ABSTRACTS OF LECTURES AND POSTERS

14-16 OCTOBER 2019

wmf MEETS IUPAC 2019

THE World Mycotoxin Forum®

IUPAC 100

LOOKING BEYOND HORIZONS

Belfast

NORTHERN IRELAND

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CONTENTS

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 presenting/corresponding author.

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WELCOME

Welcome to **WMF*meets*IUPAC** – the joint conference of the 11th Conference of The World Mycotoxin Forum® and the XVth IUPAC International Symposium on Mycotoxins – taking place in Belfast, Northern Ireland, 14-16 October 2019!

The aim of **WMF*meets*IUPAC** – the world's largest mycotoxin event – is to increase the awareness of human and animal health risks due to mycotoxin contamination. It offers a platform for the food and feed industry, science and regulatory authorities to exchange current knowledge, to promote harmonisation of food and feed safety regulations and control procedures, and to make recommendations for integrated strategies ensuring the safety and security of the food and feed supply chain.

WMF*meets*IUPAC offers an excellent way to network and to share ideas, providing a reference source for anyone involved in this field. The event includes:

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

'Looking beyond horizons' – the conference theme – alludes to both the finishing line of large-scale research programmes on mycotoxins and to the necessity to put more emphasis on countries where mycotoxins present a concern to human and animal health. 'Looking beyond horizons' requires us to consider the next steps for effective mycotoxin management along the food and feed chain.

High-quality speakers, ample time for discussions, and every opportunity to establish rewarding contacts are values **WMF*meets*IUPAC** 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!

Rudolf Krska
Chris Elliott

ABOUT THE WORLD MYCOTOXIN FORUM®

The World Mycotoxin Forum® (WMF) 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
- to promote solutions for the control of mycotoxin contamination along conventional and organic supply chains.

ABOUT IUPAC

The International Union of Pure and Applied Chemistry (IUPAC) – a scientific, international, non-governmental and objective body – serves to advance the worldwide aspects of the chemical sciences and to contribute to the application of chemistry in the service of humankind. As a scientific, international, non-governmental and objective body, IUPAC can address many global issues involving the chemical sciences. In 2019, IUPAC celebrates its 100th anniversary.

SOCIAL EVENTS

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

Sunday 13 October 2019
18:30 – 20:00

r-biopharm
www.r-biopharm.com

Where: Belfast City Hall
Donegall Square North
Belfast BT1 5GS
https://www.belfastcity.gov.uk/tourism-venues/cityhall/cityhall-about.aspx

In 1888 Queen Victoria granted Belfast the status of the city and it was agreed that a grand and magnificent building was required to reflect this new status. City Hall opened its doors on the first of August 1906, at a time of unprecedented prosperity and industrial might for the city. The new City Hall was designed by Alfred Brumwell Thomas in the Baroque Revival style and constructed in Portland stone. The incredible building cost £ 369,000 to complete, the equivalent around 128 million pounds today but remains an extraordinary beacon of success and civic pride for Belfast.

City Hall has many connections with the famous ocean liner Titanic. Viscount William Pirrie who was Lord Mayor in 1896-1897 just before City Hall's construction, was also managing director of Harland & Wolff Shipyard. He is the man credited as having the idea for both ambitious builds. He used many of his skilled workmen in the fit-out of City Hall which is why the interiors today are considered an incredible insight into the finish of Titanic's lounges and suites, the ship's carving panelling being very similar.

The World Mycotoxin Forum thanks Belfast City Council for their kind generosity for the use of City Hall.

BELFAST CITY COUNCIL | Belfast City Council

TITANIC EXPERIENCE & CONFERENCE DINNER
(reservations only)

Tuesday 15 October 2019
18:30 – 22:30

The official conference dinner takes place at Titanic Belfast, one of the world's leading visitor attractions, on Tuesday evening 15 October 2019. Step back in time to a period of luxury and elegance in the opulent Titanic Suite with a replica of the liner's Grand Staircase. Dress code: business casual.

Before dinner, you can enjoy a self-guided tour of Titanic Belfast's nine interactive galleries which use state-of-the-art technology and innovative design to tell Titanic's story from conception to construction and launch in Belfast, to its maiden voyage and subsequent place in history. Explore the shipyard, travel to the depths of the ocean and uncover the true legend of Titanic, in the city where it all began. Keep your camera ready!

