ABSTRACTS OF LECTURES AND POSTERS

THE World Mycotoxin Forum®

10TH CONFERENCE

Taking mycotoxin control to the next level

12-14 MARCH 2018

AMSTERDAM
THE NETHERLANDS

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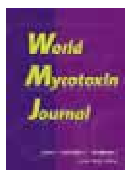

ALL ABOUT FEED

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Key to the abstracts of lectures and posters:

- the abstracts of lectures and posters are grouped separately;
- the lectures are grouped according to the daily programme; and
- the posters are grouped according to theme and then in an alphabetical order according to the corresponding author.

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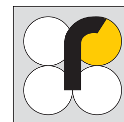

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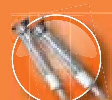

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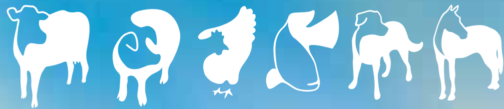
RAISING LIFE



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MYCOTOXIN MANAGEMENT: DISCOVER HOW WE ADD MORE

Nutritionists create the best possible composition and use the best raw materials. However they can't control the conditions in the field or during storage. Mycotoxins can have tremendous effect on health and growth of animals. Nutriad is the expert in solutions for controlling molds and mycotoxins in animal feed and raw materials. Nutriad offers a complete, EU-approved and hands-on range of solutions across species; such as UNIKE® PLUS, TOXY-NIL® and MOLD-NIL®. These solutions have been tested extensively and have proven reliability. Additionally, Nutriad supports its customers with mycotoxin analytical services, providing accurate information on field status, which in combination with the MYCOMAN® app, helps make the right choice of product and product dosage as quickly as possible. Supporting customers in protecting their animals and achieving higher performance.

Interested? Let's get in touch:
visit nutriad.com for your local contact.

WELCOME

Welcome to the 10th conference of **The World Mycotoxin Forum**®, 12-14 March 2018, Amsterdam, the Netherlands!

The mycotoxin contamination of various crops and derived products is a global concern because it has significant implications for food and feed safety, food security, and international trade. Despite a lot of research and available interventions, mycotoxin prevention and control remain a challenge for agriculture, and for food and feed industries. Several pre- and post-harvest measures can be taken in tackling mycotoxin exposure, however, they are not fully adequate to eliminate mycotoxins from the food and feed supply chain. In addition, climate change is increasingly affecting the occurrence of mycotoxins worldwide.

Can we ever have a mycotoxin-free supply chain? The 10th conference of **The World Mycotoxin Forum**® aims to contribute to this ultimate goal by taking mycotoxin control to the next level. The conference will offer an excellent way to network, share ideas, and formulate recommendations and conclusions on how to close knowledge gaps.

The 10th conference of **The World Mycotoxin Forum**® includes:

- presentations and discussions in plenary meetings and parallel sessions;
- poster sessions;
- company pitches covering a wide range of topics;
- workshops; and
- a concurrent instrument/manufacturers exhibition providing information on equipment, products, and services.

High-quality speakers, ample time for discussions, and every opportunity to establish rewarding contacts are values **The World Mycotoxin Forum**® wants to uphold. You are invited to take part in the discussions with participants from different disciplines and meet business relations in your area. We wish you an active and fruitful meeting!

Hans P. van Egmond
Rudolf Krska

About The World Mycotoxin Forum®

The World Mycotoxin Forum® is the leading international meeting series on mycotoxins where food and feed industry representatives meet with people from universities and governments from around the world. 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;
- to discuss strategies for prevention and control of mycotoxin contamination ensuring the safety and security of the food and feed supply, and protecting human and animal health; and
- to promote solutions for the control of mycotoxin contamination along conventional and organic supply chains.

MOBILE APP

We are pleased to introduce you to the mobile app for the 10th conference of **The World Mycotoxin Forum®**. The app is your virtual guide to the conference and has all the important information you'll need plus features to enhance your conference experience.

HOW TO DOWNLOAD?

- Follow the instructions from the leaflet (see opposite page).
- If you get a warning that your e-mail is already in use or needs to be unique: you apparently already have an account from a previous event. In that case, go to the login tab and enter your email address and password, or choose 'Forgot password?' if you don't remember the password. Once you've changed the password, you can use it to login the app. If you're logged in the app and returned to the Appendee start page, in the 'Find your event' field enter: wmf2018.
- If you encounter any problems during installation or use, please contact the WMF2018 App helpdesk: dstomp@appendee.com

APP FUNCTIONS

- Profile. Press the profile icon at the top right corner, update your profile and add your headshot! Your conversations with fellow participants will appear here (under 'Messages').
- Program. Use the program feature to view the most up to date version of the conference program. Click through each concurrent session to view the full list of presentations and speakers. Select which speakers you're interested in and create your own personalised programme by pressing the white star at the right of the speaker.
- Favourites. Your own personalised programme will appear here.
- Speakers. The full list of speakers and sessions they are involved in is available here.
- Attendees. The Attendee function allows you to view other participants' profiles and connect with them via the app messenger. Select the participant you would like to contact and press the chat icon to send a message (private in-app messaging).
- Partners. Check out our sponsors and exhibitors and view their profile.
- Bulletin board. Share and respond to interesting presentations or start discussions by posting messages and pictures on the bulletin board.
- News. Be kept up to date with important announcements before, during and after the conference. Ensure your push notifications are turned on to receive the news alerts in real time.

WiFi network: WMF2018

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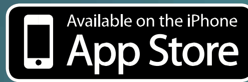

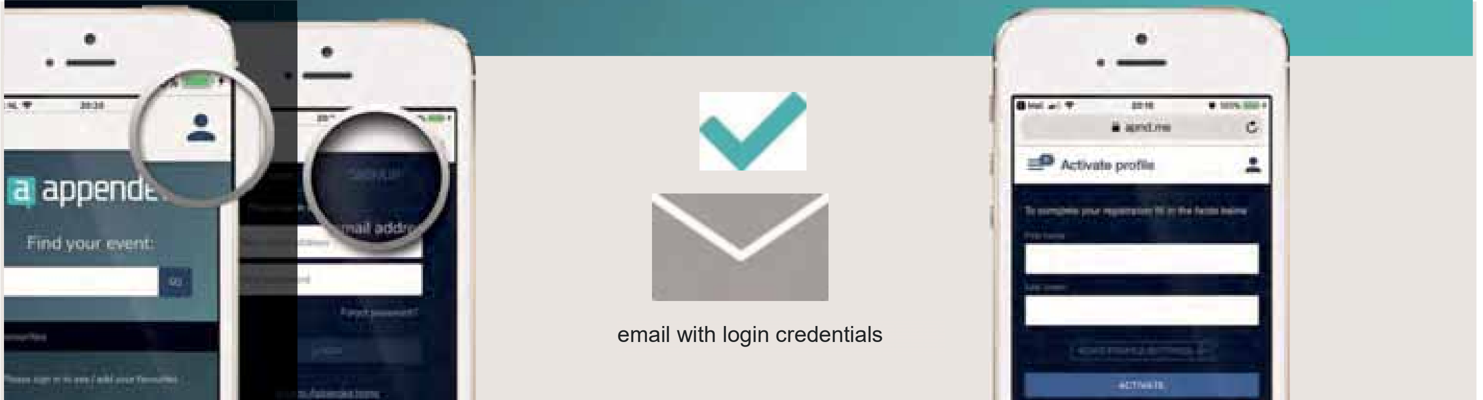

Signup

As the app is only accessible for attendees you need to signup to get access. Please make sure you use the same email address you used for the conference registration.

Verify your account

To make sure you entered the correct email address we will send you a verification email. Please click on the personal link in the email. You will be directed to the app to complete your registration.

Complete the registration process

Android: Fill in the information here, and click activate. Close the browser window and open the Appendee app. Click the profile icon and login with your e-mail address and your password.

iOS: Close the browser window without filling in anything. Open the Appendee app and fill in your first and lastname. Click activate.

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Password: mycotoxin

SOCIAL EVENTS

WELCOME RECEPTION – sponsored by R-Biopharm
(free event)

Sunday 11 March 2018
18:30 – 20:00

Where: Museum of the Canals
Herengracht 386
1016 CJ Amsterdam
http://www.hetgrachtenhuis.nl/en

The Amsterdam canals make the city. For centuries here, money has been earned, art created, feasts celebrated, and life enjoyed. This is the story that the Museum of the Canals ('Het Grachtenhuis') brings to life. The museum is situated in a monumental building on the Herengracht, where you are taken on a whirlwind tour through 400 years of history. The museum shows you not only why the creation of the Amsterdam canals was so special, but also why they're still special today. The Museum of the Canals is for everyone who loves or is just about to fall in love with the city of Amsterdam.

CANAL TOUR & CONFERENCE DINNER
(reservations only)

Tuesday 13 March 2018
19:00 – 22:30

As a special end to the 2nd day of **WMF2018**, you will be taken on a canal tour, followed by a unique dinner (with drinks sponsored by Neogen Europe) in the Saint Olof's Chapel and a historic night watch.

The Saint Olof's Chapel – the second oldest religious building in Amsterdam – was built between 1440 and 1450. Some say it was built in honour of Saint Odulphus – the patron saint of the dykes – since it is located on the Zeedijk, which translates as 'sea dyke' in English. According to others, the chapel was named after the Norwegian King Olof because of the close trade relations Amsterdam had with Norway at that time. The enlargement of the Gothic style chapel in 1644 resulted in a three-aisled church arrangement, with an irregular plan. Following the last service – which was held in the chapel in 1912 – the building has had many diverse uses.

IMPORTANT NOTES

The canal tour and conference dinner are only open to participants who registered in advance. You will find your ticket for this event at the back of your name badge.

Participants who have registered for this event must wear and show their name badge.

Participants who have registered for this event shall gather at the canal boat landing stage ('Stromma Damrak') at 19:00 sharp. The boat landing stage is a 2-minutes walk from the conference venue the Beurs van Berlage

PROGRAMME AT A GLANCE

SUNDAY 11 MARCH 2018

18:30 – 20:00	Welcome reception – sponsored by R-Biopharm

MONDAY 12 MARCH 2018

<table>
<tr><td>10:30 - 10:45</td><td>Opening of WMF2018</td><td rowspan="8">EXHIBITION</td></tr>
<tr><td>10:45 – 11:05</td><td>Introduction and overview of the conference</td></tr>
<tr><td>11:05 – 12:30</td><td>Plenary meeting
Past, present, and future challenges</td></tr>
<tr><td>12:30 – 13:30</td><td>Lunch break & poster viewing</td></tr>
<tr><td>13:30 – 15:30</td><td>Plenary meeting (continued)</td></tr>
<tr><td>16:00 – 17:15</td><td>Company pitches*</td></tr>
<tr><td>17:15 – 18:15</td><td>Speed presentations**</td></tr>
<tr><td>18:15 – 19:30</td><td>Wine tasting – sponsored by Biomin and Romer Labs
Poster viewing</td></tr>
</table>

* Short presentations by sponsors to inspire the audience to visit their booths
** Short presentation by selected poster presenters to provide an overview of their research

TUESDAY 13 MARCH 2018

<table>
<tr><td>08:30 – 10:30</td><td>Session 1
Occurrence, exposure and effects – what's up?</td><td>Session 2
Holistic analytical approaches and reduction strategies</td><td rowspan="7">EXHIBITION</td></tr>
<tr><td>10:30 – 11:00</td><td colspan="2">Networking break & poster viewing</td></tr>
<tr><td>11:00 – 12:45</td><td>Session 1 (continued)</td><td>Session 3
Managing the impact of mycotoxins – Part 1</td></tr>
<tr><td>12:45 – 13:45</td><td colspan="2">Lunch break & poster viewing
Workshops</td></tr>
<tr><td>13:45 – 15:45</td><td>Session 4
Sampling and analysis – ongoing developments</td><td>Session 5
Managing the impact of mycotoxins – Part 2</td></tr>
<tr><td>15:45 – 16:15</td><td colspan="2">Networking break & poster viewing</td></tr>
<tr><td>16:15 – 18:35</td><td colspan="2">Plenary meeting
Integrated solutions to reduce mycotoxins along food and feed chains – future expectations</td></tr>
<tr><td>19:00 – 22:30</td><td colspan="2">Canal tour & conference dinner
(reservations only)</td></tr>
</table>

WEDNESDAY 14 MARCH 2018

08:30 – 10:30	Plenary meeting *A step beyond – towards a mycotoxin-free supply chain through next level control strategies?*	EXHIBITION
10:30 – 11:00	Networking break & poster viewing	
11:00 – 12:00	Plenary meeting (continued)	
12:00 - 12:15	Best Poster Award presentation – sponsored by Nutriad	
12:15 – 12:30	Taking mycotoxin control to the next level – Top Five Answers learned	
12:30 – 12:45	Looking forward to **WMFmeetsIUPAC2019**	
12:45	Closing of **WMF2018**	

CONFERENCE PROGRAMME

MONDAY 12 MARCH 2018

PLENARY MEETING
PAST, PRESENT, AND FUTURE CHALLENGES
No progress can be made without careful consideration of the past, analysis of the present and a vision for a possible future. It is very difficult to predict the future, but it is of utmost necessity to provide a vision for such a future so as to face its challenges.

Chairs: Hans van Egmond, M.Sc. and Prof. Dr. Rudolf Krska

10:30 Opening of **WMF2018**

10:45 Introduction and overview of the conference

- A rear-view mirror perspective of The World Mycotoxin Forum
  Hans van Egmond, M.Sc., *retired from RIKILT Wageningen University & Research, the Netherlands*

- The World Mycotoxin Forum – 10th conference: Taking mycotoxin control to the next level
  Prof. Dr. Rudolf Krska, *Department IFA-Tulln, BOKU Vienna, Austria*

11:05 Global occurrence of mycotoxins in the past decade: are mycotoxin prevention measures rendering detoxification strategies obsolete?
Dr. Gerd Schatzmayr, *Biomin, Austria*

11:25 Towards integrated approaches to mitigate mycotoxins across a changing landscape
Dr. Jagger Harvey, *Feed the Future Innovation Lab for the Reduction of Post-Harvest Loss, Kansas State University, USA*

11:45 Mycotoxins and food security: are control strategies resilient under environmental change?
Prof. Dr. Naresh Magan, *Applied Mycology Group, Cranfield University, UK*

12:05 Can we foresee the future of mycotoxin testing?
Ronald Niemeijer, M.Sc., *R-Biopharm, Germany*

12:30 Lunch break
Exhibition and poster viewing

MONDAY 12 MARCH 2018

PLENARY MEETING (continued)
PAST, PRESENT, AND FUTURE CHALLENGES

Chairs: Hans van Egmond, M.Sc. and Prof. Dr. Rudolf Krska

13:30 Unravelling supply chain costs and risks due to *Fusarium* head blight (scab)
Prof. Dr. William W. Wilson, *Department of Agribusiness and Applied Economics, North Dakota State University, USA*

13:50 Stakeholders' views

- Initiatives and challenges by farmers to handle the risk of mycotoxins in the field
  Max Schulman, *on behalf of COPA-COGECA, the organisation of European farmers and agri-cooperatives*

- Mycotoxin management and raw materials – experiences in the European grain trading sector
  Dr. Johan De Meester, *on behalf of COCERAL, the European association representing the trade in cereals, rice, feedstuffs, oilseeds, olive oil, oils and fats, and agrosupply*

- Managing aflatoxin contamination in grains in Africa – the farm to fork approach!
  Dr. Owen Fraser, *Nestlé Research Centre, Switzerland*

- Controlling your feed quality: solutions for anticipating and mitigating mycotoxin risk
  Thomas Pecqueur, *Cargill Animal Nutrition, Canada*

15:10 Major challenges and needs identified
Chairs' summary of the plenary meeting

15:30 Networking break and exhibition

PLENARY MEETING
COMPANY PITCHES AND SPEED PRESENTATIONS

Chair: Dr. Franz Berthiller

16:00 Company pitches
Short presentations (5-minutes) by sponsors to inspire the audience to visit their booths
For details, see pages 27-33

17:15 Speed presentations
Short presentations (6-minutes) by selected poster presenters to provide an overview of their research
For details, see page 34

18:15 Poster viewing
WINE TASTING – SPONSORED BY BIOMIN/ROMER LABS
In the good tradition of previous years, a Wine & Cheese tasting party will be organised. A great way to meet all colleagues from the mycotoxin community and to view the posters presented.

TUESDAY 13 MARCH 2018

SESSION 1
OCCURRENCE, EXPOSURE AND EFFECTS – WHAT'S UP?
What are the main challenges in providing reliable data for supporting the risk assessment of mycotoxins?

Chairs: Dr. Vittorio Fattori and Dr. Paul South

08:30 Mycotoxins in livestock systems in developing countries
Dr. Johanna Lindahl, *International Livestock Research Institute, Kenya*

08:50 U.S. FDA mycotoxin compliance sampling and surveys: challenges and opportunities
Dr. Anthony Adeuya, *Division of Plant Products and Beverages, U.S. Food and Drug Administration, USA*

09:10 Mycotoxin exposure patterns in infants and young children in sub-Saharan Africa
Dr. Chibundu Ezekiel, *Department of Microbiology, Babcock University, Nigeria*

09:30 Ergot alkaloids: recent toxicological data on a longstanding problem
Dr. Philippe Pinton, *Toxalim Research Centre in Food Toxicology, INRA, University of Toulouse, France*

09:50 Mycotoxin cocktails in food and feed and their toxicological potential
Dr. Isabelle Oswald, *Toxalim Research Centre in Food Toxicology, INRA, University of Toulouse, France*

10:10 A review of mycotoxins toxicity to explore preventive solutions for animals
Virginie Marquis, *Phileo Lesaffre Animal Care, France*

10:30 Networking break and exhibition

11:00 Dietary intervention as a tool to manage mycotoxin-induced immune-related disorders: facts or fiction?
Prof. Dr. Johan Garssen, *Nutricia Research and Utrecht University, the Netherlands*

11:20 Biomonitoring of deoxynivalenol in human volunteers: results of an intervention study
Dr. Marcel Mengelers, *National Institute for Public Health and the Environment, the Netherlands*

11:40 Health-related risks of certain modified mycotoxins in food and feed
Dr. Hans Steinkellner, *Biological Hazards and Contaminants Unit, European Food Safety Authority (EFSA), Italy*

12:00 EFSA's risk assessment of *Fusarium* mycotoxins: a challenging endeavour
Dr. Lutz Edler, *Division on Biostatistics, German Cancer Research Center, Germany*

12:20 Probabilistic analysis in risk assessment

- An introduction to APROBA-plus, a simple tool for risk assessment visualising the quantitative uncertainties in hazard characterisation and exposure assessment
  Prof. Dr. Wout Slob, *National Institute for Public Health and the Environment, the Netherlands*

- An illustration of APROBA-plus using deoxynivalenol as an example compound
  Dr. Matthias Herzler, *Department Chemicals and Product Safety, German Federal Institute for Risk Assessment, Germany*

12:45 Lunchbreak and poster viewing
WORKSHOPS (for details, see page 18)

TUESDAY 13 MARCH 2018

SESSION 2
HOLISTIC ANALYTICAL APPROACHES AND REDUCTION STRATEGIES
Holistic approaches to mycotoxin analysis and reduction can potentially help take mycotoxin control to the next level.

Chair: Prof. Dr. Chiara Dall'Asta

08:30 Untargeted metabolomics strategy: a new predictable tool for early detection of mycotoxins
Dr. Josep Rubert, *Department of Food Analysis and Nutrition, University of Chemistry and Technology Prague, Czech Republic*

08:50 What can we learn from lipidomics experiments using mycotoxins in 3D human liver microtissues?
Prof. Dr. Gabriele Cruciani, *Department of Chemistry, Biology and Biotechnology, University of Perugia, Italy*

09:10 Metabolomics approaches to identify other relevant secondary metabolites of plants and fungi
Prof. Dr. Rainer Schuhmacher, *Department IFA-Tulln, BOKU Vienna, Austria*

09:30 Metagenomics to identify novel toxin deactivators from the soil microbiome
Prof. Dr. Chris Allen, *Institute for Global Food Security, Queen's University Belfast, UK*

09:50 *Aspergillus flavus* polyamines: small molecules with large biological implications
Dr. Raj Majumdar, *Food and Feed Safety Research, Agricultural Research Service, U.S. Department of Agriculture, USA*

10:10 Towards real-time detection of *Fusarium* spores to reduce mycotoxins in the food chain
Prof. Dr. Jonathan West, *Biointeractions and Crop Protection Department, Rothamsted Research, UK*

10:30 Networking break and exhibition

SESSION 3
MANAGING THE IMPACT OF MYCOTOXINS – PART 1
Several strategies starting in the field and continuing to storage and physical removal can help manage the impact of mycotoxins on the food and feed supply chain.

Chair: Dr. Amare Ayalew

11:00 *Fusarium* community structure revealed by metabarcoding – implications for mycotoxin contamination
Dr. Ida Karlsson, *Department of Crop Production Ecology, Swedish University of Agricultural Sciences, Sweden*

11:20 Biocontrol of aflatoxins: the pros and cons of competitive exclusion
Dr. John Pitt, *CSIRO Agriculture and Food, Australia*

11:40 The role of hermetic storage for post-harvest mycotoxin control
Dr. Charles Woloshuk, *Department of Botany and Plant Pathology, Purdue University, USA*

12:00 Effect of composting, combustion and anaerobic digestion of *Fusarium*-damaged grain and screenings on elimination of mycotoxins
Charley Sprenger, M.Sc., *Prairie Agricultural Machinery Institute, Canada*

12:20 A historical review of technologies focused on preventing and removing mycotoxin contamination in the supply chain
Dr. Gerardo Morantes, *Bühler Group, USA*

12:45 Lunch break and poster viewing
WORKSHOPS (for details, see page 18)

TUESDAY 13 MARCH 2018

SESSION 4
SAMPLING AND ANALYSIS – ONGOING DEVELOPMENTS
Despite the existence of well-established methods for sampling and analysis, some challenges remain and a number of issues require action.

Chair: Dr. Sheryl Tittlemier

13:45 Sampling of dried figs for mycotoxin: variability associated with sampling, sample preparation and analysis
Dr. Hayrettin Özer, *Food Institute, Tübitak Marmara Research Center, Turkey*

14:05 Current challenges in mycotoxin determination
Dr. Franz Berthiller, *Department IFA-Tulln, BOKU Vienna, Austria*

14:25 Quantum cascade lasers for on-site detection of aflatoxins and trichothecenes in food
Prof. Dr. Boris Mizaikoff, *Institute of Analytical and Bioanalytical Chemistry, Ulm University, Germany*

14:45 The USDA-GIPSA Mycotoxin Testing Program for grain: assuring quality in a nationwide lab network employing rapid methods
Dr. Tim D. Norden, *Grain Inspection, Packers and Stockyards Administration, U.S. Department of Agriculture, USA*

15:05 Setting-up of a proficiency-testing scheme for mycotoxins in cereals
Eric Ziegler, *BIPEA, France*

15:25 Purity determination of high purity mycotoxin standards by ^{1}H quantitative NMR
Dr. Toru Miura, *Laboratory and Specialty Chemical Division, Wako Pure Chemical Industries Ltd., Japan*

15:45 Mycotoxin analysis: the past, the present and the future of rapid testing
Dr. Kurt Brunner, *Romer Labs, Austria*

16:00 Networking break and exhibition

PLENARY MEETING
INTEGRATED SOLUTIONS TO REDUCE MYCOTOXINS ALONG FOOD AND FEED CHAINS – FUTURE EXPECTATIONS

Chairs: Prof. Dr. Rudolf Krska and Dr. Antonio Logrieco

For details, see page 16

TUESDAY 13 MARCH 2018

SESSION 5
MANAGING THE IMPACT OF MYCOTOXINS – PART 2

Processing and detoxification strategies can further reduce the impact of mycotoxins on the food and feed supply chain.

Chair: Dr. Martien Spanjer

13:45 Effects of food processing on the reduction and distribution of modified mycotoxins
Dr. Arnau Vidal Corominas, *Department of Bioanalysis, Ghent University, Belgium*

14:05 Mycotoxin transfer rates and processing factors in selected food chains
Dr. Carsten Fauhl-Hassek, *Department Safety in the Food Chain, German Federal Institute for Risk Assessment, Germany*

14:25 Transformation of mycotoxins: stories from field to process to digestion
Dr. Michele Suman, *Barilla Advanced Laboratory Research, Italy*

14:45 Atmospheric-pressure plasma: prospective tool for inactivation of fungal pathogens and degradation of mycotoxins
Dr. Lars ten Bosch, *Faculty of Natural Sciences and Technology, University of Applied Sciences and Arts, Germany*

15:05 Current research topics in mycotoxin decontamination: focus on China
Prof. Dr. Liu Yang, *Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences, China*

15:25 Effective mycotoxin detoxification strategies to ensure safer feed
María Ángeles Rodríguez Quirós, *Olmix Group, France*

15:45 Networking break and exhibition

PLENARY MEETING
INTEGRATED SOLUTIONS TO REDUCE MYCOTOXINS ALONG FOOD AND FEED CHAINS – FUTURE EXPECTATIONS

Chairs: Prof. Dr. Rudolf Krska and Dr. Antonio Logrieco

For details, see page 16

TUESDAY 13 MARCH 2018

PLENARY MEETING
INTEGRATED SOLUTIONS TO REDUCE MYCOTOXINS ALONG FOOD AND FEED CHAINS – FUTURE EXPECTATIONS

The projects MyToolBox and MycoKey funded by the European Commission aim at reducing the mycotoxin contamination throughout the food and feed chain by integrating different disciplines and research into an ICT tool that assists stakeholders in decision making.

Chairs: Prof. Dr. Rudolf Krska and Dr. Antonio Logrieco

16:15 MycoKey and MyToolBox: an example of good European cooperation
Dr. Antonio Logrieco, *Institute of Sciences of Food Production (ISPA-CNR), Italy*

16:25 Genetic basis of the resistance to *Fusarium* ear rot in maize
Prof. Dr. Adriano Marocco, *Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Italy*

16:40 Use of atoxigenic isolates in aflatoxin control in Serbia
Prof. Dr. Ferenc Bagi, *Faculty of Agriculture, University of Novi Sad, Serbia*

16:55 Control of *Fusarium* head blight with biopesticides
Prof. Dr. Simon Edwards, *Crop and Environment Sciences Department, Harper Adams University, UK*

17:10 Intervention strategies for minimising mycotoxins in the malting process
Dr. Arja Laitila, *VTT Technical Research Centre of Finland Ltd., Finland*

17:25 Feed additives for mycotoxin detoxification – efficacy and authorisation in the EU and China
Prof. Dr. Jinquan Wang, *Feed Research Institute, Chinese Academy of Agricultural Sciences, China*

17:40 Advanced analytical methods for mycotoxin detection: priorities and critical issues
Dr. Veronica Lattanzio, *Institute of Sciences of Food Production (ISPA-CNR), Italy*

17:55 The neglected issue of evaluating and reducing the impact of the lot-to-lot variation on the measurement uncertainty
David Stadler, M.Sc., *Department IFA-Tulln, BOKU Vienna, Austria*

18:10 MycoKey app for chain management
Dr. Theo van der Lee, *Plant Research, Wageningen University & Research, the Netherlands*

18:25 Integrated solutions to reduce mycotoxins along food and feed chains: future expectations
Prof. Dr. Rudolf Krska, *Department IFA-Tulln, BOKU Vienna, Austria*

19:00– 22:30
Canal tour & conference dinner (reservations only)
Join us for a very special event with an unforgettable experience in Amsterdam!
For details, see page 7

WEDNESDAY 14 MARCH 2018

PLENARY MEETING
A STEP BEYOND – TOWARDS A MYCOTOXIN-FREE SUPPLY CHAIN THROUGH NEXT LEVEL CONTROL STRATEGIES?
Current approaches, including classical breeding and pre- and post-harvest strategies, are not fully adequate to eliminate mycotoxins from the food and feed supply chain. Can incentive mechanisms, computational tools, and state-of-the-art biotechnological approaches take mycotoxin control to the next level?

Chairs: Dr. Monique de Nijs and Frans Verstraete, M.Sc.

08:30 Can incentive mechanisms contribute to realising mycotoxin-free supply chains?
Dr. Monique Mourits, *Department of Social Sciences, Wageningen University & Research, the Netherlands*

08:50 A drop in the matrix: computational tools boosting research in mycotoxicology
Prof. Dr. Chiara Dall Asta, *Department of Food and Drug, University of Parma, Italy*

09:10 Modelling the impact of mycotoxin-producing fungi on agricultural systems: issues, limits and challenges
Dr. Andrea Maiorano, *Directorate Sustainable Resources, EC Joint Research Centre, Italy*

09:30 Unravelling wheat defence against deoxynivalenol reveals novel targets for disease resistance breeding
Prof. Dr. Fiona Doohan, *School of Biology and Environmental Science, University College Dublin, Ireland*

09:50 Lowered mycotoxin accumulation in crops through investigation of *Fusarium*/host interactions: challenges and opportunities through ‘omics’
Prof. Dr. Dilantha Fernando, *Department of Plant Science, University of Manitoba, Canada*

10:10 Cutting down mycotoxins in cereals using molecular scissors
Dr. Luisa Bortesi, *Aachen-Maastricht Institute for Biobased Materials, Maastricht University, the Netherlands*

10:30 Networking break and exhibition

11:00 RNA-based strategies to control Fusarium diseases and mycotoxin contamination in agricultural crops
Prof. Dr. Karl-Heinz Kogel, *Institute for Phytopathology, Justus Liebig University, Germany*

11:20 Aflatoxin-free transgenic maize using host-induced gene silencing
Dr. Monica Schmidt, *School of Plant Sciences, University of Arizona, USA*

11:40 The regulatory landscape for plant breeding techniques – hurdles and opportunities for breeding to reduce mycotoxins
Dr. Dennis Eriksson, *Department of Plant Breeding, Swedish University of Agricultural Sciences, Sweden*

12:00 **BEST POSTER AWARD PRESENTATION**
Sponsored by Nutriad

12:15 Taking mycotoxin control to the next level – Top Five Answers learned at **WMF2018**
Hans van Egmond, M.Sc. and Prof. Dr. Rudolf Krska

12:30 Looking forward to **WMFmeetsIUPAC2019**

12:45 Closing of **WMF2018**
Take your packed lunch to eat along the way!

WORKSHOP PROGRAMME

TUESDAY 13 MARCH 2018

12:45 – 13:45

MYCOTOXIN SCREENING IN MARIJUANA: MEETING U.S. STATE REGULATIONS WITH TESTING OPTIONS

SPONSORED BY VICAM, A WATERS BUSINESS

Cannabis products may be susceptible to fungal contamination during cultivation, storage, or processing. The natural by-products of several mould species, aflatoxin and ochratoxin, present serious health risks to humans and animals – including liver toxicity, immune suppression – and are amongst the most carcinogenic naturally occurring substances known to man. U.S. State governments and the marijuana industry have been proactive in the creation of regulatory standards and requirements to establish criteria for quality and purity standards, and to build credibility with consumers and regulatory agencies. VICAM is the division of Waters Corporation dedicated to food and agricultural safety rapid diagnostics, and provides established global leadership in the development, manufacturing, and marketing of antibody-based technologies to support safety and purity regulatory compliance.

This workshop will discuss the regulatory field in the USA and beyond, and will focus on the state-of-the-art innovative testing solutions and methods provided by VICAM.

TUESDAY 13 MARCH 2018

12:45 – 13:45

EFFICIENT TESTING OF MULTIPLE MYCOTOXINS IN GRAIN: RAPID COMMON EXTRACTION AND RUN TIME

SPONSORED BY ENVIROLOGIX

Mycotoxins are natural chemicals produced by moulds that grow on and compromise various crops. When present at certain levels, mycotoxins can have harmful effects on animal health and human health and can negatively impact organisational profitably. Point-of-use testing for mycotoxins is a critical risk mitigation tool. It is very valuable for assessing contamination in various grains, particularly at delivery points and import/export terminals. Multiple aspects of mycotoxin testing procedures can impact result accuracy, which can consequently produce detrimental effects on an organisation’s operational efficiency. Fast, easy and accurate testing for mycotoxins is critical in assessing the quality of grain moving through the food chain and in driving operational efficiency into any organisation.

This workshop will discuss innovations in mycotoxin testing being delivered by EnviroLogix that improve accuracy and operational efficiency by delivering fast and easy mycotoxin tests solutions.

LECTURE ABSTRACTS

MONDAY 12 MARCH 2018

PLENARY MEETING
PAST, PRESENT, AND FUTURE CHALLENGES

Global occurrence of mycotoxins in the past decade: are mycotoxin prevention measures rendering detoxification strategies obsolete?

Gerd Schatzmayr and I. Taschl
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Since 2005 more than 75,000 samples from 95 countries encompassing 266,000 individual analyses have been performed in the frame of the BIOMIN Mycotoxin Survey Programme. The majority of samples were maize and finished feed, but also other commodities, such as wheat, barley, soybean, silage and DDGS were analysed by means of ELISA, HPLC and LC-MS/MS. Taken all results together it can be seen that more than 66% of the samples were positive for deoxynivalenol (DON), 60% for fumonisins (FBs), 46% for zearalenone (ZEN), 24% for aflatoxins (AFs) and 20% for ochratoxin A (OTA). The average of the positive samples was 947 µg/kg for DON, 1,759 µg/kg for FBs, 190 µg/kg for ZEN, 35µg/kg for AFs and 10 µg/kg for OTA. The regions with the highest prevalence for AFs were South Asia and Africa, for DON North Asia, Central Europe and North America, for FBs Africa and South America, for ZEN North Asia and for OTA Eastern Europe and South Asia. In total more than 93% of samples contained detectable concentrations of mycotoxins.

On a global level it is difficult to infer any long-term trends as climate and unusual weather events strongly influence both, types and concentrations of mycotoxins formed. For instance, heavy rainfalls in Argentina in 2017 favoured the production of mycotoxins by *Fusarium* strains. From 2016 to 2017 the prevalence of ZEN increased from 43 to 54%, DON increased from 24 to 54% and the prevalence of FBs remained high (2016: 58%; 2017 57%). Interestingly, the average concentration for DON stayed high (1,021 µg/kg in 2016 and 1,031 µg/kg in 2017), whereas the concentration of FBs increased significantly from 1,298 to 2,657 µg/kg. The increasing prevalence of FBs in Northern Europe (12% positive samples in 2016 vs. 22% positives in 2017) suggests that climate change leads to a shift of the FBs producing *Fusarium* population to the North. However, there is no dispute that mycotoxin prevention strategies are of utmost importance to reduce the mycotoxin burden. The question arises if positive effects of mycotoxin prevention measures can be seen in these data set. As the use of non-toxigenic strains of *Aspergillus flavus* to outcompete the AFs producing strains is one of the most promising preventive measures and widely used in the USA (in maize and peanuts), AFs in the US were taken as an example. Although the prevalence of AFs in the USA decreased from 17% in 2014 to 5% in 2017, no reduction of the average contamination of the positive samples (30 µg/kg in 2014 compared to 36 µg/kg in 2017) could be seen. However, even if the reduced prevalence of AFs can be attributed to the application of the biocontrol agent, supplementary intervention strategies might still be essential as the production of AFs was not entirely prevented.

Furthermore, our data clearly show that usually not only one mycotoxin occurs but the vast majority of samples (71%) contains multiple mycotoxins. So far, the simultaneous occurrence of mycotoxins is not considered in legislation and in practice this can therefore lead to a situation where mycotoxins act additively or even synergistically and exhibit detrimental effects below their individual regulatory limits or guidance values. While humans in developed countries are quite save due to mycotoxin control programmes in the food chain, animals are much more affected by mycotoxins, because grains of poorer quality are usually diverted into the animal feed stream. Therefore, the European Commission acknowledged the importance of mycotoxin binders and deactivators by establishing the legal framework under which such products can be registered and used. So far, there are three products authorised as feed additive in the EU – a defined bentonite clay for AFs adsorption, a bacterial strain for deactivation of trichothecenes (including DON) by biotransforming the epoxide ring into a double bond, and a recombinant enzyme for detoxification of FBs by cleaving the tricarballylic acid side chains. Currently, research is conducted to use enzymes during bioethanol production and other grain processing steps to reduce the concentration of mycotoxins and ideally to completely remove the toxin.

Summarising it can be said that according to this global data set of more than 266,000 analyses mycotoxins remain an issue. Analytical tools get more precise and LC-MS/MS technologies enable to

analyse for multiple co-occurring mycotoxins at reasonable costs. Although we cannot see a clear positive impact of prevention strategies in our data set yet, we strongly believe in the importance of a comprehensive approach to combat the mycotoxin burden including the application of specific, mycotoxin binding or deactivating products.

Towards integrated approaches to mitigate mycotoxins across a changing landscape

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Mycotoxins are increasingly recognised as a threat to food and nutritional security globally. Addressing mycotoxins across the global stage is dauntingly complex. Stark economic losses – current and potential – are compounded by the impact of unaddressed mycotoxins on human and animal health. Risk models predict that the geographic risk area for crop contamination is expanding with climate change, with bad years potentially costing in the billions of dollars for U.S. maize production alone. Particularly high levels of mycotoxins, highly toxigenic fungal populations, and ideal climatic conditions for mycotoxin accumulation can form a perfect storm for mycotoxin accumulation and human exposure in developing countries. Risk factors affecting mycotoxin contamination of crops can span pre-, peri- and post-harvest, touching most points along the value chain. Research spanning these areas, from Africa, Latin America and Asia will be woven into the context of integrated approaches that can be sustainably deployed across a diverse and changing landscape. This includes highlights from the activities of the Feed the Future Innovation Lab for the Reduction of Post-Harvest Loss, with mycotoxin surveys and capacity building to date conducted in Afghanistan, Bangladesh, Ethiopia, Ghana, Guatemala, Honduras and Nepal. This diverse portfolio will be used to illustrate challenges and insights into packaging mycotoxin interventions for downstream scale-up. Developing countries lie at the front lines of improving human health and livelihoods; as a community, it is incumbent on us to work with current and future leaders, to sustainably address this spectre and secure a safe harvest for all.

Mycotoxins and food security: are control strategies resilient under environmental change?

Naresh Magan, Carol Verheecke-Vaessen, Esther Garcia-Cela and Angel Medina
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Staple food production is significantly influenced by interacting abiotic and biotic factors. Under expected environmental stress in the next 25 years, the resilience of such food production systems and the control of pests, fungal diseases, spoilage moulds and mycotoxins are now under scrutiny. There may be profound impacts on the food security agenda in terms of pre-harvest yield, and post-harvest losses. In the face of such environmental changes, the question arises as to whether the minimisation strategies for mycotoxigenic moulds and mycotoxins will have the necessary resilience. While we are focussed on mycotoxins there are many associated impacts which are related and integral to the implementation of control strategies. For example, changes in pest diversity and reproduction may result in increased damage to staple crops, especially cereals, allowing increased infection by mycotoxigenic species and concomitant toxins contamination. While our focus is on mycotoxins, the nutritional and calorific value of such contaminated staple commodities is also significantly impacted.

Fungi, like many microorganisms, evolve rapidly to develop strategies for overcoming abiotic stress. Some evidence now exists suggesting that *Apergillus flavus* colonisation of maize is unaffected by climate-related abiotic factors, while aflatoxin B1 production may be increased. If this is demonstrated to be consistent, then control strategies would have to be changed to take account of these changes to

ensure efficacy. Some evidence also suggests that mycotoxigenic moulds may acclimatise to extreme climate-related abiotic factors, which may enhance virulence and, in some cases, increase mycotoxin contamination. Acclimatisation aspects for mycotoxigenic moulds needs further examination. Many mycotoxigenic moulds produce a mixture of mycotoxins, e.g., aflatoxins and cyclopiazonic acid by *A. flavus*, as well as modified (masked), but related mycotoxins. Strategies involving biocontrol of aflatoxins using atoxigenic strains have been very successful. The question arises as to whether the strains used will be resilient under climate-related environmental conditions or whether it will be necessary to modify the screening procedures or the formulations to ensure effective resilience and control of aflatoxins to be achieved in the future. Finally, in the context of climate-related abiotic stresses, the question as to whether legislation existing today may become out of step with the problems we face in the future. These aspects will be discussed in relation to the food security agenda and the implications that climate change-related scenarios may have on the resilience of control strategies for mycotoxins.

Can we foresee the future of mycotoxin testing?

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In almost 60 years mycotoxin testing evolved rapidly. Starting in the sixties of last century with analytical methods based on liquid-liquid extraction methods, followed by chromatography – first TLC, later by GC and HPLC - we now have highly sophisticated analytical tools available that allow us to detect a few hundred mycotoxins in one run. Analytical efficiency became more and more important. In times of (mycotoxin) crisis, high numbers of samples arrive in the laboratory on a daily basis. Automated analytical methods, both automated HPLC systems with auto-samplers or fully automated ELISA methods with pipetting robots, are found in many larger food testing laboratories. The demand for highly efficient, labour and time saving methods supported the development of fully automated systems. At the same time, the 'time-to-result' became a crucial parameter for mycotoxin testing. Screening methods became faster and faster, giving quantitative results in only a few minutes, but the analytical methods also became 'mobile', so mycotoxin testing is no longer restricted to the laboratory. Mycotoxin testing is now available in, or at least near the field, and became 'point-of-need' test methods.

Future of mycotoxin testing. Will the future of mycotoxin testing give us even faster, more comprehensive and even more automated methods? Certainly, when it comes to automation we will see some further developments. Automated methods, which not only cover the analytical method but also the automated sample clean-up are already available on the market and implemented in some laboratories. An additional driver for this development is standardisation of methods and commutability of the results. But in my opinion the future of mycotoxin analysis also lies in the efficient use of the analytical data and combination of these analytical data with environmental or other data.

Mycotoxins and big data? The use of mobile devices in mycotoxin analysis and sharing the analytical data in the cloud might open entirely new ways of mycotoxin data use. Analytical data about the quality of commodities may be available from all locations in real-time. Decisions to accept or reject lots will be easier to make. The analytical data may also be used in combination with other agricultural and environmental data, like weather conditions enabling to create more precise predictive models.

Unravelling supply chain costs and risks due to *Fusarium* head blight (scab)

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Fusarium head blight (FHB) has led to major economic costs for wheat and barley producers. Grain products and feed grain contaminated with deoxynivalenol (DON) (commonly known as vomitoxin) are subject to Food and Drug Administration (FDA) advisory limits and as a result, end-users place restrictions on their use. This has led to steep price discounts, as well as higher risks for producers and grain merchandisers. Research has led to development of varieties that are resistant or moderately resistant to FHB. Studies indicate combinations of genetic resistance, fungicides and some management practices (combine settings, tillage practices, etc.) can be used to decrease economic costs due to FHB.

The purpose of this study was to estimate the economic costs of scab. To do so we developed several economic models, analysed extensive data and conducted surveys of wheat flour millers, barley maltsters, and grain handlers.

The impacts of DON on growers are to increase the probability of DON being excessive, reducing yield and increasing the probability of discounts for excessive DON. Thus, any strategy that reduces DON has the opposite impacts: increasing yield, reducing probability of DON and associated price discounts. Taken together, DON mitigation strategies have the impact of increasing returns, and reducing risks relative to the technologies not being adopted. The most important direct costs are those related to increased use of fungicide, testing and increased draw areas. While reliance on fungicide is notable, it is risky. Importantly, there is growing consumer resistance to excessive chemical use in agriculture (e.g., use of pre-harvest glyphosate) and at some time may have increased scrutiny. The most important indirect costs accrued by the wheat and barley industries were the risk premium paid to induce adoption of DON reducing technologies and the value of yield forgone.

These results have implications for the wheat and barley industries. The incidence of DON has improved. However, the problems persist and have the implication of adding costs and risks to the supply chain. The impact of these vary through time, and geographically, thus impacting firms differently. There is an indirect cost of reduced production due to DON. The industry accrues an indirect cost of having to pay implicit risk premiums via the market place to induce planting and use of DON reducing technologies. Without these technologies, the cost to the industry would increase substantially. Finally, the market plays a key role in resolving problems related to excessive DON. Though not perfect, and not without pain, the market works. Important in this resolution are the combined impacts of discounts, specification limits, testing, blending and segregation and targeting shipment across end-users depending on their requirements. Though DON has improved, use of the mechanisms and processes persist in part due to inter-temporal marketing of cereals with DON.

Initiatives and challenges by farmers to handle the risk of mycotoxins in the field

Max Schulman
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The European Union (EU) currently produces some 300 million ton of cereals and is one of the main production areas for high quality wheat in the world. The EU has implemented the most comprehensive regulations for mycotoxins in food and feed. A large part of the production of cereals and cereals by-products from food and bioenergy production are used for animal feed. Detoxification of contaminated materials through simple processes and adjuvants are allowed and since July 2017 it includes detoxification processes approved under EU criteria. For food, the maximum limit levels (MLs), the non-dilution rules and lower contractual maximum limits (CMLs) represent a serious 'menace' for European farmers given that some cereal producing regions could face a permanent loss of market access and

lost revenue by the strict mycotoxin legislation. If each link in the supply chain adds pressure to the legislative limits to satisfy customers, the impact of legislation is magnified at the farm level. European farmers need a strategic approach rather than a cumulative implementation of rules which stop innovative solution.

Beyond increasing a sustainable production, the European farmers have to contend with the risk of mycotoxins development in the field. Weather conditions still represent 50% of the *Fusarium* contamination on which farmers cannot act upon. Other factors are harvest residues, type of soil, date of sowing, fungicide applications. The most impacting factors are not the same for each *Fusarium* toxin. Farmers cannot eliminate mycotoxins. They can manage the risks if risk assessment tools adapted to local conditions are made available. Most of European farmers are certified under supply chain schemes, these schemes include hygiene and good practice guidelines including mycotoxin risk management. Farmers are involved in monitoring programs and field research. However, there is still a lack of knowledge. More transfer of research information to farm level is needed. European farmers are restricted in their use of chemical control methods, a lack of authorisation of fungicides with a large spectrum is creating resistance on field level. Genetic control is therefore one of the essential ways of developing varieties which are resistant to fungal diseases. The European plant breeding sector is, however, predominantly made up of small and medium-sized enterprises. It is, therefore, vital for the European Union to impulse and unite both public and private initiatives in their attempts to identify resistant genes and multiple gene strategies, for example by funding (PPP) private, public, pre- breeding programmes.

Although there are ongoing research programs on mycotoxins under Horizon 2020, mycotoxins should be integrated as a priority area of the European research programmes, particularly on identifying genes which promote resistance to *Fusarium* mycotoxins and on developing a toolbox to help create new and more resistant varieties. The European Commission keeps postponing its communication on the legal status of new breeding techniques (NBTs). But for investments to be made and for NBTs to be developed further, breeders need legal certainty and a well-functioning EU single market. COPA and COGECA urge them to accelerate the process and clarify the legal status of NBTs to ensure that these techniques can be used in the breeding programmes and new, stronger, varieties put on the market soonest.

Mycotoxin management and raw materials – experiences in the European grain trading sector

Johan De Meester
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Grain collectors and international traders intervene after the grain is harvested, sampling and analysing the mycotoxin levels in their lots before reception, in order to ensure the safety of their products and compliance to both the regulatory and commercial requirements at European and national level. European legislation sets maximum limits for mycotoxins in food and feed products to ensure that they are not harmful for human and animal consumption. In addition to regulations, European cereal traders have to consider provisions originating from contractual agreements with business partners. This leads to a high demand in practical and reliable mycotoxin analysis methods. However, while the analytical approaches are well known and frequently reviewed, little detailed information is available on the mycotoxin management concepts of trade.

On the basis of regular surveys conducted by COCERAL amongst European cereal traders since 2007, three key issues in commercial mycotoxin management are identified. These are:

- Sampling is the major source of uncertainty in mycotoxin analysis;
- Availability and performance of validated and harmonised analytical methods; and
- Reduction factors between regulatory and contractual maximum levels, which implies additional conflict in cereal trading.

References
1. Siegel and Babuscio, 2011. Mycotoxin management in the European cereal sector. Food Control 22; 1145-1153.

Managing aflatoxin contamination in grains in Africa – the farm to fork approach!

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Mycotoxin contamination of grains pose a major challenge for food manufacturers in Africa who use them in the manufacture of cereal products. Hot and dry conditions, as well as disease, cause stressful conditions for the plants during the growing seasons; while warm and humid condition during harvest and storage, as well as further damage of the grains by pests, further increases the proliferation of the moulds that cause the contamination.

In order to reduce mycotoxin contamination of the grains entering our factories, Nestlé utilises the farm-to-fork approach, forming a fundamental pillar of the Nestlé Quality Management System, which is implemented wherever in the world the company operates. The farm-to-fork approach is essentially a scientific risk-based assessment of the supply chain, from which the knowledge obtained is used to put in place measures to eliminate the risks identified, while at the same time implementing controls to ensure that the contamination does not re-occur. This approach is reinforced by consistent testing; and extensive training of stakeholders along the value chain on best practices in farming and post-harvest management.

Recognising that good farming practices are key to mitigating aflatoxin contamination at source, ten years ago Nestlé embarked on a grains improvement programme in Nigeria and Ghana to improve the supply of suitable quality grains to its factories. At that time, half of the grains supplied (maize in this case) were rejected due to high levels of aflatoxin contamination (above 5 µg/l). The grains improvement programme was carried out in collaboration with the Ministry of Agriculture, the International Institute of Tropical Agriculture and the University of Ghana. At the heart of this programme was the concept of scientific risk-based assessment, in which critical control points in the supply chain were established and monitored. This was done in combination with extensive training of farmers and other actors on best practices throughout the supply chain. Interventions started with the selection of planting materials that were more tolerant to the environmental and disease stress while maintaining high yield, through to post-harvest handling, storage and transport. This led to remarkable improvement in the grain quality (with farm gate rejection reduced to approximately 4%) and the training of approximately 80,000 farmers – over half of them women – in 150 communities.

Controlling your feed quality: solutions for anticipating and mitigating mycotoxin risk

Thomas Pecqueur
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Research has shown that mycotoxins are becoming more prevalent as more complex ingredients/by-products are included in feed to optimise animal production costs. This, combined with worldwide grain trading, increases mycotoxin risk in feed operations. In addition to their prevalence, mycotoxins remain active even after the moulds responsible for producing them have been removed. Mycotoxins are chemically stable and survive feed processing even at high temperatures. That is why mycotoxins are considered to be among the most important feed-borne stress factors to impact animal production.

The adverse effects of mycotoxins are numerous and the financial loss associated with these invisible toxins is substantial. Recent FAO statistics indicate that a large amount of all crops is affected annually by mycotoxins resulting in financial losses of approximately US$ 5 billion in the U.S. and Canada alone. Mycotoxin contamination is difficult to trace and the usual practice of using anti-mycotoxin agents as a preventative measure is a costly approach. Being able of quickly measure the mycotoxin contamination of raw materials as well as understanding the occurrence and prevalence of mycotoxins is therefore imperative in establishing an effective control plan. Indeed, it is very important to know the level of threat to take the right action at the right time.

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ENVIROLOGIX

MONDAY 12 MARCH 2018

PLENARY MEETING
COMPANY PITCHES
Short presentations (5-minutes) by sponsors to inspire the audience to visit their booths

R-BIOPHARM
https://r-biopharm.com

The 2nd 'Habit of highly effective people' as presented by Stephen R. Covey: 'Begin with the end in mind'. What does this have to do with mycotoxin testing? Actually, quite a lot. Once I have my sample to be tested, what do I want to know? Where and when do I need the answer? Do I need to have an answer right here and right now? Or would it be more efficient to collect samples and test them all in one run? What kind of lab equipment is available? And how do I know my test method is giving me a reliable answer? Could you help me confirming this result I have? At R-Biopharm, we can help you answering all those questions. We have 30 years of experience in providing analytical solutions and are proud to offer you more than just a 'test kit'. In this short presentation, I will give you an overview of the solutions R-Biopharm can offer you for mycotoxin analysis. R-Biopharm is a leading developer of test solutions for clinical diagnostics and food & feed analysis. Since 1988, we have developed innovative products and pioneering solutions of the highest quality, safety and efficiency in Darmstadt. Our extensive product range offers best solutions for reliable food and feed analyses. A variety of different test systems enables detection of mycotoxins, allergens or illegal residues and microbiological contamination. R-Biopharm AG was founded in 1988 as a subsidiary of Röhm GmbH in Darmstadt, Germany. In 1991, it was taken over by the present managing director, Dr Ralf M. Dreher. Today, the German parent company R-Biopharm is represented by subsidiaries in the UK, USA, Italy, France, Latin America, Brazil, Spain, Australia, India and China as well as by a worldwide extensive network of more than 80 distributors. In addition, Switzerland, Austria and the Netherlands are represented by local country managers.

BIOMIN
http://www.biomin.net

'At the forefront of mycotoxin deactivation'. At BIOMIN we harness the power of science to support animal health and performance since 1983. By applying state-of-the-art and proprietary technology we deliver natural, sustainable and profitable solutions to the livestock industry. Our robust commitment to R&D in the field of mycotoxins has achieved several milestones:

- Since 2004, the BIOMIN Mycotoxin Survey programme delivers a large, global data set on mycotoxin occurrence in feed and raw commodities.
- The Mycofix® smartphone app provides instant access to mycotoxin survey data.
- BIOMIN has pioneered and commercialised novel mycotoxin deactivation strategies, such as enzymatic biotransformation, to mitigate harm to farm animals.
- The Mycofix® product line of feed additives has gained 5 EU authorisations in the group of feed additives for mycotoxin deactivation.

The 120 scientists and researchers of the BIOMIN Research Center produce one scientific and technical publication per day, maintain 200 R&D cooperations and conduct 120 feeding trials per year. BIOMIN is a part of ERBER Group. Naturally Ahead.

ROMER LABS
https://www.romerlabs.com

'Making the World's Food Safer®'. Driven by our commitment to innovation, we at Romer Labs are leading global suppliers of diagnostic solutions for the food, feed and agricultural industries. Having gathered more than 35 years of experience, we offer the most comprehensive portfolio of mycotoxin testing solutions and services to help our customers succeed in mycotoxin assessment and management. These include:

- Test kits that detect regulated mycotoxins in agricultural commodities, grain by-products and feed formulations: immunoassays (AgraQuant® ELISA tests), easy-to-use lateral flow tests (AgraStrip®), and a fluorometric test (FluoroQuant®).
- Fully accredited mycotoxin service laboratories in Austria, USa and Singapore.
- Biopure™, the broadest range of mycotoxin reference materials available.
- Clean-up columns to shorten and simplify the sample clean-up process.

Romer Labs is at the forefront of diagnostic technology and we are constantly expanding our product and service portfolio to meet your continuously evolving demands.

PATENT CO.
http://global.patent-co.com

'More than 25 years of experience in animal nutrition'. Founded in 1990 in the heart of Europe, Serbia, PATENT CO. became a global player in the feed additives industry bringing sustainable solutions on mycotoxicosis as well as on phytogenics. We are a technology-driven global company built on entrepreneurial innovation, research and teamwork with distributors and customers. Since 1993, PATENT CO. flagship product is Minazel: a clinoptilolite based product to prevent mycotoxicoses in all target species. After 8 years of experience, research in our laboratory and trials in our experimental farms, we launched in 2001 a new generation of patented mycotoxin adsorbent: Minazel Plus. However, our commitment is to provide not only best solution products but also technical support and advancement in the development of new knowledge and products to improve animal nutrition. Our product portfolio is divided into 3 sections:

- leader in mycotoxin control (Minazel, 1st generation adsorbent; Minazel Plus, premium mycotoxin adsorbent);
- phytogenics – natural based solutions (Patente Herba – 1st generation natural non-antibiotic growth promoter; Patente Herba Plus/Dysguard-S – premium natural non-antibiotic growth promoter; Ridofmite – natural non-toxic alternative to synthetic insect repellents; and
- vitamin-mineral premixes – high quality for higher profitability.

Minazel Plus is created by innovative and unique technology following the European patent. The product use leads to a broad range of action such as adsorption of all mycotoxins, polar and less polar in a very high percentage. Once adsorbed to Minazel Plus, mycotoxins are not desorbed through the intestinal tract: great speed of adsorption – the largest part of mycotoxins is adsorbed within a few minutes; does not adsorb nutrients from feed; and effective under diverse conditions with different animal species in Europe, Latin America, Africa, Asia... Minazel Plus is pH stable, retaining its mineral structure in all pH (from 1 to 10). This makes it stable in the stomach and intestines, thus favourable for use as animal feed supplement. Minazel Plus shows selectivity during adsorption. It adsorbs only mycotoxins, while leaving vitamins, minerals, amino acids in the feed! Some mycotoxins are quickly absorbed after oral intake. After 30 min they can be found in the blood, and after 60 min in the liver. Minazel Plus adsorbs more than 50% of mycotoxins in first 5 min, more than 75% in first 30 min and more than 90% in first 60 min. Minazel Plus is highly effective. It adsorbs: 99% of aflatoxin B1, 94% of zearalenone, 96% of ochratoxin A, 86% of fumonisin B1, 83% of T-2 toxin, and 97% of ergot alkaloids. Our large laboratory facilities, of which one part is the chemical analytical laboratory with an LC-MS/MS, research laboratory (analytical chemistry and microbiology), educational centre and experimental farms for broilers, pigs and ruminants makes PATENT CO. one of world's most innovative-driven companies in this field of activity. PATENT CO. has been investing continuously in innovation, research and development as the essential component of supporting the global presence and product's technical leadership. An understanding of sustainability in animal production is becoming increasingly necessary. In this context, PATENT CO. strong determination towards scientific integrity and open collaborative relationships with universities, research centres, distributors and farmers has allowed us to provide premium products for our increasingly challenging industry. Despite our growth, some things never change. We still call farms and

feed mills home. For the past 25 years, our team and business partners went together through all the challenges, ups and downs – new friendships forged, and the sheer act of improving animal production.

PHILEO LESAFFRE ANIMAL CARE
https://www.envirologix.com

'Nothing is more important than life'. Taken from the Greek verb 'to love' and associated with a spiral that illustrates the new momentum and openness to the future, Phileo has a philosophy: 'raising life'. By the year 2050, our planet will be home to more than 9 billion people. Livestock farmers have to meet the growing demand for high-quality protein food products (milk and meat) to guarantee the safety of the increasingly demanding consumers (food safety, reduction in antibiotics use, etc.), in large volume and at a reasonable price to feed the planet. They are also faced with poor cereal quality, which affects animal health and performance. Finding new solutions to meet the needs of future generations is a challenge that Phileo Lesaffre Animal Care embraces – we strive to enhance the lives of animals in order to better enhance the lives of people. Backed by more than 30 years of experience and a global staff of 140 people, Phileo is positioning itself in the health through nutrition segment located at the crossroads of the world of agronomy (focused on livestock performance through nutrition) and the world of medicine (focused on treatment using antibiotics and vaccines). Phileo has a dedicated R&D department with engineers, nutritionists and veterinarians which work closely with Lesaffre Group R&D department, a network of reference research centres and universities across the globe. In every country, our progress is led by the most advanced science as well as practical on-farm experience. Phileo provides nutritional solutions based on live yeasts, yeast fraction and yeast rich in organic selenium. These solutions are widely supported by large quantities of significant scientific research, commercial, quality testing and accreditation (such as for FAMI-QS, GMP+ B3 and other certifications). Phileo evidence based-solutions contribute to enhancing animal health and performance, including:

- improvements in digestibility and bioavailability, for better feed efficacy and performance;
- cost-effective nutritional alternatives, providing substitutes for unsustainable or limited feed sources;
- control of the risk associated with bacterial toxins and mycotoxins through binding and detoxification;
- enhancement of immune response and digestive health in preventive management;
- reduction of pathogen pressure to help limit the risk of antibiotic resistance; and
- optimisation of physiological mechanisms against stress, to support animal welfare.

ENVIROLOGIX
https://www.envirologix.com

EnviroLogix is a premier supplier of mycotoxin and GMO testing solutions based in Portland, ME, USA. Our mycotoxin test kits are designed to provide precise results on-site, eliminating the cost and delay of sending grain samples out for testing. Quantitative mycotoxin test results are obtained using the EnviroLogix QuickScan system. QuickScan is a quantification and traceability system that combines digital imaging technology with advanced mathematical processing. Using a standard PC computer platform, QuickScan provides rapid, objective and quantitative results for a variety of GMO and mycotoxin test kits. The next generation in mycotoxin testing technology is the new QuickTox Flex product line. Using the QuickScan reader platform, QuickTox Flex mycotoxin tests utilise a simple, compact strip incubator to tightly control external variables such as temperature and humidity. The dual-action incubator heats or cools the sample to optimal assay conditions, which guarantees repeatable testing conditions. Repeatable, reliable results are key to trusting a mycotoxin testing method. EnviroLogix has also developed the world's first common extraction protocol that also offers common strip run time and simultaneous strip quantification using QuickScan. **Come see us at our booth and also at our workshop on Tuesday 13 March 2018 at 12:45.**

MIXSCIENCE
http://www.mixscience.eu

'Apply industrial knowledge to develop products and services related to mycotoxin risk management'. Integration of the huge amount of knowledge, resulting from the intensive work to reduce mycotoxins risk worldwide, is one issue to improve mycotoxins management in practice. MiXscience, a company with expertise in animal nutrition and production, applies its know-how to provide various product and services to the feed industry, in several countries. The full programme developed for the French industry serves as a reference to adapt to any different market situation. The programme starts with a strict control plan of the mycotoxins contamination: every year, in the varied micro-climatic regions across France, mycotoxins levels in local cereal crops and their by-products are carefully monitored. In 2016, 443 analyses were done on wheat, barley and triticale cultivated and harvested in France for deoxynivalenol and zearalenone. According to the encountered situation, detailed recommendations are made to the technical teams of animal feed manufacturers to adjust the purchase strategy and feed formulation. This long-term experience can be adapted globally. Indeed, it is necessary to regularly evaluate feed quality as seasonal and local weather conditions during critical plant growing stages result in specific mycotoxin occurrence and concentration. In order to increase the control pressure while monitoring analytical cost, practical recommendations to use quantitative on-site testing equipments have been developed. Still in a process of a global risk management, MiXscience designed its own decision-making tool. Based on a large number of literature reviews but also thanks to its own research capacity and feed-backs from field experience of its long-term partners in feed manufacturing, a unique database has been built with critical contamination thresholds for major mycotoxins and their metabolites, for main animal species and all physiological stages. Thresholds for mycotoxin limit concentrations and their metabolites are those beyond which zootechnical and economic effects are likely, again within the framework of risk management. MiXscience has a strong capacity of research, owning its own private research centre dedicated to applied research in animal nutrition and feeding. This powerful experimental capacity, associated with expertise in sourcing and formulation, makes it possible to offer innovative and cost-effective solutions to the market, based on proprietary developments or co-operation with active ingredient suppliers. Altogether, best known practice in prevention and management of mycotoxin risk in the animal feed industry can be implemented worldwide.

