterified glucomannans (*Saccharomyces cerevisiae*) and calcium carbonate; (B) Mycosorb™ from Alltech, Inc., a formulation of brewer's dried yeast and dried *S. cerevisiae* fermentation solubles; (C) MTB-100™ from Alltech, Inc., containing brewer's dried yeast and dried *S. cerevisiae* fermentation solubles as well hydrated sodium calcium aluminosilicate and silicon dioxide; (D) Nutricell® MOS 55 from Zillo Lorenzetti, a prebiotic additive for animal feed rich in mannano-oligosaccharides and ß-glucans from *S. cerevisiae*; (E) Glucano MOS from Atis, containing ß-glucans and mannanoligosaccharides; (F) NovaSil Plus™ from Trouw Nutrition, based on a hydrated sodium calcium alumino-silicate clay; (G) Bentonite from Biomin, a clay mineral recently extensively characterized for its *in vitro* AfB1-adsorption behavior (Vekiru et al., 2006). The results indicate that products based on yeast cell wall ingredients were not able to effectively bind AfB1, although a high amount of adsorbent (0.2% w/v) was added to a relative low concentration of toxin (200µg/L AfB1). The adsorption rate slightly increased when the ash content, indicating the non-volatile inorganic matter in the products, increased (e.g., by addition of a clay mineral). The best AfB1-binding performance was observed with NovaSil Plus (F) and Bentonite (G), which were known to have an extraordinary high adsorption capacity of 0.39 and 0.42 mol AfB1 per kg adsorbent, respectively (Vekiru et al., 2006). In contrast to the bentonite, the adsorption capacity of NovaSil Plus was strongly decreased in real gastric juice, when it was applied at 0.02% w/v and 4,000µg/L AfB1. ZON was to a certain degree bound to yeast cell wall ingredients, but adsorption capacity was very low. These results were in accordance to studies done by Avantaggiato et al. (2005) and Döll et al. (2004). ZON-adsorption was independent from the product type; the specially designed toxin binder Mycosorb (B) had even a lower binding capacity than the prebiotic additive Nutricell MOS 55 (D). As expected the tested clay minerals also showed only low adsorption capacity for ZON.

Table 1. *In vitro* ability (± standard deviation, n=3) of some adsorbent materials (0.2% w/v) to adsorb AfB1 (200 µg/L) and ZON (500 µg/L) in buffer solutions and in real gastric juice.

	Product	A	B	C	D	E	F	G
[%] Adsorption of AfB1	at pH 3.0	56,6 ± 3,0	6,4 ± 1,4	16,4 ± 3,5	9,7 ± 1,9	5,2± 1,0	100	100
	at pH 6.5	92,1 ± 0,4	13,8 ± 0,5	19,6 ± 0,1	7,7 ± 0,5	2,2 ± 1,3	100	100
	gastic juice at pH 4.2	48,4 ± 2,7	10,0 ± 5,5	19,6 ± 4,6	5,7 ± 5,2	1,6 ± 1,4	96,3 ± 0,3	99,7 ± 0,2
[%] Adsorption of ZON	at pH 3.0	6,5 ± 1,1	27,8 ± 0,7	33,0 ± 2,3	49,2 ± 2,1	25,7 ± 1,6	32,0 ± 1,2	40,8 ± 2,2
	at pH 6.5	10,3 ± 0,4	35,0 ± 1,4	36,0 ± 1,0	58,4 ± 1,4	34,1 ± 1,8	52,2 ± 1,5	57,7 ± 1,0
	Ash [%]	87	5	19	4	18	85	88

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Antimicrobial activity of pyrrocidines from *Acremonium zeae* against endophytes and pathogens of maize

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Acremonium zeae produces pyrrocidines A and B, polyketide-amino acid-derived antibiotics, and is recognized as a seed-borne protective endophyte of maize which augments host defenses against microbial pathogens causing seedling blights and stalk rots. Pyrrocidine A displayed significant *in vitro* activity (MIC) against *Aspergillus flavus* and *Fusarium verticillioides* in assays performed using conidia as inoculum, with pyrrocidine A being more active than B. In equivalent assays performed with conidia and/or hyphal cells as inoculum, pyrrocidine A revealed potent activity against major stalk and ear rot pathogens of maize *F. graminearum, Nigrospora oryzae, Stenocarpella* (*Diplodia*) *maydis*, *Rhizoctonia zeae* and kernel rotting fungal pathogens *A. flavus*, *Alternaria alternata* and *Cladosporium cladosporioides*. Protective endophytes, including mycoparasites, which grow asymptomatically within healthy maize tissues, show little sensitivity to pyrrocidines. Pyrrocidine A also exhibited potent activity against *Clavibacter michiganense* subsp. *Nebraskense*, causal agent of Goss's bacterial wilt of maize, *Bacillus mojaviense* and *Pseudomonas fluorescens*, maize endophytes applied as biocontrol agents, but were ineffective against the wilt- producing bacterium, *Pantoea stewartii*. *Acremonium zeae* is recognized as a potential confounding variable in maize variety trials for resistance to pathogenic microbes and their mycotoxins.

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