NUSCIENCE
https://www.nusciencegroup.com

With more than 3,000 dedicated employees driven to excel every day, the Royal Agrifirm Group contributes to a responsible food chain for future generations. The Royal Agrifirm Group is a leading agricultural cooperative with an international network of subsidiaries in 15 countries within Europe, South America and Asia and a worldwide distribution network. By globally combining years of scientific research with local, specific know-how in the arable and livestock feed sector, the Royal Agrifirm Group offers customers worldwide the best solutions for the challenges they face every day. Nuscience, as member of the Royal Agrifirm Group, offers a wide range of innovative and effective animal feeds, customised premixes, high-performance concentrates, mineral feeds, functional feed ingredients and professional advice to the animal feed industry in over 80 countries. Ongoing research, development and testing programs are at the heart of our unique solutions. We are widely acknowledged as the industry's benchmark for increasing yields and reducing disease naturally. Enhancing animal performance in a profitable and responsible way is the main objective in modern animal husbandry. To help customers to achieve this goal, Nuscience's dedicated Health4U team has developed a broad range of innovative additives based on natural ingredients. Whether the focus is on promoting intestinal health or performance, reducing antibiotic use, protecting the animal against a specific pathogen or helping it to cope with stress, Nuscience offers a highly effective solution to ensure healthy, well-performing animals. To help the feed industry facing the increasing worldwide threat of mycotoxins, Nuscience has developed a range of highly effective mycotoxin deactivating products for feed application: Vitafix® Select, Vitafix® Plus and Vitafix® Ultra. Each product offers a powerful protection against mycotoxins supporting high levels of animal health and performance. Vitafix® Select is highly effective in binding aflatoxin. Specially selected clays in Vitafix® Plus and Ultra ensure optimal binding of both polar and nonpolar mycotoxins. Besides adsorption, toxicity of the mycotoxin deoxynivalenol is eliminated by yeast cell fractions in Vitafix® Plus and Ultra. For ultimate protection, Vitafix® Ultra reduces the negative effects of mycotoxins on animal level. Components supporting optimal liver functioning enhance endogenous detoxification while natural antioxidants

eliminate oxidative stress induced by mycotoxins. Overall, the Vitafix® range offers a cost-effective solution against mycotoxins contributing to optimal technical and financial results. For more information, go to the website (https://www.agrifirm.com/our-solutions2/vitafix) or contact our product manager Health4U, Kevin Vanneste (kevin.vanneste@nusciencegroup.com).

NUTRIAD
https://nutriad.com

'Nutriad, the global player in mycotoxin management'. Increasing knowledge of the effects of mycotoxins on animal performance and health leads to a growing need of producers to understand how this risk can be managed. Reaching customers in more than 80 countries, Nutriad has seen an increasing market penetration across all regions in the last two years stemming from continuous focus on and investments in its mycotoxin management product range. Today, Nutriad's mycotoxin deactivators are produced in Belgium and sold worldwide through a network of sales offices and distributors, supported by 4 application laboratories and 5 manufacturing facilities located on 3 continents. Key markets for Nutriad mycotoxin management range are North America, Brazil, Spain, South East Asia and China. In addition to improving its product offering, Nutriad believes it has gained a competitive edge in terms of mycotoxin screening and more insight into how to best dose mycotoxin deactivators based on known challenges, while sharing its expertise with customers. In Nutriad, we reviewed the quality of all our raw materials and decided to invest significantly in assuring only the best quality products are used in our formulations. We also worked on several trials and experiments involving dairy cows, poultry and swine where the effect of our mycotoxin deactivators against different mycotoxins was studied. The mycotoxin surveys that we executed in several countries helped our customers to evaluate the mycotoxin risks for the coming year and timely adjust their strategies. We noticed that especially in challenging circumstances our customers gained even more trust in our products, since consistently showed excellent performance at high mycotoxin levels where many alternatives failed to do so. On 12th of December 2017, Nutriad was acquired by Bluestar Adisseo Co. and by this transaction Adisseo became one of the worldwide leaders of specialty additives in animal nutrition.

OLMIX
https://www.olmix.com

'Olmix, the specialist in mycotoxin risk feed management'. A desire to provide natural alternatives to agricultural additives led to the creation of the Olmix Group in Bréhan, at the heart of Brittany, in 1995. In more than 20 years, the company has become one of the major global specialists in marine biotechnology and green chemistry. Its mission is to make effective use of an abundant untapped resource to promote sustainable-food. This approach guides the company's teams worldwide in their work of extracting value from green, red and brown algae. Innovation represents 6% of Olmix Group revenues and 12% of employees, with an R&D division comprising health and nutrition experts, along with specialists in algae, clay minerals and trace elements. In 2004, Olmix made a technological breakthrough by developing an unprecedented biomaterial. This patented hybrid material is a unique organo-clay resulting from the association of algal polysaccharides and clay. The modification of the original clay structure significantly increases its natural adsorption properties, and has enabled new applications, including binding toxins. The efficacy of Olmix technology has been demonstrated many times from experimental units to on-farm conditions. Many scientific and technical trials have been run worldwide, and Olmix is constantly looking for new opportunities to prove the efficacy of its products under diverse conditions. Olmix has also developed a deep expertise in mycotoxin risk and management. Olmix first developed the Myco'Evaluator in order to run quick mycotoxin risk audit on a farm scale. The result obtained with this tool can be confirmed with the Myco'Screen package, a tool to help customers adapting their mycotoxin analysis strategy for feed or raw materials. Lastly, Olmix launched its Myco'Calculator to take profit of mycotoxin analysis to adjust the toxin binder dosage depending on analysis results and animal's conditions. Today, the unique and efficient technology of Olmix based on algae and the deep expertise on mycotoxin risk management, make Olmix a leading company in the feed industry.

VICAM
http://vicam.com

VICAM, A Waters Business, is dedicated to developing USDA and AOAC-approved diagnostics for the detection of mycotoxins. Trusted in over 100 countries and supported by world class customer service, VICAM products are designed for real world testing environments. **Visit our workshop on Tuesday 13 March 2018 at 12:45.**

XEMA
http://xema-medica.com

'Development of immunoassay panel for detection of fungal antigens as screening tools for risk assessment of mycotoxin production in cereal material and soil extracts'. A panel of immunoassays for the antigens (Ag) of *Aspergillus fumigatus/flavus*, *Aspergillus repens* and *Aspergillus niger* species groups, *Mucorales* family, *Phoma*, *Alternaria*, *Fusarium* and *Claviceps* genus was developed with polyclonal antisera and affinity purified polyclonal antibodies. The Ag assays require very short (15-20 min) water-based buffer extraction, with the assay time compared with those of mycotoxin ELISAs (1-1.5 h). In the first experiment set with freshly obtained cereal material, we have obtained 12 samples showing elevated concentrations of one from 6 mycotoxins (aflatoxin B1, T2-toxin, zearalenone, ochratoxin, deoxynivalenol and fumonisin) confirmed by ELISA and HPLC; all 12 has shown positive in at least one of Ag ELISAs (100% concordance). In a larger experimental set of 122 random cereal samples from long term (>5 years) of storage, the concordance of positive antigen and mycotoxin detection (relative sensitivity of Ag assays) reached 88%; however, the current versions of Ag assays surprisingly showed only 30% sensitivity for the samples with 'high mycotoxin' results. We conclude that the fungal Ag assays can be used as potential fast and affordable screening method for mycotoxins in freshly collected cereals, and the sensitivity of these assays should be upgraded for long term storage material. This investigation was financially supported by the Russian Ministry of Education and Science (contract 14.604.21.0198 from September 26, 2017; unique identifier of applied research: RFMEFI60417X0198).

IMPEXTRACO
https://www.impextraco.com

Impextraco develops and produces feed ingredients that protect animal health and enhance productivity. Our goal is to deliver cost efficient solutions that protect animals and optimise their performance while respecting animal welfare and sustainability. As the animal production industry is consistently evolving, every stakeholder is facing new challenges. We strive every day to satisfy these new demands from both our customers and the authorities. That's why we created a range of premium ingredients both for protection and performance of the animals. Our products stimulate the healthy growth of the animal in an effective and sustainable way. Our ingredients are divided in two ranges. All our own products for the protection of the animal and their feed, are made with the highest quality antioxidants, mould inhibitors, mycotoxin eliminators, salmonella inhibitors, acidifiers, enzymes and prebiotics. In our performance ingredients range, we use vitamins, micro minerals, amino acids, antioxidants, growth enhancers, anticoccidials, colouring agents, enzymes and organic acid and their salts from the highest quality. To make sure you get the best feed ingredients possible, we strongly emphasise on R&D and quality control. Through advanced production facilities and experimental units, we deliver cost-effective, efficient and sustainable solutions. Alongside partnerships with universities, Impextraco runs its own research facilities and has validated animal models. One of most recent techniques, such as the use of biomarkers, are used in all our *in vivo* trials. These accurate strategies led to a unique combination of effective ingredients compiled in Elitox®, a reliable insurance policy against mycotoxins with proven efficacy in the field. Biomarkers give the opportunity to detect changes in biological parameters in a very early stage after mycotoxin contamination, including immune responses. The efficacy of Elitox® is extensively tested *in vivo* to prove its immunomodulating properties, helping the animal to overcome mycotoxicosis and hence preventing secondary infections from being manifested. The whole of our production, research, warehousing and distribution facilities comply with the highest EU and international quality control standards, such as GMP. They meet the most rigorous quality checks,

physical inspections, manufacturing best practices and comprehensive traceability requirements. What and where ever your needs are, you can safely rely on Impextraco's full commitment to your success.

MERCK
https://www.merckgroup.com

Merck is both a pharma & chemical company with 350 years of experience. Innovation is an important part of the company; 6,200 Merck R&D scientists spend €2 billion each year developing novel products and new analytical methods so that whatever your application, expertise is available to support you. Recent product developments include: aqueous compatible solid phase microextraction (SPME) for direct extraction from food substances; novel zirconia/carbon/silica based SPE tubes for effective extraction of chlorophyll and other coloured substances prior to analysis at greater sensitivity; development of flavour, fragrance and allergen reference materials; and innovative, ionic liquid GC stationary phase for the chromatographic measurement of water content. From our application laboratories, a recent multi-mycotoxin LC-MS/MS method development study investigated the selectivity for over 15 common mycotoxins on a variety of solid-core HPLC columns with different stationary phase chemistries and evaluated each for optimum resolution and selectivity. To avoid interfering matrix effects, a complementary extraction method was also developed. Through the combination of Merck, Millipore and Sigma, over 300,000 products in chromatography, reagents and reference materials are available. Access to these products, in addition to on-line articles, protocols and applications, is simple, with everything in one place on SigmaAldrich.com. One of the most advanced eCommerce platforms can be accessed from anywhere for direct on-line ordering; favourite products saved, quotes downloaded, real time stock and pricing quickly obtained, while automated warehouses deliver many products the next day. Excellent product quality, customer support, technical service all enable you to focus on your work with confidence.

TECNA
http://www.tecnalab.it

'Chorus food: automatic multi-parametric immunoassay system'. Tecna's mission is 'to create added value for the food and feed industry, enabling the rapid and cost-effective analysis of raw-materials'. The most immediate evolution of the current analytical approach to reduce assay time as well as operational costs is automation; yet this dimension of the analysis has not been fully exploited. The 'robots' that currently can be found on the market are too expensive, big and complex: a paradox. For this reason, since 2016, Tecna joint-ventured with DIESSE (Diagnostica Senese) to develop Chorus food, an 'automatic multi-parametric system with random access for single ready-to-use immunoassays'. Such a tool is already in use in clinical diagnostics, however, its application to food and feed screening would open completely new scenarios. The true innovation would be the novel format of the immunoassay, it being just a cartridge that would avoid all those annoying issues with current ELISA robots, making this instrument 100% walk-away. With a single cartridge no reagents manipulation would be required, no bottles would have to be placed and cross-contamination risk as well as disposables waste would be minimised. The plastic cartridges contain all the reagents to perform the analysis; the only manual operation will be sample preparation and pipetting it into the dedicated well. The cartridge will have to be inserted in the Chorus machine that will autonomously run the assay. Calibration of the system will rely on the already available 'B ZERO®' technology, based on a virtual master curve. The Chorus food will allow analysing any mycotoxin, just depending on the needs: one sample for aflatoxin B1, three samples for deoxynivalenol and maybe five samples for fumonins, in the same run. Nonetheless 10 samples might be analysed for zearalenone and other 10 for T-2/HT-2 toxin. Thanks to the software that manages the Chorus, the overall assay time will be dramatically lower than the sum of the single analysis and error probability will fall to minimum levels. In the meanwhile, the operator will be totally free to execute other activities in the lab.

MONDAY 12 MARCH 2018

PLENARY MEETING
SPEED PRESENTATIONS
Short presentations (6-minutes) by selected poster presenters to provide an overview of their research; the abstracts can be found in the section 'Abstracts of posters' (pages 71-163).

P11
Effects of fumonisin B1 and phytic acid on the oxidative stress: an *ex vivo* approach
Ana Paula Bracarense
Laboratory of Animal Pathology, Universidade Estadual de Londrina, Brazil

P34
Weather conditions associated with mycotoxin accumulation in Norwegian oats
Ingerd Skow Hofgaard
Norwegian Institute of Bioeconomy Research, Norway

P69
Individual and multitoxic effects of fumonisin B1, deoxynivalenol and zearalenone on rat liver and kidney phospholipids and lipid peroxidation
András Szabó[1,2]
[1]Institute of Diagnostic Imaging and Radiation Oncology and [2]MTA-KE 'Mycotoxins in the Food Chain' Research Group, Hungarian Academy of Sciences, Kaposvár University, Hungary

P86
Modulation of AFB1 cytotoxicity by natural antioxidants and PCB126 in a bovine mammary epithelial cell line
Shiva Ghadiri
Dipartimento di Scienze Veterinarie, Università degli Studi di Torino, Italy

P91
FDA Center for Veterinary Medicine: challenges and lesson learned in the regulation and approval of food additives to eliminate or reduce mycotoxins in animal food
Michael H. Henry
Center for Veterinary Medicine, Food and Drug Administration, USA

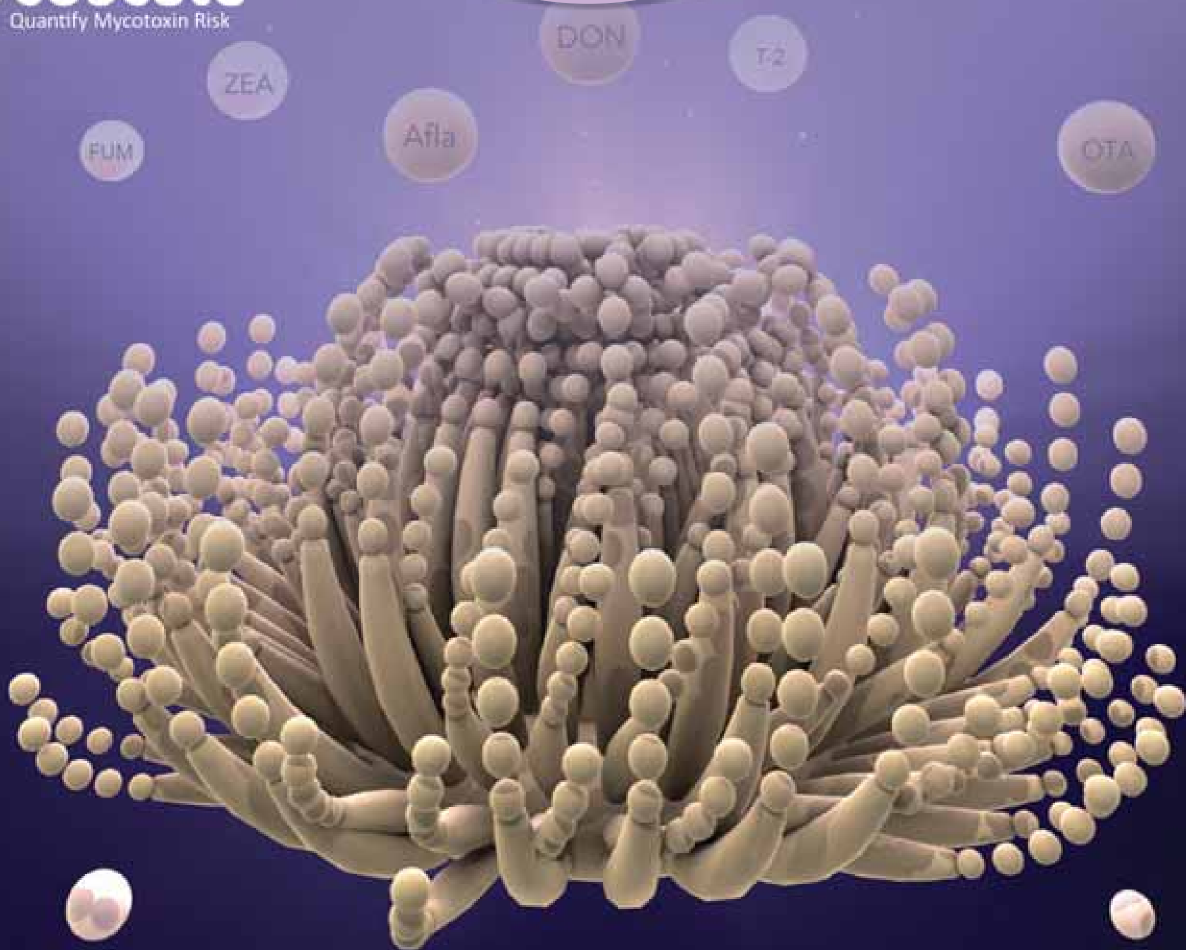
P117
Food safety risks from the conventional practice of reusing jute bags and recommendations to prevent aflatoxin cross contamination of maize
Cui Wang
Global Food Safety Center, Mars Incorporated, China

P130
A new approach employing ultra-high performance liquid chromatography coupled with orbitrap mass spectrometry for determination of *Alternaria* toxins and their conjugated forms in tomatoes and tomato-based products
Petra Jonatova
Department of Food Analysis and Nutrition, University of Chemistry and Technology Prague, Czech Republic

P136
Non-invasive cereal analysis by GC-MS detection of trichodiene as volatile mycotoxin biomarker
Maike Makowski
Bundesanstalt für Materialforschung und -prüfung, Germany

P142
European Union Reference Laboratory mycotoxins and plant toxins
Monique de Nijs
RIKILT Wageningen University & Research, the Netherlands

MYCOTOXIN RISK MANAGEMENT PROGRAM



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Avril GROUP

TUESDAY 13 MARCH 2018

SESSION 1
OCCURRENCE, EXPOSURE AND EFFECTS – WHAT'S UP?

Mycotoxins in livestock systems in developing countries

Johanna Lindahl[1,2,3] and D. Grace[1]
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Livestock feed can be classified as roughages (hay, silage, grass) and concentrates (grains, molasses, by-products such as oilcakes and bran). All these feeds can potentially be contaminated with mycotoxins, which have negative impact on the animal health and productivity. Many low and middle-income countries commonly have contaminated grains, and the lack of testing, regulations or enforcement of regulations means that the contaminated products reach both food and the feed market. Different livestock are more or less sensitive to different mycotoxins, but a common factor is that the impact most often has been assessed in experiments, or in field trials with high productivity breeds under industrialised contexts. In low and middle-income countries livestock is often not as productive as in high-income countries; the infection pressure is higher, animals are frequently kept in small-scale systems and feed is frequently suboptimal. Little is known as to the effects of mycotoxins under these conditions. In addition to the impacts on animal health and production, some mycotoxins, such as aflatoxins, are carried over into livestock products; aflatoxin in milk is a growing concern in many countries.

The International Livestock Research Institute (ILRI) has carried out research together with national partners in different countries in Asia and Africa on aflatoxins in livestock feed as well as in milk for human consumption. Results from the Kenyan dairy value chain show that aflatoxins are present in cattle feed and that up to 100% of samples collected may exceed the regulatory levels. Similarly, aflatoxin M1 was detected in many of the milk samples collected; as much as 100% of the milk samples aimed for children in low-income areas of Nairobi were positive and there was a positive association between the levels of aflatoxin M1 ingested and the degree of stunting in the children. High levels of aflatoxins in feed and milk has similarly been found in our studies in Pakistan, Ethiopia and Senegal, countries where milk production is very important for the food and nutrition security as well as the economy. In other countries, such as Vietnam and Uganda, pig production is increasing and important for both the national economy and the food supply. While more work is needed to assess which feed are most contaminated, the presence of aflatoxins in pig urine is evidence of contamination.

However, livestock may not only be a problem, but also part of the solution. Many livestock species are more resistant to the harmful effects than humans, and the use of anti-mycotoxin additives (AMA), such as clay binders, mean that animals could eat more highly contaminated feeds without risking their or human health. Our studies in Kenya indicate a high concern for aflatoxins, and a willingness to pay for safer products. In an on-going pilot study in Kenya small-holder peri-urban and urban farmers are trying AMA binders in their livestock to evaluate the effects and the feasibility of administration at household level. There is thus potential to use the livestock to improve the safety of human foods, but there is need for a better understanding of the potential impacts, and policy makers need to be made aware of the possible safe alternative uses of mycotoxin-contaminated crops.

U.S. FDA mycotoxin compliance sampling and surveys: challenges and opportunities

Anthony Adeuya
Division of Plant Products and Beverages, U.S. Food and Drug Administration, USA
anthony.adeuya@fda.hhs.gov

Mycotoxin contamination is a major threat to global food safety and food security. To protect consumers from health hazards associated with mycotoxins, many countries, including the United States, have established national maximum limits or recommended levels. The Codex Alimentarius (Codex) is a collection of internationally adopted food standards and related texts aimed at protecting consumer health and fair food trade practices. The Codex Commission works with member countries and organisations to establish maximum levels for mycotoxins and develop codes of practice for prevention and reduction of mycotoxins in foods.

This presentation will focus on how the U.S. Food and Drug Administration (FDA) regulates mycotoxins in food. Covered topics will include FDA statutory authority; the FDA Mycotoxins Compliance Program; recent developments in FDA mycotoxin analysis methods; and findings of the FDA Mycotoxins Compliance Program. In addition, FDA involvement in Codex activities will be briefly described.

Mycotoxin exposure patterns in infants and young children in sub-Saharan Africa

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Mycotoxins are toxic secondary metabolites of fungal origin that can contaminate a variety of foods and cause severe health effects when such foods are ingested. Infants and young children (IYC) are known to be highly vulnerable to the adverse effects from mycotoxin exposures. Their exposures to mycotoxins could result from mother's breast milk and/or complementary foods introduced during the weaning stage. In sub-Saharan Africa (SSA), there is sparse data on multiple mycotoxin exposure monitoring in biological fluids from children despite the evidences of frequent food contamination by diverse mycotoxins. This study therefore aimed to understand the patterns of multiple mycotoxin exposures in two IYC cohorts (exclusively breastfed infants (n=77) and children fed with complementary foods (n=42)) in Nigeria, with the view of providing useful data to promote interventions for this vulnerable population. An ultra-sensitive SIDA-based UPLC-MS/MS method for the quantification of 12 mycotoxin biomarkers and metabolites was applied to 119 first morning urine samples from the cohorts. At least one mycotoxin biomarker was detected in 96 and 100% of the urines from the breastfed infants and weaned children, respectively. Urinary biomarkers of aflatoxins (AFM1 and AFQ1) were more frequently detected than any other biomarker in breastfed infants (84%), whilst zearalenone was the most detected urinary biomarker in weaned children (83%). The urinary AFM1:AFQ1 mean concentration ratios were 1:105 and 1:10 in breastfed infants and weaned children, respectively. At least 95% of the urines from each cohort were co-contaminated with more than one mycotoxin, and the frequent combinations included AFQ1, zearelenone, deoxynivalenol, ochratoxin A, and dihydrocitrinone. This study has provided more insight into the extent of the mycotoxin problem challenging the IYC population of Africa. Partnerships aimed at driving priority interventions to mitigate mycotoxin exposures in this vulnerable group should be urgently set in motion for the safety of human lives.

Ergot alkaloids: recent toxicological data on a longstanding problem

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Over the last years, investigations in North America and Europe indicate an increase in the occurrence of ergot alkaloids contamination. These toxins are known for centuries for their effects on the circulatory and nervous systems in humans and animals. They are regulated in different countries, including Australia, Canada and Europe.

Few studies describe their effects in pigs, although this species is one of the best non-rodent model for human toxicology. In this study, three groups of 24 piglets were exposed to control feed or feed contaminated with 1.2 or 2.5 g of sclerotia/kg for 28 days. Despite the absence of major clinical signs, the two contaminated diets have significantly reduced growth performance (reduction daily weight gain up to 16%) and tended to increase the number of white blood cells and lymphocytes, which may affect the response immune. In the jejunum, exposure to contaminated feed induced a reduction in villi height, increased damage to the epithelium and decreased the number of mucus-producing cells. These alterations may compromise the physical barrier function of the intestine and its ability to absorb nutrients. In addition, they could explain the compensatory up-regulation of mRNA observed for claudin-3 and 4, occludin, JAM-A, E-cadherin, ZO-1, MUC1, ALP and PCNA. In the liver, exposure to contaminated feed led to alteration of the tissue, including development of inflammatory infiltrates, vacuolisation, apoptosis and necrosis of hepatocytes as well as presence of enlarged hepatocytes (megalocytes). Most of the genes involved in oxidative stress and lipid metabolism showed a tendency to down-regulation and SOD2 and SCD1 were significantly decreased in animals exposed to the higher dose of ergot. Overall, liver structure alteration and modulation of gene expression may disturb important functions such as detoxification and energy metabolism.

In conclusion, our results show that ingestion of ergot alkaloids, at doses close to regulatory limits, reduces growth and induces intestinal and hepatic lesions.

Mycotoxin cocktails in food and feed and their toxicological potential

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Food is contaminated by multiple contaminants, mycotoxins being the most frequently occurring natural ones. Mycotoxins co-contamination is confirmed on the one hand by the co-occurrence of these toxins in food and feed stuff and on the other hand by co-exposure monitoring survey. The co-occurrence of mycotoxins in food and feed is explained by three different reasons: (i) most fungi are able to simultaneously produce several mycotoxins, (ii) commodities can be contaminated by several fungi simultaneously or in quick succession, and (iii) the complete diet comprised different commodities. In practice, the co-occurrence of mycotoxins represents the rule and not the exception. Besides mycotoxins, food can be contaminated with other contaminants, such as heavy metals.

The toxicity of combinations of contaminants cannot always be predicted based upon their individual toxicities. The data on the combined toxic effects of mycotoxins are limited and, therefore, the health risk from exposure to a combination of mycotoxins is incomplete. Most of the studies concerning the toxicological effect of contaminants have been carried out taking into account only one compound. A synergistic effect between trichothecenes mycotoxins was observed both for intestinal cytotoxicity and inflammatory response and the synergy was already seen at low doses. The combined exposure to DON and cadmium was also studied in several human cell lines and interactions were specific to the target organ.

The importance of microbiota in intestinal health is gaining interest. With this aim, the interaction between DON and microbiota was investigated. We demonstrated that DON exacerbated the intestinal DNA damages induced by *Escherichia coli* stains producing colibactin raising questions about the synergism between food contaminants and gut microbiota. This demonstrated that mycotoxin cocktails can lead to synergistic interaction and that mycotoxin contamination should be taken in the global context of all food contaminants and the host intestinal microbiota.

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A review of mycotoxins toxicity to explore preventive solutions for animals

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Contamination of feedstuffs with mycotoxins is a persistent global problem negatively affecting animal health with a major impact on animal production. Mycotoxins have been shown to cause a broad variety of toxic effects in animals, exerting both acute and chronic toxic effects. Acute or subacute intoxication, following ingestion of high dose of toxins, are very pronounced but remain fortunately rare. Chronic exposure (repeated ingestion of low doses of toxins, representative of field situation) is of great concern because of the insidious nature and the persistence of mycotoxins. In addition, for many contaminated diets the challenge is from the possible co-occurrence of a high number of mycotoxins, in particular the interaction effects, could impact animal health at already low doses.

Here we present research data focusing on effects of mycotoxins and strategies on how to compensate the different negative effects of mycotoxin-contaminated diets in animal bodies. Mycotoxin ingestion may affect animal by reducing feed consumption, reducing nutrient utilisation, suppressing immune function, altering reproduction, irritating tissues, and causing cellular death. Biological effects include liver and kidney toxicity, central nervous system effects, and oestrogenic effects. Considering that mycotoxins interact initially with the intestinal epithelium and although mycotoxins have distinct actions, they all mediate intestinal damage through different mechanisms: (i) inhibition of protein synthesis (aflatoxins, deoxynivalenol); (ii) altered cytokines production (deoxynivalenol, fumonisins, zearalenone); and (iii) inhibition of ceramide synthase (fumonisins). Thereby, exposure to mycotoxins may induce gastro-intestinal inflammation, necrosis within the intestinal tract, affects mucosal immunity and disturbs the gut barrier function. Affecting intestinal health will increase the susceptibility of animal to intestinal (and systemic) infections and impair efficient digestion and absorption of nutrients with an associated effect on animal productivity.

Management strategies to reduce mycotoxins in animal feeds have been extensively studied and include prevention methods with pre- and post-harvest strategies to reduce mycotoxin contamination. However, practical difficulties to effectively prevent contamination using these techniques have led to the investigation on decontamination methods for feed materials. Once mycotoxins are ingested by animals, different strategies to counteract mycotoxins effects can be used: (i) decreasing the bioavailability by the inclusion of mycotoxin detoxifying agents in the feed; these detoxifiers can be divided into two different classes, namely mycotoxin binders and mycotoxin biotransforming agents; (ii) improving intestinal health and immunity, which will alleviate the negative impact of mycotoxins; and (iii) diet management.

Dietary intervention as a tool to manage mycotoxin-induced immune related disorders: facts or fiction?

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The interplay between the host and environment holds the key to health and well-being of humans and animals. The intestinal barrier serves as a first line of host defence against potentially harmful stressors from the environment ingested with food, including mycotoxins. The regulation of the intestinal barrier appears to be crucial for gut-immune homeostasis and its impairment induced by mycotoxins. For example, the trichothecene deoxynivalenol (DON), a fungal metabolite found in grain-based human diets, is one of the triggers resulting in an impairment of the intestinal tight junction network preceding an inflammatory response. Disintegration of the colonic epithelial barrier is also considered a key event in the initiation and progression of inflammatory bowel and coeliac disease and therefore exposure to the mycotoxin DON might contribute to the onset and propagation of inflammatory bowel disease. Furthermore, exposure to mycotoxins can lead to increased epithelial permeability to foodborne allergens, pathogens and toxins resulting in increased prevalence of many chronic inflammatory conditions and auto-immune diseases. Our group also showed that mycotoxins can facilitate allergic sensitisation to food proteins.

Dietary intervention with non-digestible oligosaccharides, such as galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS), is commonly used in infant formula to mimic the gut health promoting effects of human milk oligosaccharides (HMOs) via, among other things, stimulating the microbial composition. These oligosaccharides have been associated with a reduced risk of inflammatory diseases as well as allergic responses. Interestingly, GOS can also prevent the DON-induced disruption of the tight junction network and the corresponding epithelial inflammatory response *in vitro* as well as *in vivo* in experimental models. It could even be demonstrated that GOS are able to protect epithelial cells against the acetylated metabolites of DON, 3-acetyl-deoxynivalenol and 15-acetyl-deoxynivalenol. In a piglet model, dietary intervention with oligosaccharides prevents the DON-induced histomorphological alterations in the piglet small intestine. These beneficial effects of non-digestible oligosaccharides are most likely a result of a combination between effects on bacterial species, immunomodulatory effects on the immune system, inhibition of the attachment of other pathogenic bacteria and the enhancement of barrier integrity. Preventing the mycotoxin-induced intestinal hyperpermeability by dietary interventions will prevent paracellular transport of luminal antigens into the systemic circulation and thus the increased risk for various chronic (intestinal) inflammatory diseases.

Moreover, different mycotoxins can pass through the placenta during pregnancy and are present in human breastmilk, which can lead to pathophysiological effects in the offspring. Therefore, dietary interventions to prevent the adverse effects in the offspring induced by mycotoxin exposure during pregnancy warrant further investigation.

The results from these studies may lead to the identification of new indications for the utilization of protective effects of dietary interventions against fungal toxins and other environmental stressors that can jeopardise gut health.

Biomonitoring of deoxynivalenol in human volunteers: results of an intervention study

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For the first time, a human intervention study was conducted to unravel the urinary excretion profile and metabolism of deoxynivalenol (DON) and its modified form deoxynivalenol-3-glucoside (DON-3-glucoside). Ethical clearance for this intervention study was confirmed. Biokinetic models were developed to analyse the renal excretion of these compounds, including their phase II metabolites. These models were used to determine: (i) the preferred (set of) urinary biomarker(s); (ii) the preferred urinary collection period; and (iii) a method to estimate the dietary exposure to these mycotoxins.

Twenty volunteers (average age 32 years old, 55% women and 45% men) were restricted in consuming cereals and cereal-based foods for 4 days. At day 3, a single bolus of 1 µg/kg body weight of DON or DON-3-glucoside was orally administered to 16 volunteers; 4 volunteers served as control. All individual urine discharges were collected during 24 h after administration. The urine was analysed for DON, DON-3-glucoside, 3-acetyldeoxynivalenol (3-ADON), 15-acteyldeoxynivalenol (15-ADON), de-epoxy-deoxynivalenol (DOM-1), deoxynivalenol-3-glucuronide (DON-3-glucuronide) and deoxynivalenol-15-glucuronide (DON-15-glucuronide). A LC-MS/MS method for the simultaneous quantification of these compounds was used.

The urinary analysis of these compounds revealed that DON and DON-3-glucoside were rapidly absorbed, distributed, metabolised and excreted. In both challenges, DON-15-glucuronide was the most prominent urinary biomarker. Kinetic analysis revealed a complete recovery of total DON (mainly DON and its glucuronides) within 24 h after administration of DON or DON-3-glucoside. The so-called ‘reverse dosimetry’ factor was used to determine the preferred (set of) biomarker(s) and to calculate the intake of the parent compounds. The fact that DON-3-glucoside was absorbed and mainly excreted as DON and its glucuronides in humans confirms that DON-3-glucoside should be taken into account in the exposure and risk assessment of this group of mycotoxins.

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Health-related risks of certain modified mycotoxins in food and feed

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Modified mycotoxins are mycotoxins that are biotransformed in the fungus, infested plant or mammalian organism or are altered by food processing, i.e., non-enzymatic reactions, such as thermal or chemical treatment or by covalent binding to the food/feed matrix. The relevance of mycotoxin metabolites or ‘modified mycotoxins’ is an issue receiving increased attention over the last years. However, to date modified mycotoxins are not considered in EU legislation. The European Commission (EC) has tasked EFSA with an assessment on the presence of modified forms of certain mycotoxins in food and feed

which was delivered in 2014 and in which it was concluded that modified forms of *Fusarium* toxins might constitute risks both on their toxicity and their occurrence. Based on this conclusion, the EC asked EFSA if it would be appropriate to include modified forms of zearalenone (ZEN), T-2 and HT-2 toxin (T2/HT2), nivalenol (NIV) and fumonisins B (FBs) in group health based guidance values (HBGVs) with their parent mycotoxins.

In 2016, EFSA delivered a first opinion in which the tolerable daily intake (TDI) of 0.25 µg/kg bw for ZEN established earlier based on effects on the female reproductive system was confirmed. Modified ZEN can add up to an additional 100% of the concentration of ZEN in cereals. Modified forms identified were ZEN glucosides and sulfates and α-ZEL, β-ZEL, α-ZAN, α-ZAL, β-ZAL, cis-ZEN, cis-α-ZEL, cis-β-ZEL and their glucosides, sulfates and glucuronides. These are forms that are either not or not sufficiently characterised toxicologically. Since, *in vitro* studies showed that the modified forms of ZEN may act via the same mode of action as ZEN (oestrogenicity) leading likely to the same adverse outcome, results from in vivo uterotrophic assays were used to establish potency factors relative to ZEN (RPFs), that although varying widely, make it possible to include the different modified forms in a group TDI with ZEN. In the same year, an opinion on T2/HT2 was published in which the previously established TDI for the sum of T2/HT2 was revised to 0.02 µg/kg bw based on new data on haematotoxicity. In addition, an acute reference dose of 0.3 µg/kg bw was set based on emesis seen in different animal species. Modified T2/HT2 can add up to an additional 40% to the parent compounds in oats. A series of modified T2/HT2 have been identified (phase I metabolites formed through hydrolytic cleavage or phase II metabolites formed by conjugation with glucose, modified glucose, sulfate, feruloyl and acetyl groups). There are insufficient data available to derive HBGVs for these modified forms. However, *in vivo*/*in vitro* studies show that they act via a similar mode of action, protein synthesis inhibition, and that in consequence would also exert haematotoxicity and therefore can be included in the group TDI applying RPFs reflecting their toxic potencies compared to the parent compounds.

In 2017, the third opinion, on NIV and its modified forms, was published in which the previously established TDI of 1.2 µg/kg bw for NIV (based on immunotoxicity) was confirmed. Additionally, an ARfD of 14 µg/kg bw was set for NIV based on emetic effects seen in a new study. Only NIV-3-glucoside, which can occur in cereals adding up to an additional 50% to the parent compound, was identified as a relevant metabolite. There are no toxicity data for the compound but since it can be assumed that it is cleaved in the intestinal tract it can be included in a group TDI/ARfD. In those cases where a similar mode of action could be established for the different toxins and their modified forms, dose addition could be applied and inclusion in common HBGVs would be appropriate provided reliable data on relative potencies are available. Currently the last opinion within the frame of this mandate, on fumonisins B (FBs) and their modified forms, is being finalised.

EFSA's risk assessment of *Fusarium* mycotoxins: a challenging endeavour

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Contaminants have been evaluated by the European Food Safety Authority (EFSA) since its foundation in 2002 and the risk of mycotoxins from their occurrence of mycotoxins in food and feed was since then characterised in more than 20 opinions and statements by the EFSA Panel on Contaminants in the Food Chain (CONTAM) for humans and farm and companion animals. This provided risk managers of the European Commission and the EU member states with advice for their decision-making and the setting of maximum levels of mycotoxins. Among these mycotoxins are the 'big five' deoxynivalenol (DON), fumonisin, nivalenol (NIV), T2/HT2 and zearalenone (ZEN); three from the class of trichothecenes (T2/HT2 of type A and DON and NIV of type B) within the genus of *Fusariums*. Other *Fusarium* mycotoxins were systematically evaluated by CONTAM as well by the standing Working Group on Fusarium Mycotoxins since 2010. Before that, EFSA had assessed from 2003 on mostly mycotoxins in feed only.

An outcome of EFSA's enduring efforts for consumer and animal protection was the identification of highly varying types and degrees of severity of adverse health effects detected for the different mycotoxins, including the 'big five'. Whereas the ttrichothecenes were associated with chronic and fatal effects, oestrogenicity was identified as the critical effect of ZEN and fumonisin shows hepato- and

nephrotoxicity. This diversity has been a challenge for the risk assessment which follows according EFSA guidance an established structure ending in an EFSA Opinion, based on the established risk assessment paradigm that combines exposure and hazard assessment for the risk characterisation. Obviously, data gaps in any of these two pillars must cause problems. A certain amount of samples and analytical results above sensitive enough limits of detection (LOD) or limits of quantification (LOQ) are required to estimate concentration levels with sufficient precision for the food or feed categories set up by EFSA. Estimated mycotoxin concentrations, typically in cereal grains and grain-based products, are subsequently combined with intake information of EU wide dietary surveys to estimate human exposure. This also holds for feed, with the specialty that for mycotoxins units in mg/kg body weight and in mg/kg feed) might be relevant dose-metrics; as it happened recently for DON. Data gaps regarding the toxicity of a mycotoxin cause similar problems for the hazard characterisation. They are even of larger complexity and may strongly impact the quality of the risk assessment. The whole works ranging from *in vitro* and *in vivo* toxicokinetics to long-term animal studies, mode of action (MoA) and effects in combination with other mycotoxins is a fragile enterprise in presence of missing information and such an evaluation may stay incomplete (e.g., for moniliformin) or impossible, in particular, when the available data prevent dose-responses evaluation and modelling.

In addition to the more generic challenges of the risk characterisation of mycotoxins this presentation will address also other relevant issues for the evaluation of *Fusarium* mycotoxins. Most prominent has become the handling of co-occurrence of mycotoxins of the parent mycotoxin (e.g., DON or ZEN) with its modified forms. There, a manifold of questions may arise, e.g., on how to account for variations of the LODs/LOQs and how to improve and facilitate the monitoring the co-occurrence in presence of multi-toxin analytics. A second more recent and highly demanding challenge is the utilisation of biomonitoring data as support (or replacement?) of occurrence data. Last but not least, the use of group health based guidance values (e.g., group tolerable daily intake (TDI) values) needs discussion before the background of the combined effects of the parent mycotoxin and its modified forms. In case of *Fusarium*, also combined effects with other members of this genus and also other genera (e.g., aflatoxins of the *Aspergillus* genus) may be considered.

An introduction to APROBA-plus, a simple tool for risk assessment visualising the quantitative uncertainties in hazard characterisation and exposure assessment

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In current risk assessments, uncertainties are accounted for by using conservative or worst- case values. When these values are combined to obtain a final ‘risk number’ (such as the ratio of exposure to health-based guidance value, HBGV) the result will be overly conservative. On the other hand, the conservative values used in risk assessment are often based on assumptions or habits and might in reality not be as conservative as supposed. Nonetheless, when comparing the available human exposure value with the available HBGV these values are treated as very precise numbers rather than rough numbers with large uncertainties. This may lead to conclusions regarding health risks that are not warranted by the available information.

In 2014, IPCS published a general framework for evaluating the quantitative uncertainties in hazard characterisation, resulting in an uncertainty distribution of the target human dose (defined as the – unknown – dose that would result in a specified effect in a specified fraction of the human population). A lower percentile of that distribution may be used as a probabilistic HBGV, with the advantage of being much more precisely defined than the usual (deterministic) HBGV (RfD, ADI, TDI, etc), while the level of conservatism is now quantitatively specified. On the same website, IPCS published a tool that allows for a quick and approximate probabilistic hazard characterisation, called APROBA, both available as an Excel sheet and as a web application. Recently, RIVM extended APROBA by including the option to add exposure information, and the uncertainty associated with that. APROBA-plus results in a final graph that summarises the uncertainties in both the target human dose and the exposure. This final picture gives a transparent view of the overall uncertainties involved in any risk assessment. Risk management decisions will be improved by considering the overall uncertainties shown in this plot,

instead of focusing on one side of the uncertainty range, as in the current approach of comparing single (allegedly) conservative numbers.

An illustration of APROBA-plus using deoxynivalenol as an example compound

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In 2014, WHO/IPCS published a guidance document on evaluating uncertainties in hazard characterisation which has been updated recently. In the approach, the outcome of human health hazard characterisation is expressed as an interval or distribution rather than as a point estimate (e.g., RfD, ADI). In this way, potential uncertainties may be communicated more clearly, while at the same time risk management protection goals of the assessment, in particular the acceptable incidence (I) in the population and magnitude of effect (M), are made explicit in quantitative terms.

This presentation demonstrates the practical application of the approach with the mycotoxin, deoxynivalenol (DON), using an ‘approximate probabilistic analysis’ implemented in an Excel® spreadsheet tool developed by the WHO/IPCS expert group. It will be demonstrated that in this case example, a first tier analysis using NOAELs/LOAELs as the points of departure (PODs) does not produce satisfactory results in the sense that it cannot be stated with sufficient confidence that the protection goals are met. Therefore, a second tier analysis using benchmark dose (BMD) modelling is performed, and it is found that this reduces the overall uncertainties, such that it might now be stated that the protection goals are met with sufficient confidence. Potential risk management implications of the results are discussed, along with options for further refinement.

SESSION 2
HOLISTIC ANALYTICAL APPROACHES AND REDUCTION STRATEGIES

Untargeted metabolomics strategy: a new predictable tool for early detection of mycotoxins

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Food chain is step-by-step longer and more complex than ever before, and is affected by globalised trade, culture, travel and migration, changing consumer trends and habits, new technologies, emergencies, climate change and extreme weather events which are increasing foodborne health risks, especially for mycotoxins. So far, mycotoxins and their modified forms have been mainly monitored by targeted methods, such as LC-MS, in cereal and cereal-based products, however, may an early detection of mycotoxins be considered a reliable strategy?

In order to explore the early detection of mycotoxins in wheat, three standardised and targeted approaches (*Fusarium* disease severity, PCR assays for *Fusarium* spp. Identification, and mycotoxin quantification) and a novel untargeted metabolomics strategy were jointly assessed. In the first phase of this research, standardised approaches were able to quantify mycotoxins and identify *Fusarium* spp. In the second phase, an UHPLC-QTOF metabolic fingerprinting method was developed to investigate plant-pathogen cross-talk. The untargeted metabolomics method had to simultaneously detect as many metabolites as possible in wheat, in order to understand the metabolic pathways. First, the chromatographic run provided separation efficiency and good peak resolution. Consecutively, several extraction procedures were evaluated in-depth in order to extract the bulk of the information. The optimised method was able to detect hundreds of variables (m/z, RT, intensity), at this point, these variables had to be converted into more manageable information. Data processing and data pre-treatment were carried out in order to permit the identification of significant metabolites, which capture the bulk of variation between low and strong infection levels, therefore potential biomarkers.

Combining these results, the plant-pathogen cross-talk related to the early detection of mycotoxins was revealed. The outcome of our study strongly supports the key role played by lipid signalling compounds in the complex regulatory network and described the interconnection of metabolic pathways taking place in the *Fusarium* infected wheat, and how the *Fusarium* infections influence mycotoxin and other metabolites formation. To sum up, as a rapid response to fungal infection an overexpression of phosphatidic acids was discovered. By contrast, when the infection became stronger an increase of oxylipins and diacylglycerols was revealed.

What can we learn from lipidomics experiments using mycotoxins in 3D human liver microtissues?

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The study of the toxic effects of mycotoxins cannot disregard the study of the biotransformations they undergo in the organisms with which they come into contact. Indeed, there are many species that release secondary metabolites that possess chemical structure and toxicological activity different from the mycotoxin from which they originate. Therefore, to thoroughly evaluate the potential toxic effects

and mechanisms induced by a mycotoxin or a mixture of mycotoxins, to date an in-depth study of laboratory animals has been necessary. However, these studies are strongly questioned both for ethical and scientific reasons. In fact, human metabolism, kinetics and biotransformations are often not even comparable to those of animals. For these reasons, it is necessary a less invasive, ethically correct investigation procedure, to get results that are totally transportable to the in vivo situation, but also scientifically robust and appropriate to evaluate the toxic endpoints of isolated or co-presence mycotoxins.

The presentation reports a new technology, called lipidomic fingerprints, able to evaluate and quantify xenobiotic induced liver Injury for any set of xenobiotic compounds alone or in a mixture. More precisely, the 3D tissue culture of primary human hepatocytes co-cultured with Kupffer cells are used to test endpoint effect of different mycotoxins. These cells performance is stable up to 14 days in term of basal metabolism and xenobiotic metabolic capability, and they can be xenobiotic-treated for long time in sub-chronic conditions. Kupffer cells strongly regulate the hepatocyte functions, therefore, their presence in the culture give a model much more similar to the *in vivo* system. This approach represent a step forward when compared to more traditional cell-based assays, since currently used cell systems (HepG2 or HEPARG) express only partially the xenobiotic metabolised enzyme systems. This means that the effects of xenobiotics on lipid metabolism, on these cell cultures, lack the information related to the role of hepatic metabolite(s).

Since it is shown that any chemically-induced modification of a cellular function is reflected in the perturbation of the lipid profile, exposition to mycotoxin(s) is expected to also induce perturbations of the lipid profile. Therefore, we will show how the lipid profile represents a useful fingerprint to evaluate the adverse effects or potential biomarkers in human hepatocyte model to exploit negative effects and propose potential mechanism of actions.

Metabolomics approaches to identify other *Fusarium* head blight relevant secondary metabolites of plants and fungi

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Untargeted metabolomics approaches aim at probing the entire metabolic space, including substances which are currently unknown. In contrast to most state-of-the art methods, which are limited to the differential comparison between different experimental conditions, the combination of untargeted profiling with stable isotope-assisted techniques does not only offer the possibility to characterise the global metabolic composition of individual biological samples but also enables to disentangle the molecular processes underlying fungal attack and defence responses in plant-pathogen interactions.
In this study, we have successfully used a recently developed liquid chromatography-high resolution mass spectrometry (LC-HRMS) based approach which is suited to distinguish fungal metabolites from those produced by the affected plants. A time course experiment (3, 6, 12, 24, 36, 48, 72 and 96 h after infection (hai)) has been carried out in the greenhouse with the aim to investigate the attack of *Fusarium graminearum* (Fg) against two near isogenic wheat lines differing in the resistance QTLs Fhb1 and Qfhs.ifa-5A. About 100 metabolites were found to be explicitly deriving from Fg (~50 of those were common between both wheat lines). For another approximately 250 metabolites, all of which were found to be induced by Fg infection, we were able to classify as being either produced by Fg to support infection, the plant in defence or by both interaction partners. Ca. 50% of the fungal metabolites were annotated or identified including those belonging to the type B-trichothecene pathway.

Interestingly, at an early stage of infection (about 24-48 hai) some of these fungal compounds were found to be more abundant in the resistant compared to the susceptible wheat line. Our data therefore suggest that in the resistant cultivar, Fg is either ‘forced’ to produce more and higher amounts of secondary metabolites or alternatively, higher secondary metabolite formation by Fg in the resistant

wheat genotype may help the plant to induce a quicker/stronger response against the pathogen including the detoxification of mycotoxins via glycosylation. Towards the end of the monitored time window (96 hai) we observed that Fg is not only able to produce higher amounts of well-known toxins, but also to produce a much higher number of metabolites in the infected plants, as was particularly observed for the susceptible wheat line.

Metagenomics to identify novel toxin deactivators from the soil microbiome

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The discovery of mycotoxin deactivating enzymes through the application of metagenome-based enzyme discovery processes offers significant promise. The removal of mycotoxins from the food chain is a critical requirement as we seek global solutions to the present crisis in food security – for both direct human consumption and for animal feed.

Metagenome-based discovery processes are rooted in microbial ecology studies on enzymes with specific functionalities, allowing these functional genes to be identified, assembled and expressed in appropriate expression systems. This means that the potential is there to identify new enzymes with specific catalytic properties from such data sets. These may have very diverse application. Traditionally, such approaches have been successfully employed to facilitate the discovery of new enzyme biocatalysts for use in the pharmaceutical and fine chemical industries – that can then be used to access high value APIs. However, there is no reason why metagenome-based discovery pipelines cannot be also employed to facilitate the discovery of new mycotoxin-deactivating enzymes with commercial application in the food industries, In this context, we propose that the limiting factors are: (i) we must be able to identify and express enzymes with appropriate substrate specificities; and (ii) we must also be able to ensure appropriate substrate regio-specificity to ensure the target mycotoxins are effectively detoxified.

As an example, in this presentation we focus on the search for aflatoxin-deactivating enzymes from soil. New dehydrogenase enzyme sequences are identified, and proposals are given that allow us to specify substrate residues for hydroxylation in these highly functionalised molecules.

Aspergillus flavus polyamines: small molecules with large biological implications

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Aspergillus flavus is a soilborne saprophyte and an opportunistic pathogen of both humans and plants. This fungus not only causes disease in several important food and feed crops such as maize, peanut, cottonseed and tree nuts but also produces the toxic and carcinogenic secondary metabolites (SMs) known as aflatoxins. Polyamines (PAs) are ubiquitous polycations that influence normal growth, development, and stress responses in living organisms and have been shown to play a significant role in fungal pathogenesis. Biosynthesis of spermidine (Spd) is critical for cell growth as it is required for hypusination-mediated activation of eukaryotic initiation (translational) factor 5A (eIF5A). The tri-amine Spd is synthesised from the di-amine putrescine (Put) through the action of spermidine synthase (Spds). To investigate the role of *spds* in *A. flavus* growth and toxin production, we disrupted the *spds* gene (knockout). Inactivation of *spds* significantly reduced mycelial growth and sporulation *in vitro* and

addition of exogenous Spd was required to restore fungal growth and sporulation. Complementation of the *Δspds* mutant with a wild type (WT) *A. flavus spds* gene restored the WT phenotype. In WT *A. flavus*, exogenous supply of Spd (*in vitro*) significantly increased the production of sclerotia and SMs. Infection of maize kernels with the *Δspds* mutant resulted in a significant reduction in fungal growth, sporulation, and aflatoxin production compared to controls. Quantitative PCR of *Δspds* mutant-infected seeds showed down-regulation of aflatoxin biosynthetic genes in the mutant as compared to WT *A. flavus* infected seeds. Expression analyses of PA metabolism/transport genes during *A. flavus*-maize interaction showed up-regulation of PA uptake transporters in the fungus and arginine decarboxylase (*Adc*) and S-adenosylmethionine decarboxylase (*Samdc*) genes in the maize host. The results presented here demonstrate that Spd biosynthesis is critical for normal development and pathogenesis of *A. flavus* and pre-treatment of a *Δspds* mutant with Spd and Spd acquisition from the host plant, are insufficient to restore WT levels of pathogenesis during seed infection. The data presented here suggest that future studies targeting spermidine biosynthesis in *A. flavus*, using RNA interference-based host-induced gene silencing approaches, may be an effective strategy to reduce aflatoxin contamination in maize and possibly in other susceptible crops.

Towards real-time detection of *Fusarium* spores to reduce mycotoxins in the food chain

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Fusarium head blight or scab is a disease complex of small grain cereals and maize. On wheat, the disease is often caused by *Fusarium graminearum*, which produces deoxynivalenol (DON), an important mycotoxin that contaminates grain before harvest. The pathogen is dispersed by airborne spores in late spring and early summer. The occurrence of these spores can be very sporadic or localised and can be difficult to predict by weather-based models. The prediction of significant infection by mycotoxin-producing fungi is also difficult because other grain-infecting fungi, such as *Microdochium nivale*, can reduce subsequent infection by *F. graminearum*.

The presence of airborne spores is key information to inform precise decision-making for optimal crop protection using fungicides, but this information was previously not available because samples from air samplers had to be transported to a lab for DNA extraction, purification and analysis by qPCR. Recently, novel air sampling devices integrated with suitable DNA-based diagnostic methods and wireless reporting have been developed that can now provide a near real-time warning of inoculum presence to improve disease control. These methods are now possible due to the development of DNA-based diagnostic methods, including TwistDX and LAMP assays that are semi-quantitative but operate at a single temperature, which is feasible to do with field-portable equipment. As an alternative, suitable in-field diagnostic assays, can be applied manually to an air sample taken by established devices, such as the rotating-arm sampler, Burkard multivial cyclone or Burkard seven-day spore trap. In 2017, despite suitable infection conditions at the start of the wheat flowering period, the absence of *F. graminearum* and *F. culmorum spores*, coupled with presence of moderate levels of *M. nivale* spores and lack of infection conditions later in the flowering period, gave confidence that the harvested grain locally would have low concentrations of DON, which proved to be the case. Further work is on-going to expand the inoculum-based forecasting system for *Fusarium*-derived DON in wheat. Other research is monitoring airborne spores to provide an unbiased sample of a pathogen population to assess changes in genetic traits, such as fungicide resistance or the race structure and to understand what concentration of spores corresponds to a disease risk. These thresholds are likely to vary according to the sampler height above ground and should be integrated with weather-based infection models to provide accurate evidence-based spray advice.

TUESDAY 13 MARCH 2018

SESSION 3
MANAGING THE IMPACT OF MYCOTOXINS – PART 1

Fusarium community structure revealed by metabarcoding – implications for mycotoxin contamination

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We have studied the effect of different environmental and agricultural factors on *Fusarium* abundance and community composition on wheat kernels in Sweden. A protocol using 454 sequencing of EF-1α amplicons was developed. The procedure was found to preserve quantitative relationships between species when evaluated using mock *Fusarium* communities.

Winter wheat kernels were collected from 18 organically and conventionally cultivated fields in Sweden, and paired based on their geographic distance and the wheat variety grown. The newly developed protocol for 454 sequencing was used in combination with real-time PCR in order to reveal differences in community composition as well as *Fusarium* abundance among fields. In total, we identified 12 *Fusarium* operational taxonomic units (OTUs) approximating species level, with a median of 4.5 OTUs per field. Some species were split up into several OTUs, for example *F. avenaceum* where reads were distributed between four different OTUs. *F. graminearum* was the most abundant species in the wheat samples, while *F. avenaceum* had the highest occurrence. The total abundance of *Fusarium* ranged two orders of magnitude among fields. We could not detect any difference in *Fusarium* communities between the organic and conventional systems. However, agricultural intensity, measured as the number of pesticide applications and the amount of nitrogen fertilizer applied, had an impact on *Fusarium* communities and specifically *F. tricinctum*. There were also geographic differences in *Fusarium* community composition: *F. graminearum* was more abundant in the south-western part of Sweden. The application of amplicon sequencing provided a comprehensive view of the *Fusarium* community in cereals. This gives us better opportunities to understand the ecology of *Fusarium* spp., which is important in order to limit *Fusarium* head blight and mycotoxin contamination in cereals.

Biocontrol of aflatoxins: the pros and cons of competitive exclusion

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Biocontrol of aflatoxin formation by competitive exclusion is considered to be one of the most effective methods for reducing aflatoxin formation. It is widely used on maize and peanut crops in several African countries and on cotton crops in the United States. Current methodology is based on the addition of a set of carefully selected non-toxigenic strains of *Aspergillus flavus* to a carrier substrate which is then broadcast onto fields of the target crop. Moist soil then causes hydration of the carrier, usually rice or sorghum seed, permitting growth and sporulation of the fungus. The high numbers of non-toxigenic spores produced compete with the existing toxigenic spores of *A. flavus* for entry sites on the developing seed or kernel, thus reducing aflatoxin formation. The theory is clear: high enough numbers of non-toxigenic spores will simply outcompete the soilborne toxigenic spores and reduce aflatoxin formation. In practice, several factors limit the effectiveness of these processes, or render assessment of their efficacy problematic. Climatic factors are important. Heavy rain after application may result in washing away the carrier before the fungus has grown, while dry soil at the time of application will reduce or prevent fungal growth. Ungerminated *A. flavus* spores are sensitive to prolonged heat, so hot, dry

conditions after application will result in spore death. Subsequent rain may then promote the growth of the soilborne toxigenic spores, with the potential for increasing rather than decreasing aflatoxin levels in the crop. Production and application of biocontrol material will always come at a cost. Biocontrol is commonly aimed at subsistence farmers who will not take up the process without some price incentive, such as an increased price for crops with low aflatoxin. If no price incentive exists, government or granting agencies will have to provide an incentive. In many countries, little data exists on the efficacy of a biocontrol application beyond one season, so that incentive must be on an ongoing, annual, basis. A quite separate issue is whether application of a biocontrol agent is warranted. In areas with generally adequate rainfall, biocontrol makes no economic sense, yet for many countries in which biocontrol has been applied, little information exists on which are the high-risk areas, even in countries where aflatoxin is a known problem. Covering a country in biocontrol material without knowledge of risk areas is a waste of resources. A separate issue again relates to proof of the efficacy of the process. The distribution of aflatoxins in individual grains or kernels is known to vary very widely, so that adequacy of sampling plans and quality control of assay techniques are essential for verification of the reduction of aflatoxin levels by techniques such as biocontrol.

The role of hermetic storage for post-harvest mycotoxin control

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A major challenge for subsistence farmers is the storage of their harvested crops. Due to their small production and limited financial resources, the use of improved drying and storage technologies is limited. Grain, such as maize and legumes, are commonly sun-dried and stored in 50 and 100 kg woven polypropylene bags. Without the application of protectant insecticides, farmers can experience losses as much as 80% within a few months of storage. Due to security issues, many farmers store their grain-filled bags inside their home, which raises real and perceived concerns about the health impacts of these insecticides. Over the past decade, airtight plastic hermetic bags have proven to be an effective technology for grain storage. These bags create a different storage environment than traditional woven bags. The plastic material, which is impermeable to gases and moisture, allows respiratory activity within bags to increase carbon dioxide concentrations and reduce oxygen concentrations. The plastic also protects the grain from external fluctuations in humidity, which prevents rewetting or shrinkage. Multiple studies have demonstrated that hermetic bags reduce insect activity and the resultant losses, eliminating the need for insecticide application. As a result, the awareness of the bag technology has grown as well as the supply chains to farmers. The storage of maize and peanuts in hermetic bags has given rise to questions about aflatoxin accumulation during storage. Many studies have established the effects of moisture, oxygen and carbon dioxide levels on the growth of *Aspergillus flavus* and aflatoxin production, which suggest that the fungus and mycotoxin should not be a concern within the hermetic storage bags. My talk will describe efforts to obtain experimental field evidence that confirm these basic biological assumptions. What is revealed are important challenges in the handling of grain between harvest and storage.

Effect of composting, combustion, and anaerobic digestion of *Fusarium*-damaged grain and screenings on elimination of mycotoxins

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The quantity of *Fusarium*-damaged grain on the Canadian prairies has grown substantially over the past few years and is projected to continue to rise. Due to the spread of *Fusarium*, producers are experiencing grade losses, restricted marketing opportunities, added costs, and lost income. Losses in Canada are

estimated to range from $50 million to $300 million annually over the past two decades while future losses are projected to be between $75 and $325 per hectare depending on the crop and area. Some of these losses can be recovered if the grain is cleaned or disposed of in a way that generates some revenue. In cases where the grain cannot be cleaned, or the mycotoxin content is too high to be considered a blend for feed, the material needs to be disposed of. An estimated 2.7 million tonnes of grain and screenings requires disposal each year in Saskatchewan alone. The purpose of this project was to better understand how various disposal options, such as composting, combustion, and anaerobic digestion, can help agricultural producers extract value from their *Fusarium*-damaged grain and screenings and dispose of them in a way that minimises the risk of spreading the fungus. The results indicated that the composting process eliminated the mycotoxin of interest (deoxynivalenol, DON) since all composted samples tested did not contain quantifiable concentrations of DON. Anaerobic digestion and combustion both reduced the concentration of DON in the substrate but did not eliminate it. It is hypothesised that a combination of the temperatures and microbial activity that occurred throughout the composting period degraded the DON. Although DON is produced by *Fusarium graminearum*, the lack of DON in composted samples does not mean that *F. graminearum* was also eliminated. Future work will directly assess the level of *F. graminearum* in the samples.

A basic economic analysis of the composting process indicated that if the costs are in the low- to mid-range of expected composting costs and the value of the resulting product is in the mid- to high-range of the expected values, producers may be able to recover a net positive return ranging from $30 to $180 per tonne (which is equivalent to $0.81 to $4.90 per bu) of screenings or heavily damaged grain. In addition to providing some return to the producer, disposal options other than dumping or landfilling may minimise the risk of further contaminating the soil.

A historical review of technologies focused on preventing and removing mycotoxin contamination in the supply chain

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The food and feed supply chain represents one of the significant risks for mould and mycotoxin contamination. The wide variety of conditions related to climate, agricultural practices (crop rotations, irrigation, pesticide use), post-harvest practices (drying and conditioning, storage practices, and transportation means to the production facilities), which can differ specifically by crops, and regions, across the globe, and even more, cultural differences across the global supply chains' geographical regions add to the complexity of managing this risk. The regulatory requirements also have evolved over time, attempting to achieve a compromise between the need for a safe supply chain, and the economic consequences of the target action levels selected; and even though harmonisation of standards is more and more prevalent, still supply chains, countries, industry segments, customers, differ on their approach to actionable levels.

The technologies that have been developed in response to this challenges since the 1960s when the first major effects of mycotoxin contamination upon human health were public news, and the lack of a single effective solution, continues to create opportunities for innovation in this area, considering the potential impact on both human and animal health. Historically, a variety of solutions to deal with this risk in different parts of the supply chain, have been developed and implemented with different degree of success. Many of these solutions are specific to the target users and include regional differences. These solutions are developed to minimise (vs. eliminate) the risk of mycotoxin contamination in the supply chain. It is the intent of this presentation to highlight some of the technologies that historically have had a major impact (technological and economic point of view) on the progress in addressing the mycotoxin risk in the supply chains for both human and animal consumption.

Testing Solutions
AflaTest
AflaTest WB
AflaB
AflaCheck
Afla M1 FL+
Afla M1 HPLC

CitriTest HPLC

DONtest HPLC
DONtest WB
DONCHECK

FumoniTest
FumoniTest WB

OchraTest
OchraTest WB

T-2test HPLC

ZearalaTest
ZearalaTest WB

Multi-Analyte Testing Solution
AflaOchra HPLC
AOZ HPLC
DON-NIV WB
Myco6in1+
T-2/HT-2 HPLC

Rapid Testing
Vertu Lateral Flow Reader
Afla-V / Afla-V AQUA
Afla M1-V
DON-V
Fumo-V / Fumo-V AQUA
Ochra-V / Ochra-V AQUA

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TUESDAY 13 MARCH 2018

SESSION 4
SAMPLING AND ANALYSIS – ONGOING DEVELOPMENTS

Sampling of dried figs for mycotoxin: variability associated with sampling, sample preparation and analysis

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Based upon 2014/2015 crop production estimates, dried figs are produced mainly by Turkey (56% market share), Iran (18%), USA (8%), and Greece (6%) for domestic and export trade. As the world's leading producer of figs, Turkey exports approximately 90% of their dried fig production. European Union member states are the major importers of dried figs from Turkey and account for about 75% of the market share. Like many other commodities, dried figs are susceptible to the growth of aflatoxin producing moulds. Because aflatoxins are toxic and carcinogenic compounds, regulatory limits for aflatoxins and other mycotoxins have been established for food and feed products in about 100 countries. Aflatoxin limits vary widely from country to country. The current situation where aflatoxin limits vary from country to country makes international trade difficult. Exporters must be aware of each importer's aflatoxin limits and official sampling plan designs when they sample dried figs at origin. Because of the high cost of having lots rejected at destination, exporters try to determine if the product meets the importing country's aflatoxin regulations before shipping to the importer. With so much variation among regulatory limits worldwide, the Codex Committee on Contaminants in Foods (CCCF) began efforts in 2006 to harmonise aflatoxin limits and sampling plans for dried figs. The method used to evaluate the performance of mycotoxin sampling plans was used as part of the CCCF effort to harmonise aflatoxin MLs and sampling plans for various products such as peanuts [CCCF 2001; Whitaker *et al.*, 1974] and tree-nuts [CCCF, 2008; Ozay *et al.*, 2007; Whitaker *et al.*, 2006]. However, no method had been developed to evaluate and design sampling plans to detect aflatoxin in dried figs. CCCF established an electronic Working Group, chaired by the Turkey delegation to recommend an ML and sampling plan design for aflatoxin and dried figs. Studies were initiated by the TÜBITAK Marmara Research Centre (MRC) to measure the variability and distribution among replicate sample test results taken from a contaminated lot of dried figs.

An aflatoxin-sampling plan is defined by an accept/reject limit and a specific aflatoxin test procedure. The accept/reject limit is a threshold concentration that is used to classify lots into 'acceptable' and 'unacceptable' categories and is usually equal to (but not required) a defined maximum level established by customers and/or regulatory agencies. The aflatoxin test procedure for granular commodities usually consists of three steps, sampling, sample preparation, and analysis. Because of the variability associated with each step of the aflatoxin test procedure, the true aflatoxin concentration in a bulk lot cannot be determined with 100% certainty. The sampling, sample preparation, and analytical variances associated with estimating aflatoxin in dried fig lots was determined as a function of aflatoxin concentration. The total variance (relative standard deviation) associated with the aflatoxin test procedure is the sum of the variances associated with the three steps of the aflatoxin test procedure. The variability associated with sampling, sample preparation, and analytical steps of the aflatoxin test procedure account for 99.1, 0.6, and 0.3% of the total variance, respectively.

Current challenges in mycotoxin determination

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The determination of mycotoxins constantly evolved during the last decades in terms of sensitivity, selectivity, accuracy, versatility, speed and costs. After appropriate sampling, laboratory samples of food or feed can be analysed using chromatographic, immuno-analytical or spectroscopic techniques. Despite the enormous advances in methodology, analytical challenges remain, and some selected ones will be briefly discussed, including:

- the importance to improve proper and easy-to-use sampling methods;
- selection of the appropriate method, which is fit for the given purpose;
- (non-)availability of analytical methods in developing countries;
- (non-)availability of analytical standards;
- (non-)availability of matrix reference materials (e.g., for multi-toxin analysis);
- sensitivity of methods used to obtain occurrence data (left-censored data);
- dependence of results on matrix effects;
- underestimation of repeatability by validation of pooled lots;
- what is overlooked in routine analysis; and
- application of analytical methods by non-specialists.

Best and worst practice examples will be given for some of raised issues along with recommendations. Finally, the presentation aims to promote discussion whether more sensitive, cheaper, quicker and/or more accurate methods are really needed to protect consumer safety.

Quantum cascade lasers for on-site detection of aflatoxins and trichothecenes in food

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State-of-the-art sensing platforms ideally benefit from miniaturised and integrated optical technologies providing direct access to molecule-specific information. With *in situ* sensing strategies, e.g., in harsh environments or point-of-care diagnostics in medicine becoming more prevalent, detection schemes that do not require reagents or labelled constituents facilitate localised on-site analysis close to real-time. However, decreasing the analytically probed volume may adversely affect the associated analytical figures of merit, such as the signal-to-noise-ratio, the representativeness of the sample, or the fidelity of the obtained analytical signal. Consequently, the guiding paradigm for the miniaturisation of optical diagnostic devices specifically for medical/clinical applications should be creating chem/bio sensing platforms that smartly capitalise on advantageous features of integrated photonics.

Mid-infrared (MIR; 3-20 μm) photonics platforms/sensing concepts are increasingly adopted in environmental analysis, process monitoring and food/commodity analysis due to the inherent molecular specificity enabling the discrimination of molecular constituents at ppm-ppb concentration levels. Recently, emerging strategies taking advantage of innovative waveguide technologies, such as planar semiconductor waveguides shaped into sophisticated optical structures (e.g., MIR Mach-Zehnder interferometers) in combination with highly efficient light sources, such as tunable quantum cascade and interband cascade lasers (QCLs, ICLs), facilitate compact yet robust MIR diagnostic platforms for label-free chem/bio sensing in food matrices. In this contribution, we will discuss advances towards handheld IR diagnostics for on-site detection of mycotoxins in commodities and food stuff.

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The USDA mycotoxin testing programme for grain: assuring quality in a nationwide lab network employing rapid methods

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The U.S. Department of Agriculture's Federal Grain Inspection Service provides official mycotoxin testing services throughout the United States for domestic and export grains, oilseeds, and processed-grain commodities. Official testing services are available for aflatoxins, deoxynivalenol, fumonisins, ochratoxin A, and zearalenone. Testing at grain receiving locations requires rapid, simple, inexpensive, and accurate methods to effectively ensure the safety of U.S. grain. USDA evaluates and certifies the performance of quantitative rapid mycotoxin test methods according to specific criteria. Only USDA-certified rapid test methods are authorised for use in official mycotoxin testing. USDA supervises a network of laboratories located at grain shipping and receiving locations throughout the United States and carries out quality assurance programmes to support mycotoxin testing at these locations. A key quality component is the national inspection monitoring programme, which provides statistically based feedback to testing labs on the quality of their mycotoxin results. An overview of the USDA mycotoxin quality assurance programmes that support official testing will be presented, including performance evaluation of rapid test methods and inspection monitoring.

Setting-up of a proficiency-testing scheme for mycotoxins in cereals

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Bipea is a proficiency test (PT) provider, based in Paris, organising interlaboratory comparisons since 1970. The first proficiency test on mycotoxins was held in 1996. At that time, naturally contaminated products were collected and used in the test; the results for only three mycotoxins were requested. Today, Bipea organises 17 rounds a year for mycotoxins, distributed in 5 options, on various matrices, spiking the different products to allow the participants to evaluate their trueness for 16 mycotoxins. More than 140 laboratories worldwide (70% abroad) will take part this year in these proficiency tests.

To implement these proficiency tests, homogeneity and stability studies were carried out, according to the requirements of the ISO 13528 standard. The design of the proficiency tests also includes the way assigned and tolerance values are estimated. Standard deviations for proficiency assessment are especially defined as the use of the dispersion of the results can be not sufficient. The organisation of such a big number of proficiency tests allows Bipea to collect a lot of data on mycotoxins determination, such as the overall performance of the laboratories and the differences in the techniques used. Concerning the overall performance, a graph representing the coefficient of variation according to the content can especially be plotted for each mycotoxin. About the techniques, the comparison of the means and the standard deviations obtained according to the different methods used can respectively indicate if there are some systematic biases between them and how precise each method is.

Purity determination of high purity mycotoxin standards by ^{1}H quantitative NMR

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Purity determination of standard products mainly used for chromatography is generally implemented by the area normalisation method with chromatography. However, this method cannot be always considered accurate, for reasons such as the presence of undetectable impurities and the situation in which the principal component and impurities display different sensitivities. Here, we describe the application of purity determination of standard products for chromatography assays of mycotoxin by using an efficient and absolute quantification method for assuring the reliability.

Quantitative nuclear magnetic resonance using hydrogen nucleus (^{1}H quantitative NMR or ^{1}H qNMR) efficiently achieves absolute purity determination that has traceability to the International System of Units or SI and, therefore, has recently attracted considerable attention in analytical chemistry. In fact, ^{1}H qNMR was adopted by Japan's Specifications and Standards for Food Additives and Japanese Pharmacopeia to determine the purity of standards used for the chromatography of food additives and crude drugs. Moreover, as a result of a round robin test, ^{1}H qNMR was adopted by Japanese Industrial Standards, which specifies the standards used for industrial activities in Japan. We applied this method to the purity determination of standards for chromatography assays of mycotoxins. We adopted an internal standard method known as AQARI (accurate quantitative NMR with internal reference substance), which in principle is the most accurate among the multiple qNMR methods available. For internal reference substance (IRS), we utilised certified reference materials or CRMs with SI traceability of 1,4-BTMSB-*d4* or DSS-*d6* that are suitable for qNMR measurement because of emitting singlet signals close to 0 ppm where organic compounds generally have no signals; so, there is no signal overlap with the analyte. An ultra-micro balance was used to determine the accurate mass of the sample, and IRS for qNMR. A 400MHz or higher NMR instrument was used for the measurement to ensure sensitivity and resolution. In normal ^{1}H NMR measurement, the relaxation time is set to about 4 s. When the relaxation time is short, signal saturation occurs and then an accurate quantitative result cannot be obtained. Based on the aforementioned information, the relaxation time was set to 60 s or longer. From the results of this study, it was ascertained that absolute purity determination was possible while ensuring accuracy of at least two significant digits. To date, we have built a supply system of fourteen mycotoxin standards (15-acetyldeoxynivalenol, aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, diacetoxyscirpenol, fumonisin B1, fumonisin B2, HT-2 toxin, neosolaniol, sterigmatocystin, T-2 toxin, verrucarol, zearalanone) of which the purities are determined by qNMR.

Mycotoxin analysis: the past, the present and the future of rapid testing

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Several countries have introduced regulatory limits for the most important mycotoxins, including aflatoxin, deoxinivalenol, zearalenone, fumonisin and ochratoxin. Different analytical approaches have been developed, each with its own particular benefits and drawbacks. In general, two main strategies can be observed: rapid, simple screening tests and highly accurate reference methods. This talk will present a detailed overview of the different types of rapid tests for mycotoxins applied over past decades as well as the evolution of methods in mycotoxin testing, beginning with very simple screening tests and progressing to reliable quantitative on-site tests. The development of new methods was driven not only by newly available technology; changes in national regulations and customer requirements also exerted considerable influence. Currently, lateral flow devices are the most prominent and advanced rapid test systems for mycotoxins, although this technology has been in use since the 1980s. While it is true that the performance, usability, and reliability of these tests have improved significantly over the last decade, it is likely that further development of this technology will now begin to decelerate. For this reason, we can expect different, innovative technologies to emerge as alternatives in the near future.

TUESDAY 13 MARCH 2018

SESSION 5
MANAGING THE IMPACT OF MYCOTOXINS – PART 2

Effects of food processing on the reduction and distribution of modified mycotoxins

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The recent advances in analysis techniques permitted to confirm the large presence of 'modified mycotoxins' in food. These modified forms represent a major concern and potential risk for consumers because of the possible hydrolysis of modified mycotoxins into their toxic free forms during digestion. Due to the great toxicity of them, interest in knowing the effects of food processing on mycotoxins and modified mycotoxins levels has increased during the last few years. Although mycotoxins are considered extremely stable compounds, food processing can have an impact on them. Processing of raw food commodities can be considered as the application of any combination of chemical, biological or physical methods used to produce final consumer food or animal feed. Processing from harvest to the point where food is eaten by human or animal consumer involves a complex chain of actions. This may be as simple as hand grinding maize and boiling, while it can be large-scale semi-automated mills, bakeries, extrusion plants and breweries that involve many stages to produce retail product as purchased by the consumer. Cleaning, milling, fermentation and heating stages (baking, frying and boiling) have been checked for the modified forms achieving a large variability of results. Thus, at each stage, concentration of a modified mycotoxin may decrease, increase or remain unaffected. The published results pointed out that several factors could affect to the modified mycotoxins stability: temperature, time, pH, presence of enzyme, … Moreover, the efficiency of different operation varies with regard to mycotoxin. For instance, modified forms from trichothecenes showed the largest variation during processing. Understanding all these factors will assist in minimising mycotoxins formation, maximising their elimination and ensuring that, as far as possible, no toxic reaction products are produced. Thus, toxic effects of the potential breakdown products of mycotoxins to various biological systems are also clearly required before the apparent reductions in toxin levels observed during food processing can be directly equated with an overall loss in toxicity. Finally, knowing the mycotoxin behaviour during each food process would allow adjusting the processing conditions and the quality of the incoming raw materials to obtain safe products, setting then suitable performance criteria (PC), in order to achieve the desired food safety objectives (FSO), which in the case of mycotoxins usually take the values of the legislated maximum permitted levels.

Mycotoxin transfer rates and processing factors in selected food chains

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The analysis of the transfer rates of contaminants in food and feed chains will become more and more important for the identification of high risk products during an actual incident, but also preventively as indictor of critical points and products in the supply chain. Examples of such a supply chain analysis will be given by illustration of the potential effects of wheat and maize processing on deoxynivalenol (DON) and fumonisin B1/B2 (FB1/FB2) in the bread and cornflakes production, and the estimated carry-over of aflatoxins from feed to food of animal origin, based on literature data.

To protect consumers' health, maximum levels (MLs) are set by the European Union food law (Commission Regulation (EC) No 1881/2006) for several mycotoxins. MLs in cereal raw materials necessitate and promote respective control procedures at very early stages of the food chain. In

addition, lower MLs are set for the final food products. Processing factors for DON and FB1/FB2 were estimated for the production of wheat bread and of cornflakes, respectively. The considered processing steps cover dry milling, baking, pressure cooking, extrusion cooking, and/or roasting. The processing factors were further compared with legal obligations. For that, dilution effects were taken into account, since the EU MLs for DON and FB1+FB2 are defined on the product 'as is' basis.

aflatoxins contained in feed are ingested by food-producing animals and can be transferred into animal products. Once ingested by the animal aflatoxin B1 can be metabolised in the liver into aflatoxin M1, amongst other metabolites. If feed containing aflatoxins is fed to livestock, the transfer into milk is more significant than the transfer into other food, such as meat and eggs. Concentrations of aflatoxins to be expected in the milk of dairy cows, assuming different carry-over rates and feed rations for different aflatoxin concentrations in feed, are presented.

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Transformation of mycotoxins: stories from field to process to digestion

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This presentation is going to illustrate a three-years work focused on the study of specific aspects of mycotoxins, regulated and emerging, in order to provide information concerning their mitigation from field to finished product, with a particular focus on the potential impact of food processing.
Subsequently, their toxicological role, currently under discussion, has been investigated through a dedicated gastrointestinal model. Particularly, a special focus was devoted to DON, the most common contaminant in wheat, to DON-3-Glc, representing its main derivative, and to ENN B, the major compound among so-called 'emerging' mycotoxins.

The study has been divided into three sections. The first one is addressed to the development of greenhouse experiments on different durum wheat genotypes in order to better understand the detoxification pathway from DON and the resistance of the different varieties to one of the most severe disease affecting this species. The second section involved the investigation of the effective impact of different strategic production chains on mycotoxin levels in the finished product. Furthermore, a strategy of mycotoxin mitigation has been approached in order to optimise technological parameters within bakery production, still obtaining an appreciable product for consuming. In the last part of this work, investigations on the toxicological effects of these compounds have been carried out through the evaluation of their fate after human digestion.

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Atmospheric-pressure plasma: prospective tool for inactivation of fungal pathogens and degradation of mycotoxins

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Certain fungi, such as *Fusarium*, *Aspergillus* and *Alternaria*, produce mycotoxins as secondary metabolites. These toxins can be found in a wide range of food and feeds and occur especially in regions displaying hot and humid periods. For the degradation of mycotoxins different biological approaches for their reduction are under investigation, such as enzymatic degradation, degradation by different fungi as well as degradation approaches using bacteria. However, some of these methods only work for the degradation of certain toxins, although many of the currently available detoxification methods provide good results within their target group area. Therefore, new methods are still under investigation to provide a more general detoxification strategy.

This talk is concerned with the degradation of mycotoxins utilising a physical approach called cold atmospheric plasma (CAP) and the susceptibility of fungi to the process. Plasma as the so-called 4th state (non-classical) of matter provides a range of different mechanisms to interact with substrates, such as UV radiation, different reactive oxygen and nitrogen species (RONS) and strong electric fields. Many plasma treatment approaches operated within the low-pressure region are utilising radio frequency (RF) driven high power generators. The outcome of these published experiments shows the huge potential that a plasma detoxification strategy represents. However, more than the sheer ability to degrade certain mycotoxins it is necessary to pose a well-functioning treatment strategy. It has to be taken into account that an applied plasma is not only interacting with the target substance but also with the bulk material as well, leading to the necessity of an evaluation of possible effects on nutrients and an investigation of effects of the surrounding bulk material itself on the degradation process. Some of the different approaches for the plasma treatment of seeds or bulk commodities in general are briefly presented and compared with each other. This talk can give an idea of the applicability posed by the plasma technology and might be serving as a point of reference.

Current research topics in mycotoxin decontamination: focus on China

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Mycotoxins contamination in agro-food systems has been a serious concern during the last few decades. Mycotoxins are toxic secondary metabolites produced by fungi when they grow on seeds in the field, or in food products and feedstuff. The major mycotoxin producing fungi seen in China are *Aspergillus*, *Fusarium*, and *Penicillium* sp. which mainly produce aflatoxins, deoxynivalenol, fumonisins, ochratoxins, patulin, and zearalenone. Mycotoxin decontamination in food and feed products has become an urgent need to ensure food security, food safety and safeguard the national economic interests. Several surveys on the mycotoxin contamination level in cash crops and food products across China have been taken. Mycotoxin prevention at pre-harvest has been widely studied and applied in the field with good outcome. Several detoxification methods were developed to counteract the mycotoxin contamination and prevent mycotoxins to enter the food chain. Many common methods, such as extrusion cooking, are still applied in the food and feed industry. Gaseous ozonation has become one of the key methods in detoxification. Several bacterial or fungal strains and their enzymes have been employed for detoxification of mycotoxins, such as *Bacillus* sp., lactic acid bacteria, *Devosia* sp., *Pleurotus* sp., *Armillariella tabescens*, etc. In recent years, microbial-based decontamination of mycotoxins is taking the front stage to be economical, safe and cost effective.

Effective mycotoxin detoxification strategies to ensure safer feed

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Mycotoxins are secondary metabolites of various fungi commonly found in feed and foodstuffs, often co-occurring at low levels [Binder *et al.*, 2007; Schothorst and Van Egmond, 2004]. Based on their occurrence and observed effects on human and animal health, aflatoxins, fumonisins, deoxynivalenol, ochratoxin A and zearalenone are recognised as the five most important mycotoxins in animal husbandry. The most cost-effective strategy to counteract the effects of these low levels of mycotoxins in the feed industry is to use mycotoxin detoxifying agents. This paper aims at reviewing the different strategies of detoxification available in the market and their efficacy, considering the evaluation method.

We can distinguish two main categories within the wide group of mycotoxin detoxifying agents: (i) adsorbing agents, which the aim is to decrease mycotoxin bioavailability by including them in the compound feed, leading to a reduction of mycotoxin uptake; and (ii) biotransforming agents, which the aim is to degrade mycotoxins into less toxic metabolites by using microorganisms or enzymes. Within the adsorbing agents, the most commonly used are activated carbons, aluminosilicates (HSCAS), modified aluminosilicates and yeast cell walls. Within the biotransforming agents, there are a wide array of bacteria, fungi and different enzymes, although few of them are used in commercial products. The efficacy of mycotoxin detoxifying agents is very often assessed in static *in vitro* models measuring the percentage of adsorption or the degree of detoxification achieved by the agent. However, Vekiru *et al.* (2007) and Versantvoort *et al.* (2005) showed that detoxifying agents generally become less efficient when gastro-intestinal conditions are simulated. Their efficacy may be overestimated in static models making more reliable the use of dynamic gastro intestinal models. The efficacy of adsorbing agents depends on the intermolecular interactions between the adsorbing agent and the mycotoxin, such as electrostatic, hydrophobic or shape effects and the characteristics of the adsorbing agents, including atom composition, total charge and charge distribution, size of the pores or accessible surface. The efficacy of biotransforming agents depends on the activity and survival of the microorganism in the digestive tract, the specificity of the enzyme and its substrate, the time for the enzymatic reaction to occur, the toxicity of the produced metabolites and their intestinal absorption compared to the parent mycotoxin.

Many parameters influence the efficacy of a mycotoxin detoxifying agent, from their own characteristics to the evaluation method used to study them. *In vitro* results should be analysed and interpreted carefully and complementary *in vivo* trials on performance should be available to conclude on an agent efficacy.

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TUESDAY 13 MARCH 2018

PLENARY MEETING
INTEGRATED SOLUTIONS TO REDUCE MYCOTOXINS ALONG FOOD AND FEED CHAINS – FUTURE EXPECTATIONS

MycoKey and MyToolBox: an example of good European cooperation

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The European Commission funded the projects MycoKey and MyToolBox under the programme Horizon 2020 Research and Innovation topic 'Biological contamination of crops and food chain'. Both projects aim at reducing the mycotoxin contamination throughout the food and feed chain by developing new practical ICT based solutions to assist food chain players in decision making process. In addition, the projects concur to enhance the EU-China dialogue, by developing linked research activities with Chinese scientific Institutions involving actively Chinese partners in integrated research and dissemination.

The aim of this presentation is to underline the main steps made in the projects from the beginning (2016) and the possible future cooperative actions. The coordinators fostered a common ground to find synergies and tackle more efficiently the main challenges posed by this important topic. The Research Executive Agency of the European Commission encouraged this approach in order to align the overall strategy to approach the problem at EU level. The coordinators of both projects signed a memorandum of understanding (MoU) defining the potential areas of research integration and cooperation effective for reducing the risk of mycotoxin contamination in crops and along the feed and food chains. The MoU is combined to an annex document resuming synergies and distinctions between the two H2020-SFS-13-2015 projects. Both documents were shared with the consortia partners. Successively, the work packages leaders and researchers met in Gent in 2017 to discuss and define practical actions for effective cooperation in the following areas: pre-harvest reduction strategies, post-harvest reduction strategies, analytical chemistry, and ICT-tool. Finally, the common dissemination activities and cooperation with China are being organised through the participation of the two projects to the MycoKey round tables and working groups, international conferences (WMF2018, 2nd MycoKey International Conference 2018, WMFmeetsIUPAC2019, final MycoKey International Conference 2020, EU-China Mycotoxin Forum), and EU Commission EU-China events (Food, Agriculture and Biotechnology – flagship initiative between the EU and China, 2018-2020).

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Genetic basis of the resistance to *Fusarium* ear rot in maize

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Fungal infection by *Fusarium verticillioides* is the cause of a prevalent maize disease leading to substantial reductions in yield and grain quality worldwide. The breeding of resistant maize genotypes is the most effective way to achieve sustainable control of *Fusarium* ear rot (FER), and may take advantage of the identification of genes and loci responsible for natural disease resistance.

Significant advances have been made in the development of transcriptomic, genetic and genomic information for maize, *F. verticillioides* moulds, and their interactions over recent years. Findings from transcriptomic studies have been used to outline a specific model for the intracellular signalling cascade

occurring in maize cells against *F. verticillioides* infection. Several recognition receptors, such as receptor-like kinases and R genes, are involved in pathogen perception, and trigger down-stream signalling networks mediated by mitogen-associated protein kinases. These signals could be orchestrated primarily by hormones, including salicylic acid, auxin, abscisic acid, ethylene, and jasmonic acid, in association with calcium signalling, targeting multiple transcription factors that in turn promote the down-stream activation of defensive response genes, such as those related to detoxification processes, phenylpropanoid, and oxylipin metabolic pathways. At the genetic and genomic levels, several quantitative trait loci (QTL) and single-nucleotide polymorphism markers for resistance to FER deriving from QTL mapping and genome-wide association studies have been described. To guide the identification of candidate genes within the identified QTL, transcriptomic and sequencing information have been exploited. Suggestive candidate genes associated with disease resistance and pathogen related-mechanisms at the FER resistant loci have been identified on maize chromosomes 4 and 5. All these findings will contribute to identify candidate genes for resistance and to apply genomic and editing technologies for selecting resistant maize genotypes and speeding up a strategy of breeding to contrast FER disease.

Use of atoxigenic isolates in aflatoxin control in Serbia

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Outbreak of contamination with aflatoxin B1 during 2012 as well as its occurrence in individual samples in subsequent years caused significant losses in maize production in Serbia. Additionally, it gained publicity as an indicative risk for food safety. Given that Serbia is one of 10 most significant maize exporters in the world, contamination with aflatoxin B1 can seriously endanger state economic performance. In Serbia, maize is grown without irrigation and preventive measures (optimal number of plants per ha, favourable cultivation and control of European corn borer) have limited effect on infection with *Aspergillus flavus*. Therefore, contamination is primarily regulated with environmental factors. Data from the USA, various African countries and Italy show that application of locally adapted atoxigenic *A. flavus* isolates may result in prevention of maize ear infection with toxigenic isolates and decrease risk of mycotoxin contamination. Out of 109 *Aspergillus* spp. isolates that were collected from commercial maize samples in Serbia Vojvodina, 22 isolates were selected for further genome analysis. Based on incomplete AFLA1 and AFLA2 genes, which indicate fungal ability to synthesise aflatoxin B1, two isolates were selected (T1/III1, from Pivnice, Serbia and T7/I11 (from Maradik, Serbia). Since these two isolates were genetically identical, only one (T1/III1, from Pivnice) was selected and tested for toxicity profile to prove that it is atoxigenic. These analyses as well as training on biological product manufacturing were performed under supervision of Prof. Dr. Peter Cotty (USDA-ARS,) at the University of Arizona, USA.

The atoxigenic isolate was applied in trials on two localities (Bečej and Sombor) during two-year trials with and without irrigation. Results showed that in Bečej no contamination was registered in none of the tested treatments or in the untreated control. On the other side, in Sombor reduction of aflatoxin B1 during 2016 was between 62.5% in irrigated and 92.4% in non-irrigated plots, whereas in 2017 aflatoxin B1 was reduced for 59.4 and 68.7%, respectively. This isolate was deposited within the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and entered patent protection process on national level at the Office for Intellectual Protection of Republic of Serbia. It is expected to test more atoxigenic isolates in reducing aflatoxin contamination as well as their spreading capability with the aim to use them as the most effective biological product.

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Control of *Fusarium* head blight with biopesticides

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Current chemical control of *Fusarium* head blight (FHB) is limited to a single chemical group, the triazoles. This creates a large selective pressure for fungicide resistance to develop and there are currently safety concerns with triazole fungicides as they are classed as endocrine disruptors, and as such they may be removed from the market. There is also a growing interest in the use of alternative products to control plant diseases. The term biopesticide covers a wide spectrum of potential products used within plant protection and in general can be considered as any products that are not conventional synthetic pesticides used to control pests. Biopesticides include salts, plant defence elicitors, biological control agent and botanical extracts.

As part of the MyToolBox project field experiments have been conducted for wheat in the UK and oats in Norway on the efficacy of a range of biopesticides to control FHB and consequently reduce concentrations of deoxynivalenol (DON) in harvested grain. Biopesticides were selected based on previous evidence of their efficacy against FHB and/or the availability of these products for field experimentation within these countries. In 2016, 19 products were tested within inoculated field experiments in replicated block designs with untreated control plots and positive control plots treated with Proline (Bayer CropSciences, a.i. prothioconazole). Most products were tested in both countries but not all selected biopesticides were granted approval for field experiments by the national authorities. High DON was detected in untreated plots and the Proline-treated plots significantly reduced DON in both experiments. None of the biopesticides tested significantly reduced DON compared to the untreated controls. In 2017, a smaller selection of biopesticides were selected (four) based on available data and these were tested in field experiments with a range of concentrations and application timings. Results from the 2017 experiments will be reported.

Two alternative strategies to control FHB are also been investigated in the MyToolBox project. Both strategies target the pathogen on the crop debris of the previous crop, as the crop debris is known to be the principal source of inoculum. The first strategy is through competition and enhanced degradation of the crop debris and the second is the use of biofumigation. Biofumigation is the use of a brassica cover crop which is chopped and incorporated into the soil. When brassica cells breakdown, glucosinolates in the presence of the enzyme myrosinase produce isothiocyanates, which are potent biocides. Biofumigation has been shown to be effective at reducing soil-borne pests and pathogens and this study will investigate its effectiveness at reducing *Fusarium* inoculum between host crops. Initial *in vitro* studies for both these strategies have been promising.

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Intervention strategies for minimising mycotoxins in malting

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Malted barley provides the basis of most beers in the world. Approximately 13% of barley produced worldwide is processed into malt. Contamination of barley and other cereals used as raw materials in malting and brewing by toxigenic fungi is a great concern. Harmful fungal metabolites can cause failures during both malting and the brewing process. Furthermore, toxic metabolites may have severe adverse

effects on human and animal health. *Fusarium* fungi are considered as the most important group of filamentous fungi with respect to malt and beer quality and safety. The majority of fungal metabolites are produced throughput several time periods: during crop cultivation in the field, during inadequate drying after harvest or dampening during storage or transport and finally during the malting process. Preventive actions are crucially important in maintaining the quality of malting barley and in assuring safety throughout the malting and brewing process. Elimination of mycotoxins in cereals and cereal-based products may not be achievable, but reduction of toxins in every step along the cereal production chain is essential to ensure consumer and animal safety. Thus, effective means are needed to reduce the growth of fungi and hinder the production of mycotoxins at pre-harvest level as well as during processing. The first line of defence is always at farm level. The multitarget control strategies in combination with novel monitoring tools will open new possibilities for ensuring safety along the barley-to-beer chain as well as for other cereal-based products.

This presentation will provide insights into new MycoKey approaches with special emphasis on the malting barley chain. The EU MycoKey project aims to develop novel concepts for management of mycotoxins along food and feed chain. A multi-disciplinary consortium composed of scientific, industrial and association partners (32) from Europe, China, Nigeria and Argentina currently conduct the 4-year programme. The project focuses on aflatoxins, ochratoxin A, deoxynivalenol, fumonisins and zearalenone challenges in maize, wheat and barley. These crops represent nearly 60% of global cereal production. This presentation highlights activities on the most important mycotoxins and their production in the barley-to-beer-chain in order to identify the critical stages and to minimise the contamination by some effective preventive actions.

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Feed additives for mycotoxin detoxification – efficacy and authorisation in the EU and China

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Feed additives for detoxification have proved to be effective in mitigating the effects of mycotoxin contamination in animal feed. In assessing the efficacy of these substances, it is important not only to demonstrate their performance in relation to reducing the biological effects of mycotoxins for target animal species, but also to ensure there are no unintended detrimental consequences such as anti-nutritional effects. In the EU, EFSA has produced guidance for conducting animal studies as part of the risk assessment of feed additives for mycotoxin detoxification. Key to this guidance is that animal feeding studies can only be conducted at mycotoxin levels in compliance with EU maximum limits and guideline values for animal feed and that biomarkers are used as detoxification performance measures. In the MyToolBox project, work is underway to examine whether the EFSA guidance can be applied in animal studies conducted under local conditions in China to study adsorbent additives for detoxification of aflatoxin B1 in feed for dairy cows and enzyme-based additives for fumonisins in feed for pigs.

In this talk we will outline and discuss some of the practical problems and issues which have arisen in taking forward this EU-China collaboration, as well as the achievements in knowledge transfer and in moving towards establishing common standards for evaluation of feed additives for mycotoxin detoxification.

Advanced analytical methods for mycotoxin detection: priorities and critical issues

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Scientific fields are always changing, and it is important to determine the directions in which they are heading as plans are made for future research activities and research results transition to applications and policies. The MycoKey project, an EU-funded Horizon 2020 project, includes a series of 'roundtable discussions' to gather information from leading researchers inside and outside the project on directions in which the field of mycotoxicology is trending. This presentation includes a summary of the roundtable discussion on priorities and critical issues in chemical detection and monitoring of mycotoxins. The goal was to identify key elements that could impact when and how methods are used to identify mycotoxins and to increase food safety. The discussion was managed by using the nominal group discussion technique (NGT). The NGT provides for equal input from all participants and is well-known as a process for generating a large number of ideas, while also providing a mechanism for ranking them. The discussion was focused on four following key questions:

- to identify scientific and technological innovations which hold promise for development/improvement in the future (question 1);
- to identify and prioritise critical elements making methods effectively applicable in:
    - industry for autocontrol and HACCP (question 2),
    - developing countries with limited analytical capabilities (question 3),
    - official control with particular reference to multi-mycotoxin methods (question 4).

The rankings and the total list of responses provided a rich and detailed context from which particular ideas and general trends could be extracted. Test kits, usually antibody based, were one major focus of the discussions because of their many favourable features, e.g., cost, speed, and ease of use. Most important was considered the degree to which antibody-based diagnostics have become an acceptable standard in many practical applications. Much of the discussion was on how to improve these kits and to speed the detection of mycotoxins in locations where access to a well-equipped analytical chemistry laboratory is neither timely nor cost-effective. The second area of focus for this discussion was multi-mycotoxin detection protocols and challenges remaining before these protocols become methods of choice for regulated mycotoxins. This second point mirrors the first, through the increasing importance of technically highly sophisticated multi-mycotoxin detection protocols. These protocols need more standardisation and cross-laboratory validation, but are the future for many official regulatory controls, especially as the number of toxins that are regulated increases. Multi-mycotoxin assays are being developed at a large number of locations and international inter-laboratory validation and comparability of results are needed to establish credibility of results across both scientific and regulatory boundaries. Common critical factors for analyses in academic, industrial, and governmental laboratories and developing countries were speed, expense, accuracy/reliability, data management, and, particularly in developing countries, operator training. Finally, forging a partnership between scientists and well-placed communications experts was recognized as an essential step to communicating risks while retaining overall confidence in the safety of the food supply and the integrity of the food production chain.

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The neglected issue of evaluating and reducing the impact of the lot-to-lot variation on the measurement uncertainty

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In recent years, the LC-MS/MS based multi-analyte approach has been demonstrated to be a powerful technique for the simultaneous determination of mycotoxins in food and feed [1]. Quantification of mycotoxins is increasingly based on the analysis of diluted crude extracts and external- or matrix-matched calibration. In essence, the response of the analyte is compared to a calibration curve of the analyte in neat solvent or matrix and, if necessary, corrected for the method bias. The method bias, expressed as apparent recovery (RA), can arise from incomplete recovery of the extraction (RE) or signal suppression/enhancement (SSE), also known as matrix effect. In everyday practice RA is evaluated based on replicate analysis of a single lot of a matrix for each analyte-matrix combination during initial method validation. Due to the heterogeneous nature of the matrix, RA may vary for different lots of the same matrix, i.e., 'lot-to-lot variation'. Matuszewski *et al.* [2] first found differences in SSE for a compound in plasma samples from different sources, which is referred to as relative matrix effect. Also, for mycotoxins, large differences in SSE have been observed for different varieties of sorghum and rice [3,4]. However, most method validation studies neglect the lot-to-lot variation and validate the method based on a single lot of a matrix.

We hypothesised that neglecting the lot-to-lot variation during method validation can lead to an underestimation of the measurement uncertainty (U). The objectives of this study were: (i) to develop a practical procedure for the realistic estimation of U for LC-MS based multi-mycotoxin determination; (ii) to estimate the influence of the lot-to-lot variation on U; and (iii) to compare three potential strategies to reduce the impact of the lot-to-lot variation. This study presents the first calculation of the intra-laboratory U for 66 mycotoxins in figs and maize accounting for the lot-to-lot variation and differs significantly from U calculated based on repeatability studies of a single lot of a matrix.

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MycoKey app: an ICT solution to facilitate mitigation of mycotoxin risks

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The MycoKey app is developed as an ICT solution to facilitate mycotoxin risks mitigation by various stakeholders in the chain. Different work packages of MycoKey generate, validate and integrate knowledge that would provide useful information for risk assessment and would help to raise awareness, alert and specifically notify stake holders and provide options for mitigation of mycotoxin risks. This knowledge needs to be customised in order to effectively assist stakeholders. The MycoKey app, a mobile accessible platform, will deliver this customised information on a smartphone, tablet or computer. This app will generate a dashboard experience for accessing all relevant information for growers, advisors, grower associations, stakeholders in the production chain as well as policy-makers. It provides information on the risk of mycotoxins and, when required, will suggest management activities to mitigate and reduce risks. The app is user protected by a personal password and data can be private, shared with friends and advisors or anonymised and shared to other stakeholders. Governmental planners and policy makers will have access to shared, public databases and satellite data, as such biomass indices, land-use and mycotoxin risks can be estimated per region. The MycoKey app has different functionalities

for smart phone (data entry and retrieval) and computer platforms (data entry and retrieval and analysis). Recalculation using different intervention strategies allows integration of management strategies in the risk model and calculations of “what if” scenarios. We hope to demonstrate the MycoKey app for world-wide mycotoxin risk prediction.

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Integrated solutions to reduce mycotoxins along food and feed chains: future expectations

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There is still a need for fully integrated approaches from field-to-fork and beyond to effectively reduce mycotoxins in food crops [1]. Such strategies shall also consider safe use options of mycotoxin-contaminated batches, such as microbial energy conversion for efficient production of biogas, bioethanol and safe dried distiller´s grain soluble (DDGS). Up to now, different agricultural strategies including crop rotation, tillage practices, fungicide application, biocontrol and the use of resistant plant cultivars have been used to reduce the impact of fungal infection of crops and subsequent mycotoxin formation. As none of these strategies by themselves have been able to efficiently reduce the impact of fungal infection, such integrated multi-actor approaches are pursued by the recently funded EU-projects MyToolBox and MycoKey, which also take into account issues related to climate change. Consequently, these projects combine a series of integrated pre- and post-harvest measures, which could enable a 20-90% reduction (depending on the type of commodity and intervention) in losses of crops due to fungal and mycotoxin contamination. In addition, information and decision support tools shall be developed for each level of the chain and shall be integrated into web-based e-platforms that shall also be accessible over all mobile platforms. As such, the e-platform shall guide the end user to the most effective measure(s) to reduce biological contamination in crops and will provide the necessary intelligence to ensure these measures take into account the prevailing conditions such as geographical location, meteorological conditions, land-use, crop management, storage and intended end use with relevance to specific crops. Moreover, in cooperation with partners from China, a sound scientific basis for standard settings in China for authorisation of mycotoxin detoxifying feed additives is still needed, which could improve mutual market access of relevant products from the EU and China.

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PLENARY MEETING
A STEP BEYOND – TOWARDS A MYCOTOXIN-FREE SUPPLY CHAIN THROUGH NEXT LEVEL CONTROL STRATEGIES?

Can incentive mechanisms contribute to realising mycotoxin-free supply chains?

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Mycotoxins are secondary metabolites of fungi which are toxic to animals and humans upon consumption. Mycotoxin occurrence in various food crops is of major concern since it has significant implications for food and feed safety, food security and international trade. Much research has been done on agricultural prevention and control measures for mycotoxin reduction in food and feed crops, studying a wide range of measures to reduce fungal infection and to limit mycotoxin production. However, despite these huge research investments, mycotoxins still occur, and agriculture and food industries remain vulnerable to problems of mycotoxin presence. As the cost-effectiveness of mycotoxin interventions in the chain depends on the uptake level of the various actors, it is of importance to have an estimation on the expected uptake level as well as insight in the factors that will drive the level of uptake or willingness to apply. These insights provide a window of opportunities with respect to the development of incentive mechanisms aimed 'to induce the supplier to apply measures as the buyer requests' in an attempt to optimise chain control.

A variety of incentive mechanisms has already been developed to address these so-called agency problems. For instance, quality premiums and discounts to provide incentives for key product safety attributes. The use of tournaments, in which the relative performance of an individual farmer is compared to a group average, for improving efficiency when producers face significant common production risks due to external factors such as weather or disease. Another increasingly common response to agency problems in agricultural production is the use of production contracts. However, only little experience with incentive mechanisms for food safety control exists. This contribution will therefore focus on the potential of incentive mechanisms with respect to the prevention and control of mycotoxins during the primary production of feed and food crops.

A drop in the matrix: how computational tools may boost research in mycotoxicology

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In recent years, it has been shown how the integrated use of *in silico* strategies with conventional experimental approaches may significantly move ahead the knowledge on mycotoxins occurrence and toxicity. On the one side, the more and more growing number of computational toxicology studies has shown that the use of *in silico* methods makes the toxicological assessment of mycotoxins at a metabolomic scale possible. In particular, computational studies may support the experimental design, prioritising the trials in terms of which compounds and endpoints have to be tested with priority. Also, the *in silico/in vitro* interplay may lead to affordably decipher the mechanisms and modes underlying the toxic action of the metabolomes members. The integration of *in silico* and *in vitro* approaches proved also to be effective in target fishing studies aimed at discovering unexpected biological targets for mycotoxins. On the other side, innovative analytical technologies may be often integrated with computational tools to support unknown identification and to rationalise big data obtained under fully untargeted omics approaches. Recently, the addition of the collision-cross section (CCS) values

parameter to the chemical characterisation proved to be a relevant and really informative add-on to profile the mycotoxins content. In this regard, it has been demonstrated that the theoretical CCS values calculated *in silico* may virtuously support the experimental determination leading to a more precise elucidation of the 3D molecular structures. Therefore, in a changing world where mycotoxins of food origin are going to pose a more and more mutable and multifaceted menace, the joint effort of computational sciences and experimental analysis shall be among the most promising ways to probe what the lack of appropriate technologies has made elusive in the past.

Modelling the impact of mycotoxin-producing fungi in agricultural systems: issues, limits, and challenges

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Limitations by the European Union (EU) and by other nations of the world on the maximum levels of mycotoxins in cereal grain have had an important socio-economic impact on the global cereal market. Crop production with acceptable mycotoxin content and maintaining at the same time profitability has become more and more difficult, with important socio-economic consequences. The development of modelling tools to simulate mycotoxin contamination in crops during the field phase could represent a great opportunity for: (i) producers, to optimise agro-management; (ii) policy makers, to better evaluate the extent and distribution of mycotoxin contamination in Europe and thus establish safe and technically feasible mycotoxin standards in cereals; and (iii) for scientists, to better understand the biological system leading to mycotoxin contamination under climate and global change.

The pathosystems leading to mycotoxin contamination in crops are complex and the modelling of such systems is a challenge. For instance, the pathosystem leading to mycotoxin contamination in maize kernels include the maize crop, and a range of mycotoxigenic fungi (including *Fusarium* spp. and *Aspergillus* spp.), along with insect borers (i.e., the European and the Mediterranean corn borers). Modelling each element of the system, their relationships, and the consequences on the final grain contamination, on the one hand requires a thorough understanding of the biological processes involved in the system. Critical to a better understanding, for instance, are the relations between fungal infection and mycotoxins production.

The lack of standardised and shared protocols and methodologies, including standards for I/O variables, the lack of publicly available good quality field experimental data to calibrate/validate the model, the differences in the complexity of the different modelling approaches used to model the different parts of the system, the lack of transparency in the modelling solutions developed so far, the need for stronger collaboration between the different modelling communities addressing different elements of the pathosystems, are examples of issues that have slowed down the development of publicly available and effective simulation models. The AgMIP and MACSUR international networks are making efforts to foster the collaboration between the pest/diseases and the crop modelling communities, towards an improved simulation of the yield gap due to biotic stresses. Current efforts are focused on the integration of damage mechanisms in crop physiological processes, as modulated by pest/disease dynamics, aiming at reducing carbon assimilation and photosynthetic area. Although the qualitative aspect of crop productions is not targeted yet by these research programmes, it will be among the next challenges in agricultural systems modelling.

Unravelling wheat defence against deoxynivalenol reveals novel targets for disease resistance breeding

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One of the main foci of our team is to identify genes that are of benefit to breeders in terms of marker-assisted selection for *Fusarium* head blight (FHB) resistance, and at the same time elucidating the signalling mechanisms involved in the host-pathogen interaction. We used a functional genomics approach to identify genes up-regulated as part of the wheat response to deoxynivalenol (DON). Functional genomics of a population segregating for FHB resistance led us to the several genes, including the orphan gene *TaFROG*. Further studies validated its role and that of its interacting proteins, SnRK1 and a NAC transcription factor, in disease resistance. Ongoing studies are determining the allelic diversity of candidate FHB resistance genes and their promoters and developing markers for gene selection.

Using a bioinformatics approach, we have identified genomic hotspots for FHB and DON resistance, combining functional genomics, genome data and gene validation studies. One of the interesting trends emerging from our studies is that many of the DON resistance genes we identified also positively affect grain development and new research programmes aim at better understanding the impact of specific genes on yield and the relationship between grain development and DON resistance.

Lowered mycotoxin accumulation in crops through investigation of *Fusarium*/host interactions: challenges and opportunities through 'omics'

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Fusarium head blight (FHB), caused mainly by *Fusarium graminearum*, has been a devastating disease of cereal production in Canada for the past two decades. Trichothecene mycotoxins, such as deoxynivalenol (DON), are common in infected grains, which result in decreased grain quality for industry and pose health concerns for consumers. While deployment of genetically resistant varieties remains the most economically viable strategy for disease mediation, development of resistant varieties has been difficult due to complexities involving: quantitative control of resistance, high genetic variation of the pathogen and specialised chemistry required for mycotoxin quantification.

Newer biotechnological tools that are becoming increasingly affordable may offer new advantages to breeders and plant pathologists. Large data sets generated by genomics and genomic platforms (SNP assays), transcriptomics, metabolomics, and mycotoxicology may better help elucidate the *Fusarium*-cereal interaction. FHB visual symptoms are not always predictive of DON content of matured grains, where this relationship is particularly weak in barley. An Illumina 50K SNP bead-assay is under evaluation as a tool to conduct genomic selection for low DON content in barley, where preliminary results indicate this could be a cost-efficient means for resistance breeding. RNA-sequencing has been employed to investigate differential gene expression of resistance to DON accumulation, using a pairwise contrast of a malting barley variety and a resistant, doubled-haploid derivative variety developed through *in vitro* selection with mycotoxin-laden growth media. In the past decade, several studies have documented the significance of mycotoxin conjugation as means of detoxification in planta, however, conjugated products are readily hydrolysed back to principal toxic state in the animal gut thereby posing a concealed risk to consumers. The ratio of DON-3-glucoside/DON in varieties has been investigated for wheat and barley, where this ratio appeared to be associated with level of resistance in wheat but demonstrated consistency in barley. While solutions to this disease has been extremely

difficult to obtain, the importance of cereals production as a global food staple warrants further work and new biotechnological products may assist in this undertaking.

Cutting down mycotoxins in cereals using molecular scissors

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Site specific nucleases (SSNs) are molecular scissors which can be programmed to cleave the DNA precisely at a pre-determined site of choice within an entire genome. Once a double strand break is induced, different genome modifications can be achieved depending on the repair pathway and the availability of a repair template. This is called SSNs-mediated genome editing.

I will describe the different SSNs available and especially the most used CRISPR/Cas9 system, highlighting the strengths and weaknesses of this genome editing technology. I will explain how SSNs can be exploited to achieve different outcomes in plants and will illustrate possible applications (and implications) to reduce mycotoxin levels in crops.

RNA-based strategies to control *Fusarium* diseases and mycotoxin contamination in agricultural crops

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Given current trends in both food and energy demands, it will be necessary to double crop yields worldwide during the next 50 years. Moreover, food production is threatened by microbial metabolites, such as mycotoxins, and their prevalence might increase by climate change. The demonstration that agricultural pests and pathogens are killed by exogenously supplied RNA targeting their essential genes has raised the possibility that plant diseases can be controlled by lethal RNA signals. The mechanism behind the RNA's activity is RNA interference, which can be exploited to control agronomically relevant plant diseases. *In vitro* feeding of dsRNA (the precursor molecule of sRNAs) and siRNA itself can induce post-transcriptional gene silencing (PTGS) of target genes in pests, including nematode and aphids. Expression of such RNAs in plants (host-induced gene silencing, HIGS) also confers protection from fungal infection and pest infestation [1]. Moreover, we recently discovered that spraying dsRNA/siRNA targeting sterol 14α-demethylase (*CYP51*) genes of the mycotoxin-producing fungus *Fusarium graminearum* reduced infections in barley and wheat, a phenomenon called spray-induced gene silencing (SIGS) [2].

Other labs also recently showed that spray applications of RNA to plants, including *Arabidopsis*, tomato and tobacco, is rather effective in controlling plant diseases caused by microbial pathogens and viruses [3]. Moreover, optimisation of RNA delivery by certain nanostructure-based formulations was demonstrated [4]. Based on those findings and new unpublished data from our lab, we will report on advances and setbacks in controlling *F. graminearum* by dsRNA application. Altogether, we will provide information on a fundamentally new plant protection strategy, thereby opening novel avenues for improving crop yields in an environmentally friendly and sustainable manner [5].

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Aflatoxin-free transgenic maize using host-induced gene silencing

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The World Health Organization estimates nearly 25% of global crops are contaminated with some fungal produced mycotoxin. Chief among these deleterious health compounds are aflatoxins – mycotoxins produced from a few species of the opportunistic *Aspergillus* fungi. Despite numerous effects to breed for fungal resistance or deter fungal growth by limiting moisture content during storage and/or using non-toxin producing fungal strains to outcompete toxin-producing varieties, there are millions of tons of crop losses due to this mycotoxin each year. We have shown that the variation of RNAi interference technology, host-induced gene silencing (HIGS), can be successfully used to engineer maize to suppress the aflatoxin pathway in contaminating *Aspergillus*. We expressed an RNAi suppression cassette in maize kernels that would target a biosynthetic enzyme, polyketide synthase, in the *Aspergillus* aflatoxin pathway. Transgenic maize plants were produced and analysed for the incorporation and expression of the inserted RNAi cassette. During cob development, kernels of both transgenic lines that demonstrated expression of the RNAi cassette and non-transgenic control plants were subjected to infection with a known toxin-producing *Aspergillus* strain. After a month-long infection, kernels surrounding infections sites were harvested and assayed for toxin accumulation. Aflatoxin was detected in all non-transgenic kernels with levels ranging from 1,000-220,000 ppb while no toxin was detected in any of the transgenic kernels. Transgenic RNAi plants did not display any gross morphological differences. Total RNA analysis from kernels of both transgenic and non-transgenic samples were analysed to detect if the inserted RNAi caused any unintentional gene suppression events. Our analysis showed no significant differences at the total RNA level in the transgenic maize kernels compared to side-by-side growth non-transgenic kernels. This research was the first demonstration that HIGS can be used to suppress a biosynthetic pathway in a plant pathogen. It also shows that HIGS can be a powerful tool to alleviate aflatoxin contamination in crops and significantly contribute to the enhancement of global food safety and security.

The regulatory landscape for plant breeding techniques – hurdles and opportunities for breeding to reduce mycotoxins

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Emerging techniques to manage the genetic material of crop plants and pathogens are challenging the regulatory frameworks for gene techniques in several regions and countries. One example is the regulatory framework for genetically modified organisms (GMOs) in the European Union (EU), which is based on the use and stable incorporation of recombinant nucleic acids in a manner that could not have occurred without human intervention, making the regulatory status of several genome editing techniques a matter of interpretation as these may use recombinant nucleic acids in the process but creating only minor mutations in the final product. Novel regulatory approaches are emerging in some countries, such as Argentina and more recently Brazil, whereas key events for policy development are currently unfolding in the EU.

'Classical' GMO techniques, as well as emerging genome editing techniques, can be applied to develop crop plants with a higher degree of resistance to fungal infection, with a concomitant decrease in mycotoxin levels. Case studies will be presented, such as the case of Bt maize (GMO) cultivation in Italy, and the application of mutagenesis (induced and directed) in breeding for *Fusarium*-resistant oat in Sweden. This is followed by an outline of actual and potential regulatory obstacles and opportunities, with a focus on the situation in the EU but with reference also to the situation in other countries and regions.

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POSTER ABSTRACTS

P1 – P73
OCCURRENCE, EXPOSURE AND EFFECTS

P1 Solute and matric potential effects on growth and ochratoxin A production by *Penicillium verrucosum* and *Aspergillus westerdijkiae* contaminating wheat and coffee
Shaimaa Abdelmohsen, A. Medina and N. Magan
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P2 Aflatoxin contamination and microbial phylogeny of aflatoxins producing fungi from roasted cashew nuts
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P3 Risk-benefit assessment in foods: a case study involving mycotoxins
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P4 Survey of ochratoxin A, deoxynivalenol, citrinin and sterigmatocystin in craft and industrial beers
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P5 *Aspergillus versicolor* and sterigmatocystin occurrence in paddy rice during in field growth
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P6 Is coffee a significant source of mycotoxins exposure? From analytical method development to safety risk assessment
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P7 Clinical samples and threshold levels of common fungal toxins found in Western United States
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P8 Kinetic behaviour of deoxynivalenol in pigs
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[1]Nutriad International, Belgium and [2]Department of Pharmacology, Toxicology and Biochemistry, Ghent University, Belgium

P9 Systemic and immunological effects on piglets exposed to diets contaminated with deoxynivalenol and the metabolite 3-epi-deoxynivalenol
E.O. Silva1, A. Pierron[2], J.R. Gerez1, T. Zou[2], P. Pinton2, I.P. Oswald[2] and **Ana Paula Bracarense**[1]
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P10 Phytic acid modulates the oxidative stress induced by deoxynivalenol in intestinal explants
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P11 Effects of fumonisin B1 and phytic acid on the oxidative stress: an *ex vivo* approach
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P12 Impact of climate change on *Aspergillus flavus* and *Fusarium verticillioides* interaction
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P13 Occurrence of citrinin in red yeast rice from *Monascus purpureus*
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P14 Studies on the occurrence of mycotoxins and mycotoxigenic species in malting barley in central Italy
G. Beccari[1], L. Caproni[1,3], M.T. Senatore[1], F. Tini[1], U. Bonciarelli[1], A. Onofri[1], A. Prodi[2], S. Uhlig[3], M. Sulyok[4] and **Lorenzo Covarelli**[1]
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P15 Geographical effect on the occurrence of secondary metabolites and their producing fungal species in durum wheat kernels from Italy
G. Beccari[1], M.T. Senatore[1,2], L. Pedini[1], F. Tini[1], A. Prodi[2], P. Nipoti[2], V. Balmas[3], M. Sulyok[4] and **Lorenzo Covarelli**[1]
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P16 The effect of deoxynivalenol on selected populations of immunocompetent cells in porcine blood
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P17 Toxicological assessment of *Aspergillus flavus* metabolites in resistant Kenyan maize varieties
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P18 An integrated *in silico/in vitro* approach to assess the xenoestrogenic potential of *Alternaria* mycotoxins and metabolites
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P19 Toxicodynamic as a beacon to foster the hazard identification of mycotoxins in the light of the group-based approach – an *in silico* perspective on zearalenone and deoxynivalenol
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P20 Determination of aflatoxin in food supplements by means of ultra-high performance liquid chromatography coupled to tandem mass spectrometry
Alessandra Di Sante[1], E. Bafile[1], E. Galantini[1], M. Sergi[2], V. Gnagnarelli[2] and D. Compagnone[2]
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P21 Determination of mycotoxins content in feed and feed raw materials by high performance l iquid chromatography with mass spectrometric detection
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Russian State Center for Quality and Standardization of Veterinary Drugs and Feed, Russia

P22 Mycotoxins in beer and coffee obtained at the Czech market
Zbynek Dzuman, P. Jonatova, M. Stranska-Zachariasova and J. Hajslova
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P23 Occurrence of multiple mycotoxins in DDGS determined by ultra-high performance liquid chromatography coupled to tandem mass spectrometry
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P24 Carryover of dietary aflatoxin B1 to matrinxã (*Brycon cephalus*) fish tissues and effects in liver
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P25 Long term exposure of pacu (*Piaractus mesopotamicus*) fish to dietary aflatoxin B1: accumulation in tissues and hepatic changes
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P26 A preliminary study on the influence of deoxynivalenol and zearalenone on the immuno-histochemical expression of oestrogen receptors and genes encoding selected liver enzymes in pre-pubertal gilts
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P27 Chronic ingestion of deoxynivalenol induces oxidative stress in the kidney of rats
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P28 Individual and combined effects of fumonisins and deoxynivalenol on the oxidative stress in ovaries
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P29 Potential fumonisin-producing *Aspergillus* section *Nigri* species in food products from Spain
Jéssica Gil-Serna, M. García-Díaz, C. Vázquez, M.T. González-Jaén and B. Patiño
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P30 Zearalenone and α-zearalenol levels in the bile of swine exposed to zearalenone with and without antimycotoxin additive inclusion in the diet
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P31 Clinical findings after indoor trichothecenes (DON, T-2) exposure: analysis of outcomes, immuno-suppression and impaired genetic MTHFR detoxification status
Irene H. Grant, J.N. Dillard, J.B. Geller and H.Z. Sabath
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P32 Mycotoxin mixtures in food and feed: holistic, innovative, flexible risk assessment modelling approach – MYCHIF
Athanasios Gkrillas[1], C. Brera[2], K. Campbell[3], R. Palumbo[4], P. Battilani[4], I. Oswald, P. Toscano[6], A. Venancio[7] and C. Dall'Asta[1]
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P33 Oxidative stress: a link between drought and aflatoxin contamination in maize
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P34 Weather conditions associated with mycotoxin accumulation in Norwegian oats
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P35 Monitoring human exposure to aflatoxin B1 by detection of aflatoxin M1 in urine by ELISA
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P36 The effect of black point in wheat grain on deoxynivalenol production during storage
Sigita Janavičienė, A. Jonavičienė, R. Semaškienė, Y. Kochiieru and A. Mankevičienė
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P37 The effect of harvesting time on mycotoxin production in the grain of spring cereals
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P38 Biomarkers as a tool to better understand the effect of mycotoxins on gut barrier functioning and immunosuppression
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P39 Effect of climate changes on annual variation of mycotoxins profile in maize from Republic of Serbia
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P40 Comparative analysis of mycotoxin occurrence in different types of forage
Julia Laurain[1], E. Marengue[2] and M.A. Rodriguez[1]
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P41 Sicilian wheat is good for your health: survey of multi-mycotoxin occurrence in durum wheat grain from Sicily
Andrea Macaluso, V. Giaccone, L. La Scala, L. Pantano, I. Calabrese, A. Cicero, A. Vella and V. Ferrantelli
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P42 Study of correlation of aflatoxins in feed, milk and urine in dairy cows
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P43 Impacts of climate change environmental factors on colonisation and mycotoxin contamination of food commodities
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P44 A survey of mycotoxins in livestock feeds in Ethiopia
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P45 Determination of zearalenone, zeranol and other resorcyclic acid lactones in bovine and human hair
Hans Mol, M. Essers, A. Repossi, M. Groot, M. de Nijs and S. Sterk
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P46 Worldwide mycotoxin occurrence in feeds and raw materials – survey data 2017
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P47 Expression of human Th1/Th2 cytokines by human hepatocytes exposed to filamentous fungi and their combinations
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P48 Fungal and mycotoxin contamination of small-scale maize from drought affected North-West province of South Africa
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P49 Targeted approaches for ZEN biomarker discovery in prepubertal gilts
B. Grenier, **Veronika Nagl**, A. Lutz, M. Aleschko, G. Schatzmayr, W.-D. Moll and M. Thamhesl
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P50 Survey on citrinin in food and red yeast rice
Monique de Nijs[1], M. Spanjer[2], A. Pietri[3], T. Bertuzzi[3], A. Starski[4], J. Postupolski[4], M. Castellari[5], M. Hortós[5] and P. López[1,6]
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P51 Biodiversity of *Aspergillus* species and aflatoxin contamination of dairy feeds from farms around Bulawayo, Zimbabwe
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P52 Oxidative stress induced by ‘emerging mycotoxins’ in an intestinal porcine epithelial cell line
Barbara Novak, I. Reihs, G. Schatzmayr and E. Mayer
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P53 Occurrence and identification of *Aspergillus* of the *Flavi* section and aflatoxins emergence in French maize
Beatrice Orlando[1], S. Bailly[2], A. El Mahgubi[2], A. Carvajal-Campos[2], O. Puel[2], I.P. Oswald[2] and J.D. Bailly[2]
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P54 Exposure assessment of aflatoxin from dried figs in Turkey
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P55 *Alternaria* contamination of apples for food processing
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P56 Contamination of flours with deoxynivalenol in Thailand
T. Sornchumni, J. Promrat and **Awanwee Petchkongkaew**
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P57 A survey on mycotoxins detected in maize samples received from Serbia and Bosnia and Herzegovina during August to November 2017
Jog Raj, H. Farkaš, R. Čepela, I. Pol, J. Bošnjak-Neumüller and M. Vasiljević
Patent Co., DOO, Serbia

P58 Mycotoxins in commercially available beer in Lleida (Spain) and exposure assessment of the population
X. Pascari, J. Ortiz Solá, S. Marín, **Antonio J. Ramos** and V. Sanchis
Applied Mycology Unit, Food Technology Department, University of Lleida, Spain

P59 Ochratoxin A formation and detection in rye lots
Mareike Reichel, J. Gottwald, A. Merse and J.S. Mänz
Eurofins WEJ Contaminants, Germany

P60 Evaluation of deoxynivalenol-sulfate as a useful biomarker of deoxynivalenol exposure in broilers chickens
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P61 Tolerance of feed regulated mycotoxins by lesser mealworm and black soldier fly from artificially contaminated substrates
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P62 Combination of zearalenone, alternariol and genistein negatively impacts porcine embryo development *in vitro*
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P63 Oocytes from gilts are more sensitive to beauvericin than sow oocytes: a possible difference in antioxidant capacity
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P64 Occurrence of ergot and mycotoxins in Western Canada
Warren Schwab[1], T. Grusie[1] and B. Blakley[1,2,3]
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P65 Three major *Fusarium* mycotoxins modulate the barrier function of *in vitro* human placental cells.
Negisa Seyed Toutounchi[1], A.H. Hogenkamp[1,2], S. Varasteh1,2, P.V. Jeurink[1,2], J. Garssen[1,2], A.D. Kraneveld[1,3], G. Folkerts[1] and S. Braber[1,2]
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P66 Accumulation of ochratoxin A in the presence of gluconic acid contributes to pathogenicity o60 *Aspergillus carbonarius*
U. Maor[1,2], V. Zakin[1], D. Prusky[1] and **Edward Sionov**[1]
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P67 Deoxynivalenol induces nephrotoxic effects in an *ex vivo* model
Weslem G.S. Suhett, J.R. Gerez, F. Pinho, M.F.G. Santons and A.P.F.L.R. Bracarense
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P68 Deoxynivalenol induces changes in the antioxidant response in renal explants
Weslem G.S. Suhett, J.R. Gerez, M. Hohmann, W.A. Verri-Júnior and A.P.F.L.R. Bracarense
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P69 Individual and multitoxic effects of fumonisin B1, deoxynivalenol and zearalenone on rat liver and kidney phospholipids and lipid peroxidation
András Szabó[1,2], J. Szabó-Fodor[2], H. Fébel[3], M. Mézes[4], K. Balogh[4], G. Bázár[5], D. Kocsó[2], O. Ali[5] and M. Kovács[2]
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P70 Localised variability in the *Fusarium* damage – DON relationships for Canadian wheat in 2016
Sheryl A. Tittlemier[1], J. Arsiuta[2], U. Mohammad[2], C. Hainrich[3], K. Bowler[3], R. Croom[3], A. Olubodun[3], R. Blagden[1], T. McKendry[1] and K. Pleskach[1]
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P71 Aflatoxin exposure among infants and young children in the Northern Province of Rwanda
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P72 Growth and T-2 and HT-2 production by *Fusarium langsethiae* under climate change environmental factors
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P73 The influence of deoxynivalenol and zearalenone on the immunohistochemical expression of oestrogen receptors and genes encoding detoxification enzymes in pre-pubertal gilts
Łukasz Zielonka[1], M. Gajęcka[1], M. Dąbrowski[1], I. Otrocka-Domagała[2], P. Brzuzan[3] and M.T. Gajęcki[1]
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MANAGING THE IMPACT OF MYCOTOXINS

P74 The capability of plant-derived *Lactobacillus plantarum* BCC47723 to reduce mycotoxins
Saowalak Adunphatcharaphon[1], V. Dascanio[2], D. Greco[2], G. Avantaggiato[2], W. Visessanguan[3] and A. Petchkongkaew[1]
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P75 Effect of sodium meta-bisulphite (NaMBS) and selected preservatives on *in vitro* growth and aflatoxin B1 production by strains of *Aspergillus flavus* isolated from chillies
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P76 The effect of *Fusarium* mycotoxins and a mycotoxin deactivator on performance and metabolism of early lactation autumn calving dairy cows
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P77 Effect of two mycotoxin deactivators on the reduction of aflatoxin M1 levels in milk of lactating dairy cows fed aflatoxin B1
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P78 Effects of smectites clay on the growth performance and immunological function of broiler chickens fed diets contaminated with aflatoxin B1
Paul Bruinenberg[2], G. Hu[1], P. Ding[1], Z. Song[1], Z. Fan[1], S. Zhang[1], Y. Han[2], Y. Wu[2] and X. He[1]
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P79 *In vitro* adsorption of multiple mycotoxins and vitamin B1 by activated charcoal: is there a chance to improve selectivity?
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P80 Control of aflatoxin contamination in maize using host-induced gene silencing of *Aspergillus flavus* genes
Jeffrey W. Cary[1], K. Rajasekaran[1], R. Majumdar[1], M. Gilbert[1], C. Sickler[1], Q. Wei[1], C. Carter-Wientjes[1], M. Lebar[1] and Z.-Y. Chen[2]
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P81 Ochratoxin A biodetoxification experiment in a human kidney cell line
A.-N. Mohammed, C. Guti, D. Márton, B. Kriszt and **Mátyás Cserháti**
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P82 MYTOX-SOUTH: an intercontinental partnership to improve food security and food safety in developing countries
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P83 On the mitigation of mycotoxins content: the strange case of aflatoxin B1 and laccases from *Trametes versicolor*
Luca Dellafiora and G. Galaverna
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P84 Chemotyping ergot alkaloid diversity for crop selection and management
Jennifer M. Duringer[1], J. Dung[2], L. Blythe[3] and A. Morrie Craig[3]
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P85 Integrating 'omics' approaches for investigating aflatoxin production by *Aspergillus flavus* under drought stress conditions
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P86 Modulation of AFB1 cytotoxicity by natural antioxidants and PCB126 in a bovine mammary epithelial cell line
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P87 Effectiveness of essential oils and their derivatives to prevent mycotoxin production by *Aspergillus* species
M. García-Díaz, B. Patiño, C. Vázquez, **María Teresa González-Jaén** and J. Gil-Serna
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P88 Application of zearalenone hydrolase ZenA as a zearalenone-degrading feed additive in chickens
M. Aleschko, M. Killinger, A. Höbartner, B. Doupovec, J. Faas, **Christiane Gruber-Dorninger**, D. Schatzmayr and W.-D. Moll
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P89 Combination of anti-mycotoxin additives, vitamins and plant extracts in growing-finishing diets may not be beneficial to pigs when added to feed contaminated with deoxynivalenol
David Guillou, N. Lemoine, C. Messant and C. Favier
Mixscience, France

P90 The fate of *Alternaria* toxins during fermentation of whole wheat dough
Elizabet Janić Hajnal[1], D. Orčić[2], J. Kos[1] and J. Mastilović[1]
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P91 FDA Center for Veterinary Medicine: challenges and lesson learned in the regulation and approval of food additives to eliminate or reduce mycotoxins in animal food
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P93 Enzymatic detoxification of mycotoxins in the bioethanol process
Daniela Kotz, S. Rose, D. Schatzmayr and G. Schatzmayr
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P94 Efficacy of an activated and purified smectite binding agent on the toxicological effects of aflatoxins in the diets of weaned piglets
S. Rattanatabtimtong[1], T. Hongsapak[1], K. Sa-ard rak[1], L.M. Pineda[2], **Kai Kuehlmann**[3] and Y. Han[2]
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P95 Enzymatic detoxification of deoxynivalenol by epimerisation
W.-J. He[1], S.-Y. Yi[1,2], H.-P. Li[1], J.-B. Zhang[1,2] and **Yu-Cai Liao**[1,2,3]
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P96 *Fusarium graminearum* mycotoxins in maize associated with *Striacosta albicosta* (*Lepidoptera*: *Noctuidae*) injury
J.L. Smith, **Victor Limay-Rios**, D.C. Hooker and A.W. Schaafsma
Department of Plant Agriculture, University of Guelph, Canada

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Victor Limay-Rios[1], J.D. Miller[2] and A.W. Schaafsma[1]
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P98 Antifungal compounds from *Streptomyces* AS1: efficacy against *Penicillium verrucosum* growth and ochratoxin A production
A. Mohd Danial, A. Medina and **Naresh Magan**
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P99 Potential biocontrol agents for control of *Aspergillus flavus* and aflatoxin B1 production
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P100 Antifungal activity of the allyl isothiocyanate against *Aspergillus flavus* and *Penicillium verrucosum* in maize, wheat and barley
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Colm A. Moran[1], A. Yiannikouris[2], J.D. Keegan[3], K. Vienola[4] and J. Apajalahti[4]
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P104 The effect of a heterotrophically grown *Chlorella* microalgal biomass on the deposition of ochratoxin A in the liver of broiler chicks
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P105 Using a poly-omic strategy to unravel the genetic and mycotoxin profile variability of *Fusarium proliferatumin* different host plants
Antonio Moretti[1], R.H. Proctor[2], M. De Boevre[3], S. De Saeger[3], J.D. Di Mavungu[3,4], A. Villani[1], M. Haidukowski[1], S. Somma[1], T.A.J. van der Lee[5], C. Waalwijk[6], A.F. Logrieco[1] and A. Susca[1]
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P106 The impact of storage condition and their effect on animal feed quality regarding mycotoxin contamination in the North West province, South Africa
K.G Setsetse and **Mulunda Mwanza**
Department of Animal Health, North-West University, South Africa

P107 Transcriptomic analysis of the anti-aflatoxigenic property of dimethyl sulfoxide on *Aspergillus flavus*
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P108 *In vitro* mycotoxin binding studies using Minazel Plus® in artificial porcine gastrointestinal digestive juices
H. Farkaš, **Jog Raj**, J. Bošnjak-Neumüller and M. Vasiljević
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Applied Mycology Unit, Food Technology Department, University of Lleida, Spain

P110 Effect of addition of yeast cell wall to broiler diets contaminated with aflatoxin B1 on performance and relative organ weights of broiler chickens
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P111 Efficacy of an algo-clay complex on decreasing mycotoxins liver toxicity in broilers
María Ángeles Rodríguez Quirós, J. Laurain and M. Garcia Suarez
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P112 Counteracting the negative effects of zearalenone: the role of phytoestrogens' metabolites
T.E.S. Silva[1], D.B.C. Brito[1], N.A.R. Sa[1], Y.P. Silva[1], R.F. Silva[1], F. Molist[2], J.R. Figuiredo[1] and **Regiane R. Santos**[2]
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P113 Priming for protecting maize seedlings from accumulation of fumonisins in early stages of infection by the mycotoxinogenic fungus *Fusarium verticillioides*
A. Aguado[1], **Jean-Michel Savoie**[2], S. Chéreau[2], M. Aguilar[1], N. Ferrer[2], C. Ducos[2], L. Pinson-Gadais[2] and F. Richard-Forget[2]
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P114 Mycotoxin reduction by advanced grain cleaning solutions
Katarina Slettengren[1], M. Pascale[3], M. Hirschberger[1], M. Graeber[1], M. Reichel[2], B. Conde[1], G. Panzarini[3], S. Cervellieri[3] and A.F. Logrieco[3]
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Kevin Vanneste, J. Vande Ginste and K. Lannoo
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Cui Wang, F. Xu, R.C. Baker and J. Ren
Global Food Safety Center, Mars Incorporated, China

P118 Denaturation of aflatoxins in naturally contaminated peanuts using ozone
Y. Li, Y. Chang, R.C. Baker and **Guangtao Zhang**
Global Food Safety Center, Mars Incorporated, Beijing, China

P119 Clay mineral based mycotoxin binders can interfere with the analysis of aflatoxins by ELISA
H. Peng, R.C. Baker and **Guangtao Zhang**
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M. Rice[1], Q. Shen[1], M. Lochhead[2], C. Elliott[1] and **Katrina Campbell**[1]
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P121 Simultaneous determination of 42 mycotoxins in oats: a high-throughput UHPLC-MS/MS analytical method
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P122 Determination of deoxynivalenol in dry distillers grain with solubles and a comparison to mean results obtained at several analytical laboratories
Dana Dvoracek-Driksna[1], E. Bergeron[1], B. Strong[1], A. Kostin[1], F. Klein[1], P. Frasco[1], M. Gadola[1], R. Sarver[1], S. Van Hulzen[2], C. Wulf[2] and C. Nelson[2]
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Neogen Corporation, USA

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E.R. Barrey[1], O.I. Shimelis[1] **Christine Dumas**[2]
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Lynsey Finlay, C. Elliott and K. Campbell
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Marlous Focker[1], H.J. van der Fels-Klerx[1] and A.G.H.M. Oude Lansink[2]
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P128 Assessment of extraction efficiency of ergot alkaloids
Taylor Grusie[1], W. Schwab[1], D. Pollock[2,3] and B. Blakley[1,2,3]
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Jana Hajslova, V. Hrbek, F. Kratky, Z. Dzuman and M. Tomaniova
Department of Food Analysis and Nutrition, University of Chemical Technology Prague, Czech Republic

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Petra Jonatova, Z. Dzuman, J. Hajslova and M. Stranska-Zachariasova
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P131 Efficient detection and quantification of T-2 toxin in grain and animal feed
Beatrice Kachel, T.P. Huynh and W. Wolde-Mariam
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Elizabeth Manning
R-Biopharm Rhône, UK

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M. Pazdanska, R. Rhemrev, E. Marley, C. Donnelly and **Elizabeth Manning**
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Susan MacDonald1, J. Christy[1], P. Hepworth[1], S. Jardine[1] and C. Donnelly[1]
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P135 Determination of multiple mycotoxins in oats by 11+ MS PREP immunoaffinity column clean-up and LC-MS/MS
Susan MacDonald[1], J. Stratton[1], C. Milligan[2] and C. Donnelly[2]
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Maike Makowski[1], M. Werneburg[2], C. Jung[1], H. Haase[3] and M. Koch[1]
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B.M. Kunz, S. Kemmlein, A. Bahlmann and **Ronald Maul**
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P. Brown, J. McGeehan, J. Wilcox, E. Marley and **Claire Milligan**
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P140 Multi-toxin analysis using immunoaffinity column clean-up for a range of cereal samples prior to LC-MS/MS detection
J. McGeehan, J. Wilcox, **Claire Milligan** and C. Donnelly
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Hans Mol, D. van der Horst, R. van Dam and M. de Nijs
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P142 European Union Reference Laboratory mycotoxins and plant toxins
Monique de Nijs, H. Mol, P. Mulder and R. Hoogenboom
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P143 Development of a multiplex biosensor for mycotoxins in feed and food
Philana Nolan[1], S. Auer[2], A. Spehar[2], C. Elliott[1] and K. Campbell[1]
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P144 Aflatoxin M1 analysis of non-skimmed bovine raw milk with I'screen AFLA M1 ELISA kit
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P145 High-throughput microarray-based enzyme-linked immunosorbent assay
G. Rosar, F. Bravin, F. Diana and **Lidija Persic**
Tecna S.r.l., Italy

P146 Testing for cross reactivity in commercial available kits for DON and DON-like compounds in wheat and barley
Kerri Pleskach, T. Zirdum and S.A. Tittlemier
Grain Research Laboratory, Canadian Grain Commission, Canada

P147 Solid phase extraction on carbon nanomaterials followed by liquid chromatography-mass spectrometry for the determination of *Fusarium* mycotoxins in agricultural products
Ingars Reinholds[1], E. Bogdanova[1], I. Pugajeva[1], I. Rozentale[1], J. Jaunbergs[1], S. Gaidukovs[2] and V. Bartkevics[1]
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P148 Myco 10, a biochip array containing ten discrete immunoassays for the simultaneous detection of a broad range of mycotoxins in animal feed
M. Plotan, R. Devlin, J. Porter, M.E. Benchikh, **Liberty Sibanda**, M.L. Rodríguez, R.I. McConnell and S.P. FitzGerald
Randox Food Diagnostics, UK

P149 Biochip-based immunoassay for the detection of ergot alkaloids in animal feed
M. Plotan, R. Devlin, J. Porter, M.E. Benchikh, **Liberty Sibanda**, M.L. Rodríguez, R.I. McConnell and S.P. FitzGerald
Randox Food Diagnostics, UK

P150 Quantitative analyses of natural contaminants and anthropogenic residues in animal feed by LC-ESI-MS/MS: influence of absolute and relative matrix effects on the method performance
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P151 An approach to *Fusarium* regulated toxins analysis by LC-MS/MS: the complexity of quantifying different food matrices
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Laboratori de l'Agència de Salut Pública de Barcelona, Spain

P152 Validation of LC-MS/MS Based multi-toxin methods – how to manage the workload and which data are essential?
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P153 LC-MS retention index for normalised mycotoxin analysis
J.B. Renaud and **Mark W. Sumarah**
London Research & Developoment Centre, Agriculture and Agri-Food Canada, Canada

P154 Lateral flow based method – fit for the purpose of screening feed ingredients?
Ellen van 't Veer-Luiten and A. Najera
MasterLab, Nutreco, the Netherlands

P155 Fully automated mycotoxin analysis from extract to chromatogram at a sensitivity in the ppt or ppq level using not only LC-MS/MS but HPLC-FLD.
Frederik Wuppermann
LCTech, Germany

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Alternaria spp
Fusarium spp
Claviceps (Ergot)

OCCURRENCE, EXPOSURE AND EFFECTS
P1 – P73

P1

Solute and matric potential effects on growth and ochratoxin A production by *Penicillium verrucosum* and *Aspergillus westerdijkiae* contaminating wheat and coffee

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Ochratoxin A (OTA) contamination of wheat and coffee is caused by *Penicillium verrucosum* and *Aspergillus westerdijkiae*. These fungi usually survive in soil and on crop debris and colonise cereals and coffee respectively, during harvesting and storage. There is thus interest in understanding the relationship between key environmental factors which influence growth and mycotoxin production. This study has examined the effect of interacting conditions of water stress x temperature on growth and OTA production by strains of these two fungal species. The effect of non-ionic, ionic and matric stress on growth was compared at different temperatures. The impact on relative amounts of OTA which was produced on a conducive yeast extract sucrose medium (YES) and on wheat-based medium was examined for *P. verrucosum*. This showed that there were significant differences in the relative tolerance of solute and matric potentials for these two fungi. The minimum water activity (a_w) for growth in glycerol-amended medium was 0.90, for ionic solute (NaCl) 0.86, and for matric stress 0.86. The optimum a_w x temperature conditions for OTA production on non-ionic amended media by *P. verrucosum* was at 0.98 a_w and 25°C. For *A. westerdijkiae* this was also 0.98 aw and 25°C. Studies are in progress to better understand the molecular basis for the differences between these types of water stress. Studies are also being carried out to screen different natural preservatives to control growth and OTA production by these two species in wheat and coffee. Climate change impacts on contamination will also be investigated in due course.

P2

Aflatoxin contamination and microbial phylogeny of aflatoxins producing fungi from roasted cashew nuts

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Cashew nuts are one of the nuts that are highly susceptible to aflatoxin contamination. This study aimed at examining the microbial profile and phylogeny of mycotoxins producing fungi in roasted cashew nuts samples vis-a-vis its safety for human consumption. A total of 12 different composite cashew nuts meant for human consumption were examined for fungi load and identity, molecular phylogenetic analysis and total aflatoxins using the direct plating, PCR, distance and maximum likelihood (ML) phylogenetic methods and HPLC, respectively. The fungal count ranged from 0-250, 0-350 and 0-1,300 cfu/g on PDA, SDA and DCRBA, respectively. A total of 65 isolates belonging to 18 fungi genera and 34 different species were identified molecularly with Aspergillus *niger* (63.6%), *A. flavus* (54.5%) and *Penicillium* spp. (36.4%) having predominance occurrence in the sample while *Trichoderma* (9.09%) and *Curvularia* (18.18%) spp. had the least occurrence in the sample. A moderate to strong (50-99%) phylogenetic homology existed between the sequence signature of the *Aspergillus* taxa from the cashew nuts with other aflatoxin producing fungi at the gene bank. The ML tree also showed that some *Aspergillus* spp. did not align with any of the reference taxa based on their uniqueness. Hence, they could be classified as novel species with unique nucleotide signature pattern. Furthermore, the aflatoxin content of the nuts ranged from 0.03-0.77μg/kg with all the concentrations lower than the EU recommended permissible limit of 4 μg/kg for total aflatoxins. Cashew nuts should be a good substitute to other nuts that are susceptible to aflatoxins contamination.

P3

Risk-benefit assessment in foods: a case study involving mycotoxins

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Over the last years, the contamination of different foodstuffs with multiple mycotoxins has been highly reported. Data from a recent Portuguese national project that studied the toxic effects of exposure of children under 3 years old to multiple mycotoxins in infant foods (MYCOMIX) reported the co-occurrence of 21 mycotoxins and metabolites present in breakfast cereals primarily marketed for children. This study showed that almost all the analysed breakfast cereal samples (96%) were contaminated with mycotoxins. The output of this project also highlighted the knowledge gaps on the contra-balance beneficial health effect of these foods, and the need to determine the risk-benefit balance, since the evaluated food products, namely breakfast cereals, are simultaneously recognized as vehicles of food components, such as nutrients, vitamins and water soluble and insoluble fibres, which could be assumed as beneficial for children health. Health risks associated with consumption of cereal-based foods, an important source of nutrients with beneficial health effects, could increase in the near future due to climate changes in Europe (dry conditions and increased ambient temperatures), thus the dissemination and use of risk-benefit assessment (RBA) harmonized tools in Europe would be of utmost importance to support food and health policies. Can we ever have a harmonized tool that enables food and health authorities to estimate the balance between risk and benefit of foods usually contaminated by mycotoxins, such as cereals-based products? This is a question that can be raised to contribute to brainstorming under the topic of the 10th conference of The World Mycotoxin Forum. 'RiskBenefit4EU – Partnering to strengthen the risk-benefit assessment within EU using a holistic approach' is a recent European pilot project funded by EFSA and coordinated by Portugal, integrating a multidisciplinary team from health and food institutes, national food safety authorities, R&D institutions and academia from Portugal, Denmark and France. The main objectives of this project concern the development of a set of RBA tools that can estimate the overall health effects of foods, food ingredients and diets and that can be applied to data from different countries. RiskBenefit4EU aims to strengthen the EU capacity to assess and integrate food risks and benefits in the areas of microbiological, nutritional and chemical components through the development of a harmonized framework. This pilot project will validate the RBA framework created using a Portuguese case study on breakfast cereals, including results obtained under the MYCOMIX project.**Acknowledgments**. Financial support by 'RiskBenefit4EU – Partnering to strengthen the risk-benefit assessment within EU using a holistic approach' (Grant Agreement Number GP/EFSA/AFSCO/2017/01 - GA02); Projeto Incentivo de Estudos de Biomonitorização Humana de Âmbito Nacional /BioMAN, BioMAN/DAN/01) by INSA; and CESAM by the Portuguese Foundation for Science and Technology (FCT) (UID/AMB/50017/2013).

P4

Survey of ochratoxin A, deoxynivalenol, citrinin and sterigmatocystin in craft and industrial beers

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Occurrence of ochratoxin A (OTA) and deoxynivalenol (DON) in beer was reported in several studies; their contamination was probably due to storage conditions of malted grains. Other mycotoxins were rarely detected in beers. For sterigmatocystin (STC) and citrinin (CIT), EFSA recently requested data on their presence in food [EFSA, 2012. EFSA Journal 10:2605; EFSA, 2013. EFSA Journal 11:3254]; STC was detected in few samples of European beers [Veršilovskis *et al.*, 2008. World Mycotoxin Journal 1:161]. In the last decade, consumption of craft beers, a heterogeneous class of beers, sometimes brewed from unusual ingredients, increased in Europe and USA. However, craft beers are scarcely investigated for the content of undesirable components, as mycotoxins. Occurrence of both known and emerging mycotoxins was performed in this survey. Sampling of most sold 80 beers (40 craft and 40 industrial) in Italy was carried out. Three sub-samples were collected in different retail outlets in the period June-August 2017, mixed and two samples (about 100 ml each) were kept at -20°C. Before the analysis, each sample was gently shaken and degassed by ultrasonication. The analyses were carried out using both liquid and gas chromatographic instruments, coupled to fluorimeter or mass spectrometers. The limit of detection was 0.5 ng/l for OTA and STC, 4 µg/l for DON, 5 ng/l for CIT. OTA

was detected in 45.8% of the samples (28.6 and 63.4% for craft and industrial beers, respectively) showing a maximum value of 70 ng/l (craft beer); OTA level was higher than 30 ng/l only in 5 samples, while it was below 10 ng/l in 52.6% of the positive samples. DON occurred in 25.3% of samples (35.7 and 14.6% for craft and industrial beers, respectively) with an overall mean value of 7.1±16.9 μg/l (maximum value of 99 μg/l for a craft beer); DON concentration was higher than 20 μg/l in 12.0% of the samples. STC was found in 27.7% of samples (28.6 and 26.8% for craft and industrial beers, respectively) at very low levels (maximum value 18 ng/l in a craft beer). CIT was never detected. This survey showed that contamination with these mycotoxins in both craft and industrial beer occurred at low levels. **Acknowledgements**. The present work was supported by the European Foundation for Alcohol Research (ERAB).

P5

Aspergillus versicolor and sterigmatocystin occurrence in paddy rice during in field growth

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Italy is the main rice producer of Europe covering about 50% of the entire European production; rice cultivation is mostly located in Northern Italy (Piemonte, Lombardia, and Veneto) where water is relatively abundant and the rice crop can be cultivated in flooded fields. Italian rice is used for different products; in particular, rice destined to baby foods requires minimal levels of undesired substances. Recently, sterigmatocystin (STC) has often been detected in paddy rice and derived products [Mol *et al.*, 2016. World Mycotoxin Journal 9:633]. This study has been planned to evaluate the presence of *Aspergillus versicolor*, main producer of STC, and STC in paddy rice during in field growth.
Nine rice varieties, both long and round grain, and 3 sowing densities were considered in 3 fields located in the main Italian rice production region. Four varieties were cultivated for each field; 3 common varieties were cultivated in 2 fields. Soil texture and sowing period were different among the fields; while meteorological conditions (characterised by drought and high temperature) were similar among the fields because of their proximity (within 10 km). Presence of *A. versicolor* and STC was determined at 4 sampling times from flowering to harvest; moreover, for each plot, a sub-plot of rice crops was left in field at harvest and sampled 15 days after the harvest period. For each rice variety, *A. versicolor* and STC generally increased from post-flowering period to harvest, while different trends were observed between harvest and post-harvest. At harvest, incidence of *A. versicolor* in paddy rice was between 0% and 13% with respect to total fungal infection, while levels of STC ranged from 0.16 to 8.34 μg/kg. Rice produced in 1 field showed a highest *A. versicolor* incidence and STC contamination with respect to other fields. STC contamination was different among the varieties; it was lower than 1 μg/kg in 4 varieties (all round grain) and higher than 3.50 μg/kg in 3 (2 long and 1 round grain). Contamination levels of the 3 common varieties cultivated in 2 fields were slightly different. No remarkable difference was observed among the sowing density. Only for 1 rice variety (long grain) cultivated at highest density, *A. versicolor* incidence raised up to 13%; on the other hand, the STC increment was moderate. **Acknowledgements**. The present work was supported by the Lombardia region (PSR Baby Rice).

P6

Is coffee a significant source of mycotoxins exposure? From analytical method development to safety risk assessment

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Mycotoxins are secondary metabolites produced by filamentous fungi (moulds) that commonly contaminate a wide range of crops such as cereal grains, nuts, herbs and spices, fruits or oil seeds. Coffee beans are not known to be impacted by mycotoxins contamination, with the notable exception of ochratoxin A for which regulatory limits in coffee have been established in Europe (5 μg/kg in roasted coffee products, 10 μg/kg in soluble coffee products (Commission Regulation (EC) No 1881/2006). However, some recent publications have reported the co-occurrence of a large number of mycotoxins in coffee products at μg/kg or even mg/kg levels. Investigation was thus launched to evaluate the exposure to mycotoxins through coffee consumption. A new liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the detection of 31 mycotoxins in green coffee was developed and validated. Regulated but also emerging mycotoxins were considered and encompassed five metabolites of zearalanone, two metabolites of deoxynivalenol, three *Alternaria* toxins, four enniatins, beauvericin, nivalenol, diacetoxyscirpenol, neosolaniol and sterigmatocystin. Knowing that the analysis of roasted coffee is very challenging in terms of compound extractability, clean-up efficiency and matrix

effects impacting MS ionisation, the study focused only on green coffee with the ultimate goal to reach very low limits of quantification (LOQs). Such approach indeed represents a worst-case scenario, as mycotoxins are not expected to be formed during the roasting process. Our analytical procedure was based on a salting-out assisted liquid/liquid extraction with acetonitrile. Two different clean-up steps were then performed in parallel to reach a good sensitivity for all mycotoxins. Resulting extracts were then separately evaporated to dryness and reconstituted prior injection onto the LC-MS/MS system in two successive LC runs. Quantification was performed by the matrix-matched calibration approach. Positive identification of the analytes in samples was conducted according to the confirmation criteria defined in Document No. SANTE/11945/2015. LOQs ranged from 0.25 to 25 µg/kg (with the exception of nivalenol at 150 µg/kg). A comprehensive survey was performed on 85 green coffee beans from different coffee species, post-harvest processes, origins and ages. A safety risk assessment was initiated to elucidate whether the consumption of coffee is a significant source of mycotoxins exposure.

P7

Clinical samples and threshold levels of common fungal toxins found in Western United States

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The Endophyte Service Laboratory at OSU has a dual function. The first is service to seed/straw and pellet producers and veterinarians resulting in 2,700 to 3,000 samples per year. Exporters of straw require certificates that quantitate the toxins. Of particular interest is ergovaline in tall fescue, lolitrem B in perennial ryegrass samples, and any of these above toxins plus ergotamine from *Claviceps* in the products of pellet mills. After HPLC analyses, a certificate and chromatogram are sent to the producers. With high levels of any toxins, producers are advised to dilute the feed below a threshold level into safe feed. Approximately 200 to 300 samples per year are sent by veterinarians to verify or rule out these toxins as a cause of clinical disease. This requires knowledge of the threshold of the diseases in multiple species of animals. Experimental feeding trials have been done on ovine, bovine, and camelid species. Onset of clinical disease varies with the species of animal, the toxin, and with the environmental conditions. Cattle are a good example of the diversity of the clinical signs associated with environmental temperature. In hot areas such as the South-eastern United States, ergovaline levels above ~350 ppb cause ‘summer slump’, agalactia, and reproductive problems. In contrast, cattle subjected to very cold weather, i.e., near zero degrees C, and fed endophyte infected tall fescue straw at 250 ppb and above develop a fatal dry gangrene of their feet, ears and tails. The emerging problem of ergot in grasses and grains could also produce these clinical signs due to the vasoconstrictive properties of ergotamine, but thresholds have not been established. In another study, cattle eating perennial ryegrass at or above 2,000 ppb lolitrem B for 30 days will develop ryegrass staggers in 12 to 15 days. With a subsequent cattle feeding trial for 60 days, this threshold for disease dropped to levels of lolitrem at 1000 ppb or higher. Perennial ryegrass not only has lolitrem B as a toxin, but also ergovaline at approximately one-third of the concentration of lolitrem B. Camels are especially sensitive to lolitrem B in perennial ryegrass straw with ryegrass staggers seen at levels at and above 1000 ppb lolitrem B over 64 days. During late stages of pregnancy in camels, ingestion of perennial ryegrass with lolitrem B at 1000 ppb or higher and ergovaline at approximately 250 ppb, resulted in aborted or weak nonviable offspring.

P8

Kinetic behaviour of deoxynivalenol in pigs

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The objective of the study was to determine kinetic behaviour of deoxynivalenol (DON) in pigs. The study was conducted in six piglets of the same breed with females and males equally distributed, and of approximately similar body weight of 18.18 ± 1.55 kg. After two weeks of feeding a non-DON diet, an intragastric bolus of DON was administered to the animals at a dose of 0.05 mg/kg body weight. Based on the daily feed intake, the intragastric bolus resembled a DON contamination level of 1 mg/kg feed. This study focused on the plasma concentration-time profiles of DON and its metabolites de-epoxy deoxynivalenol (DOM-1) and DON-glucuronide, after an oral bolus administration of DON. Toxicokinetic modelling of the plasma concentration time profiles was done by non-compartmental analysis. The following parameters were calculated: area under the curve from time zero to the last sampling point ($AUC_{0\rightarrow 8h}$), area under the curve from time zero to infinite ($AUC_{0\rightarrow\infty}$), maximal plasma concentration

(C_{max}), time at maximal plasma concentration (T_{max}), elimination half-life time ($T_{1/2\ el}$), elimination rate constant (k_{el}), volume of distribution scaled for the absolute oral bioavailability (Vd/F) and total body clearance scaled for the absolute oral bioavailability (Cl/F). DON was rapidly absorbed from the gastrointestinal tract with maximum plasma concentration (C_{max}) 40 min after the oral administration (T_{max}). About 34, 58 and 84% of total absorbed DON appeared in blood within 2, 4 and 8 h, respectively. The plasma concentration-time profile for DON-glucuronide observed was similar to that of DON, and no DOM-1 was found in the blood serum of any experimental piglet. The half-life of serum DON ($T_{1/2\ el}$) was short and about 2 h 43 min showing rapid DON clean-up from the blood. The relative volume of DON distribution (Vd/F) was 1.7 l/kg, showing the low degree of mycotoxin distribution in body tissues rather than the plasma. The relative volume of plasma from which DON was completely removed per time unit (Cl/F) was 0.44 l/h/kg. In conclusion, orally administered DON in pigs was absorbed quickly and almost entirely rapidly metabolized in the liver to the phase II metabolite DON-glucuronide that was eliminated relatively quickly from the blood.

P9

Systemic and immunological effects on piglets exposed to diets contaminated with deoxynivalenol and the metabolite 3-epi-deoxynivalenol

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Food and feed are naturally contaminated with deoxynivalenol (DON) and its derivate modified forms. The derivate forms of DON can be the result of bacterial transformation (de-epoxy-DON, 3-epi-DON and 3-keto-DON). Data concerning DON-derived forms after bacterial transformations are scarce and the effects of these modified mycotoxins are not well established. Previous *in vitro* and *ex vivo* studies showed that 3-epi-DON induce no alteration in cell homeostasis. A better understanding of the systemic effects of 3-epi-DON using an *in vivo* model is important to the development of decontamination strategies, including bacteria/enzyme transformation of DON in metabolites with reduced toxicity. The purpose of the present study was to evaluate the morphological changes on intestine, lymphoid organs and liver, and the immunological effects through the expression of cytokines in swine exposed to acute intoxication with DON and 3-epi-DON. Eighteen 4-weeks-old weaned castrated male piglets (Pietrain/Duroc/Large-white) were used in the present study. Animals were submitted by gavage to the following treatments: control, DON (3 mg/kg feed) and epi-DON (3 mg/kg feed). Feed and water were provided *ad libitum* throughout the experimental period. After six days, piglets were euthanised and tissue samples processed for histological analysis and cytokines expression. A lesional score was established according to the intensity and severity of the histological changes. The expression of mRNA encoding for cytokines was evaluated by real time PCR. Data of the lesion scores and expression of cytokines were submitted to analysis of variance and Duncan's test considering $P \leq 0.05$. In the DON-exposed group, the main lesions in jejunum were flattening of enterocytes, villi fusion, apical necrosis and an increase in goblet cells density. In liver, disorganisation of hepatic cords, megalocytosis, cell vacuolation and mild necrosis of hepatocytes; and in lymphoid organs, lymphocyte apoptosis and hyperplasia of germinal centers were observed. Histological aspects in pigs fed 3-epi-DON diet remained similar to the control. A significant increase in the lesional score was observed in the small intestine, liver, lymph nodes and spleen of animals receiving DON-contaminated diet when compared to other diets. The expression of proinflammatory cytokines (IL-1α, IL-12p40, IL-17, CCL20, CX3CL1, TNF-α) was significantly increased in the DON group, mainly in intestinal tissue, whereas in 3-epi-DON the expression continued similar to the control. In conclusion, this study has confirmed the absence of toxicity of 3-epi-DON using pigs in an *in vivo* model, since no toxic effect was observed on intestine, liver and lymphoid organs.

P10

Phytic acid modulates the oxidative stress induced by deoxynivalenol in intestinal explants

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Phytic acid (IP6) is a natural antioxidant occurring in cereals, seeds and legumes that presents protective effects against bowel inflammatory disease and colon cancer development. Deoxynivalenol (DON) is a mycotoxin frequently found contaminating cereals and inducing toxic effects in animals. Previous studies reported a reduction in DON-induced toxicity in intestinal explants treated with IP6.

Nevertheless, the mechanisms involved in this protective effect remained unclear. The aim of the present study was to investigate the effects of DON on the induction of oxidative stress in intestinal tissue and the possible beneficial effects of IP6 exposure. Five 24-days-old piglets were used to sample jejunal explants that were submitted to the following treatments: control (only culture media), IP6 5mM, DON 1 μM, DON 10 μM and DON 10 μM plus IP6 5 mM. The explants were incubated at 37°C under orbital shaking during 4 h. After the incubation period, samples were frozen in liquid nitrogen and posteriorly stored at -80°C to the assessment of the oxidative stress. Lipid peroxidation was evaluated through thiobarbituric acid reactive substances (TBARS) level. Reduced glutathione (GSH), free-radical scavenging ability (ABTS) and ferric reducing antioxidant power (FRAP) levels were used to measure the antioxidant capacity. These parameters were evaluated by spectrophotometric assays. The data were analysed using one-way analysis of variance (ANOVA) followed by Duncan's test. P values of ≤0.05 were considered significant. Exposure to DON 10 μM induced an increase in lipid peroxidation (1.6 fold-increase in TBARs level, $P<0.05$) when compared to control, whereas the addition of IP6 to DON resulted in a significant decrease in TBARS levels in comparison to DON group. Jejunal explants exposed to 10μM of DON showed a decrease of 48% in the GSH levels compared to the control ($P<0.05$), while explants exposed to DON+IP6 remained similar to the control. A reduction in ABTS and FRAP levels occurred in DON 10 μM-exposed explants when compared to control ($P<0.05$ for FRAP). Similarly, DON+IP6 explants showed an increase in ABTS and FRAP levels in comparison to DON group. Exposure to DON 1 μM induced no significant changes in the evaluated parameters. The present data demonstrated that DON induces oxidative stress in the jejunum of piglets in a dose-dependent manner. We also verified that IP6 modulates this oxidative response, reducing lipid peroxidation and increasing the antioxidant capacity. Taken together, our results indicate that IP6 can contribute with intestinal homeostasis and animal health reducing the toxic effects of DON.

P11

Effects of fumonisin B1 and phytic acid on the oxidative stress: an *ex vivo* approach

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Fumonisin B1 (FB1) is a *Fusarium* mycotoxin frequently found in cereals showing neurotoxic, hepatotoxic, nephrotoxic, immunotoxic and enterotoxic effects in animals. In humans, FB1 has been classified as a possible carcinogen. Recent studies have associated FB1 toxicity to free radical generation and oxidative stress in spleen, liver and kidney. However, there are no data about the effect of FB1 on intestinal oxidative stress. Phytic acid (IP6) is an antioxidant present mostly in cereals that exhibits a high capacity to bind minerals and anticarcinogenic properties. The aim of the present study was to investigate the effects of FB1 on oxidative stress and the interaction with IP6 in the jejunum of pigs. Five 24-days-old piglets were used to sample explants from jejunum that were submitted to the following treatments: control (only culture media), IP6 5mM, FB1 7μM, FB1 70 μM and FB1 70 μM plus IP6 5 mM. The explants were incubated at 37°C under orbital shaking during 4 h and posteriorly frozen in liquid nitrogen and stored at -80°C. The levels of thiobarbituric acid-reactive substances (TBARS) were quantified spectrophotometrically to evaluate lipid peroxidation. The ability of the samples to resist oxidative damage was determined spectrophotometrically through reduced glutathione (GSH), ferric-reducing ability potential (FRAP) and free-radical scavenging ability (ABTS) assays. The results are expressed as nmols of GSH per milligrams of protein or as nmol of Trolox equivalent per milligrams of protein (FRAP and ABTS). The means were submitted to statistical analysis (ANOVA and Duncan's test) and significance was set at 0.05. Exposure to both doses of FB1 induced no significant change in TBARs levels when compared to control. Concerning the antioxidant capacity, a significant decrease (69%) was observed in FRAP levels in explants exposed to FB1 70 μM compared to control; however, for GSH (41%) and ABTS (23%) levels the reduction was no significant. The addition of IP6 in explants treated with FB1 increased no significantly the levels of GSH, ABTS and FRAP in comparison with explants exposed to both doses of FB1. Nevertheless, explants treated only with IP6 showed higher levels of GSH and ABTS ($P<0.05$) when compared to FB1 70 μM group. The present data demonstrated that FB1 induces oxidative stress mainly affecting the antioxidant capacity, but not lipid peroxidation. In addition, IP6 seems to present a protective effect on intestinal explants exposed to FB1 through modulation of the resistance to oxidative damage.

P12

Impact of climate change on *Aspergillus flavus* and *Fusarium verticillioides* interaction

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The influence of climate change on agricultural systems, including both animals and plants, is extensively confirmed with relevant impact on food security and safety. The occurrence of mycotoxins is supposed to be strongly affected by future climate scenarios and some confirmations are available from recent years. Temperature and CO_2 increase, variations in rain intensity and yearly distribution, besides extreme events, impact differently on fungi and determine the dominant one depending on their ecological needs. In this context, predictive models for mycotoxins gained higher consideration as support for stakeholders to face mycotoxin mitigation. In South Europe, the major mycotoxigenic fungi associated with maize are *Aspergillus flavus*, *Fusarium verticillioides* and, to a lower extend *F. graminearum*. AFLA-maize and FER-maize are two mechanistic predictive models developed and widely validated in the last decade to assess the risk of aflatoxin (AFs) and fumonisin (FBs) occurrence in maize. Worsening in model performances were recently noticed, possibly due to the effect of extreme meteorological events on fungi and their interaction. Therefore, the goal of this work was to study *A. flavus* and *F. verticillioides* interaction to obtain quantitative data on the effect of fungal co-occurrence on mycotoxin production to be included in predictive modelling. *In vitro* trials were organised with single and co-inoculum of the cited fungi on maize-base medium. They were incubated at different temperature regimes (5-35°C, step 5°C) for 21 days. Fungal growth was weekly measured, while AFs and FBs were quantified at the end of the incubation period. The dynamic of toxin production in different temperature regimes followed the same trend with single or co-inoculum; it was well described, both for AFs and FBs, with or without co-inoculum, by a Bete function. However, the co-occurrence of fungi influenced the amount of toxin detected, with a relevant temperature effect. Data collected clearly confirmed the impact of fungal co-occurrence, both on AFs and FBs production. They will be used to improve AFLA-maize and FER-maize allowing to use the 2 models jointly. **Acknowledgements**. This work was managed in the project ‘Integrated and innovative key actions for mycotoxin management in the food and feed chain – MycoKey’, GA No.678781, supported by the European Commission in the H2020 Framework Programme (www.mycokey.eu).

P13

Occurrence of citrinin in red yeast rice from *Monascus purpureus*

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Citrinin is a nephrotoxic mycotoxin produced by several species of the genera *Aspergillus*, *Penicillium* and *Monascus*. It is generally formed after harvest and occurs mainly in stored grains, but also in other plant products. Citrinin can also co-occur with other mycotoxins, such as ochratoxin A and patulin. Citrinin is an undesirable contaminant in *Monascus purpureus* fermentation products (red yeast rice). These products are used for the maintenance of normal blood cholesterol concentrations thanks to the therapeutical action of naturally present monacolin K. Commission Regulation (EU) No 212/2014 sets a limit of 2,000 µg citrinin/kg for food supplements based on red fermented rice. An analytical method for the determination of citrinin in red yeast rice supplements was developed and validated in-house at the Bologna Food Chemical Department of IZSLER (Italy), according to Commission Regulation (EU) No 519/2014. An acidic organic solvent and a mixture of salts were added to the samples to perform the extraction. Identification and determination of citrinin are performed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) using negative electrospray ionisation. The LOQ value was 50 µg/kg. Suppression or enhancement of the signal due to matrix effects are minimised by using isotope-labelled ^{13}C13-citrinin. Linearity, stability, recovery, repeatability, reproducibility, selectivity, LOQ, and LOD were determined proving that the method was fit-for-purpose. A total of 61 samples were collected from July 2016 to December 2017 to evaluate the occurrence of citrinin in red yeast rice supplements and powder. Red yeast rice powder is used by national industries to produce cholesterol control supplements. Food supplements samples were collected mainly within the Italian market while the raw materials were collected as a result of border inspection activities. One out of six samples revealed a citrinin contamination with huge variabilities in the concentrations of the mycotoxin in individual samples. Occurrence ranged from 60 µg/kg to over 2,000 µg/kg. Two food supplements exceed the limit set and a single sample of red yeast rice powder from China showed a concentration

of more than 9,000 µg/kg. A specific limit for raw red yeast rice powder has not been fixed yet. The absence of a reference point for the concentration of citrinin in such matrices could represent a difficulty for border officers to decide on the compliance of the incoming raw materials as no complete data are available on supplements manufacturing.

P14

Studies on the occurrence of mycotoxins and mycotoxigenic species in malting barley in central Italy

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Studies were carried out on 43 and 52 malting barley grain samples harvested, respectively, in the years 2013 and 2014 in the Umbria region (central Italy) to determine the presence of mycotoxins by multi-mycotoxin detection methods and to determine their producing species by fungal isolation and molecular identification by species-specific PCR assays or partial *tef1-α* region sequencing. A number of *Fusarium* strains were also tested for mycotoxin biosynthesis *in vitro*. In addition, a three-year (2011-2013) field trial was conducted on 11 malting barley varieties to detect deoxynivalenol (DON) and T-2 toxin and the presence of toxigenic fungal species. In both the surveyed years, the fungal community of barley grains was mainly composed of Alternaria species, followed by *Fusarium* species. In 2013, the *Fusarium* head blight (FHB) complex was mainly composed of *F. avenaceum*, followed by *F. graminearum*. HT-2 toxin was the most frequent mycotoxin, followed by enniatins (ENs), T-2 toxin, and nivalenol (NIV), while the number of samples contaminated with DON was low. In 2014, the predominant *Fusarium* species was *F. poae*, followed by *F. avenaceum*, *F. graminearum* and *F. tricinctum*. Secondary metabolites biosynthesised by *Alternaria* and *Fusarium* species were detected in the grains. Among those biosynthesised by *Fusarium* species, NIV and ENs were the most prevalent ones. T-2 and HT-2 toxins as well as beauvericin were also present with a high incidence. DON was present in a low number of samples. In 2014, conjugated forms, such as deoxynivalenol-3-glucoside and HT-2-glucoside, were detected for the first time in malting barley grains cultivated in the surveyed area. In addition, strains of *F. avenaceum* and *F. tricinctum* showed the ability to biosynthesise high concentrations of ENs *in vitro*. The three-year field trial showed that the high average and maximum temperatures during crop anthesis mainly favored the occurrence of *Fusarium* species but the FHB complex composition was subject to changes across the three years. The main causal agents were *F. poae*, *F. avenaceum*, *F. tricinctum* and *F. graminearum*, and some of them were associated with a specific variety and/or with specific weather parameters. T-2 toxin, in some cases, was found in kernels at levels that exceeded EU recommended values, while, low amounts of DON were generally detected. These studies highlight the importance of the combination of microbiological analyses, molecular fungal identifications and multi-mycotoxin detection to shed-light into the complexity of the toxigenic fungal community of malting barley and of their secondary metabolites.

P15

Geographical effect on the occurrence of secondary metabolites and their producing fungal species in durum wheat kernels from Italy

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Durum wheat (*Triticum durum* Desf.) grain samples were collected in 2015 in the three different Italian climatic geographic areas of Emilia-Romagna, Umbria and Sardinia, as representative of northern, central and southern areas, respectively. Kernels were analysed to: (i) detect the mycobiota infecting the grains by isolation on both potato dextrose agar and by the deep-freezing blotter method; (ii) determine the composition of the *Fusarium* head blight (FHB) complex by partial *tef1-α* region sequencing; (iii) quantify the *Fusarium* species associated with FHB by q-PCR directly in the kernels; and (iv) determine the presence of fungal secondary metabolites in the grains by LC-MS/MS. The grain mycoflora was mainly represented by *Alternaria* and *Fusarium* species. The highest *Fusarium* species incidence was found in the samples from Emilia-Romagna. The FHB complex was mainly represented by *F. poae* in all the examined areas, while, *F. graminearum* showed the highest incidence in the Emilia-Romagna samples followed by those from Umbria. q-PCR assays allowed the detection of other

Fusarium species, such as *F. langsethiae* and *F. sporotrichioides*. Secondary metabolites were correlated to the fungal community detected. Tenuazonic acid, biosynthesised by *Alternaria* species, was present in all the analysed samples, with the highest levels detected in the Umbrian ones. In addition, also alternariol, alternariol-methylether and infectopyrone were found. In general, *Alternaria* secondary metabolites were more present in the samples from Umbria and Emilia-Romagna followed by those collected in Sardinia. Considering the mycotoxins biosynthesised by *Fusarium* species, the present study showed that deoxynivalenol was mainly found in the samples from Emilia-Romagna, where its producing species were more frequent. Nivalenol, T-2 and HT-2 toxins were detected with similar levels in the samples from Emilia-Romagna and Umbria. Interestingly, HT-2 glucoside was also recovered in the analysed grains. Enniatins and beauvericin were mainly detected in the samples collected in Emilia-Romagna and Umbria. In general, considering both trichothecenes and depsipeptides, the Emilia-Romagna grains were the most contaminated samples followed by the Umbrian ones. Sardinian samples showed the lowest accumulation of these secondary metabolites. A similar gradient was also observed for moniliformin. Finally, ergot-alkaloids were also detected in a few samples from Emilia-Romagna and Umbria. In conclusion, this study indicates that, in the three considered Italian geographic cultivation areas, the different pedo-climatic conditions were very important to determine the composition of the fungal species colonising wheat kernels and, consequently, had a strong effect on the types and levels of secondary metabolites contaminating the grains.

P16

The effect of deoxynivalenol on selected populations of immunocompetent cells in porcine blood

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Deoxynivalenol (DON) is one of the most prevalent mycotoxins in Europe. Pigs are an animal species that is most susceptible to this mycotoxin. DON causes significant losses in pig production by lowering feed intake, decreasing daily weight gains, disrupting immune responses and increasing susceptibility to diseases. The aim of this experiment was to determine the influence of feed contaminated with DON at concentrations insignificantly higher than recommended by the European Commission (900 µg/kg). The experimental feed contained 1,008 µg DON/kg. The experiment was performed on 8 weaners from the same litter. The animals were randomly divided into two groups: an experimental group (M, n=4) fed contaminated feed and a control group (C, n=4) administered feed free of mycotoxins. The experiment lasted for 6 weeks and peripheral blood samples were collected from the animals for analysis of selected morphological parameters and changes in the percentages of $CD4^+8^-$, $CD4^-8^+$ and $CD4^+8^+$ lymphocytes and antigen-presenting cells (APC) with $CD14^+172^+$ (monocytes), $CD172a^{high}4^-14^-$ (conventional dendritic cells, cDC) and $CD172a^{dim}4^+14^-$ (plasmacytoid dendritic cells, pDC) phenotypes. The morphological parameters of porcine blood samples were determined by flow cytometry with non-fluorescent particle size calibration standards, and no differences were observed between groups M and C. An immunophenotyping analysis of lymphocytes and dendritic cells (DC) revealed an increase in the percentage of $CD4^+8^-$, $CD172a^{high}4^-14^-$ and $CD172a^{dim}4^+14^-$ cells and a decrease in the number of $CD4^-8^+$ cells in group M. The results of this experiment suggest that prolonged exposure to low doses of DON can change the proportions of immunocompetent cells (a shift towards humoral immunity), without affecting their overall counts. **Acknowledgements**. Supported by KNOW (Leading National Research Centre) Scientific Consortium 'Healthy Animal – Safe Food', decision of Ministry of Science and Higher Education No. 05-1/Know2/2015.

P17

Toxicological assessment of *Aspergillus flavus* metabolites in resistant Kenyan maize varieties

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The filamentous fungus *Aspergillus flavus* is one of the most important species in the *Aspergillus* genus. *A. flavus* occurs as a saprohyte in soils worldwide and has the potential to produce a diverse repertoire of secondary metabolites that may contaminate food and feed, posing a major food safety concern. *A. flavus* is mostly known for its ability to produce aflatoxins [Campbell *et al.*, 1995. Plant Disease 79:1039; Danishefsky *et al.*, 1985. Journal of the American Chemical Society 107:2474; Nicholson *et al.*, 2009. Applied and Environmental Microbiology 75:7469]. In the developing world, approximately five billion

people are at risk of mycotoxin exposure through consumption of contaminated maize and other foodstuffs [Wagacha *et al.*, 2013. Crop Protection 52:1]. In 2004, 2006 and 2008 acute aflatoxicosis outbreaks resulting in many casualties and diseased persons were reported in Kenya [Probst *et al.*, 2007. Applied and Environmental Microbiology 73:2762]. Chronic exposure to these toxins is of great concern, and yet hitherto no mitigation measures have been set to avert future loss of life [Probst *et al.*, 2007; Okoth *et al.*, 2012. Toxins 4:991; Mutegi *et al.*, 2009. International Journal of Food Microbiology 130:27]. Although aflatoxin contamination is of most concern, contamination by other secondary *A. flavus* metabolites is seldom considered in estimating the burden of exposure. Preliminary data on the metabolic profile of *A. flavus* revealed aflavinine and aflatrem as highly prevalent mycotoxins [Danishefsky *et al.*, 1985; Nicholson *et al.*, 2009; Malysheva *et al.*, 2014. Food Additives and Contaminants Part A 31:1110]. Aflatrem is a mammalian tremorgen, and aflavinine has been shown to have anti-insectan properties, however knowledge on their impact on human and animal health is scarce to non-existent. These compounds could have their own intrinsic toxicity or could act synergistically with aflatoxins. The consumption of maize in developing countries is not likely to stop, so there is an urgent need to tackle perform more in-depth research on *A. flavus* toxic metabolites. Preliminary toxicity studies suggest that aflavinine and aflatrem are potentially neurotoxins and could imply serious health issues such as mental confusion, tremors, seizures and consequent death [Nicholson *et al.*, 2009; Ehrlich, 2014. Frontiers in Microbiology 5:50]. This study aims to identify the toxicological effects of aflatrem and aflavinine through the use of an *in vivo* zebra fish model. Toxicological assessment by the use of zebrafish is gaining popularity as they have a rapid embryonic development and a well understood genome. Behavioural, morphological, and more specifically hepatocellular endpoints can be targeted. Aflavinine and aflatrem are occurring in non-aflatoxigenic strains of *A. flavus*. The use of non-aflatoxigenic strains of *A. flavus* as biocontrol agents against their aflatoxigenic counterparts are becoming commonplace. This phenomenon was demonstrated in 1990 [Cotty, 1990. Plant Disease 74:233]. Large-scale studies over the years have led to the development of biocontrol agents for commercial application based on the ability of the non-aflatoxigenic strains to reduce aflatoxin contamination in cotton seed, peanuts and maize [Amaike *et al.*, 2011. In: Annual Review of Phytopathology, vol. 49, p.107]. These formulations are based on the fact that non-aflatoxigenic strains can outcompete aflatoxigenic strains, and because of a point-mutation in the polyketide synthase gene they do not have the capability to synthesise aflatoxins [Ehrlich, 2014]. However, this approach has potential pitfalls, which should be addressed and understood before wide adaptation. A limitation is that these biocontrol agents are being selected based on their inability to produce aflatoxins, however, others could have the ability to produce other secondary metabolites detrimental to humans and animals upon consumption.

P18

An integrated *in silico/in vitro* approach to assess the xenoestrogenic potential of *Alternaria* mycotoxins and metabolites

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Endocrine disruption is an issue for public health and the dietary intake of xenoestrogens is a major source of exposure. Mycotoxins are widespread food and feed contaminants that may elicit xenoestrogenic activity causing dysfunctions and altered physiological states in humans and animals. To date, the best characterised mycoestrogens (mycotoxins eliciting an estrogenic response) comprise the *Fusarium* toxins zearalenone and congeners, but also the *Alternaria* toxin alternariol has been described to be a potential xenoestrogen as well. However, the estrogenic potential of the *Alternaria* toxins family is still poorly characterised and further data are needed to properly define health-based guidance values. In this framework, the use of in *silico/in vitro* approaches prior to animal trials can significantly focus and reduce the number of *in vivo* experiments, refine toxicological investigations and data interpretation. In the present work, an integrated *in silico/in vitro* approach has been used to evaluate how metabolic and chemical modifications may affect the xenoestrogenicity of alternariol, which is the unique *Alternaria* toxin identified so far as potential xenoestrogen. In particular, 30 alternariol toxins not tested for xenoestrogenicity yet underwent an *in silico* 3D receptor-modelling analysis to evaluate the capability to favourably interact with the oestrogen receptors. The *in silico* data integrated those from alkaline phosphatase assay on Ishikawa cells and binding activity toward the oestrogen receptors in cell-free conditions. Overall, the majority of metabolic and chemical modifications considered herein (including hydroxylations and conjugations) were found to significantly affect the xenoestrogenic potential of alternariol, while methylations have been found as critical to enhance

xenoestrogenicity. Taken together, our results have posed solid footholds to design more efficiently risk assessment studies and provided evidence-based criteria for refining further analysis.

P19

Toxicodynamic as a beacon to foster the hazard identification of mycotoxins in the light of the group-based approach – an *in silico* perspective on zearalenone and deoxynivalenol

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The toxicological assessment of mycotoxins purposing the food safety typically investigated molecules referred to as 'parentals' (i.e., those reference compounds grouping many analogues). Most of the toxicology of mycotoxins has been interpreted and inferred relying on data collected assuming such compounds as the main molecules really triggering toxicity *in vivo*. This assumption has been questioned in the recent times pointing to the need for reinterpreting mycotoxins toxicology under the group-based paradigm. Indeed, it cannot be longer neglected the assessment of all the metabolites to which the organisms are exposed as that metabolism of fungi (producers), plants (reservoirs) and animals (bystander targets) may increase the toxicity of parental compounds, and that metabolites are the forms eventually circulating *in vivo*. Therefore, the risk assessment of mycotoxins actually requires profiling those metabolites bearing toxicity (i.e., the 'metoxome'). However, the current metrological scenario cannot support such premise as most of the analogues are not still commercially available. Also, the synthesis and purification cannot be used affordably for sourcing. While this condition of low affordability is persisting, the *in silico* analysis may timely foster the toxicological assessment providing a reliable and straightforward analytical tool. In particular, profiling the metoxome toxicodynamic deepens the effects of metabolism on the molecular initiating event and provides hints on the first steps of the adverse outcome pathway. Therefore, it provides a valuable rationale to extend the analysis on mycotoxins groups and it may allow focusing experimental investigations as well. The case of zearalenone and deoxynivalenol has been presented as a proof-of-concept. The metabolites identified so far have been investigated to find additional forms possibly involved in triggering toxicity *in vivo*. Specifically, it has been used a validated molecular modelling approach computing the interaction of compounds with the toxicological targets (oestrogen receptors for zearalenone, ribosome for deoxynivalenol). The structure-activity relationship analysis figured out how metabolism may influence mycotoxins-targets interaction. Notably, the two mycotoxins differed in how the pattern of modification may affect the mycotoxin-target interaction. Beside a number of potentially toxic phase-I metabolites, among the phase-II metabolites only the glucuronides of deoxynivalenol were found possibly carrying on toxicity. Overall, our findings provided a rational for hierarchically foster the toxicological assessment of zearalenone and deoxynivalenol. Indeed, many metabolites that are currently overlooked were found possibly involved in triggering toxicity. Therefore, they should be included in further studies to better understand the group toxicity gaining a more informed perspective for risk assessment.

P20

Determination of aflatoxin in food supplements by means of ultra-high performance liquid chromatography coupled to tandem mass spectrometry

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There is an increasing concern among consumers regarding food safety, as well as a demand for high-quality foods with minimal 'bio' or 'chemical' contaminants; the occurrence of these toxins will definitely have a negative impact on the economy of the affected region/country. Mycotoxin contamination of dietary supplements represents a possible risk for human health, especially in the case of products intended for people suffering from certain health conditions. The aim of this study was to assess the extent of this problem based on analyses of a wide set of herbal-based dietary supplements intended for various purposes: treatment of liver diseases; reduction of menopause effects; and preparations for general health support. The analysis of food supplements was performed by means of ultra-high performance liquid chromatography coupled to tandem mass spectrometry using a C18 column packed with core shell particles. The UHPLC system was coupled to a triple quadrupole mass spectrometer by means of an electrospray ion source. Two MRM transitions were selected for each analyte to provide suitable identification criteria (one served as the quantifier and one as qualifier). The validation was performed following the ICH Q2(R1) guidelines. The main parameters were LOD, LOQ, linearity range, repeatability, reproducibility, accuracy, matrix effect (ion suppression). Maximum levels of mycotoxins in food supplements are not established; only the Eur. Ph. has set maximum level for AFB1 (2 μg/kg) and for the sum of AFB1, AFB2, AFG1 and AFG2 (4 μg/kg) for herbal products used as drug ingredients.

Only citrinin has a defined maximum level in food supplements that contain red rice from *Monascus purpurea* (2,000 µg/kg) About 200 samples were tested for aflatoxins B1, B2, G1, G2, and citrinin. Among the samples analysed, only 2% were positive for aflatoxins. The low LOQs obtained for aflatoxins allowed their quantification at concentrations lower than the maximum level established by current legislation. Currently, mycotoxins analysis presents several challenges that still need to be addressed and overcome. Of great importance is the evaluation of occurrence of the so called 'masked mycotoxins' in food and feed. The metabolism of some plants can generate conjugated compounds with chemical behaviours different from that of the parent mycotoxins. Another issue of great importance is the study of emerging mycotoxins derived from *Fusarium* fungi prevalently present in food from northern Europe and Mediterranean countries.

P21

Determination of mycotoxins content in feed and feed raw materials by high performance liquid chromatography with mass spectrometric detection

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Mycotoxins are the secondary metabolites of microscopic fungi with toxic properties. Favourable conditions for intensive growth of fungi contribute to the accumulation of mycotoxins in cereals, beans, sunflower seeds, vegetables, and fruits. The consumption of mycotoxins with feed leads to a slow down in growth and deterioration of animal productivity. Complete prevention of mycotoxins contamination of feeds and feed raw materials is practically impossible, thus making necessary the control of contamination of products. This study was aimed to estimate the occurrence of mycotoxins in feed and feed raw materials, which were produced in the Russian Federation. 476 samples of feed and feed raw were tested by high performance liquid chromatography with mass spectrometric detection. 251 samples of complete feed (feed for cattle, pigs, and chickens) and 225 samples of feed raw materials (cake, meal, maize, flour, oats, wheat, barley and others) were analysed. For the determination of mycotoxins, the representative samples weighing not less than 500 g were ground in a laboratory mill. Mycotoxins were extracted from 5 g of subsamples using a mixture of acetonitrile/water/acetic acid. The extract was centrifuged at room temperature for 20 min, 3000 g. After further dilution, the sample was passed through a filter with a nylon membrane. The analysis was carried out using HPLC 1200, 1290 (Agilent) and mass spectrometers API 5000, QTRAP 5500, QTRAP 6500 (Sciex). Mycotoxins were detected in 72% of the samples (91% of the feed samples and 50% of the feed ingredient samples). In total, 48 different mycotoxins were detected. The most frequently found mycotoxins were toxins from *Alternaria* spp.: tenuazonic acid was detected in 197 (41%) samples, tentoxin was found in 141 (30%) samples, alternariol was detected in 76 (16%) samples, methylether of alternariol was detected in 48 (10%) samples. 51 (11%) samples were contaminated with HT-2 toxin. 6 samples contained mycotoxins at concentrations above the maximum permitted levels set in legislation.

P22

Mycotoxins in beer and coffee obtained at the Czech market

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Determination of mycotoxins, frequent contaminants of various food crops and products thereof, often represents a challenging task, especially when present at a low content or in specific mycotoxin-matrix combinations, e.g., aflatoxin M1 in milk, ochratoxin A (OTA) in coffee, etc. Extract purification and pre-concentration is often employed in such cases to achieve sufficiently low detection limits which are in accordance with legislative regulations. Nevertheless, generic isolation approaches mainly followed by liquid chromatography and mass spectrometry (LC-MS) enabling simultaneous determination of tens up to hundreds of analytes in a single run represents the modern trend. Within this work, an extensive monitoring study focused on evaluation of contamination of popular commodities obtained at the Czech market, coffee and beer, was conducted. Altogether, 119 samples coffee (soluble, roasted and green) and 129 samples of beer (non-alcoholic, ales, lagers and specials) were analysed using and ISO 17025 multi-toxin method. A wide range of 57 mycotoxins of *Fusarium*, *Aspergillus*, *Penicillium*, *Alternaria*, *Stachybotrys*, *Claviceps* and *Phomopsis* genera was determined in beers while only predominant ochratoxin A was analysed in coffee using highly-specific clean-up enabled by immunoaffinity columns. For analytical determination, ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) was utilised. The majority of the examined coffee samples (n=94, 79 %) contained detectable OTA of which 2 samples of soluble coffee would not comply with the current EU

legislation limit (5 µg/kg). Contrary to roasted coffee, soluble coffee showed higher contamination in average, most probably due to the matrix pre-concentration within its production while green coffee contained higher OTA content which is commonly reduced by roasting. As regards mycotoxin contamination of beer, only deoxynivalenol (DON) and its conjugate, deoxynivalenol-3-glucoside (DON-3-Glc), were detected. In some beers, relatively high levels exceeding even 100 µg/l of DON and DON-3-Glc were determined. Currently, there are no maximum limits set for beer, however, the obtained results might indicate the need for some regulation. For some highly contaminated beers, the tolerable daily intake for DON (1 µg/kg bw) could be easily exceeded. Interestingly, beers with higher alcohol content showed the highest contamination, most probably due to the use of more malt extract for their production. **Acknowledgements**. This work was supported by the 'Operational Programme Prague – Competitiveness' (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503), 'National Program of Sustainability I' – NPU I (LO1601 – No. MSMT-43760/2015). This research has also received funding from the European Union´s Horizon 2020 research and innovation programme under grant agreement No. 692195 (MultiCoop).

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Occurrence of multiple mycotoxins in DDGS determined by ultra-high performance liquid chromatography coupled to tandem mass spectrometry

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Livestock production is an important part of national economies of the most countries worldwide and has a significant role in providing high quality products to consumers. Possible contamination of feedingstuffs with moulds and mycotoxins may therefore negatively affect the health of both animals and humans. Nowadays, the legislation of numerous countries requires the use of oxygenated fuels to reduce air pollution. Further significant increase of its production is expected due to the worldwide increasing demand for ethanol production. Distiller's dried grains with solubles (DDGS), the main by-product of the ethanol production, are frequently used as feeding supplement that significantly increases a nutritional value of the feed. Considerable matrix pre-concentration of processed cereals to DDGS due to the depletion of starch and relative stability of mycotoxins predetermines this feedingstuff supplement to contain high levels of mycotoxins. Thorough monitoring of mycotoxins prior the use of high quantities of DDGS as supplement should be conducted to prevent from outbreak of mycotoxicoses. Within this study, an ISO 17025 accredited method based on QuEChERS-like extraction and ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) analysis was utilised for determination of 57 mycotoxins of the genera *Fusarium*, *Alternaria*, *Aspergillus*, *Penicillium*, *Claviceps* and *Phomopsis* in a wide set of 132 maize and 27 wheat DDGS originating from Europe, Asia and Northern America. Generally, high incidence and content of mycotoxins in the analysed material was observed. Out of the 57 mycotoxins analysed, 36 analytes were found. The mycotoxins detected in the majority of the samples were primarily *Fusarium* mycotoxins – deoxynivalenol, zearalenone and their metabolites, then fumonisins, emerging enniatins and beauvericin. When comparing the contamination of DDGS according to the cereal matrix, maize-based DDGS showed significantly higher contamination as compared to the wheat-based ones. **Acknowledgements**. This work was supported by the 'Operational Programme Prague – Competitiveness' (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503), 'National Program of Sustainability I' – NPU I (LO1601 – No. MSMT-43760/2015). This research has also received funding from the European Union´s Horizon 2020 research and innovation programme under grant agreement No 692195 (MultiCoop).

P24

Carryover of dietary aflatoxin B1 to matrinxã (*Brycon cephalus*) fish tissues and effects in liver

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Depending on the sensibility of fish species, aflatoxins can be cumulative in tissues, affecting the liver. The aim of this work was to verify the accumulation of aflatoxins in tissues of matrinxã (*Brycon cephalus*) fish chronically exposed to dietary aflatoxins, also evaluating histopathological changes in liver. Aflatoxin

B1 (AFB1) was produced and incorporated to fish feed to obtain the following levels: (A) control, feed without toxin; (B) feed + 10 μg AFB1/kg; (C) feed + 20 μg AFB1/kg; and (D) feed + 50 μg AFB1/kg. Fish were daily fed with 5% of animal biomass. Each treatment had 150 animals in tanks daily cleaned and monitored. Sampling was carried out monthly over a period of six months. Samples were collected in triplicate and constituted by a pool of approximately 10 fish. AFB1 was quantified by HPLC in fish liver and muscle. Macro- and microscopic analysis were performed in liver. Muscle and liver presented levels below limit of detection in all control samples. Considering the liver, there was an effect ($P<0.05$) of treatment, time of exposure and interaction between these variables. Treatments A and B presented traces up to days 60 and 90, respectively, while treatment C presented residues as of day 30 of exposure. The maximum levels in liver reached by treatment A was 0.32 μg AFB1/kg at 150 days of exposure. Treatment B reached 0.39 μg AFB1/kg also at 150 days and treatment C presented 0.61 μg AFB1/kg at 180 days of exposure. Muscle samples presented only traces of AFB1 for all treatments. Macroscopic observation of the liver allowed noticing friable appearance and signs of jaundice from day 150 to 180 in treatments A, B and C. In the same period, microscopic evaluation showed fatty degeneration in all treatments exposed to AFB1. There was accumulation of fat in the hepatic tissue, more evident after 150 days of exposure. In all treatments exposed to AFB1, changes such as fatty degeneration, hydropic degeneration and foci of cell death were evidenced. Therefore, results indicate that chronic exposure to AF affects matrinxã fish liver, but the species is resistant to AFB1 accumulation in edible parts. **Acknowledgements**. The authors thank Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, grants 2013/23556-4; 2016/09581-4) for financial support.

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Long term exposure of pacu (*Piaractus mesopotamicus*) fish to dietary aflatoxin B1: accumulation in tissues and hepatic changes

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Deposition of aflatoxins in fish tissues can be residual and cumulative. As fish species present different susceptibility to aflatoxins, Brazilian species has been investigated for their sensitivity. The aim of this work was to evaluate effects of chronic exposure to aflatoxin B1 (AFB1) on the residues in tissues and on the occurrence of lesions in liver of pacu (*Piaractus mesopotamicus*) fish. AFB1 was produced and incorporated to fish feed to obtain the following levels: (A) control, feed without toxin; (B) feed + 10 μg AFB1/kg; (C) feed + 20 μg AFB1/kg; and (D) feed + 50 μg AFB1/kg. Juveniles of 10 to 20 cm were placed in tanks (150 animals per tank) and fed daily during 180 days with 5% of biomass. Samples were constituted by a pool of approximately 10 fish and collected monthly in triplicate. AFB1 was quantified by HPLC in fish liver and muscle. Liver was examined macro- and microscopically to find lesions. Deposition of AFB1 in the liver was observed in all treatments exposed, as of 30 days. Treatment A presented levels from 0.19 to 0.86 μg AFB1/kg. In treatment B, levels ranged from 0.18 to 0.51 μg AFB1/kg. Treatment C showed the highest levels, ranging from 0.49 to 1.16 μg AFB1/kg. There was effect ($P<0.05$) of treatment and of time of exposure. However, muscle presented trace levels of AFB1 in almost all samples, with the highest levels of 0.13 μg AFB1/kg in treatment A. Regarding the liver examination, the extraction was difficult due to the friable characteristic, with detachment of fragments in contact with the anatomical tweezers. Jaundice was observed as of day 150 in all treatments, except for the control. Fatty degeneration and hepatic lesions were observed in treatments exposed to AFB1 in the diet, besides disorganisation of the cordial arrangement of hepatocytes as of 60 days. Fatty degeneration was noted after 30 days of exposure to AFB1 in the diet. Results showed that pacu fish liver is susceptible to AFB1, however, the species did not accumulate significant amounts of AFB1 in muscle under chronic exposure at levels permitted by regulations. **Acknowledgements**. The authors thank Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, grants 2013/23556-4; 2016/09581-4) for financial support.

P26

A preliminary study on the influence of deoxynivalenol and zearalenone on the immunohistochemical expression of oestrogen receptors and genes encoding selected liver enzymes in pre-pubertal gilts

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Deoxynivalenol and zearalenone are frequently detected in plant material used for the production of feed for pre-pubertal gilts. Daily intake of small amounts of these mycotoxins with feed induces various subclinical states in pigs and influences different biological processes. The aim of this preclinical study was to evaluate the influence of low monotonic doses of deoxynivalenol and zearalenone (12 µg/kg bw and 40 µg/kg bw, respectively, applied alone or in combination; 36 animals over a period of 21 days) on the immunohistochemical expression of oestrogen receptors (ERs) in the liver and the mRNA expression of genes encoding selected liver enzymes. The immunoreactivity of ERs suggests that the presence of both mycotoxins in feed can lead to: (i) accumulation of energy in liver cells when ERα is expressed; (ii) acceleration of metabolic processes or early maturation when ERß is expressed; and (iii) metabolic regulation of hepatocytes when both ERα and ERß are activated. The mRNA expression of CYPs and GSTP1 decreased, which could indicate that low mycotoxin doses are tolerated by pre-pubertal gilts and that the accumulation of exogenous and endogenous oestrogens gradually increases due to mycotoxin-induced disruptions in hepatic oestrogen clearance. **Acknowledgements**. Supported by KNOW (Leading National Research Centre) Scientific Consortium ‘Healthy Animal – Safe Food’, decision of the Ministry of Science and Higher Education No. 05-1/Know2/2015.

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Chronic ingestion of deoxynivalenol induces oxidative stress in the kidney of rats

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Oxidative stress (OS), as a signal of aberrant intracellular mechanisms, plays a key role in maintaining cell homeostasis. The occurrence of OS due to disorders of normal cellular redox balance indicates the overproduction of reactive oxygen species (ROS) and/or deficiency of antioxidants. Deoxynivalenol (DON), one of the most common food contaminants, elicits *in vitro* nephrotoxic effects in humans and animals. *In vivo* studies focusing on the effects of DON in kidneys are scarce; therefore, the goal of this study was to investigate the effects of DON in this organ. Twenty Wistar rats (21 days-old) received a control diet (mycotoxin-free feed) or a diet contaminated with 9.4 mg DON/kg. After 30 days of treatment, blood and kidneys samples were collected to examine the serum creatinine level, histological changes and OS, respectively. A morphological score was designed to compare possible histological changes in the kidneys. The effect of DON-induced OS was evaluated by spectrophotometric assays. The reduced glutathione (GSH), ferric-reducing ability potential (FRAP) and free-radical scavenging ability (ABTS) assays were used to evaluate the renal antioxidant defence. Lipid peroxidation and superoxide anion production were evaluated through the levels of thiobarbituric acid-reactive substances (TBARS) and nitroblue tetrazolium (NBT) reduction assay, respectively. Data of the lesion scores and levels of GSH, FRAP, ABTS, TBARS and NBT were submitted to analysis of variance and Student's test considering $P \leq 0.05$. Ingestion of DON induced a significant increase (1.7 fold-increase) in the level of serum creatinine ($P=0.03$), as well as in renal lesion score (2.8 fold-increase, $P=0.003$). The main histological findings in rats fed a DON-contaminated diet were a degenerative change in tubular epithelial cells characterised as vacuolisation of the cytoplasm, infiltrate of lymphocytes with a focal or multifocal pattern and apoptosis. Also, in renal tissue the treatment with DON elevated superoxide anion production ($P=0.005$) and lipid peroxidation ($P=0.03$), when compared to the control group. The levels of GSH and FRAP remained unchanged in relation to the control. However, the exposure to DON induced a significant increase in renal ABTS level ($P=0.04$), when compared to control diet. In conclusion, our *in vivo* findings showed that DON induces renal injury and dysfunction. Furthermore, the induced overproduction of ROS and lipid peroxidation is an important mechanism involved in DON-induced toxicity in the kidneys of rats.

P28

Individual and combined effects of fumonisins and deoxynivalenol on the oxidative stress in ovaries

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Fumonisins (FBs) are mycotoxins produced by *Fusarium* genera frequently occurring in maize in combination with deoxynivalenol (DON) and zearalenone. Exposure to these mycotoxins has been linked to reproductive disorders in various animal species. Recent studies showed that fumonisin B1 (FB1) and DON change porcine granulosa cell proliferation and steroidogenesis, suggesting that these mycotoxins may compromise the normal follicle growth and oocyte survival in swine. However, the mechanisms of FB1- and DON-induced reproductive toxicity have been not extensively studied. Ovulation is an essential prelude for successful reproduction. In this process, the production of reactive oxygen species (ROS) is a vital preovulatory signalling event. In addition, considering that free radical scavenging enzymes significantly reduce the rate of ovulation in different animal species, it is interesting to evaluate the effects of mycotoxins on ovarian antioxidant capacity. Thus, the goal of this study was to determine the effect of fumonisins and DON on the antioxidant defence of porcine ovaries. Twenty-four explants obtained from six sows (5 months-old) were exposed for 48 h to the following treatments: control (DMEM medium); FBs (culture material extract containing 100 μM FB1 and FB2); DON (10 μM); and FBs+DON (100 μM + 10 μM). After the incubation period, the antioxidant potential of the samples was evaluated by spectrophotometric assays. The levels of reduced glutathione (GSH), ferric-reducing ability potential (FRAP) and free-radical scavenging ability (ABTS) assays in the samples were determined, and the results were expressed as nmol per mg of protein (GSH) and nmol of Trolox equivalent per milligrams of protein (FRAP and ABTS). In addition, the levels of thiobarbituric acid-reactive substances (TBARS), primarily malondialdehyde, were quantified spectrophotometrically to evaluate lipid peroxidation. The means were submitted to statistical analysis (ANOVA and Tukey's test) and significance was set at 0.05. The ovarian explants exposed to FBs or FBs+DON showed a significant increase in the antioxidant defence status (FRAP, ABTS, and GSH assays) ($P<0.05$). In accordance, in treatments with FBs or FBs+DON a significant decrease in lipid peroxidation was observed ($P≤0.01$). No change in parameters analysed was observed in samples exposed to DON ($P>0.05$). In conclusion, the results suggest that lipid peroxidation is not the main mechanism of DON and FBs-induced ovarian damage. However, since in the ovary the rise in ROS levels is a necessary sign for ovulation, an increase of the antioxidant potential induced by FBs or FBs+DON can compromise reproductive parameters on swine.

P29

Potential fumonisin-producing *Aspergillus* section *Nigri* species in food products from Spain

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Aspergillus section *Nigri* species are important ochratoxin A producers in foodstuffs. In the last few years, their ability to produce fumonisins has been also reported in a variety of species of *Aspergillus niger* aggregate and they are currently considered important contributors to fumonisin contamination in food products. The genes involved in fumonisin biosynthesis in these species have been also described. The aim of this work was to study the occurrence of fumonisin biosynthetic gene cluster in *Aspergillus* section *Nigri* isolates from Spain and to confirm their ability to produce fumonisins by studying the expression of *fum1*, the key gene of the pathway, by RT-PCR. An extensive sampling was performed in more than 20 matrices including the most frequently contaminated by these species. 242 isolates were obtained and identify by specific PCR protocols or sequencing. The study found 166 isolates of *A. tubingensis*, 43 *A. welwitschiae*, 25 *A. niger*, 3 *A. brasiliensis*, 3 *A. carbonarius* and 2 isolates of *A. aculeatus*. The presence of the fumonisin biosynthetic cluster was tested in *A. welwitschiae* and *A. niger* isolates by detection of all the genes by PCR. Three genetic variations were found. Some of the isolates presented the complete cluster, others presented a truncated version and only the 3' end of the cluster was present (*fum15*, *fum19* and *fum1* genes) whereas other isolates did not present any of the genes. Most *A. welwitschiae* isolates (70%) presented the truncated version although the intact version was also found in 28% of the isolates. On the other hand, the intact cluster was found in almost all *A. niger* isolates (24 out of 25), only one presenting the truncated version. No correlation was found between the origin of the isolates and the presence of the intact or truncated cluster. The functionality of the cluster was also tested by analysing the expression of the key gene *fum1* by RT-PCR in both *A. niger* and *A. welwitschiae* isolates which presented the intact and the truncated cluster versions. The results showed that if the complete cluster is present, *fum1* is expressed at very high levels indicating that they are

potential fumonisin producers. However, isolates with the truncated version did not expressed *fum1* gene in any case. The high occurrence of potential fumonisin producers of *A. niger* and *A. welwitschiae* might be carefully studied in Spain to prevent contamination of these toxins in food products. **Acknowledgements**. Work supported by AGL2014-53928-C2-2-R.

P30

Zearalenone and α-zearalenol levels in the bile of swine exposed to zearalenone with and without antimycotoxin additive inclusion in the diet

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This work aimed to measure the levels of zearalenone (ZEN) and α-zearalenol (α-ZEL) present in the bile of swine fed a mycotoxin-contaminated diet in order to use these molecules as an indicator of the efficiency of an antimycotoxin additive (AMA) to decrease ZEN absorption. Ninety-six 21-day weaned pigs (± 6 kg) were allotted in a randomised complete block design experiment with four treatments, six replications, and four animals per experimental unit (pen). Treatments were arranged in 2x2 factorial design: T1, basal diet; T2, basal diet with inclusion of AMA; T3, basal diet with inclusion of AMA (0.15%) and addition of mycotoxins (fumonisin B1 and B2, ZEN and deoxynivalenol); and T4, basal diet without AMA inclusion and with mycotoxins addition. Feed and water were offered *ad libitum* during a 42-day nursery feeding experiment. The bile was collected from one animal per pen, total of 24 animals. Mycotoxin concentrations were measured by HPLC with fluorescence detection, using immunoaffinity columns to purify the extract. Data were analysed with MIXED procedure of SAS®. Tukey test was used to compare treatment means ($P \leq 0.05$). As expected, the bile from T1 and T2 did not present detectable levels of the studied molecules. The ZEN levels detected in the bile of animals from T3 (245.2 ng/ml) and T4 (242.7 ng/ml) were not significantly different; therefore, AMA inclusion in T3 was not able to decrease ZEN absorption. On the other hand, the α-ZEL levels found in the bile of animals from T3 (166.0 ng/ml) were higher ($P \leq 0.05$) than the levels from T4 (86.7 ng/ml). The same behaviour was noticed for the sum of ZEN and α-ZEL levels (SUM) in the bile from T3 (411.2 ng/ml) and T4 (329.9 ng/ml). A possible explanation for this unexpected behaviour – the increase of α-ZEL and SUM with the inclusion of AMA in the diet – may be due to some factor present in the AMA formula that was able to modify the liver function by making it more efficient to turn ZEN into α-ZEL. The AMA used in this trial has on its label only adsorbent material and extracts declared. This possible induced enhancement of the liver biotransformation capacity is a concern, since α-ZEL has been shown to be 3 to 100 times stronger than ZEN in the oestrogenic agonist effect.

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Clinical findings after indoor trichothecenes (DON, T-2) exposure: analysis of outcomes, immuno-suppression and impaired genetic MTHFR detoxification status

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Chronic indoor water intrusion allows *Stachybotrys* to proliferate and accumulate trichothecenes (Ts), including deoxynivalenol (DON or vomitoxin) and T-2. Readily-airborne, persisting in dust, fungal fragments, mite faeces, these extremely cytotoxic, inert, nanoparticulate sesquiterpenoid epoxides of *Stachybotrys*, *Fusarium* and *Trichoderma*, remain toxic despite disinfection. Biocides may transform them to even more toxic ‘daughter’ particulates. They inhibit protein synthesis damaging skin, mucosa, immune phagocytes, nerves, trigger cell destruction exposing nuclei potentiating autoimmunity. Human data are sparse. The objective of this study was to define clinical presentations, risks, indoor hazards, and biomarkers. Methods: (a) prospective observational cohort analysis of 45 patients exposed to 18 Ts-contaminated indoor environments; (b) environmental: visual-inspection/spore counts/ microscopy/ fungal-specific PCR/ERMI analyses/dust-mycotoxin (MCT) testing (Real-Time Laboratories, Inc.); (c) exposure analysis: timing/type/activity/protection/severity/duration/mould spp./MCTs; (d) medical: symptoms timing (onset/progression/resolution), diagnoses/disability/immunosuppression risks, physical skin/mucosal injury/lymphatic (hyperplasia/adenopathy/tonsil nodules/splenomegaly); fungal quantitative-IGGs, urine MCTs (ppb); aflatoxin (AF)/ochratoxin (OTA)/Ts, leucocyte counts/lymphocyte subsets/IGG-IGA(total/subclasses)/IGM/IGE/deficiencies (protein/vitamin D/zinc); genetic methylene-tetrahydrofolate reductase (MTHFR) C677T, A1298C variants (glutathione production/cellular detoxification); (f) biostatistical analysis (Pearson correlation coefficients): outcomes, clinical parameters (mucosal injury/fungal IGGs/MCTs/MTHFR-defects). The results can be summarised as follows. Indoor contamination: *Aspergillus/Penicillium* in all 18 environments (56% *A. niger*)/94% *Chaetomium*/67% *Stachybotrys*/61% *Mucor*/44% *Alternaria*/33% *P. brevicompactum*; *Fusarium* not detected in any. Of 45

trichothecenes-exposed patients (22 male, 21 female, 2 embryos), 38 in 9 family clusters, 7 solo-exposed, 5 pregnant women; 20 children 12 years (exposure-ages:12 *in utero* (7 1st trimester (2 miscarried) /2 2ndTrimester / 2 3rd trimester), 4 <3 years, 1 <3-6 years, 3 6-12 years). Outcomes: disabled 54% (23% permanent/16% temporary), neurologic 67% (female predominance), ear/nose/ throat 30%, pulmonary 21% (male predominance), dermatologic 12%. Urinary MCTs (32/43 tested): 6% AF/31% OTA/97% Ts detectable (trace 38%, elevated 46%). All extremely ill excreted Ts (none excreted AF). Higher Ts predominated in females (46 vs. 24%), trace levels males predominate (38 vs.18%). Disease severity (DS)-exposure analysis: The strongest DS predictors were mucosal injury (P=0.03e-6), environmental contamination (P=0.01e-6), unprotected exposure (P=0.01e-5) especially with MTHFR defects (P=0.08e-6), positive fungal IGGs (P=0.09e-5). Detection of any urine Ts correlated significantly with DS (P=0.01e-5). Those minimally ill were not very exposed. In conclusion, Ts exposure appears highly associated with permanent damage. Disease severity correlated with exposure parameters coupled with immunosuppression (*in utero*/young age/pregnancy/malnutrition/steroids), genetic MTHFR-detoxification defects; upper respiratory mucosal injury the strongest clinical predictor. Ts appear to cause toxic injury (mucosal, neuron, leucocyte), miscarriage, foetal/placental/congenital defects, prematurity, developmental delay. Mycotoxin-contaminated breast milk appears hazardous. Human mycotoxin testing is an important biomarker advance in identifying exposures.

P32

Mycotoxin mixtures in food and feed: holistic, innovative, flexible risk assessment modelling approach – MYCHIF

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Mycotoxins as mixtures are produced mainly by fungi of the genera *Aspergillus*, *Penicillium* and *Fusarium*. Their omnipresence in many food commodities, represents a challenge to the health of humans, animals and the environment. The main objective of MYCHIF is to develop innovative methods for the risk assessment of mixtures, using various mycotoxins case studies from a comprehensive approach, covering their synthesis at the primary production to adverse effects in human health and environment. The MYCHIF consortium provides a holistic perspective of food and feed safety and security framework by capitalising on cooperation, collaboration and knowledge exchange within multidisciplinary partners. This will be accomplished first by extensive literature searches (ELS) and data collection on the key environmental conditions and factors that influence the biosynthesis and occurrence of mycotoxin mixtures in plants of relevance to food and feed safety. These ELS should review also TK and toxicity data for single and combined mycotoxins in humans, livestock and fish. The data collected on both occurrence and toxicity should be combined using modelling techniques (i.e., Monte Carlo modelling) and the outcome will be described by their frequency distributions and associated probability to model uncertainty and statistical reliability. The developed MYCHIF method based on open source tools will be used collectively with any other tool based on the needs and operations of the European Food Safety Authority or other international agencies.

P33

Oxidative stress: a link between drought and aflatoxin contamination in maize

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Drought stress results in exacerbated aflatoxin contamination in maize and has been the focus of research efforts for pre-harvest aflatoxin contamination since the 1970s. However, the identification of components of this host-pathogen interaction which explain this increase in contamination under drought stress has proven difficult. Here, we have utilised proteomics and metabolomics in addition to biochemical analyses to examine the drought stress responses of kernel and leaf tissues of maize with the objective of identifying biomarkers including proteins, metabolites, and compounds which contribute to exacerbated aflatoxin production by *Aspergillus flavus* during infection of drought stressed maize. Initially, seedlings and mature plants of breeding germplasm were examined for drought sensitivity.

Following this screening, the lines B73 (drought sensitive with high aflatoxin contamination) and Lo964 (drought tolerant with reduced aflatoxin contamination) were selected for further examination. An isobaric tag for relative and absolute quantification (iTRAQ) proteomics approach was used to compare the proteomes of B73 and Lo964 kernel tissues following drought treatment compared to well-watered controls. B73 exhibited more diverse and vigorous responses to drought compared to Lo964. Antioxidant enzymes and pathway components were also found to accumulate higher in Lo964 compared to B73. Based on this, a biochemical examination of seedling leaf and developing kernel reactive oxygen species (ROS) accumulation and antioxidant enzyme activity under drought stress was performed. B73 accumulated higher levels of ROS than Lo964 in both leaf and kernel tissues under drought which correlated with observed aflatoxin accumulation at maturity following A. flavus inoculation. Metabolomic analysis also found that B73 accumulated higher levels of simple sugars and oxylipins in developing kernel tissues than Lo964. These observations are interesting considering that ROS, oxylipins, and carbohydrates are required for and have been shown to stimulate aflatoxin production by *A. flavus in vitro* and *in vivo*. This suggests that these components, ROS in particular, may function in cross-kingdom communication between maize and *A. flavus* during infection of a drought stressed host resulting in exacerbated aflatoxin contamination. Ongoing experimentation based on this hypothesis will explore the use of transgenic and/or genome editing approaches to enhance the antioxidant capacity of maize tissues to improve drought tolerance and mitigate aflatoxin contamination under drought stress.

P34
Weather conditions associated with mycotoxin accumulation in Norwegian oats
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High concentrations of the mycotoxins deoxynivalenol (DON), produced by *Fusarium graminearum*, and HT-2 and T-2, produced by *F. langsethiae*, are sometimes detected in Norwegian oats. In order to identify oat grain lots at potential high risk, we aimed to identify weather conditions associated with mycotoxin accumulation in Norwegian oats. First, a mathematical model was developed and used to estimate developmental stages in oats (tillering, flowering, etc.) based upon weather data. Thereafter, weather summarisations within specific oat developmental stages were calculated for a number of oat fields. In order to assess the association between weather conditions and mycotoxin accumulation, a Spearman rank correlation factor was calculated between mycotoxin contamination in oats at harvest and the weather summarisations within each developmental stage. The most important weather variables identified were included in the empirical models with the aim of predicting the risk of DON and HT-2 and T-2 accumulation in harvested oats [Hjelkrem *et al.*, 2017. European Journal of Plant Pathology 148:577; Hjelkrem *et al.*, 2018. European Journal of Plant Pathology, https://doi.org/ 10.1007/s10658-017-1394-3]. The models should be useful to authorities and industry representatives to identify grain lots with potential food safety problems.

P35
Monitoring human exposure to aflatoxin B1 by detection of aflatoxin M1 in urine by ELISA
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Aflatoxin B1 is a highly carcinogenic metabolite derived from the fungal genus *Aspergillus*, which has been found to contaminate a wide assortment of crops, such as maize, wheat, and sorghum. Consumption of aflatoxin B1 leads to its subsequent metabolism into a variety of less harmful by-products, including aflatoxin B1-albumin (AF-alb) found in blood and the hydroxylated metabolite, aflatoxin M1, secreted into urine or milk. AF-alb has been used as a biomarker for aflatoxin B1 exposure because it has a long half-life and represents integrative exposure over time, though the sampling procedure is more invasive requiring a blood draw and is more indicative of chronic exposure. Previously, we developed an enzyme-linked immunosorbent assay (ELISA) for quantifying aflatoxin M1 in urine, which involves easy sample collection and is representative of acute aflatoxin B1 exposure. We review validation data showing that the aflatoxin M1 in urine marker strongly correlates with AF-alb measured by ELISA. Additionally, the detection of aflatoxin M1 in urine correlated with consumption of aflatoxin B1 contaminated maize. We summarise data from several epidemiological studies identifying susceptible populations in Tanzania, Bangladesh, Bolivia, Kenya, Malaysia, and Ethiopia. Together these findings demonstrate the utility of measuring aflatoxin M1 in urine by ELISA as an important tool to determine the extent of human exposure to the more potent aflatoxin B1. Knowledge from such survey

studies will support the development of strategies to mitigate consumption of foods naturally contaminated with aflatoxin B1.

P36

The effect of black point in wheat grain on deoxynivalenol production during storage

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In 2016, cereal harvesting was very complicated in Lithuania due to abundant rainfall and provoked a lot of discussion concerning the quality of purchased grain. Black-pointed grains are associated with *Fusarium graminearum* Schwabe, teleomorph *Gibberella zeae* (Schw.) Petch. It is not clear how *G. zeae* persists on grain during storage and if the concentrations of deoxynivalenol (DON) do not change (increase) in the grain samples with higher incidence of black-pointed grain, particularly if the moisture content in stored grains is elevated. To answer these questions, 32 grain samples were selected and analysed for black point and *Fusarium*-damaged grain incidence and DON concentration. The grain was sampled according to Commission Regulation (EC) No 401/2006. Deoxynivalenol (DON) was determined by the ELISA method using Ridascreen® (R-Biopharm AG, Germany) diagnostic test kits. The grain samples for analyses were selected according to the abundance of grains with black points and *Fusarium*-damaged ones with a moisture content ranging from 16.2 to 19.5%. The samples were kept for 1 month in the thermostats Binder (Germany) at different temperatures, 4°C, 16°C, 20°C and 28°C. DON analyses were done in duplicate: after 14 days and 30 days of storage. Analyses showed that in the samples from commercial enterprises grains with black points accounted for from 0.30 to 13.58%. Of the 32 grain samples analysed, 79% had black points. The samples of grain with higher incidence of black points (4.2-13.6%) did not form more *Fusarium* spp. colonies on the nutritional agar medium than those of grain without black points. This suggests that black points do not have effect on the *Fusarium* spp. infection level. It was found that storage of grains with elevated moisture content (about 17%) and in which *Fusarium*-damaged ones account for more than 1 percent poses the greatest risk in terms of DON concentration increase. In such grain, DON concentrations increase by on average 25% during storage. In the samples with more than 10% of black-pointed grain, DON concentrations did not change regardless of grain moisture, storage conditions, storage duration, initial grain moisture and abundance of black-pointed grain.

P37

The effect of harvesting time on mycotoxin production in the grain of spring cereals

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Grain samples of spring oat (*Avena sativa* L.), spring triticale (× *Triticosecale* Wittm.) and spring wheat (*Triticum aestivum* L.) were collected in Lithuania in 2017. The weather conditions at the end of August and beginning of September were exceptionally unfavourable for harvesting, as the soil moisture was 2.38 times higher than usual. Due to the adverse meteorological factors, spring cereals were harvested later than normal, which favoured production of high levels of mycotoxins. It is important to note that a delay in harvesting resulted in co-occurrence of several mycotoxins. The grain samples were harvested in three stages: after the crop had reached full maturity, 8 days and 14 days after full maturity. In this study, a total of 36 samples of spring oats, triticale, and wheat were analysed for co-contamination with deoxynivalenol (DON), zearalenone (ZEN), and T-2 toxin (T-2) by the ELISA method. The obtained findings showed that during the period from the 1st grain harvesting stage to the last, the concentrations of DON, ZEN and T-2 toxin in spring wheat increased 3-fold, 21-fold and 2-fold, respectively. In spring triticale grain harvested at the 3rd stage, the concentration of DON and ZEN was 4-fold and 14-fold higher compared with fully mature grain. Within 14 days after full maturity, the level of DON in spring oats increased twice and the concentration of T-2 toxin exceeded the allowable limit; however, the harvesting time did not significantly influence the concentrations of ZEN and T-2 toxin. Harvesting time was found to increase the concentration of T-2 in spring wheat and triticale but its level was within the permitted limit. The highest concentration of DON was detected in spring triticale grain and that of ZEN in spring wheat grain, which was 12.8 and 10 times respectively greater than the permitted level. Spring wheat and triticale were the most susceptible to co-contamination with ZEN and DON.
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P38

Biomarkers as a tool to better understand the effect of mycotoxins on gut barrier functioning and immunosuppression

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Intestinal gut health is key for overall performance and health and can be impaired by different factors. One of these factors is the presence of mycotoxins in the feed. Mycotoxins are immunosuppressive leading to higher susceptibility to secondary infections and production losses. The indirect effect of mycotoxicosis on performance losses finds its origin into impaired gut barrier functioning and decreased gut health (bacteria dislocation) with an altered immune response as a consequence. To gain information on the effect of mycotoxins on the animals' immune reaction, biomarkers were used as a tool in this *in vivo* experiment. Biomarkers give the opportunity to detect changes in biological parameters in a very early stage after contamination, including immune responses. Ninety-six one-day-old broiler chickens were used to evaluate the effect of feeding naturally contaminated rations with low levels (17 ppm) of fumonisins and the protective effect of a commercial mycotoxin eliminator on circulating and intestinal immune cells, blood biochemistry and haematological variables. Contamination with fumonisins lead to a significant increased sphinganine to sphingosine ratio, decreased haematocrit value, decreased total leukocyte count and an increased ratio of albumin to globulin in the blood biochemistry. All these signs are correlated with a decreased general health status. The immune response to fumonisins was clearly demonstrated by a significant effect on the amount of circulating helper T-lymphocytes, regulatory T-lymphocytes and terminally activated cytotoxic T-lymphocytes. The number of circulating monocytes and macrophages was also significantly decreased by contaminating the broilers, whereas the use of an effective mycotoxin eliminator resulted in higher levels of available immune cells again and hence give immune support to the animal to overcome not only mycotoxicosis but also secondary infections. In conclusion, there is still much to reveal on the effect of mycotoxins in the gastro intestinal tract. However, in this research, it is clearly demonstrated that mycotoxins, fumonisins in this particular case, affects immune responses orchestrated by impaired gut barrier functioning and metabolic changes. Biomarkers, such as circulating lymphocytes, are a great tool to better understand how mycotoxins cause production losses by immunosuppression.

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Effect of climate changes on annual variation of mycotoxins profile in maize from Republic of Serbia

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The Republic of Serbia represents a leader in terms of maize production and exports in Europe and among the top ten maize exporters in the world. Therefore, presence of mycotoxins in maize should be recognized as a significant concern in Serbia, since those contaminants may potentially affect human and animal health and could also cause great economical losses. The presence of mycotoxins in maize strongly depends on weather conditions during maize growing season. Even though climate conditions in Serbia are mainly described as moderate-continental, during the recent years significant weather conditions changes were noted. The objective of this study was to investigate the influence of weather conditions, recorded during maize growing seasons in a period 2012-2015 on annual variation of the following mycotoxins: aflatoxins (AFs), ochratoxin A (OTA), zearalenone (ZEN), deoxynivalenol (DON) and fumonisins (FUMs). Extremely hot and dry conditions followed by extreme drought were noted during maize growing season in 2012 while 2014 year had the highest amount of rainfall since meteorological observations exist in Serbia. Furthermore, high air temperatures and low amount of precipitation were recorded during the maize growing seasons in years 2013 and 2015. Analysis showed that 72, 26 and 25% of the examined maize samples from 2012, 2013 and 2015 years were contaminated with AFs, respectively. AFs from maize further caused presence of AFM1 in milk and dairy products. Presence of AFs in food chain during year 2013 has led to the so-called 'aflatoxins crisis' in the country. On the other hand, none of the maize samples from the rainy 2014 year was contaminated with AFs. OTA was detected only in maize samples from year 2012 with a contamination frequency of 22%. Extremely rainy weather conditions in maize growing season 2014 caused DON and ZEN presence in 95% and 85% of maize, respectively. However, in the years characterised by hot weather, DON as well as ZEN were detected in less than 12% of the maize samples. Even though significant differences were noted between weather conditions in investigated period, FUMs were detected in maize samples from each of the examined four years, with contamination frequency between 40 and 95%. Extreme weather conditions recorded during the investigated period have influenced significant differences in mycotoxins profile. Furthermore, the obtained findings indicate that occurrence of

mycotoxins in maize is variable from year to year. According to global climate change prediction, it could be assumed that presence of mycotoxins may become a big future challenge for maize producers in Serbia.

P40

Comparative analysis of mycotoxin occurrence in different types of forage

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Feed raw materials show different profiles of mycotoxin contamination depending on the type of culture and storage conditions. In previous studies, we highlighted the difference of mycotoxin occurrence in different types of cereals and maize materials. Ruminant diets include high levels of various forages, the aim of the present study was to compare the occurrence of mycotoxin in the most frequent types of forage: maize silage, grass silage, hay and straw. This study used the LABOCEA database composed of chromatography analyses run with LC-MS/MS from 2013 to 2016 and including 931 forage samples. This database has the unique characteristic of quantifying 45 mycotoxins per sample, which is very rare in the available data on forages. Data show that maize silage is the most poly-contaminated forage as 78% of the samples contain more than 5 mycotoxins on the contrary to straw, hay and grass silage, with 46, 15 and 11%, respectively. As shown on other feed materials, fusariotoxins are also the most frequent mycotoxins in forages, but on the contrary to grains, deoxynivalenol (DON) is not always the most frequent. Mycotoxins, such as zearalenone (ZEN), can be more often positive in grass silage and hay than DON. Nevertheless, the median level of DON is always the highest in all types of forage samples, maize silages having higher DON median level of contamination (1,090 ppb) than straw (345 ppb), grass silage (70 ppb) and hay (25 ppb). Apart from fusariotoxins, our data also illustrate the specificity of grass to have endophytic toxins like ergot alkaloids (6 alkaloid toxins are quantified) on the contrary to maize silage and straw. In this study, *Alternaria* mycotoxins are not as rare as described in other studies as tenuazonic acid contaminates more than 20% of grass silage and hay samples. Regarding storage mycotoxins, as demonstrated in other studies, hay and straw are a good substrate for the development of *Aspergillus versicolor*. Consequently, the contamination in sterigmatocystin is the highest in these types of forage whereas aflatoxins and ochratoxins were almost not detected in the four types of forage tested. This unique wide study on mycotoxin contamination in forages is a first step in the identification and understanding of the different profiles of mycotoxin contamination in the main forages.

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Sicilian wheat is good for your health: survey of multi-mycotoxin occurrence in durum wheat grain from Sicily

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The aim of this study was to survey the presence of multi-mycotoxin in durum wheat grain from Sicily. A total of 176 samples of durum wheat grain and 124 samples of white flour produced in Sicily in the years 2016-2017 were collected. A rapid multi-mycotoxin analysis method based on liquid chromatography coupled to triple quadrupole mass spectrometry was validated and applied for the determination of twelve mycotoxins, including fumonisins B1, B2 and B3, aflatoxins B1, B2, G1 and G2, zearalenone, deoxynivalenol, T-2 and HT-2 toxin, and ochratoxin A. After extraction with acetonitrile, the toxins were detected by electrospray ionisation in positive ion mode and multiple reaction monitoring (MRM), achieving the separation in 6 min. Only a small number of samples were contaminated with at least one of these mycotoxins. None of the durum wheat grain samples exceeded the established regulatory limits in the European Union. Results of this study suggest that Sicilian wheat-based products are almost mycotoxin-free and do not represented any risk to consumer health.

P42

Study of correlation of aflatoxins in feed, milk and urine in dairy cows

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The aim of this study was to assess the level of aflatoxins intake and excretion to evaluate in milk and urine in dairy cows. Five cows in milk production were identified from two different farms in the Ngaka Modiri Molema District, South Africa. Daily feed, milk and urine were collected simultaneously for five

consecutive days. Collected samples were analysed using ELISA kits (R-Biopharm) and confirmed on HPLC. Results obtained showed the mean concentrations of aflatoxins total in feed at 12,154.85 ng/kg in feed samples collected from farm1 and 16,760.64 ng/kg in feed samples collected from farm 2 over a period of 5 days. The average carry-over in milk was of 0.12 ng/l in dairy cows from farm 1 and 0.04 ng/l in cows from farm 2, while the average carry-over in urine was of 4.42 ng/l in animals from Farm1 and 1.22 in animals from farm 2. Results obtained showed that the stress condition of animals influenced significantly the metabolism of aflatoxins excretion in milk and urine. In addition, these results agreed with those of from other studies regarding mycotoxin concentrations in feed while the carry-over in milk and urine was lower than the reported ones. In addition, it was found that the levels of aflatoxin M1 were above the MRL in milk from both farms. The presence of these toxins in both feed and milk can be controlled through management by monitoring the storage conditions of feed as well as environmental factors.

P43

Impacts of climate change environmental factors on colonisation and mycotoxin contamination of food commodities

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In the recent years, there has been a significant interest in the impact that climate change factors may have on mycotoxigenic fungi. We have now studied, for the first time, the effect that three-way interactions between water availability, temperature and elevated CO_2 have on different mycotoxin producing fungal species, including *Aspergillus* spp., *Fusarium* spp. and *Penicillium* spp. In this work, we will present the main results obtained for the different species and then we will focus on our newest results with *Aspergillus flavus*. For *A. flavus* we have studied effects on (i) growth, (ii) the relative expression of all genes in the aflatoxin gene cluster using both RT-qPCR and RNAseq, and (iii) the phenotypic aflatoxin B1 (AFB1) production on both conducive media and stored maize. Fungal growth, AFB1 production and expression of the all genes in the aflatoxin gene cluster by RNAseq were studied. The results in culture media showed growth was relatively unaffected. In contrast, the three-way interacting conditions had a profound effect on AFB1 production both in media and maize grains. Under slightly elevated CO2 conditions there was a stimulation of AFB1 production. Furthermore, we studied the temporal evolution of the AFB1 accumulation during the first 10 days of the incubation, sowing that the nutrients available in the media have a high impact on toxin accumulation. In stored maize grain, differential expression of several genes in the aflatoxin gene cluster where found in relation with these interacting factors. AFB1 production increased under elevated CO_2 conditions at both temperatures and aw tested. RNAseq data studying transcriptomic effects of the three-way interaction between elevated CO_2, temperature and water availability will be presented and discussed.

P44

A survey of mycotoxins in livestock feeds in Ethiopia

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Mycotoxins in feeds are a risk factor in the production of livestock due to potential adverse health effects in exposed livestock, and the potential of human exposure through animal-derived foods. A survey of commodities commonly used in livestock feeds in Ethiopia was conducted to provide information on the risk potential associated with feed commodities. Mycotoxins included in the survey were aflatoxin, fumonisin, ochratoxin, and vomitoxin. Levels of concern (LOC) were defined as 20 ppb for aflatoxin, 5 ppm for fumonisin, 100 ppb for ochratoxin, and 5 ppm for vomitoxin. A total of 108 samples were analysed from 22 feed distribution locations situated in major livestock-producing regions in the states of Amhara, Oromia, SNNPR, and Tigray. LOCs were reached in 31, 0, 6, and 0% of samples for aflatoxin, fumonisin, ochratoxin, and vomitoxin, respectively. LOCs were not associated with specific regions, but there was an association between LOCs and oil seed cakes. Following manufacture, oil seed cakes are distributed widely in Ethiopia, explaining the lack of regional bias in LOCs. Future studies should investigate the production chain of oil seed cakes in Ethiopia to identify mycotoxin production risk factors and potential risk mitigation opportunities.

P45

Determination of zearalenone, zeranol and other resorcyclic acid lactones in bovine and human hair

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Zearalenone (ZEN) is a resorcyclic acid lactone (RAL) mycotoxin with oestrogenic activity produced by several *Fusarium* species. Zearal'en'one' contains an alkene double bound ('en'), and a keto group ('one') which after reduction can lead to the corresponding alkane ('an') and alcoholic hydroxyl group ('ol'), and through that in formation of five other RALs: α-zearalenol (α-ZEL) and β-zearalenol (β-ZEL), α-zearalanol (α-ZAL) and β-zearalanol (β-ZAL), and zearalanone (ZAN). These five RALs may occur as reductive phase I metabolites formed by fungi, plants or mammals. In the field of veterinary drugs, α-ZAL is known as zeranol and commercially produced for use as hormonal growth promotor in cattle outside the EU. Due to the oestrogenic activity of ZEN and its metabolites (reduced forms and their phase II conjugates), ZEN is regulated in the EU in food (Commission Regulation (EC) No 1881/2006) and guidance values have been established for feed (Commission Recommendation 2006/576/EC). These (guidance) limits apply to ZEN only although it is recognised that phase I and II metabolites, when present in food and feed, significantly add to the overall exposure [EFSA, 2016. EFSA Journal 14:4425]. Exposure assessment of human and livestock to ZEN and metabolites is mostly limited to monitoring of ZEN in food and feed. Although phase I metabolites are also measured to some extent, the plant conjugates (e.g., glucosides) are typically not included, partly due to lack of analytical standards. Consequently, food/feed monitoring data may give an incomplete picture of overall exposure to ZEN and its toxicologically relevant metabolites. Biomonitoring is an alternative way to assess exposure in which biomarkers of the compound of interest are measured in a biological matrix. Based on animal and (limited) human data on the toxicokinetics of ZEN, it appears that excretion of ZEN and its major metabolites are suitable biomarkers of ZEN exposure in human [Mally *et al.*, 2016. Archives of Toxicology 90:1281]. In beef cattle, biomonitoring using urine [Le Bizec et *al.*, 2009. Journal of Chromatography A 1216:8016] and also hair [Ramboaud *et al.*, 2007. Analytica Chimica Acta 586:93] has been used to test for illegal use of zeranol. In biomonitoring, urine is the most widely used matrix. However, it typically provides information on recent exposure only. Hair is an alternative matrix providing information on exposure covering a longer time window (months). The aim of this work was: (i) to develop a method for quantitative determination of RALs in human and bovine hair; (ii) to carry out a first inventory of the occurrence of RALs in human hair as biomarkers for ZEN exposure; and (iii) to investigate RAL-profiles in hair of beef cattle and the ability to distinguish between RAL background caused by mycotoxin exposure through feed and (illegal) treatment with zeranol. For the LC-MS/MS-based method, extraction and clean-up were optimised. The final method involved hair pulverization, extraction with methanol, IAC clean-up and quantification using isotopically labelled standards. The limit of detection was in the range 0.1-0.25 pg/mg in both bovine and human hair. In human hair, no RALs were detected in the 23 samples analysed. In bovine hair (calf/cow) on the other hand, RALs were found in virtually all the 26 samples analysed (predominantly ZEN, α-ZEL and β-ZEL; 0.3-45 pg/mg). In an experiment in which a dairy cow was additionally exposed to high levels of ZEN, concentrations in the hair increased. In a calf treated with zeranol, the profile was distinctively altered and α-ZAL and β-ZAL were detected at 0.8-2.1 pg/mg hair in samples collected 33 days after starting (16 days after stopping) treatment.

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Worldwide mycotoxin occurrence in feeds and raw materials – survey data 2017

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Due to the natural co-occurrence of mycotoxins, the toxicity of contaminated feed cannot be accurately estimated by determining the concentration of one or two toxins only. The following study represents an effort to further broaden the knowledge on mycotoxin occurrence and co-occurrence in feed. The results hereby presented include data from samples sourced worldwide from January to September 2017. These samples were analysed using liquid chromatography-mass spectrometry/ mass spectrometry (LC-MS/MS, Spectrum 380®) at IFA-Tulln, according to Vishwanath *et al.*, 2009 [Analytical and Bioanalytical Chemistry 395:1355]. The analytical method was transferred to a more sensitive mass spectrometer (QTrap® 5500) and extended to cover more than 380 metabolites [Malachova *et al.*, 2014. Journal of Chromatography A 1362:145; Streit *et al.*, 2013. Journal of the Science of Food and Agriculture 93:2892]. For practical relevance, a cut-off level for all mycotoxins was established at >1 ppb (except aflatoxin at >0.5 ppb). A total of 802 feed and feed raw materials samples were analysed for more than 380 mycotoxins and other secondary metabolites. On average, 31 different metabolites were

detected per sample. 97% of samples contained 10 or more mycotoxins and metabolites. One of the most common fungal metabolites detected were moniliformin (detected in 76% of samples, with a mean average concentration of positive samples of 169 ppb and a maximum concentration of 2,900 ppb). Other common metabolites included beauvericin (74% of samples, mean 25 ppb, max 509 ppb), aurofusarin (74% of samples, mean 341 ppb, max 8,664 ppb), culmorin (71% of samples, mean 257 ppb, max 5,099 ppb), deoxynivalenol (65% of samples, mean 381 ppb, max 5,646 ppb), zearalenone (64% of samples, mean 46 ppb, max 2,748 ppb), fumonisin B1 (55% of samples, mean 974 ppb, max 18,990 ppb) and fumonisin B2 (51% of samples, mean 338 ppb, max 8,144 ppb). Enniatin compounds were also common; including B1 (62% of samples, mean 47 ppb, max 848 ppb), B (55% of samples, mean 51 ppb, max 1,056 ppb) and A1 (50% of samples, mean 20 ppb, max 366 ppb). Thanks to the increased sensitivity of mycotoxin analysis a greater number of mycotoxins and fungal metabolites have been reported. Performing multi-mycotoxin analysis is leading to the need for more research to evaluate the practical impact of most of these metabolites including emerging mycotoxins on animals and humans.

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Expression of human Th1/Th2 cytokines by human hepatocytes exposed to filamentous fungi and their combinations

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Th1 and Th2 cytokines produced by T helper cells are essential for maintaining innate immunity in the human body, particularly in the presence of infection or injury. Interferon gamma IFN-γ, tumour necrosis factor (TNF), interleukin 10 (IL-10), interleukin 5 (IL-5), interleukin 4 (IL-4) and interleukin 2 (IL-2) are some Th1 and Th2 cytokines that play important roles in the innate immune system. Healthy human hepatocytes were exposed to 4, 9 and 14 days old individual filamentous fungi and their combinations *in vitro* for 3 to 24 h and cytokine expression determined using a cytometric bead array (CBA) human Th1/Th2 cytokine kit and flow cytometry. Results obtained revealed that hepatocytes exposed to individual filamentous fungi elicited production of cytokines IFN-γ, TNF, IL-10, IL-4 and IL-2 at a range of 0.017-4.863 pg/ml, 0.023-0.460 pg/ml, 0.103-0.367 pg/ml, 0.107-4.183 pg/ml, and 0.203-2.680 pg/ml, respectively. *Fusarium* species induced the highest level of cytokine production by hepatocytes compared to other filamentous fungal genera used in the study. Filamentous fungal combinations also elicited the production of five cytokines (IFN-γ, TNF, IL-10, IL-4 and IL-2) at a range of 0.010-4.720 pg/ml, 0.020-2.093 pg/ml, 0.017-0.623 pg/ml, 0.020-3.693 pg/ml, and 0.037-3.217pg/ml, respectively. Combinations of two fungal species induced production of high levels of cytokines whereas combinations of more than two fungal species induced low levels or no production of cytokines at all. This study revealed that filamentous fungi has the ability to induce production of cytokines from hepatocytes *in vitro* when the cells are infected; the production of cytokines is an innate immune response by the hepatocytes to combat the effects of micro-organisms

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Fungal and mycotoxin contamination of small-scale maize from drought affected North-West province of South Africa

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Drought stress and high temperatures are two environmental factors that directly impact maize and other grains. Prevalence varies geographically, as does the risk of mycotoxin contamination of grains. Often climate extremes may lead to altered composition of fungal species infecting maize, which invariably could alter the mycotoxins contaminating infected grains. Contamination of foods by fungi and mycotoxins has been linked to various health and economic implications to both man and animals. This study evaluates the incidence of fungal species and mycotoxins contaminating small-scale maize from North-West province of South Africa. A total of 100 maize samples were randomly collected from small-scale farmers across the province. Samples were investigated for fungal contamination using conventional and molecular methods to identify fungal species. Mycotoxin analysis was done using IAC, TLC, HPLC and ELISA. Percentage incidence of different genera revealed the predominance of *Fusarium* (82%), *Penicillium* (63%) and *Aspergillus* species (33%). Among the species, *Fusarium verticilloides* had the highest incidence of 76% while *Penicillium digitatum* had 56% total incidence and *Aspergillus fumigatus* 27%. Mycotoxin analysis revealed that fumonisin B1 was the most contaminant mycotoxin with incident rate of 100%. Aflatoxins contamination occurred in 26.7% while ochratoxin A had a high incident rate of 97.8% and ranged from 3.60 to 19.44 µg/kg. Zearalenone occurred in 50%

of the samples. Results showed that maize from small-scale farmers may contribute to dietary exposure to mycotoxins. Although little can be done to influence weather, farmers can make agronomic management decisions to minimise the impact of drought on maize quality.

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Targeted approaches for ZEN biomarker discovery in prepubertal gilts

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Zearalenone (ZEN) is a frequent contaminant of cereal grains and acts as full and partial agonist on oestrogen receptors α and β, respectively. In pigs, clinical signs include ovarian atrophy, enlargement of uterus, swelling of the vulva, decreased fertility and stillbirth. Although biomarkers of exposure have been found to appropriately assess ZEN exposure under controlled conditions [Thanner *et al.*, 2016. Mycotoxin Research 32:69], biomarkers of effect might be more suitable for ZEN diagnosis in the field. Thus, the aim of our study was to identify potential ZEN biomarkers of effect by employing targeted approaches for biomarker discovery. To this end, a dose-response experiment was conducted in prepubertal gilts. Twenty-four female piglets (four weeks old) were randomly assigned to one out of four dietary treatments, receiving either control feed or feed artificially contaminated with 0.17, 1.45 or 4.6 mg/kg ZEN, respectively. After four weeks of exposure, serum, uterus and jejunum samples were collected, and effects of ZEN on oestrogen receptor response, immune system and plasma metabolome were investigated. Based on the presence of oestrogen-responsive element in their nucleotide sequences, a defined set of genes (EBAG9, OVGP1, IGFBP4, GJA1, LTF) was selected for subsequent qPCR analysis in uterus samples. Although the expression of some of these genes was significantly affected at the highest ZEN level, these effects were neither pronounced nor dose-dependent. Regardless of the dose, ZEN did not influence immune response of piglets, as assessed by gene expression of pro-inflammatory cytokines (IL-6 and IL-1β) in jejunum. Using a targeted metabolomics approach, no effect of ZEN exposure on 179 metabolites in plasma, including hormones, amino acids, biogenic amines, sugars and bile acids, could be observed. Although the mode of action of this mycotoxin is well known, there is still no appropriate biomarker of effect available for ZEN diagnosis in the field. Similarly, our targeted approaches did not result in the identification of suitable candidates. Hence, the use of non-targeted metabolomics or RNA sequencing might facilitate biomarker discovery in the future.

P50

Survey on citrinin in food and red yeast rice

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Citrinin is a mycotoxin produced by several species of the fungal genera *Aspergillus*, *Penicillium* and *Monascus*. Citrinin is nephrotoxic and is usually formed after harvest during storage as a result of mould growth [EFSA, 2012. EFSA Journal 10:2605]. To obtain insight in exposure of the population to citrinin, EFSA funded a project to select and analyse food samples across the EU [López *et al.*, 2017. EFSA supporting publication EN-1177, p.47]. A total of 1,195 samples of industry cereals (n=390; wheat, barley, rye, oats, rice), cereal-based products (n=510; flour, rice retail, bread and bread rolls, pasta (dry), breakfast cereals (flakes and muesli)), beans (n=68), fruit (n=66) and vegetable juices (n=69) and red yeast rice food supplements (RYR; n=92), were analysed for the presence of the mycotoxin citrinin. The samples were collected from industrial premises, retail stores and the internet (RYR), between September 2015 and November 2016, in France, Germany, Italy, Lithuania, the Netherlands, Poland, Spain, and Sweden. Samples were analysed by liquid chromatography-tandem mass spectrometry. The limit of quantification (LOQ) was 10 µg/kg for RYR and 1 µg/kg for the other matrices. Citrinin was detected at concentrations above the LOQ in 6% of the industry cereals, 3% of the cereal-based products, and in 26% of RYR samples. No citrinin was detected in beans and (fruit and vegetable) juices.

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Biodiversity of *Aspergillus* species and aflatoxin contamination of dairy feeds from farms around Bulawayo, Zimbabwe

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The presence of moulds especially of the genus *Aspergillus* in food commodities is often associated with aflatoxin contamination. This study aimed at determining the diversity of *Aspergillus* species and the level of aflatoxin contamination in feeds given to dairy cows around Bulawayo in Zimbabwe. Moulds were isolated by culturing on potato dextrose agar (PDA) using the plate dilution method. *Aspergillus* isolates were screened based on colony colour followed by the amplification and sequencing of their ITS region. The sequences were blasted into the GeneBank to determine the identities if the isolates. The isolates' ability to produce aflatoxins was determined using ammonia test after growing the *Aspergillus* on yeast extract sucrose (YES) agar and amplification of the major genes in the aflatoxin biosynthetic pathway *aflR*, *omt*, *mor* and *ver*. Quantification of aflatoxins in the feed was done using HPLC. Phylogenetic relationship of the isolates was analysed using the neighbour-joining method and the evolutionary analysis was conducted in MEGA7. A total of 96 samples were collected from 13 farms during the period (August-October 2016) and consisted of dairy feed concentrates, mixed ration, brewers spent grain and grass. Out of the 112 presumptive isolates, 73 were positively identified as *Aspergillus* using molecular methods. They showed 99-100% similarity to the following species, *Aspergillus niger*, *A. awamori*, *A. tubengensis*, *A. flavus*, *A. nomius*, *A. oryzae*, *A. parasiticus*, *A. fumigatus*, *A. foetidius* and *A. citratus* with 47% of the isolates testing positive for aflatoxin production on YES agar. Presence of aflatoxin biosynthetic genes were as follows: *nor* (53%), *ver* (15%), *omt* (22%) whereas only one isolate showed the presence of the *aflR* gene. Aflatoxin concentration in the feeds ranged from 0.1-251 µg/kg. This study showed the presence of aflatoxigenic *Aspergillus* species and aflatoxins in the feeds given to dairy cows and some of the samples had concentrations above the stipulated Zimbabwean limit of 20 µg/kg. This means that the population may be exposed to aflatoxin poisoning through the consumption of animal food products, such as milk. Therefore, there is need for the government authorities to put strict legislature in terms testing food commodities used in feed formulation for aflatoxin contamination.

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Oxidative stress induced by 'emerging mycotoxins' in an intestinal porcine epithelial cell line

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The formation of intracellular reactive oxygen species (ROS) is an indicator of oxidative stress. Continued oxidative stress can damage cell constituents as lipids, DNA, proteins, and carbohydrates resulting in tissue damage and further mediate various diseases. Farm animals are frequently exposed to different sources of oxidants by ingesting high-concentrate feed with possible pro-oxidant compounds, such as pesticides, organic solvents, and mycotoxins. Especially, many novel and less-investigated fungal metabolites, often called 'emerging mycotoxins' are found in feed samples with unknown toxicological profile. Those compounds often co-occur with regulated mycotoxins (e.g., deoxynivalenol, fumonisins) and their collective effects are non-considered. The aim of the study was to investigate the effects of those compounds on their capability of ROS production in an intestinal porcine epithelial cell line, IPEC-J2. Therefore, an *in vitro* method was used in which ROS convert the non-fluorescent dye DCFH to the fluorescing metabolite DCF measurable by a photometer. Gallic acid, a strong antioxidant, was used as a positive control. Emerging toxins were tested in a concentration range from 0.156 to 5 µM. Up to now, only tryptophol and alternariol showed an increase in ROS production at the highest tested concentration of 5 µM after 1 h of incubation. Tests are currently ongoing.

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Occurrence and identification of *Aspergillus* of the *Flavi* section and aflatoxins emergence in French maize

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Aflatoxins are secondary metabolites produced by *Aspergillus* of the *Flavi* section during their development, especially on maize. It is usually admitted that aflatoxin B1 (AFB1) is a major contaminant in tropical regions where climate favours *Aspergillus flavus* development. However, global warming could lead to the appearance of aflatoxins in maize produced in Europe. In 2015, in France, climatic conditions were favourable for aflatoxin production and surveys revealed the contamination of some maize. This study aimed to decipher the origin of this contamination by characterising the mycoflora of contaminated samples with a special emphasis on *Aspergillus* section *Flavi*. In order to better analyse the mechanisms leading to aflatoxin contamination, some samples, produced in the same geographic and climatic conditions but with no aflatoxin, were analysed in parallel. Thirty-one samples issued from fields (7 AFB1+ and 24 AFB1-) and 12 issued from silos just after harvest (all AFB1+) were analysed. All samples displayed comparable overall fungal loads whatever their origin, with mean fungal load of $3.5x10^5$ and $2x10^5$ cfu/g for fields and silos samples, respectively. *Fusarium* was the most predominant genus, observed in all samples. The mycological analysis also revealed the frequent presence of *Aspergillus* of the *Flavi* section and 67 strains were isolated from samples, some being contaminated with several different strains. Such isolates were observed, as expected, in all AF+ samples but also in almost 40% of AF- samples, demonstrating the presence of these potent toxin producers in soils in France. It has to be noted that *Aspergillus* section *Flavi* isolates were present at lower counts in AF- samples. Since these samples were cultivated in comparable climatic conditions, it suggests that, for AF- samples, agricultural practices may have limited the development of *Aspergillus* section *Flavi* and avoided toxin production. The identification to the species levels of isolates was done by morphological and molecular approaches. *A. flavus* was found to be the main contaminant, representing 69% of the strains. However, surprisingly, our survey revealed that *A. parasiticus* was also a frequent contaminant of French maize since it represented 28% of the isolated strains, mostly from AF+ samples. This finding is in agreement with the presence of AFG in some of those samples. The widespread distribution of *Aspergillus* section *Flavi* observed in this study and the sporadic contaminations with aflatoxins measured in maize highlight that, in a context of climate change, it could rapidly become an important safety issue in France.

P54

Exposure assessment of aflatoxin from dried figs in Turkey

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Dried figs, with their rich mineral content and nutritious ingredients, are among the most commonly consumed dried fruits by people of all ages. Dried figs are susceptible to aflatoxin (AF) production and AF can occur at high levels. There are several factors that affect the aflatoxin formation in dried figs because the fruit structure, formation, harvesting, and drying of figs differs than other dried fruits. Besides its high sugar content, AF contamination can occur easily as it has a fleshy skin which does not provide any protection. Since the toxin content of even a single fig can reach very high levels (e.g., a single fig can contain about 4,000 mg AF/kg), mycotoxin presence in figs is considered to be a great hazard for human health. Aflatoxin B1 (AFB1) is known to be a potent hepatocarcinogen and classified as carcinogenic to humans (Group I) by the International Agency of Research on Cancer. Due to its high toxicity and carcinogenic properties, legal tolerance levels in the EU are low for aflatoxin in foods that are destined for human consumption (8-10 ppb). The Codex Alimentarius Commission has adopted on 2 July 2012 new maximum levels for aflatoxins in ready-to-eat dried figs (6 µg/kg for AFB1 and 10 µg/kg for total aflatoxins). In the present study, AF analysis of dried figs results in Turkey were used to estimate of daily intake of Turkish population. In order to make this evaluation monitoring data from the Turkish Ministry of Food, Agriculture and Livestock between the years of 2012-2016 in Turkey were used. Samples were analysed for the presence of AFs using an immunoaffinity column for clean-up and HPLC with fluorescence detection. Food consumption data in Turkey are not available. Production data were used to calculate consumption data. Intakes are estimated by multiplying consumption data by the concentrations of AFs present in the dried figs. These intakes are expressed per kg of body weight using

the average body weight in Turkey (71.5 kg), according to the results of the National Institute of Statistics.

P55

Alternaria contamination of apples for food processing

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Argentinean apple production is destined both to internal commerce and export trade. More than 40% of the fruits, particularly those falling below quality and safety standards, are turned into fruit concentrates, which become supplies to different food industries. They are exported to China, USA, and Europe, where they are further processed to produce various apple-derived foods, such as juices, ciders, purées and infant foods. *Alternaria* causes the mouldy core rot in apples, a disease in the centre of the fruit without external symptoms, which usually remains undetected by the visual inspection commonly performed in the Argentinean fruit concentrate industries. Long-term storage in refrigerated chambers increases the incidence of mouldy core. *Alternaria* is known to produce a wide variety of toxic secondary metabolites that resist the thermal treatments applied in traditional food processing; they can, therefore, accumulate in the final products. *Alternaria* contamination in Argentinean apples for food processing was evaluated, including the incidence of fungal disease, metabolomic capacity of the strains and mycotoxin contamination. Hundred apple fruits were analysed for mouldy core rot. *Alternaria* strains were isolated in DCMA and morphologically identified according to Simmons, 2007 [CBS Biodiversity Series 6:1]. The metabolite profiling was done on DRYES (14 days, 25°C) by micro-extraction [Andersen *et al.*, 2015. Chromatography 2:277]. Detection was performed by UHPLC-HRMS in ESI+ and ESI-. The presence of alternariols (AOH and AME) and tenuazonic acid (TeA) was investigated in the fruits by HPLC-UV (modified from Da Cruz Cabral *et al.*, 2016. European Journal of Plant Pathology 145:363]. From the 100 apples for food processing analysed, 83 were affected by mouldy core, 10 showed external lesions caused by *Alternaria,* and only 7 were undamaged. The *Alternaria* isolates from the apples belonged to *A. tenuissima* (74), *A. arborescens* (1), and *A. gaisen* (1), and 7 remained classified as *Alternaria* sp. The main metabolites produced *in vitro* were altertoxin-I (78%), TeA (68%), tentoxin (61%), AOH (58%), AME (58%), altertoxin-II (56%), altenuene (33%), and altertoxin-III (33%). 93% of the apple fruits were contaminated with either AOH, AME or TeA, and 7% were simultaneously contaminated with the three mycotoxins. The toxin concentration ranges were 28-1,809 µg/kg for AOH, 113-21,764 µg/kg for AME, and 10-55 µg/kg for TeA. These results suggest a substantial risk of the presence of toxic metabolites in apple concentrates and by-products. The need for regulatory guidelines on *Alternaria* metabolites in apple-derived foods should be evaluated by the food safety authorities.

P56

Contamination of flours with deoxynivalenol in Thailand

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Deoxynivalenol (DON) is a naturally occurring mycotoxin mainly produced by *Fusarium* spp. that frequently infect maize, wheat, oats, etc. DON poses a potential health risk to humans and animal causing acute temporary nausea, vomiting, diarrhoea, abdominal pain, headache, weight loss, severe gastroenteritis, and immunosuppression. In Southeast Asia, DON is the third most prevalence mycotoxin (60% contamination) followed by fumonisin (83% contamination), and zearalenone (63% contamination) [BIOMIN, 2015. Mycotoxin Survey]. In Thailand, the average wheat flour consumption is 30 kg per person per year which tend to increase yearly. Unfortunately, there is no scientific report about DON contamination in wheat and flour in Thailand. Therefore, this research aims to investigate DON contamination in flours sold in Thailand. A total of 38 flour samples (9 maize starch flours, 6 glutinous flours, 6 rice flours, 8 all-purpose flours, 6 wheat flours for cake, and 3 wheat flours for bread making) were collected from the supermarket and further analysed for DON contamination using an enzyme linked immunosorbent assay (ELISA; Romer Labs®) with a limited of detection of 200 µg/kg. The results indicated that 95% of the samples were contaminated with DON. The highest contamination was in wheat flour for bread making, whereas the lowest contamination was in glutinous flour. In addition, 43% of the samples exceeded the maximum limit established by the EU cereal-based foods for infants and young children.

P57
A survey on mycotoxins detected in maize samples received from Serbia and Bosnia and Herzegovina during August to November 2017
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The aim of the present study was to screen maize samples received from Serbia and Bosnia and Herzegovina during August to November 2017. The samples were analysed by UHPLC-based multi-mycotoxin method for the determination and quantitation of all mycotoxins (aflatoxin B1, B2, G1 and G2, ochratoxin A, zearalenone, deoxynivalenol, fumonisin B1 and B2, T-2 and HT-2 toxins) regulated in feed (Directive 2002/32/EC, Commission Recommendation 2006/576/EC, and Commission Recommendation 2013/165/EU) by liquid chromatography coupled with tandem mass spectrometry. The method is based on 'dilute-and-shoot' principle. It involves two-step extraction and centrifugation of the extracts. To compensate the matrix effects in electrospray ionisation, the extracts are mixed with ^{13}C-labelled internal standards for each group of mycotoxins (U-[13C17]-aflatoxin B1, U-[13C15]-deoxynivalenol, U-[13C18]-zearalenone, U-[13C20]-ochratoxin A, U-[13C34]-fumonisin B1, and U-[13C24]-T-2) before injection onto LC-MS/MS (Agilent 6460c LC-MS/MS). A total of 113 samples were received for analysis at Patent Co., Mišićevo, Serbia. Out of these, 53% samples were found contaminated with mycotoxins and 28% of these contaminated samples were found to contain more than one mycotoxin. Aflatoxin B1, fumonisin B1 and B2, and HT-2 toxin were detected in 13%, 44%, 24% and 8% samples, respectively. Aflatoxin B1 ranging from 0.59 to 5,644 ppb, HT-2 toxin ranging from 9 to 66 ppb, fumonisin B2 ranging from 53 to 2,540 ppb, and fumonisin B1 ranging from 48 to 8,623 ppb were detected in maize samples. Therefore, this survey concludes that maize harvested in 2017 has high levels of aflatoxin B1, fumonisin B1 and B2 in Serbia and Bosnia and Herzegovina regions.

P58
Mycotoxins in commercially available beer in Lleida (Spain) and exposure assessment of the population
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The aim of the study was (i) to evaluate the incidence of mycotoxins in 64 beers purchased in Lleida (Spain), using a MS/MS detector for the simultaneous monitoring of 23 mycotoxins, and (ii) to evaluate the exposure to mycotoxins through beer consumption. Samples varied by their origin, brewing technology, alcohol content, etc. The results showed that 20.3% of the samples contained one or more of the following mycotoxins: zearalenone (ZEN), deoxynivalenol (DON), deoxynivalenol-3-glucoside (DON-3-G), fumonisin B1 (FB1) and HT-2 toxin. None of the mycotoxin positive samples contained mycotoxin levels overpassing the legal limits. Also, none of the alcohol-free samples (17% ot total samples) were contaminated with mycotoxins. The most frequently occurring toxin was ZEN, being quantified in 65% of the positive samples, with levels ranging from 8.24 to 62.96 µg/l. Regarding the co-occurrence of mycotoxins, three samples were found to contain two or more mycotoxins simultaneously, namely DON, DON-3-G and FB1. A deterministic approach was used to evaluate the contribution of beer consumption to mycotoxin daily intake and the proportion of the established tolerable daily intake (TDI) for ZEN and DON and its metabolite DON-3-G. According to the available data on national beer consumption (an average of 120 ml/day/person), the consumers are not at risk for none of the monitored toxins, although there might be a certain risk in the case of heavy drinkers (>0.5 l/day/person).
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P59
Ochratoxin A formation and detection in rye lots
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In North and Central Europe contamination of grain with ochratoxin A (OTA) mostly results from post-harvest *Penicillium* infection. Some *Penicillium* species need water activities not higher than 0.8 to grow. In particular, rye is susceptible to fungal growth as sorption isotherms are flat. This means that sufficiently free water for OTA production is already available after a little increase in relative humidity, e.g., from 14 to 16%. Variations in temperature in a silo can already cause zones with such conditions. The resulting OTA contamination is often confined to a small area in the lot. To avoid cost-intensive

recalls, mills have increased OTA control. However, even dynamic sampling and a huge number of tests per lot resulted in non-satisfying detection of OTA hot spots in raw material. Insufficient performance of on-site tests but first of all limitations in sampling might be a reason. Dust sampling was to be evaluated for its potential to identify grain lots with OTA hot spots. However, a new method can never be better than a method you compare it to and the real contamination of a huge grain lot or whole silo can never be completely ascertained. Hence, the biggest challenge to develop a new strategy for OTA monitoring is to generate reliable reference data. To gain more knowledge about OTA formation in grain silos and to test the potential of dust sampling to detect it, uncontaminated rye lots were infected with *P. verrucosum*. A commercial strain was first pre-cultivated. Its potential to form OTA was assured by HPLC-MS/MS measurements of culture extract and infected single rye kernels. Beside OTA, also further toxins as aflatoxins were present in the culture. Single kernels showed tremendously high OTA loads, confirming the theory that few single kernels can cause a contamination above the legal limit in a whole lot. Batches of 10 kg of rye were prepared with and without dust and with and without water addition. They were infected by either spore suspension or infected kernels. After a sufficient growing period, dust of the lots was separated and the kernels were completely milled. Both dust and kernel samples were analysed by HPLC-MS/MS. First results and experiences to set up OTA data models for dust sampling of rye as well as the evaluation of study designs to prove reliability of sampling methods were deduced.

P60

Evaluation of deoxynivalenol-sulfate as a useful biomarker of deoxynivalenol exposure in broilers chickens

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The goal of this study was to evaluate the blood kinetics of deoxynivalenol (DON) and its phase I and II metabolites in broiler plasma samples after a single oral administration of the toxin. Three experimental doses of oral DON application were tested: 0 (T-0), 0.75 mg DON/ml (T-1) or 2.25 mg DON/ml (T-2) (16 chickens 21-day-old per dose). Blood samples were collected from 1 bird of each pen (8 birds per treatment) after 15, 30 and 45 min, and 1, 2 and 4 h post administration. DON and its phase I and II metabolites in broilers plasma were analysed using liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS), after their extraction with an acetonitrile/water/acetic acid (59/40/1) solution. The limit of quantification (LOQ) of DON was set at 5 ng/ml using the LC-HRMS instrument. The results revealed that DON and C13-DON (internal standard), after a single oral administration, were below the LOQ and therefore could not be quantified in the samples. The phase II metabolite deoxynivalenol-sulfate (DON-S) was detected in all samples after oral application which, suggests that DON-S is an appropriate biomarker for DON exposure in broiler chickens. Since no commercial standard of DON-S is available, the results were evaluated using the absolute chromatographic peak areas. Average chromatographic peak areas were calculated per treatment and per time point. The maximum areas of DON-S were 16,873 and 37,274 for treatments T-1 and T-2, respectively, at 15 min (t_{max}).The mean area under the curve (AUC_{0-4h}) for treatment T-1 was 1,042,209 counts and the mean AUC_{0-4h} for treatment T-2 was 3,436,293. The ratio of counts between T-1 and T-2 was close to 1:3, similar to the ratio of doses of DON applied. Results of the study suggest that DON-S is a good biomarker of DON exposure. According to these results the optimum time for the evaluation of DON metabolism was 15 min after oral bolus.

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Tolerance of feed regulated mycotoxins by lesser mealworm and black soldier fly from artificially contaminated substrates

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The aim of the study was to investigate whether feed material contaminated with aflatoxin B1 (AFB1), deoxynivalenol (DON), ochratoxin A (OTA) and zearalenone (ZEN), either individually or as a mixture thereof, can be used as a primary feed source for insects. Two species were investigated: lesser mealworm (*Alphitobius diaperinus*, LMW) and black soldier fly (*Hermetia illucens*, BSF) larvae. Blank larval feed was spiked with the parent compounds, with concentrations ranging from 1 to 25 times the EC maximum limit for all feed materials for AFB1 [Directive 2002/32/EC] and EC guidance values for

complete feeding stuffs for DON, OTA, and ZEN [Commission Recommendation 2006/576/EC). Both larvae and the residual material after harvesting the larvae were tested with a validated chromatographic/mass spectrometric analytical method for parent compounds and relevant metabolites: aflatoxicol, aflatoxin P1, aflatoxin Q1 and aflatoxin M1, 3-acetyl-DON, 15-acetyl-DON, DON-3-glycoside, and α- and β-zearalenol. LMW and BSF larvae development and survival rates were not influenced by the contaminated feed material. No mycotoxin accumulation was observed in LMW larvae and BSF larvae contained only small traces of mycotoxins when compared to the mycotoxin concentrations in feed. Metabolisation of the four parent mycotoxins varied between LMW and BSF larvae. For example, LMW larvae metabolised AFB1 at rates less than 40% whereas the metabolisation rate for AFB1 in BSF larvae exceeded 80%. Mass balance calculations revealed that only minimal amounts of traces of metabolites were encountered, except for α- and β-zearalenol in BSF larvae, where substantial metabolisation was detected (average maximum of 86% of the overall mass balance). Furthermore, no differences were observed when the larvae were subjected to the individual mycotoxins or to the mycotoxin mixture.

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Combination of zearalenone, alternariol and genistein negatively impacts porcine embryo development *in vitro*

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The adverse effects of mycotoxins in pigs, especially the *Fusarium* mycotoxin zearalenone (ZEN) is well known. However, most information is based on single contamination at concentration levels above EU guidance limits, i.e., >0.1 ppm. Recent findings indicate that co-occurrence of mycotoxins can lead to additive or even synergistic effects, which may have a negative consequence on animal performance. Besides ZEN, other compounds with oestrogenic activity are found in feedstuffs used in the pig diet, the so-called phytoestrogens. Isoflavones are the most common phytoestrogens, and they are mostly found in soybeans in the form of genistein, which presented synergistic activity with ZEN and with the *Alternaria* mycotoxin alternariol (AOH). Here we evaluated if these mycotoxins and the phytoestrogen genistein (GEN) can interact, and if this interaction can affect porcine embryo development. Concentrations were selected based on previous studies indicating no toxicity of each isolated compound at such levels. Exposure to ZEN 1 μM + AOH 5 μM + GEN 1 μM (ZAG) occurred during *in vitro* maturation (IVM) of oocytes or during IVM, fertilisation and embryo culture *in vitro*. After six days, the rates of blastocysts formation were determined. As a control, exposure during IVM or IVM and embryo culture was performed in the presence of oestradiol 3.14 μM. It was observed that, as expected, exposure to oestradiol decreased ($P<0.05$) the percentages of blastocyst formed from oocytes. Exposure to ZAG only during IVM did not ($P>0.05$) affect embryo development. However, when embryos were also exposed to this combination during culture, a decrease ($P<0.05$) in the percentages of formed blastocysts was observed. These results indicate that a combination of ZEN 1 μM + AOH 5 μM + GEN 1 μM negatively affect embryo development. The next step consists in evaluating the interaction between the three compounds.

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Oocytes from gilts are more sensitive to beauvericin than sow oocytes: a possible difference in antioxidant capacity

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Deoxynivalenol (DON) and beauvericin (BEA) are secondary metabolites produced by *Fusarium* and are associated with growth retardation and fertility problems in stock-breeding. Both chemicals have a wide range of toxicity, and pigs demonstrated to be most sensitive for these toxins. Both chemicals demonstrated to induce oxidative stress in somatic cells. Oocyte maturation is a precisely orchestrated process where formation of reactive oxygen species (ROS) needs to be minimised. To prevent damage of ROS, intracellular glutathione (GSH) gradually increases in the oocyte during maturation. Developmental potential of sow oocytes is higher than that of gilt oocytes, and sow oocytes are expected to have higher intracellular GSH levels and to be more resistant against oxidative stress. The objective was to evaluate the toxicity of DON, BEA and ROS and the antioxidant role of GSH during *in vitro* maturation of sow and gilt oocytes. Cumulus oocyte complexes (COCs) were collected from sow and gilt ovaries by aspiration of 2-4 mm follicles and cultured for 44 h at 38.5°C and 5% CO_2 in air. During

the entire culture period, oocytes were continuously exposed to 0.02-2.0 µM DON, 0.5-5.0 µM BEA or 10-100 µM H_2O_2 in 0.1% DMSO (control). After culture, oocytes were fixed, stained for chromatin-tubulin and examined with fluorescence microscopy for progression through meiosis. In addition, in matured gilt and sow oocytes from the control group, oocytes were determined for GSH. In both sow and gilt oocytes, exposure to BEA and H_2O_2 resulted in degeneration of oocytes at prophase stage thereby preventing oocytes to progress to the metaphase II (M2) stage. However, higher percentages of sow oocytes were at the M2 stage compared with gilt oocytes (5 µM BEA: 66 vs. 0%; 100 µM H_2O_2: 61 vs. 7%). When exposed to DON, aberrant meiotic spindles were observed both in sow and gilt oocytes with similar percentages of M2 oocytes (2 µM DON: 24 vs. 19%). Intracellular GSH levels in gilt oocytes were similar to those in sow oocytes (sows: 6.7±2.5; gilts: 8.0±3.0 pmol GSH/oocyte). Different toxicity of BEA in sow and gilt oocytes may be due to a difference in oxidative stress resistance. GSH is considered as the primary defence against ROS, but gilt and sow oocytes were found to have similar GSH levels in the ooplasm suggesting that resistance to oxidative stress is also due to other defence mechanisms.

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Occurrence of ergot and mycotoxins in Western Canada

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Ergot alkaloids and mycotoxins are toxic secondary metabolites produced by fungus which contaminate a variety of food and feed sources. These toxins cause a wide range of effects and disorders when consumed. Prairie Diagnostic Services (PDS) offers analysis of grain and feed samples for farmers and producers. There are currently two diagnostic panels in place. The first panel detects 6 ergot alkaloids (ergosine, ergocornine, ergocristine, ergocryptine, ergotamine, and ergometrine). The second panel detects 14 mycotoxins (DON, NIV, 3-ADON, 15-ADON, DAS, ZEN, T-2, HT-2, α-ZEL, β-ZEL, OTA, AFB1, FB1 and FB2). High performance liquid chromatography tandem mass spectrometry was used to determine both ergot and mycotoxin contamination. The current limit of detection for each ergot alkaloid is 1.25 ppb (µg/kg). Mycotoxins can be detected at 25 ppb (µg/kg) with the exception of α-ZEL and β-ZEL which are detected at 66 ppb (µg/kg) or greater. The majority of the samples received for testing of ergot and/or mycotoxins came from Western Canada (Saskatchewan (58%), Alberta (22%) and Manitoba (16%)). Within this geographical area, ergocristine had both the highest concentration and frequency of detection of the 6 ergot alkaloids followed by ergocryptine and ergotamine. With respect to the 14 mycotoxins, DON, 3-ADON, 15-ADON, NIV, T-2 and H-T2 were the most commonly detected mycotoxins above detection limits. In 2016, it appears that the elevated Saskatchewan rainfall in May and June led to an increase in the number of sample submissions. The continued monitoring of the number of samples submitted for these analyses will help predict the future trends related to ergot and mycotoxin contamination in Western Canada that may influence international market demands.

P65

Three major *Fusarium* mycotoxins modulate the barrier function of *in vitro* human placental cells.

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Deoxynivalenol (DON), T-2 toxin and zearalenone (ZEN) are the major *Fusarium* mycotoxins in human food and animal feed on a global level. Moreover, these mycotoxins can readily pass through the placenta during pregnancy and are present in human breastmilk, which can lead to patho-physiological effects in the offspring. However, the effect of mycotoxins on the placental cells itself has hardly been investigated. The aim of this study is to measure cytotoxicity, cytokine release and barrier function after concentration-dependent administration of DON, T-2 and ZEN to human placental cells. BeWo cells (human placental choricarcinoma cells) were exposed to different concentrations DON (1, 2, 4, 8 µM), T-2 (1, 2, 4, 8 nM), ZEN (2, 4, 8, 16 µM) for 24 h *in vitro*. Cytotoxicity of these mycotoxins was determined via the lactate dehydrogenase (LDH) release. The effects of non-toxic concentrations of mycotoxins on immune markers were measured using an ELISA for IL-6 and IL-8. To determine the effects of these mycotoxins on barrier function, mRNA expression (RT-qPCR) and protein levels (western blot analysis, immunofluorescence staining) of highly expressed tight junctions in BeWo cells were quantified. Increasing concentrations of DON, T-2 and ZEN all affected the cell viability, although T-2 toxin was

more toxic compared to the other mycotoxins. Non-toxic concentrations of mycotoxins increased IL-6 release in BeWo cells concentration-dependently, whereas no significant effect on IL-8 release was observed. Protein and mRNA levels of occludin, ZO-1 and claudin-1, -3 and -4 were modified due to the exposure to different non-toxic mycotoxin concentrations. In conclusion, low mycotoxin concentrations alter the expression of tight junction proteins *in vitro*. This could be a direct effect of these mycotoxins but could also be explained by the local release of IL-6. The release of IL-6 and associated change in barrier function may even further enhance the passage of mycotoxins or other pathogens through the placental barrier during pregnancy, which hence may lead to pathophysiological effects in the offspring.

P66

Accumulation of ochratoxin A in the presence of gluconic acid contributes to pathogenicity of *Aspergillus carbonarius*

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Aspergillus carbonarius, the main species causes severe post-harvest decay of vine fruit, is considered as the major source of ochratoxin A (OTA) contamination of grapes and derived products. Our previous findings indicated that production of organic acids, such as D-gluconic acid (GLA), *by A. carbonarius* in the growth medium or in the decayed fruit tissue was directly related to ambient pH reduction. Involvement of GLA in pathogenicity has been suggested but the mechanism of OTA accumulation and its contribution to *A. carbonarius* pathogenicity remain unclear. Deletion of the gene encoding glucose oxidase (*gox*) in *A. carbonarius* was carried out to suppress the conversion of glucose to GLA with the aim to investigate the roles of GLA and OTA accumulation in *A. carbonarius* pathogenicity. The obtained results showed that the GLA accumulation was completely inhibited in grape berries infected with Δ*gox* knockout mutant, concomitant with a reduction in decay development compared to the wild-type strain, suggesting that tissue acidification significantly contribute to *A. carbonarius* pathogenicity. Functional characterisation of Δ*gox* mutant showed a significant decline in relative expression level of nonribosomal peptide synthetase gene (*nrps*), which is involved in OTA biosynthesis. Indeed, impairment in the ability to produce GLA was accompanied by a drastic reduction in the OTA production. Present results indicate that elimination of GLA production, and not low pH, affected OTA accumulation, suggesting that GLA production is the driving force for activation of OTA synthesis and, together, these compounds contribute to the enhanced pathogenicity of *A. carbonarius* in fruit.

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Deoxynivalenol induces nephrotoxic effects in an *ex vivo* model

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Kidneys play an essential role in the maintenance of homeostasis, so knowledge about the toxic effects of xenobiotics on the kidneys as well as the mechanisms of injury is of scientific relevance. Ochratoxin A and citrinin have often been associated as renal injury promoters, however, data concerning the effects of deoxynivalenol (DON) on the kidneys are scarce. Thus, the aim of the present study was to investigate the potential use of renal explants as an *ex vivo* model and to evaluate the effects of renal exposure to DON using this model. A total of 180 kidney explants from ten 24-day-old pigs were evaluated. The explants were divided into: control 0 h (0 h), control 4 h (4h), and a group exposed to DON (10 μM) (DON). The groups 4 h and DON were incubated for 4 h at 37°C in culture medium (DMEM) (4 h) or DMEM+DON (DON). Explants from 0 h group were not incubated. After incubation the explants were fixed and processed for histological analysis. A lesion score was established according to the intensity and severity of the histological changes. Samples of the incubated mediums were used to evaluate gamma glutamyltransferase (GGT) and creatinine levels using an enzyme activity test of high sensitivity and colorimetric assay, respectively, in order to assess renal function. Data of the lesion scores and biochemical analysis were submitted to statistical analysis (analysis of variance and Tukey's test) considering $P \leq 0.05$. The main histological findings in both control groups were congestion (62% in 0 h group) and cytoplasmic vacuolisation (41% in 4 h group). No significant difference in the lesional score was observed between 0 h (3.19) and 4 h (4.67) control explants. This result indicates that renal homeostasis is preserved during the period of incubation, maintaining tissue viability. DON induced a significant increase in lesion score (8.25) compared to 4 h group. An increase in the frequency and severity of cytoplasmic vacuolisation (72%) was observed in this group. Concerning biochemical analysis, a significant increase in GGT level was observed in DON group (9.52 UI/L) compared to control

(4 h) (6.87 UI/L). No significant change in creatinine level was observed between the treatments. Taken together, our results denote that renal explants are a suitable model for toxicological analysis. In addition, the nephrotoxic action of deoxynivalenol, even in low concentrations, was demonstrated. The damage to renal cells can affect the renal function and the homeostasis of the organism.

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Deoxynivalenol induces changes in the antioxidant response in renal explants

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Deoxynivalenol (DON) is one of the most frequent trichothecene contaminant of grains worldwide. Toxic effects were associated with inhibition of protein synthesis and generation of free radicals. DON induces tissue damage in renal explants; meanwhile the mechanisms involved remain uncertain, leading to the interest in investigating DON-induced oxidative stress. Antioxidants substances are largely known for the management of oxidative stress. Considering that phytic acid (IP6) modulates DON toxicity in intestinal tissue, it is interesting to evaluate the potential beneficial effects of this antioxidant in DON-induced renal changes. The aim of this study was to investigate the effects of DON on oxidative stress in renal tissue and the efficacy of IP6 in DON-induced toxicity. Renal explants from five piglets (24 days-old) were exposed for 4 h (37°C) to the following treatments: control (DMEM medium), DON (10 µM), IP6 (5 µM), and DON plus IP6 (10 µM + 5 µM). A total of 40 explants were analysed. The levels of thiobarbituric acid-reactive substances (TBARS) were quantified spectrophotometrically to evaluate lipid peroxidation. The ability of the samples to resist oxidative damage was determined through ferric-reducing ability potential (FRAP) and free-radical scavenging ability (ABTS) assays. The results are expressed as nmol of Trolox equivalent per milligrams of protein in both assays. The reduced glutathione (GSH) levels in renal explants were determined using a spectrophotometric method and the results presented as mmols of GSH per milligrams of protein. The means were submitted to statistical analysis (ANOVA and Duncan test) and significance was set at 0.05. The levels of TBARS in renal explants showed no significant changes when compared to control ($P>0.05$). Concerning the ability to resist oxidative damage, explants treated with IP6 showed a significant increase in FRAP and ABTS levels when compared to control. DON induced a significant increase in FRAP level compared to control. DON and IP6 induced no significant changes in GSH levels compared to control, whereas DON + IP6 resulted in a significant increase in GSH level compared to DON group. The results suggest that lipid peroxidation is not the main mechanism of DON-induced renal damage. Oxidative stress can also result from imbalance of antioxidant systems. A tendency to a decrease in GSH level was verified in this study leading to the hypothesis that DON affects this mechanism of defence, which may involve the inhibition of protein synthesis. Furthermore, the increase in GSH levels in explants exposed to DON + IP6 reinforces this hypothesis.

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Individual and multitoxic effects of fumonisin B1, deoxynivalenol and zearalenone on rat liver and kidney phospholipids and lipid peroxidation

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A 14-day *in vivo*, multitoxic rat experiment was conducted with zearalenone (ZEN; 15 µg/animal/day), deoxynivalenol (DON; 30 µg/animal/day) and fumonisin B1 (FB1; 150 µg/animal/day), as single mycotoxins, binary (FD, FZ, DZ) and ternary combinations (FDZ). All mycotoxins were administered as a single bolus (1 ml water solution) per day and compared to a toxin-free control. Somatic parameters, membrane lipid fatty acid (FA) profile (liver and kidney), oxidative stress markers and blood biochemicals were recorded and analysed by ANOVA, Bliss independence test (interactions), and principal component analysis (PCA). Body weight was unaffected, liver (ZEN↑ vs. DON) and kidney weight (ZEA and DZ ↑ vs. FDZ) increased. Hepatocellular membrane lipids referred to ceramide synthesis disturbance (C20:0, C22:0), and decreased unsaturation (C22:5 n3 and unsaturation index), mainly induced by DON and to a lesser extent by ZEN. The DON-FB1 interaction was additive (C20:0). In renal phospholipids, ZEN had the strongest effect on the FA profile, affecting the saturated and the n6 FAs; ZEN was in most of the cases in an antagonistic relationship with FB1 (C18:0) or DON (C18:2 n6, C20:1

n9). Hepatic oxidative stress was the most expressed by the FD treatment (reduced glutathione and glutathione peroxidase ↑), while nephrotoxic effect was proven by malondialdehyde in the DON treatment. Serum creatinine was the highest in the FDZ group, with significant FB1↔DON, DON↔ZEA and FB1↔ZAE antagonism results. PCA was effective in the identification of the FAs contributing with the highest relative power to the overall variance (liver and kidney, similarly: C16:0, C18:0, C18:2 n6, C20:4 n6). Results refer to multiple interactions of the analysed fusariotoxins, needing further analysis.

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Localised variability in the *Fusarium* damage – DON relationships for Canadian wheat in 2016

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In the 2016 growing season, *Fusarium* head blight (FHB) was widespread in western Canada. Approximately 70% of harvest samples submitted by producers to the Canadian Grain Commission's Harvest Sample Program contained visible evidence of *Fusarium* damage (FUS DMG) in 2016, versus 9% in 2017. Visual symptoms of FHB in wheat include bleached heads and shrivelled chalky or pinkish-coloured kernels. On the Canadian Prairies in western Canada, the predominant *Fusarium* species is the DON-producing *Fusarium graminearum*. Therefore, the incidence of visible FUS DMG is correlated with the concentration of DON measured in wheat. Due to the high incidence of FUS DMG in wheat grown in 2016, the FUS DMG-DON relationship was studied on a more localised basis, in addition to the Prairie-wide assessment in 2016. Samples from the two largest classes of wheat grown in western Canada – Canada Western Red Spring (CWRS), and Canada Western Amber Durum (CWAD) were used in this work. Four crop districts from across the Prairies were selected for each class. All samples of wheat grown in these districts (or 50 randomly selected samples, if more than 50 were available) were analysed from each crop district. The FUS DMG was quantified in each sample by experienced inspectors, and then the entire mass of samples was ground, sub-sampled using rotary sample dividers to avoid bias, and analysed for DON using ELISA. The Prairie-wide assessment consisted of 50 samples of wheat from each class that were randomly selected from the pool of submitted samples. These samples were inspected and processed as described above, however analysis was performed using a multi-mycotoxin UPLC-MS/MS method. Linear correlations were observed between FUS DMG and DON for the Prairie-wide assessment of CWRS and CWAD. For CWAD, the FUS DMG-DON relationships observed in the four crop districts were consistent with the Prairie-wide assessment. For CWRS, samples from the crop district in southern Alberta did not show a relationship between FUS DMG and DON; the relationship was consistent between the Prairie-wide assessment and the other three crop districts though. For CWRS from the Albertan crop district, the DON concentrations measured in samples were much lower than expected based on the degree of FUS DMG observed. There was no indication from the multi-mycotoxin UPLC-MS/MS analyses that a non-DON producing *Fusarium* species was responsible for the FUS DMG, suggesting that the visible symptoms of damage were not produced by *Fusarium*.

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Aflatoxin exposure among infants and young children in the Northern Province of Rwanda

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Aflatoxins contamination of food commodities is still a serious problem in Sub-Saharan Africa. Maize and peanut are known to be highly susceptible to aflatoxins contamination. This poses a constant risk of exposure to young children as maize and peanut are part of the complementary diets fed to them. The aim of this research was to analyse the total aflatoxins contamination of maize and peanut flours that are used as part of complementary feeding of children and estimate the exposure to aflatoxins in infants and young children. A cross-sectional survey was conducted in Musanze District located in the Northern region of Rwanda, with children aged 5 to 30 months. The maize and peanut flours samples were collected from households and in the local centres from which local families buy their maize and peanut flours. Aflatoxins content of the flours was analysed using a single-step lateral flow assay (Reveal Q+) and the results were validated using thin layer chromatography. Dietary intakes of maize and peanut flour were estimated using a one day 24-hour recall questionnaire. Aflatoxins exposure of children was estimated from the maize and peanut intake of children and the aflatoxins content of the flours. In total,

145 children participated in the study. A high percentage of children (48%) consumed maize on a daily basis, while for peanut the consumption per week varied, with most children (33%) consuming it twice or once per week. Maize flour was fed to children as porridge, and peanut flour was included in local dishes or cooked in a soup. All the samples of maize and peanut flour analysed tested positive for aflatoxins. The median aflatoxins content of maize and peanut was 3.02 µg/kg and 181.4 µg/kg, respectively. Peanut flour was highly contaminated with all the samples exceeding the maximum tolerable limit for aflatoxins in the European Union (4 µg/kg) and in East-Africa (10 µg/kg). The exposure to aflatoxins ranged from 0.00-580 µg/kg bw/day; with the highest mean exposure coming from peanut 16.62 µg/kg bw/day and from maize to a lesser extent 0.59 µg/kg bw/day. We conclude that children in the northern region of Rwanda are chronically exposed to aflatoxins through the consumption of contaminated maize and peanut flours. Community based methods to reduce the contamination level of maize and peanut, such as increasing the awareness of the local population about aflatoxins and use of improved storage methods to reduce the contamination levels, are needed.

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Growth and T-2 and HT-2 production by *Fusarium langsethiae* under climate change environmental factors

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Since the species was named in 2004, *Fusarium langsethiae* has been found to be an increasing problem in Nordic countries, central Europe and the UK/Ireland. This symptom-less fungus can lead to a high amount of T-2 and HT-2 contamination in oats, barley and wheat. T-2 toxin is considered the most toxic trichothecene and has immunosuppressive effects. Although, there is no current information with regard to the effects that forecasted environmental conditions due to climate change will have on the growth and toxin production by this fungal species. In this work, the first objective was to study the effect of water activity (a_w; 0.995-0.90) and temperature (15-25°C) in an oat-based medium on the growth rate and T-2 and HT-2 production of 3 newly isolated strains from the UK and the recently sequenced *F. langsethiae* Fl201059. The results were compared with our previous study on strains isolated in UK, Norway, Finland and Sweden. The most interesting strains were selected for complementary ecophysiological studies including boundaries temperature and a_w conditions. The second objective was to study the impact that climate change environmental fluctuations will have on these strains. The effect of elevated CO_2 (400 and 1000 ppm) was studied under different a_w (0.95/0.98) and temperatures (25/30/34°C) simulating the global climate change predictions. These results will provide the first information of the impact of climate change on *F. langsethiae* and its production of T-2 and HT-2 toxins. **Acknowledgements**. BBSRC-SFI-Oats for the future project BB/P001432/1.

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The influence of deoxynivalenol and zearalenone on the immunohistochemical expression of oestrogen receptors and genes encoding detoxification enzymes in pre-pubertal gilts

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Plant-based materials used in the production of pig feed are very often contaminated with deoxynivalenol and zearalenone. Daily intake of small amounts of these mycotoxins with feed induces various subclinical states in gilts and influences different biological processes. Mycotoxins intensify or inhibit proliferation and apoptosis, induce changes in the steroid hormone balance and participate in metabolic processes in colonic enterocytes which probably prevent preclinical states characterised by intensified proliferation. The aim of this preclinical study was to evaluate the influence of low monotonic doses of zearalenone and deoxynivalenol (40 µg/kg bw and 12 µg/kg bw, respectively, over a period of 42 days) on the immunohistochemical expression of oestrogen receptors (ERs) in the intestinal tract and the mRNA expression of genes encoding selected colonic enzymes. The immunohistochemical expression of ERα was observed in the colon, but its intensity varied in different weeks of exposure. A minor increase in ERβ expression was noted in the colon, whereas the expression of mRNA CYP1A1 and mRNA GSTP1 decreased. The results of this study suggest that the risk of loss of control over the biotransformation and biological activity of the parent compounds in distal intestinal mucosa is delayed.
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MANAGING THE IMPACT OF MYCOTOXINS
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The capability of plant-derived *Lactobacillus plantarum* BCC47723 to reduce mycotoxins

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Mycotoxins are the toxic secondary fungal metabolites mainly produced by certain filamentous fungi in genus *Aspergillus*, *Penicillium*, and *Fusarium*. Mycotoxins can contaminate various agricultural commodities either before harvest or under post-harvest conditions. FAO reported that 25% of the world's crops are affected by mould or fungal growth with losses of around 1 billion metric tons of foods and food products annually. Several attempts have been made to develop methods either to remove mycotoxins from agricultural products and feeds or to degrade mycotoxins into less toxic compounds. Biological method is an alternative to use for mycotoxins removal. Lactic acid bacteria (LAB) are a Gram-positive bacteria group which numerous reports indicating that these bacteria isolated from gut, rumen animal and dairy products can reduce mycotoxins from liquid medium. In contrast, LAB from vegetable fermented foods have never studied before. Nowadays, plant-derived LAB are the popular group to use in food industries, such as fermented food manufactures and beverage manufactures, due to its properties: producing high acids, resisting acid and bile, inhibiting pathogenic bacteria, and producing some antioxidants. These properties are the reason that plant-derived LAB are interesting to apply for mycotoxins removal. The aim of this study is to assess the capability of plant-derived LAB (*Lactobacillus plantarum* BCC47723) to remove mycotoxins *in vitro*. The strain was isolated from wild spider flower pickle, a fermented vegetable product typically found in Thailand. The strain was tested for its ability to reduce the main mycotoxins (AFB1, ZEN, FB1, OTA, DON, T-2 toxin and HT-2 toxin). The results show that the strain can reduce OTA with the highest percentage removal 82.8%), T-2 toxin (77.8%), ZEN (69.6%), AFB1 (41.3%), FB1 (7.7%) and HT-2 toxin (3.3%). Unfortunately, this strain did not reduce DON.

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Effect of sodium meta-bisulphite (NaMBS) and selected preservatives on *in vitro* growth and aflatoxin B1 production by strains of *Aspergillus flavus* isolated from chillies

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Seven different potential preservatives were compared for potential control of growth and aflatoxin B1 (AFB1) production by strains of *Aspergillus flavus* isolated from chillies. The efficacy of the 7 compounds (sodium meta-bisulphite (NaMBS), potassium sorbate (PS), propyl paraben (PP), calcium propionate (CP), trans-cinnamic acid (TC), ferulic acid (FE), and fumaric acid (FM) were examined at three water activity (a_w) levels in a 10% chilli powder medium to calculate the ED_{50} values and the minimum inhibitory concentrations (MIC) required for growth and AFB1 control at 30°C. Of these compounds, NaMBS was the most effective compound in controlling growth and AFB1 production by strains of *A. flavus* at 0.93, 0.98 and 0.995 a_w. In general, mycelial growth and AFB1 production of the three strains were gradually inhibited by increasing NaMBS concentrations. No growth and production of AFB1 occurred at 500, 1,250 and 2,500 ppm at 0.93, 0.995 and 0.98 a_w, respectively. One *A. flavus* strain (DAJ1) was more sensitive to NaMBS (ppm) when compared with the other two strains. The ED_{50}, ED_{90} and MIC for NaMBS and other preservatives were calculated for both mycelial growth inhibition and AFB1 control. The best compound was NaMBS followed by PS, PP, TC, CP, FE and FM, respectively. Statistical analysis showed that NaMBS (ppm), a_w and their interaction had a significant effect ($P<0.05$) on mycelial growth and AFB1 production for the three strains. There was also a significant effect of preservatives (ppm), a_w and their concentration ppm x a_w on growth and AFB1 production by *A. flavus* (DAJ1). AFB1 production was unaffected by a_w in the PP, FE and TC preservative treatments. Studies are in progress to examine *in situ* control of *A. flavus* growth and AFB1 production on stored chillies.

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The effect of *Fusarium* mycotoxins and a mycotoxin deactivator on performance and metabolism of early lactation autumn calving dairy cows

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The objective of this research was to investigate the effects of *Fusarium* mycotoxins (FM) and a mycotoxin deactivator (MD) (UNIKE Plus, Nutriad) in dairy cow diets on performance and metabolism of dairy cows in early lactation. Twenty-eight Holstein Friesian cows were allocated to one of two dietary treatments (n=14) in a randomised complete block design. T1 (control) maize contained low FM levels (deoxynivalenol (DON) 163 µg/kg TMR DM and zearalenone (ZEN) 19 µg/kg TMR DM), and T2 maize contained high FM levels (1966 µg DON/kg TMR DM and 366 µg ZEN/kg TMR DM). Cows were blocked on calving date and balanced for parity, predicted milk yield and body condition score. Cows started the trial 28 days post calving and there were 4 dietary periods within each treatment: P1= acclimatisation (10 days); P2=low vs. high FM TMR (28 days); P3=low + MD (15 g/cow/day) vs. high + MD (15 g/cow/day) FM TMR (28 days); P4=low vs. high FM TMR (28 days). In P2, there was no difference observed between T1 and T2 for DMI (T1=20.97 vs. T2=20.52 kg DM, P=0.64), milk yield (T1=37.49 vs. T2=36.75 kg, P=0.38), milk fat and protein (T1=2.61 vs. T2=2.59 kg, P=0.47). Similarly, there was no difference in liver enzymes alanine aminotransferase (T1= 24.82 vs. T2=24.13 u/l, P=0.46) or aspartate aminotransferase (T1=34.62 vs. T2=29.23 u/l, P=0.28) between treatments. The inclusion of the MD in P3 resulted in differences in T2 cows having higher milk fat kg (T1=1.39 vs. T2=1.51kg, P= 0.03), milk fat (T1=4.48 vs. 4.78%, P=0.04), protein corrected milk (T1=34.00 vs. T2=35.32 kg, P=0.05), and GGT (T1=21.62 vs. T2=25.09 u/l, P=0.02) compared to T1. In P4, when the MD was removed, values remained higher for T2 GGT (T1=24.32 vs. T2=27.90 u/l, P=0.02) while no difference in other performance and metabolic parameters was observed. In conclusion, at the level of contamination reported in this study, adding FM contaminated maize to the TMR had no significant effect on performance or metabolism of early lactation autumn calving dairy cows. However, the 0.75 l/cow/d decline in milk yield due to feeding a DON-contaminated ration can represent a significant reduction in farm gate income. The use of a MD in dairy diets contaminated with FM has shown potential to mitigate the negative effect of FM contamination and merits further research.

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Effect of two mycotoxin deactivators on the reduction of aflatoxin M1 levels in milk of lactating dairy cows fed aflatoxin B1

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The objective of the study was to evaluate the efficacy of two mycotoxin deactivators in the reduction of aflatoxin M1 (AFM1) concentrations in the milk of dairy cows challenged with dietary aflatoxin B1 (AFB1). Thirty-two mid-lactation Holstein cows were randomly assigned to receive one of four treatments: (i) 2.8 mg AFB1/cow/day (positive control, PC); (ii) 2.8 mg AFB1 + 100 g TOXY-NIL® (TN)cow/day; (iii) 2.8 mg AFB1 + 100 g UNIKE® Plus (UP)/cow/day; and (iv) no AFB1 and no mycotoxin deactivator (negative control, NC). The study comprised three 7-day periods; an acclimatisation period from days -7 to -1, an experimental period during which AFB1 and mycotoxin deactivators were fed to cows as per treatment design (days 1 to 7), and a recovery period during which all cows received the NC diet and were monitored until levels of AFM1 in milk were undetectable (days 8 to 14). Treatments had no effect on feed intake, milk yield, milk composition, or somatic cell count (SCC) ($P \geq 0.10$). Both mycotoxin deactivators significantly reduced the percentage of AFM1 transferred into milk from 2.74% in PC to 1.00 and 1.27% for TN and UP, reductions of 63 and 54%, respectively ($P<0.001$). A review of the data indicated that reduction efficiencies associated with concentrations of AFB1 fed (2.8 mg daily) and concentrations of adsorbent added to the diets (0.37%) were higher in this experiment compared to previous studies with other products. Supplementation with mycotoxin deactivators also reduced the time required post-challenge for AFM1 concentrations to drop. Feeding high concentration of AFB1 to lactating dairy cows resulted in few differentially expressed genes in blood leukocytes but elicited numerous changes in gene expression in milk somatic cells, many of which were modulated in cows fed TN or UP.

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Effects of smectites clay on the growth performance and immunological function of broiler chickens fed diets contaminated with aflatoxin B1

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The effects of smectite clay on growth performance and inflammatory responses in broilers fed with aflatoxin B1 (AFB1)-contaminated feed were investigated. A total of 480 one-day-old AA male broilers were randomly distributed to 6 treatments with 8 replicates and 10 broilers per replicate. The birds were fed diets containing 0, 100 or 200 ppb AFB1 and 0 or 2.5 kg smectites clay per tonne as a 3 × 2 factorial study design. The experiment lasted for 42 days with two feeding phases (1~21 days and 22~42 days). At the end of each phase, one bird was randomly chosen from every replicate for sample analysis. The growth performance, organ index, intestinal morphology, sIgA of jejunal mucosa and cytokines of liver and spleen were determined. The results showed that AFB1 addition in feed significantly decreased feed intake at the early phase ($P<0.05$). Smectites clay tended to increase final weight ($P=0.069$) and average daily gain ($P=0.060$) of birds in the last phase. Smectites clay addition tended to decrease the thymus index which was enlarged by AFB1 at day 42 ($P=0.072$). For the intestinal morphology, supplementation of smectites clay significantly increased the crypt depth of duodenum, villous height and crypt depth of jejunum at the early stage and the villous height, crypt depth and villous height/crypt depth of duodenum in the later period ($P<0.05$). Compared to the control group, 100 ppb AFB1 reduced the secretion of jejunum mucous sIgA ($P<0.05$) at the early stage, but this negative impact was relieved by adding smectite clay ($P<0.05$). Furthermore, smectites clay significantly increased jejunal mucosa secretion of sIgA at the later stage ($P<0.05$). The level of relative mRNA expression of liver IL-1β in the first phase and IL-6 in the last phase was significantly raised up by 200 ppb AFB1, while they were declined after smectites clay addition ($P<0.05$). The interaction of AFB1 and smectites clay was observed for IFN-γ transcription both in liver and in spleen at the early stage ($P<0.05$). In conclusion, feed contaminated with 100 or 200 ppb AFB1 has adverse effects on growth performance and immune function of broilers. Supplementation of smectites clay to the diet can alleviate the negative effects induced by AFB1.

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In vitro adsorption of multiple mycotoxins and vitamin B1 by activated charcoal: is there a chance to improve selectivity?

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The capacity of an adsorbent compound to adsorb a specific mycotoxin may be influenced by other compounds present in animal diets, which can compete for adsorption points in the adsorbent structure. At the same time, it is well known that different kinds of mycotoxins may occur simultaneously in animal feed. Thus, when a compound is evaluated to be used as a possible mycotoxin adsorbent, it is important to evaluate its capacity to bind a specific mycotoxin, taking into account the presence of other mycotoxins in the test solution. Among several compounds that have been considered to be used as mycotoxin adsorbents, activated charcoals have shown good capacity to adsorb some mycotoxins. However, their unspecified adsorption of nutrients present in animal diets has been pointed out as a disadvantage, despite the lack of published literature that corroborates this fact. The present research studied the adsorption capacity for aflatoxin B1 (AFB1), fumonisin B1 (FB1), zearalenone (ZEN), and deoxynivalenol (DON) of two commercial activated charcoals (CA and CB), considering the presence of each mycotoxin individually and combined in the test solution. Additionally, their adsorbent behaviour towards vitamin B1 individually was also checked by HPCE. The test solutions simulated gastrointestinal conditions, such as gastric juice (pH 3.0) and intestinal juice (pH 6.0), according to the method of Lemke *et al.*, 2001 [Animal Feed Science and Technology 93:17]; mycotoxin concentrations were measured by HPLC-FL/DAD. The adsorption results were evaluated by a central composite design (CCD) configuration. The porosity of the charcoals was characterised by the conventional nitrogen adsorption isotherm 77 K, and the superficial areas were estimated by the BET (Brunauer-Emmett-Teller) method. Scanning electron microscopy was also used to study charcoal morphology. CB presented adsorption equal or higher than CA for the four mycotoxins, which is probably a result of its superficial area (2.6x larger) and micropore volume (3.75x larger). The pH influenced the absorption of the mycotoxins, except for ZEN, which was not influenced. However, the type of charcoal and the presence of other mycotoxins influenced the pH effect. There was a significant interaction between pH and other mycotoxins affecting DON adsorption for both charcoals. For vitamin B1, there was 45.4 and 83.9% adsorption in pH3 for

charcoals CA and CB, respectively. In pH 6, it increased to 100 and 95.8%, respectively. However, as these two charcoals showed significant differences in their physical features and mycotoxin adsorption capacity, we believe that it is possible to explore charcoals with different chemical or physical features that can offer lower nutrient adsorption and good mycotoxin adsorption.

P80

Control of aflatoxin contamination in maize using host-induced gene silencing of *Aspergillus flavus* genes

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Pre-harvest aflatoxin (AF) contamination of maize is a very complex problem affected by a multitude of genetic, environmental and nutritional factors. In addition to biocontrol and marker-assisted breeding strategies, transgenic approaches are being utilised by our group as a means to control pre-harvest AF contamination of maize. Host-induced gene silencing (HIGS) is a promising new control technology in which the pathogen is directed by the host plant to down regulate the expression of its own genes. HIGS technology does not require that the host plant express a foreign protein, so food and feed produced from resistant lines of transgenic maize should be more acceptable to the consumer. During HIGS, RNA interference (RNAi) is the process by which specific target genes are silenced before they are translated into protein. To this end, we are targeting *Aspergillus flavus* genes that are critical to growth, development and AF production. These include the *veA* and *nsdC* genes, both global regulators required for normal *A. flavus* development and AF production and the alpha-amylase (*amy*) gene required for starch degradation by the invading fungus. We are currently evaluating transgenic maize kernels expressing the hairpin form (RNAi) of the *A. flavus amy* gene as well as the *veA* and *nsdC* genes using a GFP-expressing *A. flavus* strain. Fluorescence emanating from the fungus was measured in ground kernels which is directly proportional to fungal growth. Aflatoxin levels were also quantified from the infected kernels. An *in vitro* kernel screening assay (KSA) with kernels indicated significant reduction in both fungal growth and AF levels in several *amy*-RNAi transgenic lines compared to control lines. Results of quantitative real-time PCR of *A. flavus amy* gene expression correlated well with those *amy*-RNAi maize lines that demonstrated the greatest reductions in *A. flavus* growth and AF production. The levels of *A. flavus* growth and AF production during infection of maize RNAi lines targeting *veA* and *nsdC* will also be presented.

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Ochratoxin A biodetoxification experiment in a human kidney cell line

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Ochratoxins are the metabolites/by-products of the *Aspergillus* and *Penicillium* sp., which are threatening the animal and human health through the contamination of feed and food. Ochratoxin A (OTA) is a nephrotoxic, genotoxic mycotoxin, which is occurring on grains, fruits and other food materials. Our department isolated a *Cupriavidus basilensis* bacteria strain (ÖR16), which is able to biodegrade 98% of 2 and 10 mg OTA/l during 5 days in LB medium. For the quality measurement of the biodegradation process, we used 786-O human kidney cell line as a biotest for the analysis of OTA by-products from the biodegradation process. We used bacteria-free, filtered inoculum after 5 days of biodegradation; the exposure of the cells was 48 h. The following genes expression level was measured by RT-PCR: *gadd 45*, *gadd 153* (DNA damage reporter), *clusterin* (kidney tumour marker), and *annexin2* (DNA-repair marker) genes. For control, the following household genes were used: *β-actin* (conservative, cytoskeleton peptide coding gene), *hprt* (playing a role in the purine peptide developing), *gapdh* (glycolysis ruler gene). Methyl methanesulfonate (MMS) was used as a genotoxic control. In the case of ÖR16, the target gene expression was less than in the case of OTA. According to these results, OTA biodegradation by-products have less toxic effects on the 786-O cell line, than 2 and 10 mg OYA/l. The ÖR16 bacteria is able to biodetoxificate OTA in high concentrations, without any harmful by-products for the human kidney cell line. **Acknowledgements**. This research was supported by NVKP_16-1-2016-0035, Bolyai Foundation of the Hungarian Science Academy, and the Aquafuture VKSZ-12-1-2013-0078 grant.

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MYTOX-SOUTH: an intercontinental partnership to improve food security and food safety in developing countries

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Food security remains a major challenge in developing countries, particularly in sub-Saharan Africa, where the highest prevalence of undernourishment occurs. Worldwide, cereal-based crops are spoiled by toxigenic moulds and the mycotoxins they produce [Dao and Dantigny, 2011. Food Control 22:360; Bennet and Klich, 2003. Clinical Microbiological Reviews 26:497]. Weather extremes associated with climate change further deteriorate and complicate the situation [Medina *et al.*, 2014. Frontiers in Microbiology 5:7; Miraglia *et al.*, 2009. Food and Chemical Technology 47:1009]. Food spoilage by mould and mycotoxins, not only reduces the amount of the available food, but also adversely affects the ability of the South to trade with the rest of the world. For instance, there was an export-collapse of African groundnuts from 90% (1960) to <5% (2005) due to aflatoxin-regulated markets, leading to economic crises in affected countries (e.g., Malawi) [https://comtrade.un.org/]. Since contamination can take place at different stages in the food chain, mycotoxin mitigation requires a multifaceted and multi-disciplinary approach. In developed countries, a lot of efforts are made to tackle the mycotoxin problem and the necessary measures to protect the population are being implemented. African countries, however, lack awareness and have limited competences and infrastructures for the implementation of prevention strategies and legislation, and the execution of mycotoxin analysis for monitoring and control purposes. MYTOX-SOUTH broadens and consolidates the scientific network between South and North partners around the theme of mycotoxins and toxicogenic moulds with the ultimate goal of strengthening the capacity of the Southern partners, making collaborations and research output more sustainable. MYTOX-SOUTH is structured to bring together researchers with complementary expertise in different areas of the mycotoxin issue and includes 35 professorial/post-doc research partners from Europe, USA, China and different African countries/universities. MYTOX-SOUTH intends to harness the expertise and infrastructure to strengthen the capacity of the Southern partners to tackle the mycotoxin problem and the associated food safety and food security issues. MYTOX-SOUTH is an intercontinental partnership to improve food security and food safety through mitigation of mycotoxins at global level with the following long term goals: (i) building human capacity through training, student/staff exchange programmes and joint PhDs which can contribute to an increased capacity of partners in the South and the identification of new research areas to reduce mycotoxin risks; (ii) bridging the gap between research and the development of more safe food and feed, different actors including farmer organisations, NGOs such as mycotoxicology associations, the private sector and policy makers; and (iii) creating a sustainable network. A stimulating environment for a fruitful public-private partnership is a pre-requisite for long-term and sustainable innovation.

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On the mitigation of mycotoxins content: the strange case of aflatoxin B1 and laccases from *Trametes versicolor*

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Aflatoxins are mutagenic, genotoxic and carcinogenic mycotoxins produced by *Aspergillus* spp. primarily contaminating cereals, maize, oilseeds and nuts. Aflatoxin B1 (AFB1) has been classified as a class-I carcinogen by IARC being among the most harmful natural food contaminants in terms of acute and chronic toxicity. Besides health concerns, AFB1 causes significant losses in terms of veterinary costs and managing of incompliant food and feed batches. Therefore, the mitigation of AFB1 in food and feed is critical for facing the forthcoming challenges in view of the sustainability and global trade. Indeed, reducing the contamination in food and feed ultimately ameliorates the health and welfare of humans and animals. Also, the implementation of strategies for recovering contaminated batches may concretely allow the weaker markets to re-enter the global trade. A wide number of strategies for the mitigation of mycotoxins content are currently under consideration. The strategies relying on microbial/biochemical detoxification of incompliant products have the potential advantage of reducing and/or reusing waste. The enzymatic transformation is among the most promising tools for the mitigation in situ and the laccase enzymes seem effective in degrading AFB1. However, whether or not they can be used for mitigating AFB1 in real matrices is under discussion basically because of the unclear mechanism of degradation. The suspected need of third party molecules to trigger the reaction (i.e., mediators) and the possible low specificity rise major issues. Focusing on the laccases from *Trametes versicolor*, this

work aimed at investigating the role of mediators and gaining insights on the specificity of reaction. AFB1 was incubated with laccases and degradation was monitored in a 5-days kinetic by using LC-MS/MS and LC-FL/UV. The degradation rate was negligible when mediators were missing. Conversely, the almost complete degradation was observed when the commonly used mediator ABTS was added to the reaction, sustaining the role of mediators in the degradation process. Although ABTS cannot be considered in a real usage on food and feed, an aqueous maize extract succeeded in triggering the degradation pointing to the presence of 'natural' mediators in matrices susceptible to AFB1 contamination. Laccases were found able to degrade AFB1 in naturally contaminated maize as well, supporting a valuable use on real matrices. Some hints on the reaction specificity were derived as no degradation was observed for other co-incubated natural compounds (e.g., rutin and quercetin). The tentative identification of degraded products was done combining MS and spectroscopic analysis.

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Chemotyping ergot alkaloid diversity for crop selection and management

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The composition and quantity of secondary metabolites produced by a fungus within a plant host is collectively called its chemotype, which he can vary by fungal species, genotype and environmental factors. Ergot is a seed replacement disease whereby *Claviceps* fungi invade the seed head to replace it with a black sclerotium, decreasing seed yield by 5-10%. In addition, the fungus produces a diversity of ergot alkaloids that have varying vasoconstrictive potential which can negatively impact livestock production, thereby decreasing the safety, value and usefulness of grass products intended for livestock feed. Our laboratory has seen an increase in requests for characterisation of the ergot alkaloid profile in harvested and processed feed/food recently; plants infected with *Claviceps* and their sclerotia have been a particular focus. A survey of 8 Kentucky bluegrass and 39 perennial ryegrass sclerotia pools collected from commercial seed lots for ergot alkaloids via LC-MS/MS showed α-ergocryptine (531 μg/g, 32.0%), ergocornine (494 μg/g, 29.8%), ergocristine (312 μg/g, 18.8%) and ergotamine (273 μg/g, 16.5%) and their epimers to be dominant in Kentucky bluegrass, whereas ergotamine (679 μg/g, 38.8%), ergocryptine (505 μg/g, 28.9%) and ergocornine (360 μg/g, 20.6%) and their epimers were highest for perennial ryegrass. A smaller sampling of chewings fescue, hard fescue and colonial bentgrass sclerotia (n=2 each) contained 599/6187/2686 μg/g ergocristine (55.1/16.5/42.1%), 153/12840/2170 μg/g ergotamine (14.0/34.3/34.0%), 153/8983/1207 μg/g ergocryptine (14.1/24.0/18.9%) and 152/8597/304 μg/g ergocornine (14.0/23.0/4.8%), respectively. A preliminary comparative study of malted versus unmalted rye (n= each) showed that the malting process decreased or eliminated the ergot alkaloids present in a rye crop intended for beer brewing. Chemotype screenings such as these will help identify cultivars and/or ecologic and processing conditions that are less conducive to mycotoxin production. This will aid in crop/pathogen management as more rigorous food safety standards are implemented, and climate change pushes niche boundaries of fungal-plant host relationships, threatening food security for an increasing proportion of the globe.

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Integrating 'omics' approaches for investigating aflatoxin production by *Aspergillus flavus* under drought stress conditions

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Identifying the causes behind the stimulation of aflatoxin contamination of host plants by drought stress during *Aspergillus flavus* infection is critical to mitigating this issue. Recently, our group has employed 'omics' approaches, including genomics, transcriptomics, proteomics, and metabolomics, to investigate the responses of multiple *A. flavus* isolates with varying levels of aflatoxin production to drought-related reactive oxygen species (ROS) accumulation and subsequent oxidative stress. This oxidative stress has been found to correlate with both drought stress in host plant tissues and with increased *in vitro* aflatoxin production by *A. flavus*. These expression-related analyses have shown similar results, that carbohydrate, amino acid, and lipid metabolic pathways, enzymatic accumulation, and gene expression regulatory networks are important in *A. flavus* oxidative stress responses. Carbohydrates and lipids serve as a primary source of biologically important compounds such as acetyl-CoA and malonyl-CoA

which are used by polyketide synthases for the initial construction of secondary metabolites including aflatoxin. Their accumulation was significantly affected in response to oxidative stress. These compounds also serve as energy sources with less aflatoxigenic isolates showing increased demand to remediate oxidative stress compared to more aflatoxigenic isolates. Highly toxigenic isolates also showed elevated expression of lytic enzymes important to host pathogenicity and microbial competition in response to oxidative stress. Atoxigenic biological control isolates also showed greater antioxidant and redox damage remediation capabilities compared to atoxigenic isolates not selected as biological controls. These results also suggest that the production of aflatoxin and other secondary metabolites, such as aflatrem, cyclopiazonic acid, and kojic acid, may provide antioxidant benefits to these fungi and may provide a partial explanation for the production and evolutionary conservation of aflatoxin production. Ongoing genomic analyses will explore these evolutionary relationships, and causal mutations and differences in genome architecture between A. flavus isolates to explain why they respond to stress differently. By correlating these findings with the application of similar approaches studying drought stress in host plants, it is possible to identify components of this host-pathogen interaction which can be manipulated through transgenic or genome editing to improve host resistance. Currently, we are employing these methods to study the link between ROS accumulation and aflatoxin contamination under drought by manipulating host antioxidant gene expression.

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Modulation of AFB1 cytotoxicity by natural antioxidants and PCB126 in a bovine mammary epithelial cell line

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Aflatoxin B1 (AFB1), a widespread food and feed contaminant, is bioactivated by drug metabolising enzymes (DME), mainly CYP1A and 3A families, yielding highly cytotoxic and carcinogenic metabolites, such as AFB1-exo-8,9-epoxide and AFM1 [Abrar *et al.*, 2013. Critical Reviews in Food Science and Nutrition 53:862]. The latter undergoes mammary excretion contaminating dairy products [Prandini *et al.*, 2009. Food and Chemical Toxicology 47:984]. Factors modulating DME are therefore expected to affect AFB1 kinetics and toxicity. For instance, dioxin-like (DL) compounds, via the activation of the AhR pathway, can up-regulate the expression of CYP1A1. Conversely, a number of natural antioxidants have been reported to inhibit CYP1A1 and/or to induce detoxifying and antioxidant enzymes with the potential to reduce the generation and/or increase the inactivation of AFB1 metabolites [Qin *et al.*, 2015. PloS ONE 10:e0127551; Nishiumi *et al.*, 2007. Archives of Biochemistry and Biophysics 466:267]. The aim of the study was to evaluate the role of selected natural antioxidants (i.e., curcumin, curcuminoids, quercetin and resveratrol) as well as of PCB126, a DL-compound mostly involved in the contamination of dairy milk [Bertocchi *et al.*, 2015. Environmental Science and Pollution Research 22:9775], in the modulation of AFB1 toxicity in the bovine species. Such an issue has been addressed in vitro using an immortalised bovine mammary epithelial cell line (BME-UV1), in the light of the active role played by mammary DME in the generation of AFB1 metabolites [Caruso *et al.*, 2009. Toxicon 53:400]. Cells were incubated with increasing concentrations of AFB1 (96-750 nM) after a pre-incubation of 16 or 24 h with each antioxidant (5 μM) or PCB126 (10 nM), respectively. Cell viability was evaluated by the WST-1 or neutral red uptake assays after 24 and 48 h of AFB1 incubation. The modulation of CYP1A1 by curcumin was evaluated incubating cells with increasing concentrations of the antioxidant (0.6-5 μM). CYP1A1 expression and activity was assessed at different time-points (from 4 to 48 h) by both real-time PCR and the CYP1A-mediated EROD assay, respectively. Statistical analysis was performed by 1-way ANOVA followed by Dunnett's or Bonferroni's post hoc test. As expected, AFB1 cytotoxicity occurred in a time- and concentration-dependent manner and was enhanced by PCB 126 to a variable extent ($P<0.001$). By contrast, quercetin ($P<0.01$) and, to a lesser extent, resveratrol ($P<0.05$) or curcuminoids ($P<0.01$) were able to afford protection against AFB1 cytotoxicity at all tested concentrations. This was not the case for curcumin, which surprisingly slightly amplify the effects of AFB1 ($P<0.01$). To investigate the possible mechanisms of such a phenomenon, we checked the ability of curcumin in activating the AhR pathway, as suggested by some authors [Shehzad and Lee, 2013. Biofactors 39:27]. However, in our model curcumin failed to induce CYP1A1 at both gene and protein level. Further studies are ongoing to test the protective effects of the same natural antioxidants against AFB1 in other tissue cell lines.

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Effectiveness of essential oils and their derivatives to prevent mycotoxin production by *Aspergillus* species

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Mycotoxins are fungal secondary metabolites that pose a serious risk to food security and cause high economic losses, contaminating crops in the field and during storage. These toxins are produced on food mainly by *Aspergillus*, *Fusarium*, *Penicillium* and *Alternaria* species. The best strategy to prevent mycotoxins in food products is to avoid fungal growth. Chemical fungicides are effective but more eco-friendly and safer methods are necessary. In recent years, research has been focused on alternatives based on natural products, such as essential oils (EOs). In this work, seven plant EOs (*Rosmarinus officinalis*, *Thymus vulgaris*, *Satureja montana*, *Origanum majorana*, *O. vulgare*, *O. virens* and *O. majoricum*) and their hydrolates (distilled plant water derivatives) were tested *in vitro* towards *Aspergillus steynii* and *A. flavus* to evaluate their effect on growth and ochratoxin A (OTA) and aflatoxins (AFs) production, respectively. The experiments were performed using EOs and hydrolates corresponding to two harvests (2015 and 2016). The tests were performed on CYA medium supplemented with EOs (10, 100, 500, 1,000 ppm) and their corresponding hydrolates (100, 1,000, 5,000, 10,000 ppm in 2015, and 50,000 and 75,000 ppm in the second year). In all cases, fungal spore suspensions were placed in the centre of the plate and incubated at 28ºC for 5-8 days. Colony diameter was measured every day to calculate growth rate and mycotoxin production was evaluated at the end of the experiment by HPLC. Statistical analysis showed that all EOs tested affected to some extent fungal growth and mycotoxin production, being *O. virens* EO the most effective in the first year, with reductions in growth up to 75% and close to 100% in mycotoxin production in both fungi at 1000 ppm. In 2016, EOs from *O. virens* and *S. montana* were the most effective with reductions in growth up to 100% in both fungi at the highest concentrations tested. The hydrolates studied showed little or no effect in both years. Multiple linear regression showed an influence of all the parameters tested including the year of production of the EOs. Taking into account that the composition of the EOs varied depending on the year, it is necessary to standardise their composition to be able to successfully apply them to food products. In conclusion, some EOs are effective to prevent mycotoxin production *in vitro* although more studies are needed to study their application in food matrices. **Acknowledgements**. This work was supported (AGL 2014-53928-C2-2R).

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Application of zearalenone hydrolase ZenA as a zearalenone-degrading feed additive in chickens

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The application of mycotoxin degrading enzymes as feed additives is a promising strategy for the removal of mycotoxins from feedstuffs. Previously, our group investigated the bacterial enzyme zearalenone hydrolase ZenA (ZENzyme®). ZenA exhibits lactonase activity against zearalenone (ZEN), thereby converting ZEN to hydrolysed ZEN (HZEN). HZEN is subsequently converted to decarboxylated HZEN (DHZEN) by spontaneous decarboxylation. In this study, we evaluated the efficacy of ZenA as a feed additive for the gastrointestinal degradation of ZEN in broiler chickens. In a 14-day feeding trial groups of chickens (n=8) received: (i) feed artificially contaminated with 400 ng ZEN/g (positive control group); (ii) feed contaminated with 400 ng ZEN/g and supplemented with ZenA (ZenA group); and (iii) uncontaminated feed (negative control group). On day 14 of the trial, digesta samples from the crop and the gizzard were collected. In these samples as well as in crop and gizzard samples taken from animals (n=8) before the start of the trial, ZEN, HZEN and DHZEN concentrations were determined by liquid chromatography tandem mass spectrometry analysis. We found that ZEN levels were significantly lower and HZEN and DHZEN levels significantly higher in digesta from the ZenA group compared to the positive control group. ZenA activity was detected already in the crop. In conclusion, when administered as a feed additive, ZenA effectively transformed ZEN to HZEN in the crop of chickens. ZenA is therefore suitable for application as a ZEN detoxifying feed additive in chickens.

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Combination of anti-mycotoxin additives, vitamins and plant extracts in growing-finishing diets may not be beneficial to pigs when added to feed contaminated with deoxynivalenol

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Pigs are often considered highly sensitive to mycotoxins, because of dramatically reduced feed intake of contaminated feed. As a preventive measure, premixes combining anti-mycotoxin additives with vitamins and plant extracts to manage reactive oxygen species are often added to pig feed. In order to evaluate the interest of such a premix based on sepiolite, bentonite, betaine and plant extracts a trial was undertaken with fattening pigs fed *ad libitum* with a computerised feeding system, recording individual feed intake. 108 pigs were selected based on weight at 69 days, sex and sire ancestry. They were all housed in one single pen equipped with a sorting scale and four electronic feeders, all connected to three feed bins, allowing to feed each pig the feed assigned according to the protocol. A 10 days pre-experimental period was allowed for pig adaptation to the equipment; 9 pigs failed to adapt and needed to be removed from the pen. Then, three dietary treatments (growing feed, finishing feed sequence) were compared during 105 days: positive control (PC), formulated with ingredients containing deoxynivalenol (DON) below 0.3 ppm; negative control (NC), deriving from PC, substituting maize (20% of diet) with maize naturally contaminated by *Fusarium* mycotoxins (DON, 7.0 ppm; deoxynivalenol-3-glucoside, 0.6 ppm; nivalenol, 0.6 ppm; and zearalenone, 0.5 ppm); anti-mycotoxin premix added to NC (AMPX). Individual data from each visit of each pig to the sorting scale and to the feeders were used to analyse the pig response to treatment. Growth curve and cumulated feed intake curve were fitted to a quadratic response to days in trial, with a random effect of individual pig, and fixed effect of sire ancestry, sex and treatment. Effect of treatment on health records was analysed with khi² statistics. Eleven pigs did not complete the trial (4, 1 and 6 in PC, NC and AMPX, respectively, not significant, P-khi²>0.10). Cumulated feed intake was significantly reduced in NC (-3.3%) and AMPX (-2.1%) compared to PC (P<0.001), and so did average daily gain (962 vs. 920 and 936 g/day in PC, NC and AMPX, respectively, P<0.05). The effect of treatment on weight was significant for interaction with days-on-trial, not as main effect, describing a higher difference between PC and other treatments in the early phase of the trial. This could explain why the performance reduction, although significant, was low compared to forecast (-11% expected from literature). In this context, no benefit of anti-mycotoxin premix could be demonstrated during the whole fattening period.

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The fate of *Alternaria* toxins during fermentation of whole wheat dough

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Besides *Fusarium* species, which remain a main source of mycotoxin contamination of wheat, other mycotoxigenic fungi have been recognised as important wheat contaminants, such as fungi of the genus *Alternaria*. Its flexibility to different climate conditions is responsible for the apparent contradiction related to *Alternaria* diseases, which may develop both at high and low temperature, different humidity, and under multiple combination of environmental factors. Various *Alternaria* species are capable of producing a variety of toxins. Taking into consideration their possible harmful effects on human and animal health and the fact that scarce information is available worldwide about the behaviour of *Alternaria* toxins in food and feed during processing, the aim of this study was to investigate the fate of the most common *Alternaria* toxins found in wheat – tenuazonic acid (TeA), alternariol (AOH), and alternariol monomethyl ether (AME) – during sourdough processing. For this purpose, spiked whole wheat flour (100 µg/kg of each TeA, AOH, and AME in flour), sourdough starter, and water were used as raw materials. Spiked whole wheat dough was fermented for 4, 8, 12, 24 and 48 h at 25ºC, and at each point the fermented dough samples were taken, frozen, lyophilised, grounded, and stored until further analysis. To study the effect of sourdough processing on TeA, AOH and AME content, a validated method of high performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) for these mycotoxins was used. A more intensive reduction of examined *Alternaria* toxins was observed only after 12 h of fermentation. The maximum reduction of TeA, AOH and AME levels was archived at 48 h of fermentation of dough at 25ºC compared with dough after kneading (0 h). Under these conditions, a reduction of the toxin levels of 60.3, 41.5 and 24.1% was observed for TeA, AOH and AME, respectively. This study represents the first report about behaviour of *Alternaria* toxins during fermentation of whole wheat dough. **Acknowledgments**. This paper is a result of the research within the project III 46001 financed by the Ministry of Education, Science and Technological Development, Republic of Serbia.

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FDA Center for Veterinary Medicine: challenges and lesson learned in the regulation and approval of food additives to eliminate or reduce mycotoxins in animal food

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Since the discovery of aflatoxins in 1962, the FDA Center for Veterinary Medicine has been dealing with health risks associated with the presence of mycotoxins in animal food. The presence of mycotoxins in animal food has both human and animal health consequences as well as economic cost to the animal industry, the consumers, and the government. In the USA, mycotoxins are considered to be an added poisonous or deleterious substance. Depending upon the mycotoxin level in the food or feed, the human and animal food could be considered to be adulterated under section 402(a)(2) of the Federal Food, Drug, and Cosmetic Act (the Act) (21 U.S.C. 342(a)(1)). Section 402(a)(2) of the Act states that a food is deemed to be adulterated if it bears or contains an added poisonous or deleterious substance "which may render [the food (or feed)] injurious to health". The classification of mycotoxins as added poisonous or deleterious substances gives the FDA the ability to establish regulatory limits for mycotoxins in human and animal food, and it also affects how the FDA approves and regulates agents to eliminate or reduce the risk of mycotoxins hazards in animal food. This poster presentation describes the challenges of regulating and approving food additives intended to eliminate or reduce mycotoxins in animal food under the food additive regulations and the lessons learned for over 4 decades. The discussion on lessons learned covers: (i) insights on the application process; (ii) how claims made affect the regulatory process followed; (iii) the dilemma associated with various types of food additives proposed to eliminate or to reduce the risk of mycotoxins in animal food; and (iv) what can and cannot be approved.

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Farm(er) characteristics driving the adoption of pre-harvest mycotoxin management measures in the Netherlands

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Mycotoxin management aims to increase the safety of grains, such as wheat. Because of the inherent difficulty to remove mycotoxins down the chain, mycotoxin management mainly focusses on reducing initial fungal infection and production of mycotoxins in the field and during storage. Farmers can apply various pre-harvest measures to reduce fungal infection and mycotoxins in wheat, such as selection of a resistant wheat variety, fungicide use, soil cultivation and crop rotation. Since farmers play a key role in the prevention and control of mycotoxin contamination, it is important to understand their behaviour regarding mycotoxin management to get a better insight in how to stimulate a future change in behaviour. The aim of this study was to identify the (sets of) pre-harvest measures that Dutch wheat farmers currently apply and to understand related farm(er) characteristics. Based on literature and expert expertise, a list of pre-harvest measures was composed. By means of a structured questionnaire, Dutch wheat farmers were asked to indicate which pre-harvest measures were applied during the last growing seasons and to characterise their farming situation based on different farm (e.g., arable size, main crop) and farmer (e.g., age, education, risk perception) characteristics. Survey data were analysed by a multivariate probit model to identify those characteristics that drive farmers' decisions to adapt specific pre-harvest measures related to mycotoxin management. **Acknowledgements**. This study is performed as part of the MyToolBox project, which received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 678012.

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Enzymatic detoxification of mycotoxins in the bioethanol process

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The U.S. ethanol industry surpassed the 1 million barrels of ethanol produced per day mark at the beginning of 2017 (U.S. Energy Information Administration). As 1 barrel of ethanol leads to 98 kg of distiller dried grains with solubles (DDGS) [Bothast and Schlicher, 2005. Applied Microbiology and Biotechnology 67:19] substantial amounts of DDGS are produced, which is an interesting by-product for the feed industry. Mycotoxins present in the raw materials for the bioethanol production are not degraded but even concentrated in the process. The use of DDGS in the feed industry therefore increases the likelihood of exposing animals to higher mycotoxin levels. In the animal mycotoxin exposure can lead to

adverse health effects, decreased animal performance and economic losses. The latest results of the BIOMIN Mycotoxin Survey showed that all 79 U.S. DDGS samples tested were at least positive for one of the three major mycotoxins (deoxynivalenol, fumonisin or zearalenone) and 96% of the samples were contaminated with more than one mycotoxin emphasising the existing issue of mycotoxins in this feed compound. Possibilities to counter high mycotoxin concentrations in DDGS comprise rejection of highly contaminated raw materials or dilution by blending high and low contaminated materials. Actual degradation of mycotoxins in the bioethanol production process by application of mycotoxin-degrading enzymes offers a new possibility to tackle mycotoxin contaminations. Lab-scale tests with mycotoxin degrading feed additives showed the degradation of fumonisin B1 (FB1) and the formation of the degradation product hydrolysed FB1 proving the detoxification of the mycotoxin during the process. FUMzyme® added either before liquefaction or before fermentation (10 U/kg maize and 100 U/kg maize) during the bioethanol production process with naturally contaminated maize (4,879 ppb FB1) led to a ≥96% reduction of FB1 in the mash. Mycotoxin degrading additives can be used to produce high quality DDGS with low mycotoxin levels thereby benefitting livestock producers and boosting bioethanol industry's revenues.

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Efficacy of an activated and purified smectite binding agent on the toxicological effects of aflatoxins in the diets of weaned piglets

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In a 2-phase experiment, the efficacy of toxin binder containing an activated and purified smectite against the toxicological effects of aflatoxins in piglets was investigated. A total of 400 mixed sex (1:1 ratio of males and females) crossbred piglets at 28 days of age were randomly assigned to four groups with 10 replicates of 10 pigs per pen. In phase 1 (day 1-14), piglets were dietary challenged with pure aflatoxin B1 (P-AFB1) and each pen received 1 of the 4 diets: an AGP-basal diet without AFB1 and toxin binder (control); control + 0.5 ppm AFB1 (AFB1); control + 0.5 ppm AFB1 + 2.0 g/kg toxin binder (AFB1+MX2) or control + 0.5 ppm AFB1 + 4 g/kg toxin binder (AFB1+MX4). In the second phase (day 15-35), all piglets were fed with a standard commercial pig diet. Body weight was measured at day 1, 14 and 35, while the feed consumption was recorded weekly and FCR was calculated. At day 14, one pig per pen was randomly selected and sacrificed for organ collection and tissue sampling for histological analysis. Under aflatoxin challenge, the dietary inclusion of toxin binder in AFB1+MX2 and AFB1+MX4 improved body weight gain (+9.0%, $P<0.05$) and feed intake (+4.3%, $P<0.05$) compared to AFB1, and matched the performance of piglets in control ($P>0.05$). Improvement in FCR was not significant, but 5% lower in toxin binder supplemented groups. In the second phase, there were no significant differences in ADG and FCR, but the final weight was higher for piglets in AFB1+MX2 and AFB1+MX4 compared to AFB1 (+3.4%, $P<0.05$). Overall, the ADG was 4.2% higher ($P<0.05$) and FCR was 3.3% lower ($P>0.05$) in AFB1+MX2 and AFB1+MX4 in relation to the AFB1 group. Kidney weight was higher for piglets in AFB1+MX2 ($P<0.05$), whereas the weights of other organs were comparable among the groups ($P>0.05$). The histological findings showed normal condition of heart and spleen, while abnormal lesions in liver and kidney were observed in all the pigs. In conclusion, dietary inclusion of 2.0 and 4.0 g/kg of toxin binder can effectively support weaned piglets to mitigate the toxicological effects of aflatoxin B1. Supplementation of the smectite concept in the diet of piglets can improve body weight gain and feed efficiency during aflatoxin challenging conditions and has a carry-over effect on the overall performance of piglets at the end of the weaning period.

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Enzymatic detoxification of deoxynivalenol by epimerisation

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Deoxynivalenol (DON) is one of the most abundant mycotoxins produced by *Fusarium* pathogens that infect wheat, barley, maize and other small grain cereals in the field. DON directly accumulates in the harvested grains and enters food/feed chains, posing a serious threat to human and farm animals. A new detoxification pathway was proposed where DON is catabolised into 3-keto-DON and 3-epi-DON, both of which exhibit little to no toxicity. Recently we have reported that a novel aldo/keto reductase superfamily member, AKR18A1, is responsible for the selective oxidation of DON at the C3 position, converting DON into 3-keto-DON in an aerobic bacterial *Sphingomonas* S3-4, and the recombinant

AKR18A1 protein catalysed the reversible oxidation/reduction of DON. Our results demonstrated that the oxidation of DON into 3-keto-DON catalysed by AKR18A1 is the first enzymatic step of the two sequential reactions in the DON epimerisation pathway. To clone genes encoding enzymes for the second reaction, we selected one strain with the highest biocatalytic activity from a collection of aerobic bacterial strains that were isolated from different environments and capable of catabolising DON into 3-epi-DON. This strain had the capacity to detoxify DON at a rate of 150 µg/h/10^8 cells, 50-fold higher than other strains reported in the literature. Based on comparative analysis of genome and transcript sequences, genes for DON epimerisation were cloned and heterologously expressed in *Escherichia coli*. Affinity-purified recombinant proteins retained the high capacity to transform DON into 3-epi-DON. Thus, these enzymes could be directly utilised to eliminate DON in food and feed products.

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Fusarium graminearum mycotoxins in maize associated with *Striacosta albicosta* (*Lepidoptera*: *Noctuidae*) injury

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Western bean cutworm (WBC), *Striacosta albicosta* (Smith) (*Lepidoptera*, *Noctuidae*) has become a key pest of maize, *Zea mays* (L.), in Ontario, Canada which is challenging to control due to its lack of susceptibility to most Bt-maize events. Injury by WBC may exacerbate *Fusarium graminearum* (Schwabe) infection through provision of entry points on the ear. The objectives of this study were to: investigate the relationship between injury by WBC and deoxynivalenol (DON) accumulation; evaluate non-Bt and Bt-maize hybrids, with and without insecticide and fungicide application; and determine optimal insecticide-fungicide application timing for reducing WBC injury and DON. Incidence of injury by WBC and ear rot severity were found to increase DON under favourable environmental conditions for *F. graminearum* infection. The Vip3A × Cry1Ab event provided superior protection from WBC injury compared to non-Bt or Cry1F hybrids. Insecticide application to a Vip3A × Cry1Ab hybrid did not reduce injury further; however, lower severity of injury was observed for non-Bt and Cry1F hybrids when applied at early VT or R1 stages. DON concentrations were reduced with prothioconazole fungicide tank-mixed with insecticide at late VT (before silk browning) or when insecticide was applied at early VT followed by prothioconazole at R1. Application of an insecticide/fungicide tank-mix is the most efficient approach for maize hybrids lacking high-dose insecticidal proteins against WBC and *F. graminearum* tolerance. Results demonstrate that reducing the risk of DON accumulation requires a strategic approach to manage complex associations among WBC, *F. graminearum* and the environment.

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Risk factors for hotspots of the growth of *Penicillium verrucosum* and ochratoxin A in grain storage bins in the Great Lakes Region.

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The growth of *Penicillium verrucosum* and ochratoxin A (OTA) contamination were studied in a total of 250 samples collected from 90 storage bins on farms across Southwestern Ontario over a four-year period. Grain and grain clump samples positive for *P. verrucosum* and OTA were related to moisture resulting from either condensation or migrating moist warm air in the bin or areas where precipitation including snow entered the bin even when grain entered storage below the 14.5% threshold of moisture. Twenty on-farm storage bins were setup with 5 sensors that recorded relative humidity and temperature for 3 years (2011-13). Temperature and moisture gradients were measured from three of the sensors placed at 1.5 m and 20 cm below and 1 m above the surface of the grain in the centre of the bin. Two additional sensors were placed in each bin, oppositely just inside the north and south walls 30 cm above the bottom of the storage bin. The base of these sensors was attached to the bin wall with the sensor extending into the grain. Dew point temperatures for each sensor location were calculated from measured relative humidity and the temperature. Moisture migration and condensation occurred in the grain mass naturally due to temperature gradients. Alternatively, condensation was caused by convection currents generated by the difference between temperature of the grain and the ambient temperature of the bin headspace. Normal practice has aeration beginning when ambient temperatures fall well below freezing in late fall/early winter to cool the grain. However, aeration from November to January had the highest risk of condensation on bin surfaces, on grain, as well as under bin roofs that could drip on top of the grain surface. Additional aeration during the period from bin fill at harvest to the end of October reduced the amount of moisture inside the bins and reduced the temperature difference

between the grain mass and ambient air. This resulted in less convection that could lead to condensation and OTA contamination in hotspots. To prevent condensation in or on the grain bulk, an active ventilation system of the headspace is also recommended.

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Antifungal compounds from *Streptomyces* AS1: efficacy against *Penicillium verrucosum* growth and ochratoxin A production

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There is significant interest in the use of natural secondary metabolites from microorganism to control *Penicillium verrucosum* and ochratoxin A (OTA) in staple food chains. However, most of the previous studies only examined the effect of metabolites on growth of *P. verrucosum*, and not on OTA production. The objective of this study was to identify the bioactive antifungal compounds produced by *Streptomyces* AS1 and to study their efficacy against *P. verrucosum* growth and OTA production. The antifungal compounds from AS1 supernatant was extracted three times with ethyl acetate (EA). The solvent was removed, and the compounds present analysed by LC-MS/MS. The dried compounds were dissolved in DMSO (0-5 µg/ml) under sterile conditions and screened for efficacy against *P. verrucosum* growth and OTA production at 0.95 water activity (a_w) and 25ºC for 7 days using the agar spot assay. Four major compounds with the concentration >1µg/g AS1 biomass were present: valinomycin (149 µg/g biomass), cyclo(L-Pro-L-Tyr) (22 µg/g biomass), cyclo(L-Pro-L-Val) (10 µg/g biomass) and brevianamide F (3 µg/g biomass). The efficacy study showed that EA extract at concentration ≥2.5 µg/ml completely inhibited OTA production and reduced the growth rate by 64-84% as compared to the control. More detailed studies have determined the concentration of each compound in 5 µg/mL EA extract: 22.6, 3.2, 1.6 and 0.4ng/ml for valinomycin, cyclo(L-Pro-L-Tyr), cyclo(L-Pro-L-Val) and brevianamide F, respectively. Subsequently, two major compounds; valinomycin and cyclo(L-Pro-L-Tyr) were selected to study the efficacy of individual, and combinations of the selected compounds and that naturally produced by the *Streptomyces* AS1 (EA extract) on *P. verrucosum growth* and OTA production. Naturally produced EA extract (5 µg/ml) inhibited the growth of *P. verrucosum* by 88% when compared to the control and totally inhibited production of OTA. Interestingly, no inhibition of growth and OTA production was observed in chemically derived individual compounds alone (cyclo(L-Pro-L-Val, 4 ng/ml; valinomycin, 22 ng/ml) or combination of the two compounds (4:22 ng/ml). In conclusion, four major compounds were identified as valinomycin, cyclo(L-Pro-L-Tyr), cyclo(L-Pro-L-Val) and brevianamide F. The use of naturally produced combination of these antifungal compounds (EA extract, 5 µg/ml) was very effective in controlling growth and OTA production by *P. verrucosum*. This was possibly due to the additive or synergistic effect of major/minor compounds present in the EA extract from the *Streptomyces* AS1 strain, which had better control efficacy. Studies are in progress to treat cereals to examine the potential for in situ efficacy under different storage regimes. **Acknowledgements**. We are very grateful to Dr M. Sulyok, Department IFA-Tulln, BOKU Vienna, Austria, for analysis of the AS1 extract. A.M-D is grateful to MARDI, Malaysia for a PhD scholarship.

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Potential biocontrol agents for control of *Aspergillus flavus* and aflatoxin B1 production

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A potential option for *Aspergillus flavus* management in the field has largely been focused on the use of atoxigenic isolates of *A. flavus*, which can compete with the toxigenic strains by displacing them and reducing aflatoxin B1 (AFB1) contamination. The aim of this study was to (i) isolate atoxigenic *A. flavus* strains from GM and non-GM maize hybrids for insect resistance and/or herbicide tolerance from Brazil and (ii) competitiveness and control of AFB1 production *in vitro* and in stored maize GM- and non-GM hybrids. Competitiveness between toxigenic and atoxigenic strains was examined under different water activity (a_w) at 30ºC on a maize-based matrix. This showed that toxigenic *A. flavus* strains were largely dominant against other potential BCAs including atoxigenic *A. flavus* strains. Different conidial ratios of toxigenic and atoxigenic strains were used and spread plated on maize-based media using maize grain from a GM Bt hybrid P30F53H (gene Cry1.F, Herculex®I) and the isogenic non-GM hybrid (P30F53). AFB1 content was evaluated by HPLC-FLD after 7 and 14 days incubation. All four atoxigenic (AFL-) strains selected were able to reduce the AFB1 production at both 0.98 and 0.95 aw treatments after 7/14 days at 30ºC. The ratios of 50:50 and 25:75 of AFL+: AFL- strains were not statistically different at 0.95 a_w and the overall relative reduction of AFB1 was between 46-100%. There was no significant difference in control of AFB1 when comparing the GM Bt hybrid with the non-GM maize as substrate. A

multiplex PCR was employed to identify the deletion of key biosynthetic genes for AFB1 production by the 4 atoxigenic *A. flavus* examined. This method uses primers developed for 32 markers spaced approximately every 5 kb from 20 kb proximal to the aflatoxin biosynthesis gene cluster to the telomere repeat. One strain had deletion of 6 genes in the aflatoxin cluster, two strains had 5 genes deleted, and one of the AFL- strains had all the biosynthetic genes. The AFL- strains with the larger number of biosynthetic genes deleted are being examined for *in situ* biocontrol of AFB1, and the resilience under potential future climate change environmental conditions. **Acknowledgements**. This research was supported by CAPES Foundation, Ministry of Education of Brazil – Project BEX 12937/13-4.

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Antifungal activity of the allyl isothiocyanate against *Aspergillus flavus* and *Penicillium verrucosum* in maize, wheat and barley

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Isothiocyanates (ITCs) are bioactive substances characteristic of the plants of the *Brassicaceae* family. The antifungal activity of the ITCs is due to the strong electrophilic properties of these compounds; they can also react easily with nucleophiles such as amines, amino acids, alcohols, water, and sulphites during food treatment and under physiological conditions and with several functional groups of many mycotoxins. The aim of this study was to evaluate the antifungal properties of the bioactive compound allyl isothiocyanate (AITC) against *Aspergillus flavus* (8111 ISPA) aflatoxins producer and *Penicillium verrucosum* (D-01847 VTT) ochratoxin A (OTA) producer on maize, barley and wheat. The experiments were carried out in a lab scale silo system that was composed by a glass jars containing 300 g of cereals barley and wheat (contaminated with $1x10^4$ spores of *P. verrucosum* per g) and maize (contaminated with $1x10^4$ spores of *A. flavus* per g). The cereals were treated with a 12% hydroxyethyl cellulose gel disk containing 500 µl of AITC, hermetically closed and incubated during 30, days at 21ºC. The cereals control group did not receive any treatment. At 1 and 30 days, the fungal population, the mycotoxin content and the AITC content in the samples were determined. The reduction in fungal growth after 1 day of treatment was 1.5, 1.20 and 0.9 log for *A. flavus* (maize) and *P. nordicum* (barley and wheat), respectively, having practically disappeared in all the samples with the treatment at 30 days. In maize contaminated with *A. flavus*, the amount of aflatoxin B1 detected in the controls and the samples treated were 8.07 and 0.12 ppb, respectively. Likewise, in the barley contaminated with *P. verrucosum*, the amount of OTA present in the controls and the treated samples were 0.28 and 0.09 ppb, respectively. Wheat samples contaminated by *P. verrucosum* did not show a significant reduction in the presence of OTA.

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Reduction of ochratoxin A by microorganisms isolated from Tempranillo grape in a wine system

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Ochratoxin A (OTA) is a very important mycotoxin from a toxicological point of view, the presence of which is often reported in different foods as well as in beverages, such as grapes, grape juices and wines. Detoxifying this toxin is therefore of prime importance in protecting consumer health, and biological approaches have been the most promising methods. This work aims to investigate the performance of 12 strains isolated from Tempranillo grapes to reduce OTA during vinification. On the one hand, the capacity of different strains to degrade OTA present in tryptic soy broth (TSB) was evaluated. The isolated strains were grown in TSB pH 3.5 and 6.5, contaminated with 0.5 mg OTA per l. The tubes were kept at 25ºC for 48 h. After that, the content of OTA was determined by HPLC-FLD. For the study of OTA reduction during vinification, 4 strains with the highest reduction of OTA in culture medium were used. The strains were inoculated (10^8 cfu/ml) in triplicate in a test tube containing 10 ml of must artificially contaminated with OTA at 0.6 mg/l. The microvinifications were performed at 25ºC for 18 days. On days 0, 1 ,4, 6, 8, 11 and 15, the wines were analysed to determine the residual concentration of OTA. Microbial identification was carried out at the Spanish Type Culture Collection (CECT) by PCR amplification and subsequent sequencing of the reference genes for taxonomic purposes. The results obtained showed that the OTA concentration in TSB suffered a reduction to 10-51% when the pH was 3.5 and 12-64% at pH 6.5. In microvinification, OTA reduction is proportional to the fermentation time employed. The strain that showed the highest potential in the OTA reduction was the microorganism *Lactobacillus rhamnosus* JCM 1136 with a reduction of the OTA at 18 days of 33.62%. Mass spectrometry associated to the linear ion trap method identified ochratoxin alpha m/z=256.1 and phenylalanine m/z=166.1 as the major metabolites of OTA degradation by the strains

isolated. In wine production, these strains could be used as starter cultures during alcoholic or malolactic fermentation as a biological strategy for the reduction of OTA in the final product.

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Potential utilisation of yellow and oriental mustard flours in loaf bread for shelf life improvement against mycotoxigenic fungi

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In this study, the antifungal activity of yellow (YMF) and oriental mustard flour (OMF) extracts was tested against 14 strains of fungi on solid medium. The water extract and the lyophilised water extract of the flours, autoclaved or not autoclaved, were studied. The minimum inhibitory concentration and minimum fungicidal concentration were calculated for the extract with the highest activity. Not autoclaved lyophilised YMF extract showed antifungal activity against 13 out 14 strains studied. The lyophilised autoclaved YMF extract and the lyophilised not autoclaved OMF extract were active against two strains, whereas the other extracts did not show any antifungal activity. Furthermore, shelf life improvement of loaf bread contaminated with *Aspergillus flavus* (8111 ISPA) or *Penicillium nordicum* (2320 CECT) and treated with YMF was evaluated, and the reduction of aflatoxin B1 (AFB1) was studied in the bread contaminated with *A. flavus*. A significant reduction of the fungal population was observed employing 6 and 8 g YMF/kg both in bread contaminated with *A. flavus* and *P. nordicum*. A shelf life improvement of 2 days was observed in bread contaminated with *P. nordicum* and prepared with 4g YMF/kg in comparison with the bread control, whereas no fungal growth was observed in breads with 6 and 8g YMF/kg during the experiment. A shelf life improvement of 2 days was observed in bread contaminated with *A. flavus* and prepared with 6 g YMF/kg in comparison with the bread control, whereas no *A. flavus* growth was observed in bread with 8g YMF/kg during the 10 days storage period of the study. A reduction of 78% of AFB1 was obtained using 6 g YMF/kg, whereas no AFB1production was observed employing 8 g YMF/kg in bread preparation.

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The effect of Mycosorb A+® on the uptake of zearalenone from the digestive tract of growing pigs

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Preliminary work indicated that zearalenone (ZEN) (200 μg/kg feed) could be detected with its metabolite α-zearalenol (α-ZEL), along the digestive tract and in the faeces of pigs. The aim of this experiment was to use this swine model to test the efficiency of the mycotoxin binder Mycosorb A+ (MSA+). The hypothesis was that addition of MSA+ would increase the recovery of ZEN and α-ZEL from faeces as it would inhibit uptake into the digestive tract. Three experimental diets were designed: Feed 0 (F0), the base diet, commercial pig feed amended with 10% cracked wheat and Celite™, a digestibility marker; Feed 1 (F1), the base diet combined with ZEN (200 μg/kg); and Feed 2 (F2), the base diet combined with ZEN (200 μg/kg) and MSA+ (4kg/t). Twelve pigs were individually housed and provided with F0 for an adaptation period of one week, followed by a four-week period in which six pigs were each randomly assigned to F1 or F2 treatments. At the end of this period, four pigs from each group were sacrificed. The four remaining pigs were then provided with F0 for a washout period of two weeks, after which they were sacrificed. Faecal samples were taken twice weekly for ZEN analysis. Samples for ZEN and α-ZEL analysis were prepared using the Zearala test (VIACAM®, USA). ZEN and α-ZEL were quantified using HPLC with reversed phase column (Cortex 2.7 μm C18, 4.6 x 150 mm, Waters, USA), 65% methanol/0.01 M acetic acid isocratic eluent and fluorescence detector with wavelength 274 nm for excitation and 450 nm for emission (column temperature = 40ºC, injection volume = 50 μl, eluent flow = 0.8. ml/min). The amount of ZEN recovered in the faeces was expressed as a percentage of the amount of ZEN added to the diet. Animals receiving F2 had a significantly higher rate of ZEN + α-ZEL recovery than animals receiving F1 after two (29.3 vs. 46.1%), three (29.1 vs. 48.1%) and four (30.5 vs. 54.7%) weeks. MSA+ significantly reduced the accumulation of both ZEN (77 vs. 15 μg/kg) and ZEN + α-ZEL (89 vs. 17 μg/kg) in the reproductive tract after five weeks. Similar levels of ZEN (23 vs. 16 μg/kg) and ZEN + α-ZEL (25.5 and 20.1 μg/kg) were detected in both groups following the washout period. These results indicate that MSA+ reduced ZEN uptake and metabolism in the pigs and increased its passage through the digestive tract.

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The effect of a heterotrophically grown *Chlorella* microalgal biomass on the deposition of ochratoxin A in the liver of broiler chicks

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The aim of this experiment was to test the effect of dietary inclusion of a heterotrophically grown microalgae, *Chlorella* (CHL), on the deposition of ochratoxin A (OTA) in the liver of broilers. The trial was conducted over a 21-day period using male Ross 508 hatchlings. Chicks were randomly assigned to one of five treatment groups. The study was conducted using 40 pens, providing eight replicates per treatment with six birds per pen. A wheat-soy-based mash served as the basal diet (D1) which was provided to all birds for the first seven days. The following experimental diets were introduced from day 8: D2 = basal diet + OTA (90 µg/kg); D3 = basal diet + OTA (90 µg/kg) + CHL (0.5 kg/t); D4 = basal diet + OTA (90 µg/kg) + CHL (1 kg/t); and D5 = basal diet + OTA (90 µg/kg) + CHL (2 kg/t). Chicks were individually weighed on days 1 and 21. On day 21, all birds were euthanised and their livers removed and weighed. Four livers per pen were homogenised and then used to generate two pools of two livers. The liver homogenates (5 g) were then analysed by HPLC equipped with a fluorescent detector. The mobile phase was a mixture of acetonitrile, deionised water and acetic acid (99:99:2). HPLC separation was performed on a Phenomenex Luna C18(2) 3 µm, 150 × 4.60 mm column and Gemini C18 4 × 3mm SecurityGuard pre-column at a flow rate of 0.8 ml/min. Injection volume was 40 µl and column oven temperature was set to 30ºC. The dietary treatments did not significantly affect any of the measured performance parameters. For D1, the mean OTA concentration detected in the livers was less than 1 ng/kg. For D2, the mean OTA concentration detected in the livers was 72 ng/kg. The addition of CHL resulted in the detection of OTA at a level of 63, 47 and 45 ng/kg for treatments D3, D4 and D5, respectively. The addition of CHL to broiler feed at 0.5, 1 and 2kg/t resulted in a 13, 34 and 35 % decrease in liver OTA concentration, respectively, when compared with the control containing no mycotoxin binder. D4 and D5 resulted in a significant decrease in liver OTA concentration, although D5 was no more effective than D4. These results indicate that CHL reduced the deposition of OTA the liver of broiler chicks.

P105

Using a poly-omic strategy to unravel the genetic and mycotoxin profile variability of *Fusarium proliferatumin* different host plants

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Fusarium proliferatum, a member of the *Fusarium fujikuroi* species complex, is a plant-pathogenic fungus able to both colonise a wide range of plant hosts and produce a relevant number of mycotoxins, among which fumonisins are the most important in terms of their toxicity and worldwide occurrence on several crops. In the last decade, many studies, devoted to investigate the genetic variability of *F. proliferatum* occurring on different hosts, have been performed. These investigations were done in order to understand the capability of this fungus to adapt in a widely different array of ecological niches, and therefore exerts its pathogenicity on different plants. Along with genetic studies, many efforts have been addressed to evaluate the possible variability of the mycotoxin profile of *F. proliferatum* strains isolated from the different plants, mainly to evaluate a possible specificity in the secondary metabolite production according to the crop origin. *F. proliferatum* is considered as the major fumonisin producer worldwide, as we have proved for strains isolated from several other hosts, such as maize, asparagus, onion and wheat. However, strains isolated from fig in Southern Italy and Turkey, and from date palm in Iran, appear to lack the capacity to produce fumonisins. Recently, we gained more insight into the gene content and organisation of the fumonisin gene (*FUM*) cluster, and data revealed no significant differences in gene occurrence, orientation and genome location among producing and non-producing fumonisin *F. proliferatum* strains. Also, phylogenetic analyses were performed for identifying possible clades within the species based on distinction using DNA barcodes, but only slight differences were provided. Thus, a more in-depth investigation of both the genetic variability and metabolic production of *F. proliferatum* by generating and analysing genomes and by metabolic profiling was performed. This

research underpinned that a poly-omic approach is a powerful tool for unravelling the genetic and mycotoxin profile variability of *F. proliferatum* from different host plants.

P106

The impact of storage condition and their effect on animal feed quality regarding mycotoxin contamination in the North West province, South Africa

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The improper storage system of feed is also a factor influencing the presence of mycotoxin contamination. The aim of this study was to collect animal feed samples from emerging farmers and their commercial supplier, analyse and compare the ascertaining if there were any toxigenic levels of mycotoxin contaminants present in the collected feeds from storage facilities where they store their feedstuff. A total of 100 animal feed samples (60 samples from 12 emerging farms and 40 samples from 3 commercial suppliers). A survey and interview were done during collection to find the view and level of knowledge of farmers and suppliers about the right way of animal storage and the importance to avoid feed contamination during storage. Fungi were detected by serial dilution technique using MEA, PDA and SDA agar and about 43% of the screened fungi produces several species of the mycotoxins. In this study, it was found that samples were contaminated with major mycotoxin-producing fungal strains, such as *Aspergillus* spp. as the most predominant, followed by *Penicillium* spp. and other similar mycotoxin strains, whilst *Fusarium* spp. (*Fusarium oxysporum*) was only found in hay fed horses. ELISA was used as a screening test for four major mycotoxins (aflatoxin, fumonisin, ochratoxin A and aearalenone) and SPE was used as a clean-up method quantifying fumonisins. About 97.7% of the samples collected from storages of emerging farms and a commercial supplier were positive for aflatoxin (B1, B2, G1, G2). Fumonisin was present in about 1.1%, in samples from an open storage of an emerging farmer; fumonisin was the least present in commercial supplier storage. Ochratoxin A was detected at a maximum tolerable level (MTL) in 16.7% of feed collected at emerging farm storage and 6.7% from a commercial supplier. Zearalenone was present at both storages, 25.6% were positive. TLC was done of aflatoxin (B1, B2, G1, G2). The standard showed R_f=0.69, and the samples tested had an R_f of 0.89; about 85.3% were positive. HPLC was used as a confirmatory test, whereby the detection of MTLs was determined based on ppb units. Aflatoxins (B1, B2, G1, G2) were mostly detected in commercial farm feed samples and fumonisins (B1, B2) were detected more in emerging farm feed samples, although at a low concentration. Zearalenone was present at a low range, between 39.9 and 51.6 ppb, in samples from emerging farms and a commercial supplier, respectively. The results revealed a correlation between storage types and mycotoxins contamination in feed. There is a need for training of emerging farmers as well as the improvement of storage conditions to reduce mycotoxin contamination.

P107

Transcriptomic analysis of the anti-aflatoxigenic property of dimethyl sulfoxide on *Aspergillus flavus*

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Aflatoxins are synthesised through a 27 enzymatic reaction process, occurring in a cluster localised on the chromosome 3 in *Aspergillus flavus*. The cluster activation is principally regulated by two internal genes named *aflR* and *aflS*. However, a lot of studies demonstrated that aflatoxin B1 (AFB1) production can be modulated by others external regulators which are themselves under the control of environmental stimuli. Understanding mechanisms involved in the regulation of AFB1 biosynthesis could allow conceiving new strategies against aflatoxin contamination of foods, based on targeting key genes located upstream to the cluster. To study mechanisms involved in the regulation of the aflatoxins production, we characterised the effect of an anti-aflatoxigenic compound, dimethyl sulfoxide (DMSO), on various developmental parameters of *A. flavus*. We then studied the impact of DMSO on the transcriptome using high-throughput RNA-sequencing assay (RNA-seq). The exposition of two doses of DMSO during 4 days on fungal cultures leads to 63 and 100% inhibition of AFB1 production, respectively. A dose-dependent depigmentation with no significant impact on fungal growth was also observed macroscopically. Transcriptomic data analysis showed that 4,891 genes were differentially regulated (FDR<5%) in response to DMSO (46% of studied transcripts). Among these genes, 95% were specifically regulated in response to the strongest dose of DMSO, while only 19 genes were modulated after exposure to the lowest dose. The genes of AFB1 cluster were the most negatively modulated genes after the strongest dose treatment. Globally, secondary metabolites clusters genes were widely affected by the DMSO at high dose, with 91% of clusters impacted. Among these, 27% of the clusters

were up-regulated, whereas 13% were down-regulated, and 51% showed a mixed modulation (both up and down-regulated genes). We also noted a modulation of the expression of several global transcription factors (*veA*, *mtfA*) or regulators correlated with fungal secondary metabolism (*laeA*). Our data illustrated the major impact of the DMSO on the expression of genes involved in the secondary metabolism of *A. flavus* and suggests a complex regulation network between mycotoxins biosynthesis and other secondary metabolites pathways.

P108

In vitro mycotoxin binding studies using Minazel Plus® in artificial porcine gastrointestinal digestive juices

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A liquid chromatography-tandem mass spectrometer (LC-MS/MS) was used for evaluation of the *in vitro* binding efficacies of Minazel Plus® for aflatoxin B1 (AFB1), and zearalenone (ZEN) in artificial porcine gastrointestinal digestive juices. The pig feed (grower for pigs ST II Plus) and maize was digested by artificial gastric (pepsin 3.2 g/l, sodium chloride 2 g/l, concentrated hydrochloric acid 7 ml/l, pH=2.0±0.5) and intestinal juices (trypsin 10 g/l, porcine bile salt 3 g/l, monopotassium phosphate 6.8 g/l, pH =6.8) *in vitro*, and then the mycotoxins (2 ppm) and the adsorbent (10 mg/ml) were mixed in the tubes with 10 ml gastric and intestinal digested juices. After incubation and centrifugation, the supernatant was diluted 10-fold and then analysed by LC-MS/MS. The two mycotoxins were separated on a reversed-phase C18 column using a gradient elution programme. Eluent A was composed of water/formic acid (99.9:0.1, v/v) and eluent B of methanol/formic acid (99.9:0.1, v/v); both contained 5 mM ammonium formate. Quantitative analysis was carried out by adding ^{13}C isotope internal standards. The results are summarised in the tables below.

Table 1. Adsorption of AFB1 and ZEN by Minazel Plus in buffer and gastric juices.

	AFB1	ZEN
Buffer	99%	72%
Blank gastric juice	94%	62%
Maize feed in gastric juice	92%	58%
Pig feed in gastric juice	78%	56%

Table 2. Adsorption of AFB1 and ZEN by Minazel Plus in buffer and intestinal juices.

	AFB1	ZEN
Buffer	99%	72%
Blank intestinal juice	98%	61%
Maize feed in intestinal juice	97%	57%
Pig feed in intestinal juice	90%	52%

In conclusion, Minazel Plus retained more than 72 % of its adsorption efficacies for AFB1 and ZEN tested at 2 ppm in the presence of maize and pig feed in gastrointestinal juices. The obtained results are in correlation with results obtained in in vitro binding studies with 0.1M phosphate buffer: 99% for AFB1 and 72% for ZEN. These results have shown 10-20% decrease in AFB1 adsorption and 22-27% decrease in adsorption capacities of Minazel Plus for ZEN in gastrointestinal juices. Similar reports on charcoal, HSCAS and a series of bentonites have also shown that adsorption for AFB1 strongly decreased in the presence of swine gastric juice. The percentages adsorbed dropped from 88 down to 35% for activated charcoal, from 98 to 72% for HSCAS, and generally by more than 15% for a series of bentonites [http://onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2009.EN-22/epdf; Wang *et al.*, 2015).

P109

New efficient multi-mycotoxin adsorbents based on bentonites for animal feed decontamination

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On a worldwide level, 30-100% of feed samples are co-contaminated with mycotoxins. Three *Fusarium* mycotoxins (deoxynivalenol (DON), zearalenone (ZEN), and fumonisins (FBs)) are the major contaminants, while aflatoxins (AFs) and ochratoxin A (OTA) are less detected. In animal feed, only aflatoxin B1 (AFB1) is limited through EU regulation. As mycotoxins cause serious diseases in farm animals, the EU approved the use of mycotoxin adsorbents, as feed additives, to prevent mycotoxicoses on farm animals (Commission Regulation (EC) No 386/2009). In the present work, different silicate clays have been characterised by their adsorbent capacity against mycotoxins. Firstly, 31 inorganic adsorbents (tested at 0.02% w/v) from different geographical origins were studied *in vitro* using a buffer solution (pH 5) at 37°C against 5 mycotoxins tested separately (AFB1, 4 mg/l; DON, 10 mg/l; ZEN, 5 mg/l; OTA, 1 mg/l; FB1, 10 mg/l) by ELISA. Subsequently, the 7 best adsorbents (bentonites, 0.02% w/v), were subjected to an *in vitro* adsorption test against 6 concentrations of AFB1 (0.02-4 mg/l), ZEN (0.1-5 mg/l), OTA (0.05-1 mg/l), FB1 (1-10 mg/l), and DON (2-12 mg/l), by using simulated gastro-intestinal juices (pH 1.2 and pH 6.8) and successively analysed by HPLC-FID/DAD. Mycotoxin

adsorption was calculated by nonlinear regression methods. Then, the 2 most efficient bentonites, with a multi-mycotoxin adsorbent profile, were modified and studied in depth. Increasing percentage of calcium carbonate (0-5%) and moisture (5-11%), and different particle sizes (<100, <150, <200 µm) were tested to get the best performance of bentonites (0.02% w/v) against 4 mycotoxins (ZEN, 0.5 mg/l; AFB1, 4 mg/l; OTA, 0.25 mg/l; FB1, 6 mg/l). Finally, increasing doses of the two studied bentonites (up to 0.2% w/v) were tested. In vitro adsorption experiments showed that AFB1 was the best adsorbed mycotoxin (close to 100% adsorption), followed by FB1 (although only in acidic conditions), and by ZEN and OTA (close to 50%), whereas the adsorption of DON was negligible. Adsorption increased inversely to the toxin concentration and the Langmuir and Freundlich isotherms models were fitted to the data. The physical-chemical parameters tested did not affect the adsorption, however, the 10-fold dose increase of bentonites rendered a more effective adsorption of ZEN, reaching almost 95% of adsorption. The present work has characterised 7 highly effective bentonites and has selected one specific bentonite for the adsorption of multiple mycotoxins (AFB1, ZEN, OTA and FB1) as a practical and feasible solution to detoxify multi-contaminated farm animal feed. **Acknowledgements**. The authors are grateful to the Spanish government (projects RTC-20153508-2 and AGL2014-52648-REDT) and to XaRTA (The Catalonian Reference Network in Food Technology) for funding. P. Vila-Donat thanks MINECO and the University of Lleida for her postdoctoral contract.

P110

Effect of addition of yeast cell wall to broiler diets contaminated with aflatoxin B1 on performance and relative organ weights of broiler chickens

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A study was conducted to determine the effect of aflatoxin B1 (AFB1) (40 ppb) on chicken performance and relative weight of organs and to evaluate the efficacy of yeast fractions-based feed additive in diminishing the toxic effects of AFB1 added to chicken diets. Sixty one-day-old male broiler chicks (Ross 308) were randomly distributed into 3 experimental groups (4 replicated cages of 5 birds) for 21 days. The dietary treatments were: (i) non-contaminated control; (ii) naturally contaminated diet with 40 ppb AFB1; and (iii) contaminated diet with AFB1 (40 ppb) plus the feed additive (yeast cell wall-based product (YCW)) at 0.2%. Performance parameters were evaluated for the overall period and at the end of the study the relative weight of organs was measured. The results revealed that the inclusion of AFB1 without or with the detoxifier additive in the diet did not affect body weight gain and feed conversion ratio ($P>0.05$). The feed intake was higher for broilers receiving AFB1 compared to those fed the control diet ($P<0.05$). The relative weight of proventriculus, pancreas, spleen and thymus were not affected by the experimental treatments. The relative weight of the liver was numerically increased for the AFB1 group and the addition of YCW decreased this parameter to values comparable to birds fed the control diet. There was a significant decrease of the relative weights of the bursa of Fabricius ($P<0.03$) for the animals fed the AFB1-contaminated diet plus YCW. A tendency ($P<0.08$) was found for the relative weight of gizzard, which was higher in broilers receiving AFB1-contaminated diets. This study shows that exposure to AFB1 leads to deleterious effects on broiler chicken without impacting the performance of broilers at 21 days. The addition of YCW to AFB1-contaminated diets significantly reduced the negative effects of the mycotoxin on the relative weight of the bursa of Fabricius.

P111

Efficacy of an algo-clay complex on decreasing mycotoxins liver toxicity in broilers

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The aim of this study was to measure the efficacy of an algo-clay complex on T2- and HT2-toxins, fumonisins and aflatoxins individual liver toxicity. Three trials were conducted by the Samitec Institute of Analytical (Brazil). 1080 broilers chickens (Cobb 500) were used in total for the 3 trials. 360 animals were allocated to 5 treatments with 6 replicates within each of the 5 tests treatments or 12 replicates within the control treatment. Each group contained 10 animals. The study was run from day 1 to day 21. 3 trials were set up allowing to test each mycotoxin individually at a contamination level of 2.8 ppm for aflatoxins, 100 ppm for fumonisins and 2 ppm for T-2/HT-2 toxins. Treatments differed by the presence of each individual mycotoxin, alone or with inclusion of the algo-clay complex at 2.5 or 5 kg/t. Performance and liver parameters were measured: feed intake (FI), body weight (BW), individual relative liver weight (RWL), sphinganine-to-sphingosine ratio (Sa/So) for fumonisins, Lamic/Samitec Index (LSI) for aflatoxins and total plasma proteins (TPP). The inclusion of 0.50% algo-clay complex in the diets

containing mycotoxins significantly improved FI and BW compared to the diets containing mycotoxins alone (P≤0.05). In each study, the RWL of the birds receiving 0.50% algo-clay complex was significantly improved when compared to those receiving mycotoxins alone. The inclusion of 0.50% algo-clay complex in the diets containing 2.8 ppm aflatoxins improved significantly the LSI compared with those diets containing aflatoxins alone (P≤0.05). The inclusion of 0.25 and 0.50% algo-clay complex in the diets containing 100 ppm fumonisins diminished significantly the Sa/So compared with those from the birds fed with fumonisins alone (P≤0.05). The inclusion of 0.25 and 0.50% algo-clay complex improved TPP (P≤0.05). The algo-clay complex decreased significantly (P≤0.05) the deleterious hepatic effects and performance losses caused by very high levels of the three types of mycotoxins in broilers.

P112

Counteracting the negative effects of zearalenone: the role of phytoestrogens' metabolites

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Zearalenone (ZEN) is a non-steroidal oestrogenic mycotoxin that can naturally contaminate feedstuffs and, consequently, be present in animal diets. Apart from this xenoestrogen, phytoestrogens can also be present in the diets. Isoflavones (found in soybeans) and lignans (found in cereals) are the most common ones, usually being represented by genistein (GEN) and matairesinol (MAT), respectively. It is known that ZEN presents synergistic activity with GEN and, consequently, the same is expected with MAT. However, GEN and MAT are mostly metabolised by the host microbiota into equol (EQL) and enterolactone (ENL), respectively, both also oestrogenic. The aim of this study was to evaluate if and how ZEN will interact with GEN, MAT, EQL, ENL, or various combinations thereof, and if such interaction will affect female reproduction. To study this, ovine female gametes at the earliest stages (enclosed in preantral follicles) were *in vitro* exposed to these combinations during a 3-days culture to assess their survival and ability to develop. Follicles were also divided into primordial, which are quiescents, and developing when start to grow. Each compound was used at a concentration of 1 μM, except for the negative control, where exposure during culture was performed in the presence of oestradiol (E2) 3.14 μM. Percentages of normal PAFs were negatively affected when they were exposed to E2, ZEN, EQL and ENL separately. GEN and MAT decreased the rates of normal PAFs only when combined with ZEN. Importantly, when gametes were cultured in the presence of ZEN, medium supplementation with the metabolites EQL and ENL improved the rates of normal PAFs. Follicles were able to develop only when cultured in control medium or added by GEN alone. When in presence of ZEN, development was observed when medium contained EQL or ENL, independently on the presence of GEN and MAT. These results indicate that the phytoestrogens' metabolites EQL and ENL may play a role in counteracting ZEN effects. Considering that they are mainly produced by the host microbiota, the next step consists in evaluating if these findings can be translated to the *in vivo* situation, and to assess how microbiome modulation helps to eliminate/minimise the negative effects of ZEN.

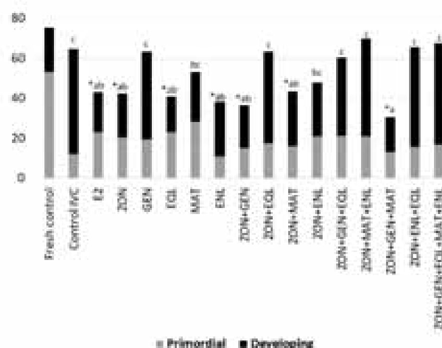

Figure 1. Mean percentages of normal PAFs before (fresh control) or after *in vitro* culture (IVC). *differs from fresh control (P< 0.05); a-c, different letters indicate significant difference (P<0.05). Grey bars represent non-developing and black bars developing follicles.

P113

Priming for protecting maize seedlings from accumulation of fumonisins in early stages of infection by the mycotoxinogenic fungus *Fusarium verticillioides*

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Systemic infection through the seed is one of the routes used by mycotoxigenic pathogen *Fusarium verticillioides* for colonising maize plants. Induction of a priming state in maize seeds might affect their susceptibility for contamination by the fungus and accumulation of fumonisins. A natural fertilizer was applied on maize seeds from two varieties for provoking a priming state. The treatment accelerated seed

germination. Eight days after inoculation of *F. verticillioides* on germinating seeds, the colonisation by the fungus and the accumulation of fumonisins B1, B2, and B3 were significantly lower in seedlings coming from treated seeds than from controls. However, after sowing, the number of plant that stop their development due to necrosis was stimulated by the treatment. The consequence on the yield of whole plant was checked in a field trial. Despite a lower plant density, the level of biomass at harvest was not affected. Soaking maize seeds in a natural fertilizer diluted at 0.01% for 20 h before sowing could contribute to the control of *F. verticillioides* development from seed infection, and the accumulation of fumonisins, without affecting the final crop yield, in absence of contamination.

P114

Mycotoxin reduction by advanced grain cleaning solutions

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Grain cleaning is the most effective post-harvest measure to reduce high levels of mycotoxins due to the efficient removal of mould-infected grains and contaminated grain fractions on the basis of features such as size, density and optical properties. Several studies were performed during the last years to investigate the reduction of e.g. deoxynivalenol in wheat, ergot in rye, and total aflatoxins in peanuts. In this study, the reduction of total aflatoxins (AFB1, AFB2, AFG1, AFG2) in naturally contaminated maize was tested in three cleaning steps using industrial-scale cleaning Bühler machines. The first step included (i) mechanical size separation and dust removal by aspiration with the 'Grain Plus', the second (ii) separation based on density differences with a 'Concentrator', and the third (iii) optical sorting with 'Sortex'. Four batches of maize (about 3 tons each) with different levels of aflatoxin contamination were used for the trials. Furthermore, different process settings and cleaning intensities were tested. Sampling (3 replicates/batch) was performed according to the Commission Regulation (EC) No 401/2006 and the collected samples were analysed by HPLC-FLD with photochemical derivatisation. In addition, the incoming material was analysed by the Eurofins' Rapidust® system for on-site sampling and analysis of mycotoxins in grains. Firstly, trials once again highlighted the difficulties of sampling for aflatoxins. Samples showed a large variability with respect to aflatoxin level. However, high levels of aflatoxin contamination were observed in the removed product streams, with values up to 250 µg/kg. Consistent results were achieved by calculating the aflatoxin level of the incoming material from the removed products taking into account the mass balance. These values compared well with the analysed levels from the Rapidust® system. Aflatoxin levels were reduced from about 10 and 20 µg/kg to 3-4 and 2-3 µg/kg for the low and high contaminated material, respectively. In conclusion, the combination of the tested cleaning machines could allow a total aflatoxin removal of 60-90% in maize. **Acknowledgements**. This work was supported by the MYCOKEY project which has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 678781.

P115

Prevention of mycotoxin absorption by piglets using a mycotoxin binder: when an *in vivo* toxicokinetic study confirms *in vitro* studies

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The negative effects of mycotoxins on animal health and performance are widely known and mycotoxin binders are commonly added to animal feed to prevent these negative effects. A first screening method to evaluate the binding efficacy of a mycotoxin binder is an *in vitro* adsorption test. According to the European Food Safety Authority, the efficacy of mycotoxin-detoxifying agents should be investigated based on *in vivo* trials, measuring specific parameters such as the toxin or a metabolite of the toxin in plasma or other tissues. Therefore, an *in vivo* toxicokinetic study was performed in addition to an *in vitro* adsorption test, to evaluate the effect of a mycotoxin binder on the absorption of zearalenone (ZEN) by piglets. A mycotoxin binding efficacy test for ZEN (protocol developed by Kemin Europa NV) was performed at pH 3 and 7 to evaluate a specifically designed mycotoxin binder including a patented organic compound highly active against ZEN (Toxfin™ XL, Kemin Europa NV). Results showed a 99.9 and 96.5% binding efficacy for ZEN, respectively. To confirm the efficacy *in vivo*, twelve Belgium Landrace x Piétrain piglets of 19.5 ± 3.5 kg body weight (bw) at the start of the experiment were divided into 2 groups: a positive control group and a treatment group. At day 10 the animals were administered

3 mg ZEN/kg bw. Besides the oral bolus of mycotoxins, the pigs in the treatment group also received 150 mg/kg bw of the mycotoxin binder on day 10 via the same intragastric tube (immediately prior to the mycotoxin challenge). Blood was collected at several time points from the vena jugularis externa and plasma levels of ZEN were quantified using UPLC-MS/MS. As the ZEN levels in plasma were low, its metabolite zearalenone glucuronide (ZEN-GlcA) was used as biomarker and was semi-quantified using UPLC-HRMS. Toxicokinetic modelling of the plasma concentration-time profiles was done by Excel pharmacokinetic functions (ZEM-GlcA). The addition of the mycotoxin binder significantly reduced the relative oral bioavailability of the ZEN-GlcA ($AUC_{0\text{-}6\ h\ ZEN\text{-}GlcA\ treatment}/AUC_{0\text{-}6\ h\ ZEN\text{-}GlcA\ control}$) biomarker to 37.6%, proving its ability to adsorb ZEN in the gastro-intestinal tract and consequently decrease the absorption of ZEN by piglets. In conclusion, the present toxicokinetic trial shows the suitability of the mycotoxin binder for the prevention of ZEN in piglets.

P116

Elimination of oxidative stress caused by deoxynivalenol leads to a better performance

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Due to the wide occurrence of deoxynivalenol (DON) in feed cereals and its toxicological effects in the animal, DON has attracted a lot of attention. Besides other toxic effects it is known that by increasing the concentration of free radicals, DON induces an imbalance of the antioxidant capacity in the animal. The aim of this trial was to show the effect of a mycotoxin binder on the oxidative blood parameters and the performance of DON contaminated pigs. In this experimental trial, 252 piglets were weaned at 26 days and divided into 3 groups of 84 pigs each. The first group was not challenged (negative control) whereas the other two were challenged with a 0.9 ppm DON feed contamination. This level of contamination is just below the European legal limit and without co-contamination of other mycotoxins. One of the challenged groups received 3 kg/t Vitafix® Ultra. At day 35 of the trial, the performance was measured and blood samples were collected whereof 3 oxidative parameters were determined. The blood parameters related to oxidative stress measured were the half haemolysis time of the red blood cells (HT50), the concentration of malondialdehyde (MDA) in the blood plasma and the concentration oxidised glutathione (GSSG) over total glutathione. Compared with the other challenged group, the addition of the mycotoxin binder resulted in an increase of ADG and FI with 4.1 and 4.5%, respectively. This ensures a body weight gain of 700 g (24.11 vs. 23.40 kg). The blood parameters showed a tendency that less oxidative stress occurred in the animal. Compared with the other challenged group, HT50 with the mycotoxin binder was significantly higher (99.63 vs. 87.51 min; $P<0.05$). MDA concentration and the ratio oxidised glutathione/total glutathione showed a numerical improvement (MDA, 6.50 vs. 6.62 mmol/ml plasma; GSSG, 40.00 vs. 44.86). The group with the mycotoxin binder added showed similar results with the negative control group. It can be concluded that reducing the oxidative stress due to a DON contamination leads to an improvement of the performance parameters in piglets.

P117

Food safety risks from the conventional practice of reusing jute bags and recommendations to prevent aflatoxin cross contamination of maize

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Aflatoxins are a confirmed carcinogen and a major food safety risk in both foodstuffs and animal feed. This is a global issue in food supply chains and a particular challenge in certain markets, e.g., a high rejection rate (30-50%) of maize loads in India due to aflatoxin contamination has been noted. This may result in part from the reuse of jute bags for storage and transportation of maize in many areas of India. This study determined the impact of this conventional practice on aflatoxin contamination of maize. A total of 122 samples were collected from new and reused jute bags in India. The reused bags included samples from bags that had contained maize rejected because of high aflatoxin concentration (average 67 ppb, range 14 to 188 ppb) (rejected) and samples from bags containing maize that had been accepted for factory use (aflatoxin concentration <12 ppb) (accepted). Aflatoxigenic fungi were determined in the samples. An average of 2,736 cfu of aflatoxigenic fungi conidia were found in samples from rejected bags, 4.4-fold higher than the number from accepted bags and 27.0-fold the number from new bags. A batch of 'clean' maize (in which aflatoxigenic fungi could not be detected) was stored in reused (both from accepted and rejected maize batches) and in new jute bags. After four weeks in conditions optimised for aflatoxigenic fungal growth, 25 cfu/g aflatoxigenic fungi conidia were detected from the maize packed in bags from rejected samples which was 2-fold higher than maize packed in 'accepted' bags. No aflatoxigenic fungi could be detected in maize packed in new bags. The effect of

four treatments intended to minimise the risk of reusing jute bags were compared. Shaking the bag, UV light, shaking combined with UV light and the effect of ozone treatment all decreased the number of residual aflatoxigenic fungi in the reused jute bags. This is the first systematic study assessing the effect of reusing jute bags on fungal population and aflatoxin contamination risk. It revealed the cross-contamination risk of reusing jute bags for raw material storage and packaging. Moreover, the application of readily applied treatments by farmers and suppliers will help to minimise aflatoxin contamination.

P118

Denaturation of aflatoxins in naturally contaminated peanuts using ozone

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Contamination of crops by aflatoxins (AFs) is unavoidable, even with the best agricultural practices. Once aflatoxin has formed in a commodity, it is difficult to remove and its concentration should be reduced to a food safe level. Ozone represents one chemical approach to detoxifying AFs, however, most studies were conducted using spiked materials and application conditions were inconsistent. In this study, ozone conditions for reducing naturally contaminated peanuts were investigated.

P119

Clay mineral based mycotoxin binders can interfere with the analysis of aflatoxins by ELISA

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The contamination of animal feed with mycotoxins is a continuing food safety issue leading to economic losses. Mycotoxin detoxifiers are widely used by industry as additives for the decontamination of animal feed. Mycotoxin detoxifiers can be divided into two groups based on their functional mechanism: mycotoxin binders which aim to reduce the adsorption of mycotoxins, and mycotoxin modifiers which aim to modify the chemical structure of mycotoxins. Most published research is focused on the development of novel mycotoxin detoxifiers and the evaluation of their efficacy through in vitro or in vivo experimentation. There are few studies investigating the impact of mycotoxin detoxifiers on the analytical determination of mycotoxins, and one concern is that mycotoxin binders may mask mycotoxins leading to underestimation of the true mycotoxin content of a raw material. The objective of this study was to investigate the interference of mycotoxin binders with the analysis of mycotoxins using ELISA. This study focused on aflatoxins (amongst the most toxic mycotoxins) in maize and maize gluten as these materials are frequently contaminated. Three types of commercially available mycotoxin binders, yeast cell wall, clay mineral, and a mixture of clay mineral and yeast, were investigated at inclusion levels of 0.0, 0.1, 0.2 and 0.4%. The binders were added to maize and maize gluten contaminated with aflatoxins at concentrations between 6.8 to 20 µg/kg. The samples were analysed using commercially available ELISA kits. The yeast cell wall binder did not impact the ELISA results at any of the tested inclusion levels. Addition of either the clay mineral or mixed clay mineral and yeast binders resulted in significantly lower ($P<0.001$) detection levels of aflatoxins by the ELISA test. The greatest underestimate (80.9%) was observed with the inclusion of 0.4% of the clay mineral based binder. The study demonstrated that clay mineral based mycotoxin binders can result in underestimation of mycotoxin contamination of maize and maize gluten when tested by ELISA.

SAMPLING AND ANALYSIS
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P120

Development of biosensor approaches for the detection of phomopsin

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Phomopsin, a secondary metabolite produced by *Diaporthe toxica*, is a mycotoxin that has been shown to contaminate crops and affect animal and human health upon consumption. The main host for the fungus and thereby phomopsin occurrence is in lupin and lupin-derived food. The majority of lupin seed is used in animal feed, however, lupin products may also increasingly be used for human consumption. Therefore, whole lupin seed and flour may be a source of human exposure to phomopsins, which have been shown to be stable to processing and cooking. Australia and New Zealand have set a regulated maximum permitted concentration of phomopsin in lupin seeds at 5 ng/g but currently no legislation has been established for this mycotoxin in Europe. Consequently, there are limited methods of analysis applied. The aim of this study was to develop a rapid biosensor approach for the determination of phomopsin. A monoclonal antibody was raised to phomopsin keyhole limpet haemocyanin and characterised by indirect competitive ELISA to phomopsin ovalbumin to have a titre of 1/32000 with an IC_{50} of 0.5 ng/ml. The antibody was used to develop two different immunological biosensor approaches for the detection of phomopsin in lupin flour. The first biosensor approach was lab-based utilising a label free real-time surface plasmon resonance (SPR) instrument and the second approach was a portable planar waveguide fluorescence technique (MBio). Both methods were developed as fit for purpose to detect phomopsin below the current maximum permitted limits of 5 µg/kg for lupin seeds and products thereof. Both methods developed were quantitative with the SPR method taking 8 min for the analysis of each sample whereas the analysis time by the Mbio method was 10 min per sample. A comparison of the methods will be described.

P121

Simultaneous determination of 42 mycotoxins in oats: a high-throughput UHPLC-MS/MS analytical method

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Mycotoxins are secondary metabolites produced by fungi that can readily colonise crops in the field or during storage. These compounds can cause a wide range of toxic effects in humans or animals, such as vomit and diarrhoea, protein synthesis inhibition and cancer, representing a serious problem for public health and possibly leading to significant economic losses. A multi-residue analytical method was developed and validated for the confirmatory and quantitative analysis of 42 mycotoxin residues in oats using ultra-high performance liquid chromatography coupled to tandem mass spectrometry detection (UHPLC-MS/MS). The method includes all the mycotoxins listed under Commission Regulation (EC) No 1881/2006, the emerging mycotoxins enniatins and the masked metabolites deoxynivalenol-3-glucoside (D3G) and T-2-glucoside (T2G). A rapid sample preparation procedure was optimised based on the QuEChERS methodology, consisting of a double extraction with acidified water and acetonitrile, evaporation and reconstitution of a sample aliquot, prior to filtration and injection into the UHPLC-MS/MS system. Validation studies showed good reproducibility of the method. For the majority of analytes, accuracy ranged from 70 to 130%, while precision (expressed as RSD%) was $<23\%$ under reproducibility conditions, fulfilling the performance criteria currently established by Commission Regulation (EU) No 519/2014. The limit of quantifications (LOQs) ranged from 0.5 to 100 µg/kg, depending on the sensitivity of each analyte. The high-throughput method developed in this work allows the simultaneous determination of 42 compounds in 50-55 samples by a single analyst in one day.

P122

Determination of deoxynivalenol in dry distillers grain with solubles and a comparison to mean results obtained at several analytical laboratories

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An inter-laboratory study (ILS) coordinated by POET was completed to quantitate deoxynivalenol (DON) in 14 samples of dry distillers grain with solubles using the services of 8 analytical laboratories with established mycotoxin analytical methods. Neogen's analytical laboratory participated in the ILS and also collected Reveal Q+ for DON and Veratox for DON 5/5 data on the samples. The ILS was conducted with samples submitted in 4 rounds, precision improved during the rounds with a mean CV of 29.6% for round 1 to a mean CV of 11.3% for round 4. Results from one laboratory (ILS laboratory #734) were significantly different than the other 7 laboratories and were not included for final comparison to the mean analytical ILS results. Neogen's analytical, Q+ and Veratox results were not significantly different from the mean ILS analytical results. Absolute values of the Z-scores for Neogen analytical results were all <1.2 with a mean Z-score of 0.44 and mean percent difference of 6%. Mean percent difference of Veratox and Q+ results from the ILS mean were 4 and -3%, respectively.

P123

Inter-lab study for fumonisin detection: a technical services chemical residues comparison

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Neogen Corporation collaborated with three additional labs to detect naturally contaminated fumonisin in maize. The purpose of the study was to compare different analytical methods across labs. Using their preferred method, each lab analysed the samples and reported the results to Neogen. The study included triplicate analysis of 3 blinded ground corn reference samples containing 0.5, 1.6 and 4.0 ppm fumonisin and a liquid fumonisin stock sample by 4 analytical laboratories on 3 different weeks. The overall mean of the inter-laboratory data for the 0.5 ppm fumonisin reference sample was 0.49±0.12 (coefficient of variation (CV)=24.5%) ppm fumonisin and ranged from 0.30 to 0.70 ppm. The mean for the 1.6 ppm fumonisin reference sample was 1.55±0.23 (CV=14.8%) and ranged from 1.1 to 2.06 ppm fumonisin. The mean result for the 4.0 ppm sample was 3.95±0.55 (CV=13.9%) and ranged from 3.1 to 5.3 ppm. Variation in the inter-laboratory results was most evident in the 0.5 ppm reference sample with a range of 0.4 ppm fumonisin. Intra-laboratory results showed better precision with all laboratory CVs<12% for the 0.5 ppm sample, <6.5% for the 1.6 ppm sample and <7.3% for the 4.0 ppm sample. A Z-score analysis was conducted to compare each labs results to the mean.

P124

Analysis of Multiple Mycotoxins by LC-MS/MS: in-depth analysis of column selectivity

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Mycotoxins are toxic secondary metabolites produced by fungi, which can exist in food as a result of fungal infection of crops. Their strong resistance to decomposition and digestion cause mycotoxins to remain in the food chain. The analysis of mycotoxins in food and animal feed has been a challenge mainly due to the complexity of food matrices and desired low detection limits. In recent years, significant advances in the analytical techniques were applied to the detection of mycotoxins. There has been an increasing need for a method to detect multiple mycotoxins with a single sample preparation and analysis method. Previous research concentrated on an LC-MS/MS method for multi-mycotoxin analysis as mass spectrometry provides appropriate selectivity and sensitivity for detection. This study investigated the selectivity for over 15 common mycotoxins on a variety of solid-core HPLC columns with different stationary phase chemistries. The results of these analyses were evaluated for optimum resolution and selectivity. The separation of analytes from matrix was also important as often no sample clean-up is performed during analysis and matrix effects are highly probable. The choice of column chemistries will be presented with emphasis on overall method performance.

P125

Systematic review of methods of analysis for mycotoxin detection in the food supply chain

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Mycotoxins are a diverse group of biologically active toxic secondary metabolites produced mainly by filamentous fungi and found as contaminants in almost all agricultural products worldwide, before or after harvest, during transportation or storage. The key toxins are identified in families of structurally similar chemical compounds whereby aflatoxins are the most regulated. Other family groups include ochratoxins, fumonisins, trichothecenes, zearalenones and ergot alkaloids. Currently, there are over 300 known mycotoxins and these may co-occur in feed or food but knowledge of co-occurrence of the toxins is limited due to the methods of analysis applied. The main aim of this study was to conduct a systematic review of the methods of analysis for mycotoxin analysis whereby co-occurrence can be determined and to rank these methods in suitability detection to limit exposure but also to rank for use in defined fields based on suitability for the end user as a prevention and mitigation tool. A systematic literature review was performed from various databases, internet searches and through the examination of policy papers to identify the different mycotoxins and mycotoxin mixtures affecting food and feed on a global scale and in varying climates and the methods of analysis used to detect them. The literature has identified that there are many methods of analysis that can be used for mycotoxin detection incorporating physicochemical, immunological and molecular techniques. The methods of analysis were ranked and a road map developed for their suitability in use for the detection of multiple mycotoxins. It is imperative, nowadays, to develop methods of analysis or strategies based on risk for multiple mycotoxin determination whereby the simultaneous exposure of the multiple mycotoxins within a product can be prevented. The current lab state of the art technique is liquid chromatographic coupled to tandem MS (LC-MS/MS) though this method is not field deployable and affordable in all regulatory environments. A highly specific yet sensitive method of analysis is required which is capable of multiple mycotoxin detection yet that is economically viable to monitor the future global food production. However, one (bio)analytical method may not be suitable for all circumstances and whereby a roadmap of the current methods of analysis available would enable strategies for analysis to be implemented in different scenarios.

P126

Cost-effective sampling and analysis for deoxynivalenol in wheat and aflatoxins in maize

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Sampling and analysing cereal batches for mycotoxin contents has to deal with a lot of uncertainty due to the fact that mycotoxins are inhomogeneously distributed throughout the batch. The aim of this research was to find the optimal sampling and analytical (S&A) plan for mycotoxins in a batch of grain given certain budgets. Different S&A approaches were considered. One approach was to have the samples sent to a laboratory: samples are collected from the batch, combined into one aggregated sample, which is sent to a laboratory and analysed with LC-MS, as prescribed by the European Commission, or alternatively, the multiple aliquots from the aggregate sample are analysed with a faster and less expensive method, being ELISA (enzyme-linked immunoassay). A second approach was the on-site detection: multiple samples are collected and each analysed with a LFD (portable lateral flow device) directly. Operating characteristic (OC) curves showed the probability to accept the batch for different concentrations, which depends on the accuracy of the S&A plan, which was determined with the variances associated with collecting samples form the batch, preparing the samples, and analysing the samples. With a typical distribution of mycotoxin concentrations among batches, and the OC curves, a percentage of correct decisions was estimated for different S&A plans. This was done for deoxynivalenol in wheat, and for aflatoxin B1 in maize. An optimisation model was used that maximised the percentage of correct decisions, subject to a budget constraint, by changing the number of samples taken, the number of samples analysed and the detection method used. Results showed that, at lower budgets, LFDs are most suitable for estimating the batch concentration. However, using LC-MS allows to take more samples and leads therefore to the highest percentage of correct decisions at a higher budget. The use of LFDs is more suitable for deoxynivalenol in wheat since this mycotoxin does not grow during storage and will be more homogeneously distributed and therefore, the sampling variability has a relatively smaller influence and taking less samples is less penalised than for aflatoxins in maize.
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P127

Monitoring of CO_2 levels in stored wheat grains for early detection of *Fusarium graminearum* colonisation and zearalenone and derivatives accumulation

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Cereals are commonly colonised by *Fusarium* species pre-harvest. At present, temperature (T) and sometimes relative humidity (RH) sensors are being used in silos to monitor grain quality and detect changes that may be related to spoilage and therefore quality deterioration of the grain due to mould growth and insect infestation. However, although there are some ecophysiological studies on *Fusarium graminearum* ecology, the boundary conditions for growth and zearalenone (ZEN) production by this species are scarce. This study focused on the ecological profile in terms of T x water activity (a_w) of two *F. graminearum* strains isolated from British wheat. The a_w (0.88-0.995) x T (6-35ºC) profile for optimum and minimum growth and ZEN production was determined on wheat-based matrices for the first time. The growth data was used to develop probabilistic models. The ZEN temporal production kinetics in relation to these interacting factors were quantified over 30-day periods. The shift from ZEN to the main derivate compounds (zearalenone-sulfate, alpha-zearalenol and beta-zearalenol) on inoculated grain was studied under grain storage-like conditions (a_w 0.90-0.95 x T:10-25ºC). Interestingly, *F. graminearum* was able to grow under 30°C although the a_w was a limiting factor. No fungal growth was observed at 0.91a_w except at 25°C and under 0.97 a_w at 6°C. This data has been combined with respiration of *F. graminearum* in wheat grain stored under similar interacting environmental conditions. Overall, respiration in wheat at 0.90a_w and 10°C was constantly very low throughout the 15-day storage period. However, at 15°C increase of respiration was observed in the wettest conditions. Respiration rates increased with time and humidity at 20 and 25°C showing a good relationship between fungal growth and CO_2 levels. The respiration data (mg CO_2/kg/h) was utilised to identify optimum dry matter losses (DMLs). DMLs in wheat colonised by *F. graminearum* represented up to 3-4% depending on storage conditions. This has been combined with ZEN production patterns to correlate the relationship between DMLs and ZEN production which will exceed the legislative limits set by the EU and other countries. High risk to ZEN contamination was observed with DML higher than 0.5%. The thorough understanding of *F. graminearum* ecophysiology is of paramount importance in order to improve the evaluation of the potential risks of ZEN and derivative compounds. The use of such biological models may facilitate the use of boundary conditions and respiration/DMLs to predict the relative risk of ZEN contamination in stored cereals.

P128

Assessment of extraction efficiency of ergot alkaloids

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Ergot alkaloids produced by the fungus *Claviceps purpurea* contaminate over 600 species of cereal crops and grasses. Analysis using high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) can be used to identify these alkaloids. This study was conducted to determine the most efficient procedure to extract ergot alkaloids from a ground sample. Four factors of the extraction process were evaluated in three experiments: (i) extraction solvent ratio; (ii) mixing time; and (iii) solvent volume combined with multiple extractions of a single sample. All three experiments examined a low (approximately 100 ppb (µg/kg)) ergot contaminated ground wheat sample. The other experiments also examined mid and high (approximately 1,500 and 7,500 ppb, respectively) ergot contaminated ground wheat samples. The currently used 85% acetonitrile/15% 10 mM ammonium acetate was compared to 90/10, 80/20, 70/30 and 60/40 acetonitrile/10 mM ammonium acetate extraction solvent mixture. The second experiment compared a standard 10 min mixing time to an extended 60 min mixing time using the 85/15 extraction solvent mixture. The third experiment evaluated the use of six extractions on a single sample at 25, 50 and 75 ml extraction solvent (85/15) volumes and a 10 min mixing time for each extraction. The 85% acetonitrile/15% 10 mM ammonium acetate (control) extraction solvent ratio resulted in the most efficient extraction of ergot alkaloids from the sample with 90/10, 80/20 extraction solvents not being significantly different from the control. The 70/30 and 60/40 extraction solvent mixtures extracted 50 to 60% less alkaloids compared to the control. The mixing time (10 vs. 60 min)

did not alter the alkaloid extraction efficiency at any concentration. For all concentrations, two extractions at 50 and 75 ml were able to extract 83-89% of the ergot alkaloids, whereas two extractions at 25 ml only extracted 78-79% of the ergot alkaloids. All volumes extracted 97% or more of the ergot alkaloids by the fifth extraction. These experiments demonstrated that six extractions using a 50 ml solvent extraction volume is optimal to extract the maximal amount of ergot alkaloids in a sample. However, from an economical perspective, considering time, consumables and amount of ergot extracted, two extractions at 50 ml is the most efficient procedure to extract ergot alkaloids from a sample for diagnostic purposes.

P129

Supercritical fluid chromatography coupled with mass spectrometry, a novel approach for a rapid aflatoxins analysis in high fatty matrices

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Aflatoxins, highly toxic (carcinogenity group 1 according to the IARC) secondary metabolites produced by *Aspergillus* species, represent one of the most important mycotoxins groups impacting both human and animal health. For these reasons, aflatoxins are regulated by relevant authorities worldwide and their content in food and feed must be controlled. Aflatoxins are commonly occurring in improperly stored commodities, such as cereals, seeds, and a variety of spices originating especially from countries with warm climate of Africa, Southeast Asia, etc. For the control aflatoxins, a wide range of analytical methods has been developed until now. Currently, a reverse phase liquid chromatography coupled with mass spectrometry (RPLC-MS) is generally a preferred technique. In case of high fatty matrices, such are nuts and seeds, the presence of triacylglycerols (TAGs) in the final extract represents a problem due to their strong retention in the RPLC system. Within this study, a novel strategy based on supercritical fluid chromatography coupled with high resolution mass spectrometry (SFC-HRMS), is demonstrated to overcome this issue. Since carbon dioxide, representing non-polar mobile phase (normal chromatography), is used in the SFC-HRMS system, TAGs are easily eluted. An analytical procedure for the determination of the major aflatoxins B1, B2, G1 and G2 has been developed. Peanuts (and walnuts) have been selected as representatives of high fatty matrices (more than 60% fat), with relatively often incidence of aflatoxins. For the purpose of validation, aflatoxins were quantified in the extracts by a QuEChERS-like approach. The recoveries obtained for peanuts and walnuts at two spiking levels, 20 and 4 µg/kg, were in the range of 75-111 % (RSD 1-5 %). Satisfactory accuracy for all measurements was achieved by employing matrix-matched calibration. The experimentally established limit of quantification (LOQ) was 2.5 µg/kg for all analytes, which is in compliance with EU legislation (Commission Regulation (EC) No 1881/2006; Commission Regulation (EU) No 165/2010).
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P130

A new approach employing ultra-high performance liquid chromatography coupled with orbitrap mass spectrometry for determination of *Alternaria* toxins and their conjugated forms in tomatoes and tomato-based products

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Alternaria ssp., also known as black moulds, belongs to a genus of worldwide occurring fungi producing a variety of potentially dangerous mycotoxins which can contaminate the food chain. Several tens up to hundreds of known secondary metabolites are produced by *Alternaria* species, however, the molecular structures of only a few of them have been elucidated so far. In addition to the free *Alternaria* toxins, also their conjugated forms originating during the plant detoxification processes have been emerged. Within this study, a new analytical method based on ultra-high performance liquid chromatography (UHPLC) coupled with high-resolution tandem mass spectrometry (HRMS/MS) employing Q-Exactive^{TM} Plus (hybrid quadrupole-orbitrap) was developed for targeted screening of more than 70 different *Alternaria* metabolites and their conjugates in tomatoes and tomato-based products. For the purpose of analytical method optimisation, extracts of tomatoes with visible black *Alternaria* moulds were used

(commercial availability of pure analytical standards is limited). As concerns the sample preparation, QuEChERS-like extraction together with simple aqueous extraction were compared. In this method, a reverse-phased analytical column Acquity UPLC® HSS T3 (100 mm × 2,1 mm; 1,8 µm) was used and two different buffered aqueous mobile phases with or without addition of formic acid were tested. As a 'strong' mobile phase, methanol was utilised. Within tuning the detection conditions, different ionisation modes, ion source settings (auxiliary/sheath gas flow, spray voltage, heater temperature, capillary temperature, S-lens value) and detection parameters (maximum inject time, automatic gain control) were tested with respect to final intensity of particular analytes. Identification of *Alternaria* metabolites was realised through the measurement of the exact masses and elemental composition calculation. As the confirmatory criteria, the agreement of isotopic patterns and detection of specific fragment ions in high resolution, were taken. The poster presents the results of screening of the above discussed analytes in tomatoes and tomato-based products from the Czech market. **Acknowledgements**. This work was supported by the 'Operational Programme Prague – Competitiveness' (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503), 'National Programme of Sustainability I' – NPU I (LO1601 – No. MSMT-43760/2015). This research has also received funding from the European Union´s Horizon 2020 research and innovation programme under grant agreement No 692195 (MultiCoop).

P131

Efficient detection and quantification of T-2 toxin in grain and animal feed

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T-2 toxin belongs to the type A trichothecene mycotoxins and is one of the most toxic secondary metabolites produced by several fungi of the genus *Fusarium*. Due to the typical stability of the trichothecene family, T-2 toxin is able to withstand processing procedures and can commonly be found in animal feed and food. Its contamination has been implicated to be responsible for health problems primarily because of its ability to inhibit protein synthesis in both human and animal and as a result cause cell death. T-2 toxin can be the source for symptoms such as weight loss or reduced weight gain, diarrhoea, dermal necrosis, and dyspnoea. Therefore, we developed a T-2 toxin enzyme linked immunosorbent assay (ELISA) which provides a quantitative method for the determining the presence of T-2 toxin in cereals and animal feed. ELISAs have proven to be a valuable tool in the determination of mycotoxin levels as they allow for a simple, quick, and cost-effective high-throughput detection of trace amounts of mycotoxins. In addition, ELISAs are convenient and easily executed in any setting. We will present inter- and intra-assay performance showing that with a CV of less than 5% this assay is a reliable method for the detection of T-2 toxin. Moreover, we validated the accuracy of the assay measurements with experiments consisting of spike recovery from several commodities and correlation of recoveries from reference materials that have also been quantified by HPLC. These collective studies demonstrate that our ELISA can be an efficient tool for screening a variety of complex commodities for T-2 toxin contamination.

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Use of core-shell columns in conjunction with Immunoprep® Online immunoaffinity cartridges for the automated clean-up and analysis of aflatoxins, ochratoxin A or zearalenone

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R-Biopharm Rhône Ltd. offers a novel technology, which enables the automatic and selective sample clean-up by immunoaffinity combined with on-line quantitative analysis by HPLC. This technology is ideal for routine testing of a high number of samples. However, in order to fully exploit the potential of this technology, analytical columns with improved separation characteristics are required. For instance, with current UPLC technologies, rapid analysis in combination with high sensitivity is possible. Alternative columns, which do not require these high pressures, are the core-shell columns. In conjunction with our Immunoprep® Online cartridges for the automated clean-up of respectively aflatoxins B/Gs, ochratoxin A, aflatoxin M1 or zearalenone, various core shell columns (C18, biphenyl, pentafluorophenyl, F5 and phenyl hexyl) have been tested and chromatographic methods have been defined. The results, in terms of improvement in resolution, sensitivity, sample throughput and waste disposal, have been compared with currently used porous columns.

P133
Determination of aflatoxin M1 in milk products using an automated immunoaffinity cartridge system
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Aflatoxin M1 is a major metabolite of aflatoxin B1 in humans and animals and may be present in milk from animals fed with aflatoxin B1 contaminated feed. Current European legislation is in place for aflatoxin B1 in animal feed in order to reduce the risk of aflatoxin M1 in the food chain. For aflatoxin M1 legislative levels are often very low therefore suitable methods are required. R-Biopharm Rhône has developed a patented, online immunoaffinity cartridge which can be used prior to HPLC for analysis of aflatoxin M1. The cartridges are used together with the Rida®Crest handling system to combine automated online sample clean-up with quantitative analysis. Each cartridge is calibrated and can be used for up to 12 samples before the cartridge is automatically removed and replaced with a new one. The cartridges contain monoclonal antibodies attached to a polymer making them highly specific and sensitive. A key advantage of this process is that during the LC run of one sample, the next sample is simultaneously passed through the affinity cartridge, reducing the time taken for subsequent sample clean-up to almost zero. Here, methods and results will be presented for the automated, rapid determination of aflatoxin M1 in various commodities, including milk, infant milk formula, cream, yoghurt and cheese.

P134
Determination of aflatoxins in spices by immunoaffinity column clean-up and fluorescence detection – an international multi-laboratory validation study
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A method was developed for the determination of aflatoxins B1, B2, G1 and G2 and total aflatoxins (sum of B1, B2, G1 and G2) in spices by high performance liquid chromatography (HPLC) with post column derivatisation (PCD) and fluorescence detection (FLD) after immunoaffinity column clean-up. The method uses extraction with a mixture of acetonitrile, methanol and water with the addition of salt. After filtration, extracts are cleaned-up by immunoaffinity column and analysed by HPLC-FLD, using post column bromination, either by use of a Kobra® cell or addition of pyridinium bromide perbromide (PBPB). The method was validated in-house for the spices for which EU maximum level are in force (other than paprika), i.e., ginger, turmeric, chilli, nutmeg, pepper and mixed spice. The method was then validated in an international multi-laboratory validation study. A range of test samples that comprised: ginger; pepper; nutmeg; turmeric; mixed spice; chilli; and three mixtures of pepper:chilli:nutmeg; mixed spice:ginger, and turmeric:ginger were used for the study. Twenty samples, analytical standards and immunoaffinity columns were distributed to fourteen international laboratories. The validation was carried out over the following concentration ranges: aflatoxin B1=1 to 16 µg/kg, B2=0.5 to 8 µg/kg, G1=0.5 to 9 µg/kg, G2=0.5 to 6 µg/kg and total aflatoxins=2 to 35.6 µg/kg. Method performance was acceptable for all spices and mixtures over all concentrations for aflatoxin B1 and total aflatoxins. HorRat values of 0.55 to 1.18 were found for aflatoxin B1 in individual spices, and 0.57 to 1.59 in spice mixtures. For total aflatoxins, HorRat values were 1.03 to 2.0 for individual spices and 0.84 to 1.58 for spice mixtures. The project was funded by the European Commission by Mandate M520 addressed to CEN for standardisation for methods of analysis for mycotoxins in food. The method can be applied for control of aflatoxin B1 and total aflatoxins in the spices capsicum, pepper, nutmeg, ginger, turmeric and mixtures as regulated by Commission Regulation (EC) No 1881/2006.

P135
Determination of multiple mycotoxins in oats by 11+ MS PREP immunoaffinity column clean-up and LC-MS/MS
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New multi-mycotoxin immunoaffinity columns have been developed by R-Biopharm Rhône Ltd. The columns (11+ MS PREP immunoaffinity column) can be used to clean-up samples for aflatoxins B1, B2, G1 and G2 ochratoxin A, zearalenone, deoxynivalenol, T-2 toxin, HT-2 toxin, and fumonisins before analysis by LC-MS/MS. The columns have been validated for wheat, barley, maize and some feed types. This poster describes the single laboratory validation of a method for oats using these columns. Oats can be problematic during analysis, as samples absorb a lot of extraction solvent, extracts can be more

viscous than other types of cereals, and diluted extracts can contain a lot of particles, all these issues can result in difficulties during clean-up and analysis caused by matrix components. The extraction procedure, and maximum loading potential of the columns using oat extracts will be assessed. The potential of the columns to retain other related mycotoxins of interest will also be assessed. Repeatability, reproducibility, recovery, and reporting limits will be determined and assessed against recognised performance criteria (Commission Regulation (EC) No 401/2006 and CEN TC275WG5 Criteria document) to determine the method's applicability for use to support mycotoxin regulatory controls.

P136

Non-invasive cereal analysis by GC-MS detection of trichodiene as volatile mycotoxin biomarker

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Due to the increasing consumption of cereals worldwide, the monitoring of growth, storage and processing is becoming more and more crucial. Particularly when stored, infested grains breed fungal clusters ('hot spots') in which mycotoxins greatly exceed allowed maximum levels. Because of their unpredictable presence, current sample drawing and procedures for mycotoxin analysis represent a complex challenge for operators, involving invasive and cost intensive steps. Therefore, new time- and labour-saving mycotoxin control methods including sampling and analysis steps are needed. A possible approach is the non-invasive analysis of the homogeneous gas phase above the crops, instead of analysing random samples. However, this procedure requires microbial volatile organic compounds (MVOCs) being released by the samples and representing the present mycotoxins. Previous investigations revealed trichodiene to be a precursor in trichothecenes biosynthesis – one of the largest mycotoxin groups with over 180 compounds. Due to its non-functionalised sesquiterpene structure, trichodiene has already been quantified using headspace GC-MS methods [for instance, Becker *et al.*, 2014. Journal of Agricultural and Food Chemistry 62:5226]. Thereby, it can possibly be used as a biomarker for trichothecene contamination in foodstuff. However, further investigations are necessary. The correlation between trichodiene concentration in the gas phase and trichothecenes mass fraction in the sample must be examined closely to draw conclusions about the exact trichothecene content within samples. Realising this idea would widely extend the applicability of trichodiene and enormously simplify trichothecene quantification. Hence, this first step of an ongoing study aims to develop a laboratory reference method using trichodiene as volatile biomarker to quantify trichothecenes in cereals. Static headspace and SPME-enrichment coupled to gas chromatography with mass spectrometry (GC-MS) were employed. In a second step, this reference method is intended to validate new approaches for fast on-site screening of trichodiene in cereals.

P137

Biosynthesis of zearalenone conjugates by fungi

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Zearalenone (ZEN) and its sulfate and glucoside conjugates have been detected in a broad variety of food and feed commodities [De Boevre *et al.*, 2012. World Mycotoxin Journal 5:207]. Both conjugated derivatives are formed as part of fungal or plant secondary metabolism and thus, belong to the group of modified mycotoxins [Rychlik *et al.*, 2014. Mycotoxin Research 30:197]. After consumption of contaminated foodstuff, the conjugates can be hydrolysed by human intestinal microbiota leading to liberation of ZEN that implies an underestimation of the true ZEN exposure. In order to include ZEN conjugates in routine analysis, as well as for toxicological investigation reliable standards are needed. The objective of the present study was to develop a simple and economic method for biosynthesis of ZEN conjugates. Preceding experiments on the biotransformation of ZEN by *Rhizopus* and *Aspergillus* species showed a mixed metabolite formation [Brodehl *et al.*, 2014. FEMS Microbiology Letters 359:124]. Therefore, these known ZEN conjugating fungal strains were screened for their potential to selectively synthesise the ZEN derivatives ZEN-14-sulfate (Z14S), ZEN-14-glucoside (Z14G) and ZEN-16-glucoside (Z16G). The screening was conducted by adding ZEN to liquid fungal cultures. Cultivation conditions and ZEN incubation time were varied. All media samples were analysed for metabolite formation by HPLC-MS/MS. Z14S was exclusively formed by *Aspergillus oryzae*. Under optimised

conditions, a specific biosynthesis of Z14G by *Rhizopus oryzae* and Z16G by *R. oligosporus* was achieved. After liquid-liquid-extraction and preparative chromatographic clean-up 1H-NMR purities of ≥73% for Z14S, ≥82% for Z14G and ≥50% for Z16G were obtained. In addition, a consecutive biosynthesis was developed by first using *Fusarium graminearum* for ZEN biosynthesis on rice based medium. After inactivation of *Fusarium*, the subsequent conjugation reaction was conducted utilising *Aspergillus* and *Rhizopus* species under the various optimised conditions. In this study, an easy and cost-efficient biosynthesis for Z14S, Z14G and Z16G was developed. The developed biosynthesis could also be used for other metabolites, such as ZEL conjugates. Our results of the *in vitro* screening indicate also the formation of a ZEL-glucoside and α-ZEL-sulfate as major metabolites by *R. oryzae*. In sum, under optimised cultivation conditions, fungi can be easily utilised for a targeted and stereospecific synthesis of ZEN conjugates.

P138

Method development and market sample screening for mycotoxins in legumes

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Although the popularity of legumes as part of the global nutrition is increasing continuously and 2016 was even claimed as the ‘year of pulses’ by the FAO, information on the occurrence of mycotoxins in legumes is scarce. However, some studies indicate that zearalenone (ZEN) is a contaminant in soy products [Schollenberger *et al.*, 2007. International Journal of Food Microbiology 113:142]. Fumonisins have been reported for peas [Waskiewicz *et al.*, 2013. Toxins (Basel) 5:488] and aflatoxins and ochratoxin A (OTA) can be formed as post-harvest contamination, e.g., on beans [Fakoor Janati *et al.*, 2011. Bulletin of Environmental Contamination and Toxicology 87:194; Maringe *et al.*, 2017. Food Additives and Contaminants Part B 10:21]. Following the development of highly sensitive and fast LC-MS/MS instruments, it is becoming more convenient to establish quantification methods for the analysis of various mycotoxins in different commodities. However, the quantification of a multitude of mycotoxins in only one run poses a significant challenge due to the very different chemical properties of mycotoxins, such as patulin, fumonisins or the peptidic enniatins. Moreover, the impact of the sample matrix on the extractability and ionisation is immense. Consequently, standardisation bodies like CEN make strong efforts to establish multianalyte methods to facilitate mycotoxin analysis for certain toxin groups with similar properties. The poster will present results of a method developed at the German National Reference Laboratory for Mycotoxins aiming to screen and quantify a set of relevant mycotoxins in grain legume using HPLC-MS/MS analysis utilising the stable isotope dilution approach. The method is based on the current draft for the multimethod for the screening of OTA, aflatoxin B1, deoxynivalenol, ZEN and fumonisin B1 and B2 in foodstuffs (EU mandate EN M520 item 5) extended by aflatoxins B2, G1 and G2 as well as T-2- and HT-2-toxin. Partial validation according to Commission Regulation (EC) No 401/2006 showed good linearity, precision and sufficient sensitivity to cover all important toxins including aflatoxins and OTA in the range of the MLs applicable for cereals according to Commission Regulation (EC) No 1881/2006 (cereals are used for orientation as no MLs are presently established for legumes). Moreover, data from a screening of more than 30 pea, bean (*P. vulgaris*), soy, lupine and lentil (*L. culinaris*) samples from local retail markets will be shown. This investigation revealed a detectable ZEN contamination in 2 out of 7 green lentil samples and the presence of significant amounts of OTA in a pea sample. Although, only few samples showed detectable amounts of the investigated mycotoxins, legumes should be further investigated to obtain a more comprehensive picture of their contribution to the overall human exposure to mycotoxins.

P139

Determination of citrinin in red yeast rice: a comparison of immunoaffinity clean-up with HPLC and LC-MS/MS

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Citrinin is produced by a number of *Aspergillus* and *Penicillium* fungi and has been found in a variety of foods, such as grains, cheese and red yeast rice, the latter of which is regulated in the EU (Commission Regulation (EU) No 212/2014). Although legislation is currently in place for only red yeast rice there is considerable interest in Europe with regards to levels in foods including cereals as this toxin is considered as a potential issue during storage often occurring simultaneously with ochratoxin A with both toxins considered as potential agents of Balkan endemic nephropathy. R-Biopharm Rhône have developed a new immunoaffinity column that selectively isolates and concentrates citrinin from a wide range of commodities, including cereals and red yeast rice. Red yeast rice is available as a loose powder

and also as a food supplement prepared in tablet or capsule form. Samples were analysed using immunoaffinity clean-up with HPLC and the results compared with an LC-MS/MS method.

P140

Multi-toxin analysis using immunoaffinity column clean-up for a range of cereal samples prior to LC-MS/MS detection

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EU regulations for mycotoxins are highly complex with varying limits applied to specific commodities. There are limits in place for a number of mycotoxins for cereals and cereal products therefore a multi-toxin method targeting a wide range of toxins is of particular interest. In addition, baby foods and animal feeds are composite in nature and again are regulated for a number of toxins, therefore, a multi- toxin immunoaffinity column can offer many advantages. R-Biopharm's new immunoaffinity column, 11+Myco MS-PREP® utilises a single extraction method for the analysis of 11 mycotoxins (aflatoxins B1, B2, G1, G2, ochratoxin A, fumoninisins B1, B2, deoxynivalenol, zearalenone, T-2 and HT-2) prior to detection by LC-MS/MS. In the study, a number of cereals and cereal samples were analysed in order to determine recoveries and results demonstrate that the use of an immunoaffinity column enables the concentration of the mycotoxins prior to detection, improving sensitivity and eliminates the use of isotopic labelled standards.

P141

Comparison of different methods to determine the limit of detection in mycotoxin analysis

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The LOD (limit of detection) is an important performance parameter of analysis methods. It specifies the lowest concentration at which a mycotoxin can be detected. Often, it also determines the LOQ (limit of quantification) since in many protocols the LOQ is a multiplication of the LOD (often 3.3x). When an analysis does not result in a numerical value, i.e., is reported as '<LOQ' or '<LOD', the LOQ and LOD values are used for calculation of the median and N^{th} percentile lower/middle/upper bound concentrations in risk assessment. Hence, the LOD and LOQ can have a strong impact on the outcome of a risk assessment exercise. The fact that there are various ways to determine and calculate the LOD (and LOQ), with different outcomes, is therefore a serious issue. Recently, an attempt has been made to harmonise the way to establish the LOD of a method [Wenzl *et al*., 2016. EUR 28099, Publications Office of the European Union, ISBN 978-92-79-61768-3, doi:10.2787/8931]. This still resulted in three options: (i) estimation of LOD via blank samples, (ii) estimation of LOD via paired observations, and (iii) estimation of LOD via the calibration approach. In this work, these three options plus a simpler but often used S/N approach have been compared for a multi-mycotoxin LC-MS/MS method. This was done for two example mycotoxins (aflatoxin B1 and deoxynivalenol) in two example situations: a single commodity (wheat) and a none-defined commodity ('feed ingredients'). The effect of the different methods, moment of measurement, normalisation or not against a labelled internal standard, and effect of determination across a non-defined group of matrices (feed ingredients) was investigated. The methods of determination resulted in differences of a factor of 2 to 5, with paired observations generally resulting in higher LODs and the S/N method giving similar results as the 'blank samples' method. The heterogeneous matrix 'feed ingredients' resulted in an increase of the LOD by a factor of 2 to 6 compared to wheat. Repeated analysis of the same extracts 4 weeks later resulted in differences of a factor 1.5-2. The outcome of the investigation was that there is no such thing as the LOD of a method. Providing an LOD-range rather than a fixed value would do more justice to reality than a single fixed value.

P142

European Union Reference Laboratory mycotoxins and plant toxins

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RIKILT Wageningen University & Research hosts the European Union Reference Laboratory mycotoxins and plant toxins in food and feed as of 1 March 2018. With that, RIKILT will continue the tasks for mycotoxins that were carried out by JRC (Geel, Belgium) during 2006-2017. Tasks and activities of the EURL are laid down in Regulation (EU) 2017/625. Area of work is to provide technical and scientific assistance to the national reference laboratories in the EU and the European Commission.

Proficiency tests will be organised among the NRLs and a training using LC-MS/MS equipment will be organised in 2018. Focus components in 2018 are deoxynivalenol and its metabolites and pyrrolizidine alkaloids.

P143
Development of a multiplex biosensor for mycotoxins in feed and food
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Naturally occurring food and feed contaminants are inevitably unavoidable making them a major issue in global food security, particularly those which pose serious health concerns to humans and animals. Mycotoxins are one such example, mainly entering the food chain as a result of fungal colonization of pre-harvest susceptible crops, during the time between harvesting and drying or during storage. Whilst visual and reader-based ELISA and lateral flow assays are the main current mycotoxin detection methods used for direct on-site analysis by importers, traders, and food and feed manufacturers, these methods require expensive labelling of reagents. This project aims to develop a novel rapid, handheld, multi-mycotoxin detection device based on mass sensitive micro-array (MSMA) technology, where detection of analytes is by mass making it both a label free method and cost efficient. The sensing platform consists of an array of microscopic weighing scale pixels that can be used as a functionalised surface for the immobilisation of reagents such as antibodies or hapten conjugates. The technology will be evaluated and assessed using key antibody reagents for important mycotoxins: deoxynivalenol, HT2-/T2-toxin, zearalenone and fumonisins that have been developed at Queen's University, to show in principle the MSMA technology performance in buffer reagents. A novel sample preparation method will be developed for the simultaneous extraction of these toxins. The technology will be evaluated for feed and food matrix interference effects as well as limit of detection (LOD) and results will be compared to other biosensor platforms or commercial lateral flow devices for its suitability for use.

P144
Aflatoxin M1 analysis of non-skimmed bovine raw milk with I'screen AFLA M1 ELISA kit
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Aflatoxin M1 analysis in milk is one of the most performed analysis in the dairy industry in Europe, with particular attention to the Mediterranean area where aflatoxin B1 incidence, and consequently M1, are higher due to climate conditions. Till now, aflatoxin M1 screening by ELISA kits currently on the market require defatting of milk by centrifugation and subsequent careful samples management, an operation that is quite time consuming. Within this context, 166 bovine raw milk samples (collected from a number of Italian farms) were firstly analysed by I'screen AFLA M1 kit with the traditional sample preparation procedure for raw milk. This procedure requires centrifugation of the milk for 10 min to separate the fat from the aqueous phase; after removing the fat layer, the skimmed milk can be added to the reaction wells. After the analysis, the samples turned to contain an aflatoxin M1 contamination level ranging from <5 to 100 ppt. The same milk samples were analysed during a second analytical run, by the same kit, without the centrifugation step, by direct addition of the raw milk into the reaction wells. The two data sets were compared through statistical analysis (Wilcoxon Test) and the result ($P=0.1757$) showed that data populations were not statistically different. Furthermore, the correlation coefficient R^2 was higher than 0.95, showing an almost complete correspondence between the results of skimmed and non-skimmed samples. Thanks to this experiment, Tecna confirmed that I'screen AFLA M1 ELISA kit, used worldwide since about 15 years, can allow an even faster monitoring of bovine raw milk. This improvement is of outstanding value for the whole dairy sector as the overall analytical time, sample manipulation, error probability and consumables needs can be significantly reduced.

P145
High-throughput microarray-based enzyme-linked immunosorbent assay
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The continuing use and evolution of enzyme-linked immunosorbent assay has led us to explore miniaturised, highly multiplexed, microarray formats. In this study we demonstrate the feasibility of simultaneous and specific analysis of six mycotoxins (aflatoxin B1, fumonisins, zearalenone, deoxynivalenol, T-2 and HT-2 toxins, ochratoxin) using a 96-well, microarray-based ELISA. Antigens

(mycotoxin-carrier protein) are spotted by a non-contact spotter and spots are passively adsorbed on white polystyrene plates; subsequently, plates are blocked and dried. In the assay procedure, there is a first 20 min incubation at 30°C, where free mycotoxin molecules in the sample and the spotted surface-bound mycotoxin antigens compete for their mycotoxin specific antibody binding sites. After a washing step, HRP-labelled secondary antibodies are added (15 min) and after a washing step, these remain bound to the solid phase through binding to the specific antibodies. The bound enzyme activity is determined by addition of a precipitating chromogenic substrate (20 min), leading to visible coloured spots, which are registered by an image analyser and measured. The assay is calibrated to measure fumonisins in the range of 200-5,000 ppb, zearalenone in the range 20-1000 ppb, aflatoxin B1 in the range of 1-30 ppb, deoxynivalenol in the range of 150-4,000 ppb, T-2/HT-2 toxins in the range of 15-1000 ppb; regarding ochratoxin, the work is still in progress. The use of a breakable 96-well plate as solid phase makes possible low as well as high sample throughput. The assay performance has demonstrated to be highly precise and lot-to-lot reproducible; there is no interferences between different mycotoxin spots and the signal-to-noise ratio is reduced to minimum. By the application of a very simple mycotoxins extraction method, the assay has been validated with wheat and maize. In reference materials with mycotoxin concentrations lower in respect to the above-mentioned measuring ranges, no matrix effect was observed, demonstrating high specificity of the assay. Wheat and maize reference materials with mycotoxin concentrations inside the above-mentioned range were chosen to verify dosage along all the measuring range. The analysis results showed high accuracy and precision of dosage. Limits of quantifications for all assay components were confirmed. The application of this assay prototype for the analysis of other molecules families, such as biotoxins, allergens, pesticide and veterinary drug residues, is to be expected.

P146

Testing for cross reactivity in commercial available kits for DON and DON-like compounds in wheat and barley

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Many crops are susceptible to *Fusarium graminearum*, which produces deoxynivalenol (DON) and other DON conjugates or DON-like compounds, such as 15-acetyl deoxynivalenol (15-ADON), 3-acetyl deoxynivalenol (3-ADON), deoxynivalenol-3-glucoside (DON3G) and nivalenol (NIV). In Canada, DON can be monitored throughout the transport chain; from the time the producer offloads their grain at the elevator to the time the company loads the vessel for export. Depending on where in the world the grain is going to be consumed and how the grain will be processed, the maximum limit for DON can vary. The Codex Alimentarius Commission has adopted a maximum limit (ML) for DON at 2 ppm for commodities destined for further processing. Grain buyers or processers may set their own maximum limit, lower than the MLs from Codex. Our lab has tested a few commercially available ELISA and lateral flow device kits for testing DON and the cross reactivity with DON-like compounds. Our lab tested these kits for cross reactivity by running individual standards of DON, 3-ADON, 15-ADON, DON3G and NIV at various concentrations, as well as mixtures of the standards at various concentrations to get an idea of the cross reactivity of each mycotoxin. It was found that DON3G and the ADONs had an effect on the DON reading. We also tested matrix matched standards for wheat and barley, in which we found no difference between the matrices, but found that cross reactivity still occurred. Lastly, we ran a suite of wheat samples that had known amounts DON and DON-like compounds, previously determined by LC-MS/MS. From the kits we have tested so far, we have found that when a sample has a read out of >2.0 ppm from the kit, and there are DON-like compounds in the sample, there is a positive bias and cross reactivity from these DON-like compounds usually gives a higher reading for DON than what is actually in the sample. This is important to know, when the samples are being tested for DON during transport, that DON3G, and the ADONs can increase the DON value that is actually in the sample.

P147

Solid phase extraction on carbon nanomaterials followed by liquid chromatography-mass spectrometry for the determination of *Fusarium* mycotoxins in agricultural products

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The uncertainties about fungal contamination of crops leading to increased presence of mycotoxins in agricultural products have been recognised as one consequence of climate change [Medina *et al.*, 2017.

Current Opinion in Food Science 18:76]. Reliable management of health risks posed by mycotoxins requires improved analytical methods with low detection limits. This can be achieved by improving instrumental capability for fast and highly sensitive detection, commonly performed using separation by liquid chromatography (LC) coupled to detection by time-of-flight (TOF) and Orbitrap high resolution mass spectrometry (HRMS) or triple quadrupole tandem MS/MS instruments. Some of the most studied contaminants in recent years are *Fusarium* mycotoxins, which also include emerging mycotoxins – beauvericin and enniatins [Medina *et al.*, 2017]. A crucial factor for the efficiency of analytical method is the sample preparation procedure. Carbonaceous materials have been recently developed as sorbents for planar molecules, such as polycyclic aromatic hydrocarbons [Wang et al., 2014. Environmental Science and Technology 48:4817], and other contaminants, such as heavy metals, pesticides, and drugs [Ibrahim *et al.*, 2016. Critical Reviews in Analytical Chemistry 46:267] but have been less studied for the determination of mycotoxins [Dong, 2015. Journal of Chromatography A 1423:177; Yu and Fan, 2017. Food Additives and Contaminants Part A 34:273]. In the present study, we tested pristine multi-walled carbon nanotubes (CNTs) and graphene (G), as well as magnetite-coated multi-walled carbon nanotubes (m-CNTs) and magnetite-coated graphene (m-G) as solid phase extraction sorbents for the enrichment of ten mycotoxins from standard solutions and real cereal extracts, followed by analysis using ultra-high performance liquid chromatography-time-of-flight mass spectrometry (UHPLC-TOF-MS). According to an enrichment study, 95-100% of the less polar mycotoxins (beauvericin, enniatins) were adsorbed on m-G (5 mg), whereas the adsorption effectiveness was >50% in the case of very polar compounds (HT-2 and T-2 toxins). In the case of CNTs, the adsorption efficiency was below 50% for most of the compounds, unless higher amounts of sorbent (>20 mg) were used. The superiority of G compared to CNTs for the enrichment of analytes can be primarily attributed to the larger surface area indicated by BET diagrams. However, the matrix effects from cereal samples required additional steps to prevent the adsorption of matrix components. The elaborated method was optimised for rapid and convenient preparation of cereals for mycotoxin analysis. **Acknowledgements**. The study was funded by the Latvian State Education Development Agency, within the Activity No. 1.1.1.2. 'Postdoctoral Research Aid', project proposal No. 1.1.1.2/VIAA/1/16/219.

P148

Myco 10, a biochip array containing ten discrete immunoassays for the simultaneous detection of a broad range of mycotoxins in animal feed

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Biochip array technology allows the simultaneous screening of multiple analytes from a single undivided sample which leads to test consolidation, an increased result output and a reduction of samples to be assessed by confirmatory methods. The application of this technology to the detection of mycotoxins is beneficial as it maximises the screening of these undesirable contaminants of cereal products, which can cause adverse health effects in humans and animals. In Europe, maximum permitted limits for aflatoxin B1 have been set and guidance values for fumonisin, ochratoxins, trichothecenes A (T-2 toxin, HT-2 toxin), trichothecenes B (deoxynivalenol) and zearalenones have been recommended. This study reports the analytical performance of Myco 10, a biochip array designed for the simultaneous screening of the most prevalent mycotoxins from a single undivided sample of animal feed to accommodate the lowest guidance limits established for a particular feed. Ten discrete simultaneous competitive chemiluminescent immunoassays, arrayed on the biochip surface, were applied to the evidence investigator analyser. This system incorporates dedicated software to process and archive the multiple data generated. Mycotoxins were extracted from feed by a single generic liquid/liquid extraction. Screening results are semi-quantitative. Myco 10 exhibited a broad specificity profile, allowing detection of aflatoxins (B1, B2, G1, G2), nineteen ergot alkaloids, fumonisins (B1, B2, B3), ochratoxin A, paxilline, trichothecenes A (T-2 toxin, HT-2 toxin, diacetoxy-scirpenol), trichothecenes B (deoxynivalenol, 3-acetyldeoxynivanelol, 15-acetyldeoxynivanelol), and zearalenone (zearalanone, α-zearalenol, α-zearalanol, β-zearalenol, β-zearalanol). The limits of detection ranged from 0.25 ppb for aflatoxin B1 and ochratoxin A to 100 ppb for deoxynivalenol. Analysis of 12 animal feed samples from the FAPAS proficiency testing programme showed values within the range assigned by FAPAS (Z-score ± 2). Analysis of 24 FAPAS QC samples showed a recovery ranging from 78 to 124. Myco 10 revealed samples presenting single or multi-mycotoxin contamination. In conclusion, Myco 10 allows the simultaneous screening of the most prevalent mycotoxins from a single undivided sample of animal feed and enables the detection of single or multi-mycotoxin contamination. The application of this biochip array increases the detection capacity in test settings and reduces the number of samples requiring confirmatory analysis.

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Biochip-based immunoassay for the detection of ergot alkaloids in animal feed

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The determination of ergot alkaloids in feed and food has gained importance for food and feed safety as they are undesirable contaminants and can cause adverse health effects in humans and animals. The use of a screening method allowing the detection of ergot alkaloids in animal feed facilitates the detection process in this matrix as only samples presenting positive results after screening require further analysis with a confirmatory method. Biochip array technology allows the simultaneous screening of multiple analytes from a single undivided sample as multiple discrete immunoassays take place at the same time on the biochip surface. This methodology is flexible, the mycotoxins to be screened can be customised according, for instance, to the most prevalent in a particular geographical region. This study reports the analytical performance of the biochip-based immunoassay for the detection of ergot alkaloids in animal feed. A competitive chemiluminescent immunoassay, defining a discrete test site on the biochip surface, was applied to the evidence investigator analyser. This system incorporates dedicated software to process and archive the data generated. Ergot alkaloids were extracted from feed by a single generic liquid/liquid extraction. Screening results are semi-quantitative. The assay was standardised to ergotamine and detected other ergot alkaloids (including ergocristine, ergocryptine, ergocornine, ergometrine, ergosine and their corresponding -inine forms) with cross reactivity values ≥43%. The cross reactivity with paxilline was <1%. The limit of detection was 10 ppb. The intra-assay precision, expressed as CV (%) was ≤ 6%. In conclusion, data indicate applicability of biochip array technology to the screening of ergot alkaloids in animal feed. The application of the biochip-based immunoassay to the evidence investigator analyser enables the screening of multiple samples as 54 biochips can be handled at the same time. Furthermore, the flexibility of this technology enables multi-mycotoxin detection by incorporating other immunoassays alongside the reported biochip-based immunoassay. This application facilitates the testing process when monitoring the presence of these undesirable contaminants.

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Quantitative analyses of natural contaminants and anthropogenic residues in animal feed by LC-ESI-MS/MS: influence of absolute and relative matrix effects on the method performance

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Influencing factors, such as climatic parameters, storage conditions or processing steps, enable the occurrence of a variety of contaminants, such as mycotoxins or plant toxins, in the food chain. Animal livestock is particularly endangered by the consumption of contaminated feed and thus show disease symptoms, such as oestrogen syndrome or feed refusal. In addition to these naturally occurring contaminants, anthropogenic agricultural inputs, such as pesticides or veterinary drugs, the latter due to the development of antibiotic resistance, pose further threats to human and animal health. In this work, a liquid chromatographic method with mass spectrometric coupling was developed first to allow the simultaneous quantification of around 700 secondary fungal metabolites, 500 pesticides and 100 veterinary drugs. The performance of the method was tested in a set of complex feed matrices (poultry, cattle, pork and fish) and additionally provides first impressions of analytical characteristics of these sample types. The focus is on the assumption that significant differences in signal suppression and enhancement (SSE) as well as in the extraction efficiency (RE) within different lots of a sample type are substantially influencing the method performance. Spiking experiments on different concentration levels were conducted to determine analytical characteristics, such as SSE, RE and overall process efficiency (RA), with 7 different lots of each matrix. Constant extraction efficiencies combined with a high variation of intra-matrix effects suggest that relative matrix impacts are the limiting factor of method validation processes in terms of precision and sensitivity.

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An approach to *Fusarium* regulated toxins analysis by LC-MS/MS: the complexity of quantifying different food matrices

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Fusarium toxins are an increasing health problem for the international trade. The Laboratory of the Public Health Agency in Barcelona is involved in the official control of the internal market as well as in the control of goods imported from countries outside the EU. An LC-MS/MS method has been implemented in the laboratory in order to determine and quantify 6 EU regulated *Fusarium* mycotoxins in cereal-based food commodities. The different samples are extracted using a QuEChERS (quick, easy, cheap, rugged and safe) approach. The analytical method has been validated and the results fulfill the requirements of Commission Regulation (EC) 401/2006 and subsequent modifications. The method robustness has been evaluated participating in proficiency tests with satisfactory results. The local government surveillance programmes include many different types of cereal-based samples for *Fusarium* regulated mycotoxins. One of the most important challenges in order to get an accurate result is how the matrix effect can be minimised. The use of the surrogate matrix method approach and the role of the internal standards is discussed. The Laboratory of the Public Health Agency in Barcelona has a flexible scope of analysis and results for all kind of foodstuff matrices have been reported under ISO/IEC 17025 accreditation.

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Validation of LC-MS/MS Based multi-toxin methods – how to manage the workload and which data are essential?

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Most of the guidelines that are available on proper method validation have been designed for assays targeting only one or very few analytes. In case of multi-analyte analysis, following these guidelines without any modification is impractical in view of the workload resulting from the number of replicates, number of concentration levels and number of measurements for the within-laboratory reproducibility. In addition, it is unrealistic to expect that compliance to all performance criteria (e.g., a target range of 70-110% for recovery) might be obtained for all analytes in case of methods covering a diverse set of target substances. As a consequence, guidelines for multi-analyte determination (such as SANTE/11945/2015 for pesticides residues) allow for some flexibility considering both performance criteria and experiments that are foreseen for method validation. This presentation tries to identify the part of the experiments that might be skipped during validation in order to reduce the workload for LC-MS/MS based methods covering several hundreds of fungal metabolites. In particular, matrix effects as well as recoveries of the extraction step seem to be independent of the concentration, which would allow for spiking at a single high level, thus facilitating data evaluation. However, our results indicate that an additional effort to characterise relative matrix effects is an essential part of the validation process. This is not foreseen in current guidelines and might lead to an underestimation of the methods uncertainty.

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LC-MS retention index for normalised mycotoxin analysis

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One of the biggest challenges for LC-MS analysis is the difficulty in comparing data between research groups and instrument manufactures. Identification of mycotoxins and fungal metabolites is complicated by the fact that many are not commercially available. Screening for these compounds in crops, food products and fungal cultures is generally accomplished using the precursor m/z, product ions and retention time (RT). Although precursor m/z and product ions are intrinsic physicochemical properties for the compounds, RTs are difficult to compare between laboratories as they are impacted by many factors including: choice of mobile phase solvents, gradient, pH, temperature, and column type. To help normalise comparison of chromatographic properties, the National Research Council in Halifax, Canada has designed a new, electrospray compatible retention index (RI) system using 20 N-alkylpyridinium-3-sulfonates (NAPS) of differing chain length. This series of compounds spans RI units from 100 to 2,000, are UV active, have both positive and negative fixed charges for ionisation in either mode and possess a common fragment. We analysed NAPS standards and calculated the RIs for 95 mycotoxins and fungal

secondary metabolites under 20 different LC conditions including pH, column, gradient, and temperature. This allowed us to calculate the potential RT shifts and propose an appropriate secondary correction for optimised normalisation. The utilisation of the NAPS RI system will greatly enhance the sharing of compound data between laboratories and improve dereplication efforts. We are proposing to conduct a multi-laboratory analysis to compare the impact of different systems and conditions on RT. This will involve expert laboratories that routinely analyse for mycotoxins. Each laboratory will be asked to use their standard LC-MS method and report the RTs of their analytes in addition to the RTs of the NAPS RI standards, which we will provide. These data will be compiled as a 'white page' report with the goal of providing a tool for normalising LC-MS data for inter- and intra-laboratory comparisons within the mycotoxin community. This will allow for easier database searching, especially when standards are not commercially available, and it can be used to help establish scheduled SRM windows for LC-MS methods [Quilliam, M.A., Retention index standards for liquid chromatography, Patent WO2013/ 134862A1].

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Lateral flow based method – fit for the purpose of screening feed ingredients?

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Lateral flow mycotoxin testing is increasingly used in the quality control of animal feeds and feed materials. Lateral flow testing has generally speaking a higher measurement uncertainty than official methods, such as HPLC. Whether a method is fit for screening purposes fully depends on the level of interest. This has led the European Union to describe specific performance requirements for screening tests based on quantitative methods. These requirements have been laid down in Commission Regulation (EU) No 519/2014. In this study, Mycomaster, a lateral flow based method for testing mycotoxins in animal and feed ingredients, has been validated according the above-mentioned regulation for deoxynivalenol and zearalenone. The performance of the test was evaluated using natural contaminated wheat at screening target concentrations (STC) close to EU maximum permitted or indicative levels. The intermediate precision, sensitivity and specificity were established by testing 20 wheat samples on 5 different days. Additionally, the robustness of the test was evaluated by setting up a ring test based on ISO / IEC 17043. For this ring test, three natural contaminated samples were sent out to 22 quality control laboratories in Europe, Asia and America. The laboratories used different methods: ELISA (9x), lateral flow (11x) and HPLC (2x). The samples were natural contaminated with aflatoxin, fumonisin, deoxynivalenol and/or zearalenone. The performance of the participating laboratories was evaluated comparing the obtained analysis results with the assigned value based on the homogeneity testing using HPLC. The Z-scores were calculated using the standard deviation per mycotoxin according to the performance criteria described in Commission Regulation (EU) No 519/2014. 80.3% of all analysis results were satisfactory as the Z-score was 2 or lower. There was no difference noticeable between the laboratories using either the lateral flow or an ELISA method.

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Fully automated mycotoxin analysis from extract to chromatogram at a sensitivity in the ppt or ppq level using not only LC-MS/MS but HPLC-FLD.

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Highly regulated mycotoxin analysis needs special clean-up or high-end analytical devices. Combine both to increase analytical speed, sample throughput, and to reduce costs by highly selective clean-up and full automatisation. The FREESTYLE ThermELUTE™ system allows fully automated sample processing from the crude extract to the final chromatogram at low detection range. It combines highly selective immunoaffinity clean-up, a thermal denaturation of the antibody-toxin-complex and quantitative injection directly into HPLC, thus an ultimate sensitivity down to the ppt or even ppq level is achieved. A benefit of the technology is the high capacity and selectivity of the immunoaffinity columns, which allows matrix clearance and direct injection of up to 10 g matrix equivalents (aflatoxin M1 in milk) into the HPLC. This leads to two aspects, a lower sample amount increasing the analytical speed and a higher sensitivity of HPLC-FLD or MS/MS, respectively. Not only standard matrices, such as hazelnut and maize. but also spices or other difficult matrices can be analysed using the system down to baby food regulated levels for mycotoxins. The technology reduces solvent amounts for extraction and clean-up by using miniaturised affinity columns with high loading capacity and excellent matrix clearance. High flow rates and low sample volumes allow a sample turnover time within less than 30 min, including chromatography. For aflatoxins, B/G the lower ppt range quantification is possible and for aflatoxin M1

even in the ppq range reliable analytical results are found. Ochratoxin A and zearalenone analysis can be performed using the same technological approach. The LODs were determined for aflatoxins B1/B2/G1/G2 (7, 2, 8 and 2 ppt, respectively), the LOQs were determined ranging from 6 to 24 ppt. For ochratoxin A analysis, an LOD of 10 ppt and an LOQ of 31 ppt allow a sensitive and precise analysis. The analysis of aflatoxin M1 especially in milk, where up to 10 ml could be loaded, revealed an LOD of 0.7 ppt and an LOQ of 2.2 ppt. The quantification of zearalenone can be achieved with an LOD below 1 ppb and an LOQ of 2.6 ppb. Overall, the FREESTYLE ThermELUTE™ in combination with dedicated immunoaffinity columns increases sample throughput and saves time and money by overlapping processing of sample and analytical measurement.

Thank you!

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