IMPORTANT NOTES

- **The conference dinner is 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 18:30 sharp at the bus stop in Oxford Street, which is in front of ICC Belfast, the conference venue.**

PROGRAMME AT A GLANCE

SUNDAY 13 OCTOBER 2019

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

MONDAY 14 OCTOBER 2019

10:00 – 10:15	Opening of **WMF*meets*IUPAC**		EXHIBITION
10:15 – 10:30	Introduction and overview of the conference		
10:30 – 12:30	PLENARY SESSION Looking beyond horizons		
12:30 – 13:45	Lunch break & poster viewing		
13:45 – 15:15	SESSION 1 Controlling plant disease and mycotoxin formation	SESSION 2 Process optimisation to reduce mycotoxin contamination of food and feed	
15:15 – 15:45	Networking break & exhibition		
15:45 – 17:15	Company pitches*		
17:15 – 18:00	Speed presentations**		
18:00 – 19:30	Poster viewing Wine tasting – sponsored by Biomin and Romer Labs		

***** 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 15 OCTOBER 2019

08:30 – 10:30	SESSION 3 Mitigating the negative impact of mycotoxins	SESSION 4 Smart strategies for effective mycotoxin management along the chain: toward food & feed 4.0 Part 1. MyToolBox	EXHIBITION
10:30 – 11:00	Networking break & poster viewing		
11:00 – 12:30	SESSION 5 Fate of free and modified forms of mycotoxins	SESSION 4 (continued)	
12:30 – 13:45	Lunch break & poster viewing Workshops		
13:45 – 15:30	SESSION 6 Mycotoxins: occurrence, exposure and effects	Smart strategies for effective mycotoxin management along the chain: toward food & feed 4.0 Part 2. MycoKey	
15:30 – 16:00	Networking break & poster viewing		
16:00 – 17:30	SESSION 6 (continued)	SESSION 8 Mycotoxin mitigation at the front lines: Feed the Future Innovation Labs and global collaborations	
18:30 – 22:30	Conference dinner (reservations only)		

WEDNESDAY 16 OCTOBER 2019

08:45 – 10:30	SESSION 9 Update on (multi-)mycotoxin analysis	SESSION 10 Improving food security and safety at the global level – Mytox-South	EXHIBITION
10:30 – 11:00	Networking break & poster viewing		
11:00 – 12:40	PLENARY SESSION Looking at mycotoxins from a different angle		
12:40 – 12:50	Best Poster Award presentation		
12:50 – 13:10	**WMF*meets*IUAPC** – Top Five Answers learned		
13:10 – 13:15	Looking forward: **WMF*meets*ASIA** and **WMF*meets*ITALY**		
13:15	Closing of **WMF*meets*IUPAC**		
14:00 – 16:00	Excursion (optional)		

CONFERENCE PROGRAMME

MONDAY 14 OCTOBER 2019

PLENARY SESSION: LOOKING BEYOND HORIZONS
The horizon is visible. It represents the edge of what is known or manifest. It does not, however, define the limit of what exists or of what is possible. Think about it this way: the horizon is the beginning of a new challenge. Are you ready to take mycotoxin management and control to a new level?

Chairs: Prof. Rudolf Krska, BOKU Vienna, Austria and Prof. Chris Elliott, Queen's University Belfast, UK

10:00 Opening of **WMF*meets*IUPAC**

10:15 Introduction and overview of the conference
Prof. Rudolf Krska, Department IFA-Tulln, BOKU Vienna, Austria
Prof. Chris Elliott, Institute for Global Food Security, Queen's University Belfast, UK

10:30 Creating a common language for chemistry: looking beyond IUPAC's 100th anniversary
Prof. Diane Purchase, Middlesex University London, UK – on behalf of the International Union of Pure and Applied Chemistry (IUPAC)

10:45 The grand challenge of climate change – beyond mycotoxins
Prof. Manfred Grasserbauer, Vienna University of Technology, Institute for Chemical Technologies and Analytics, Austria

11:10 Agriculture 4.0 and mycotoxin management
Prof. Josse De Baerdemaeker, Division of Mechatronics, Biostatistics and Sensors, KU Leuven, Belgium

11:30 Vertical farming: solution to old problems?
Prof. Nigel Scollan, Institute for Global Food Security, Queen's University Belfast, UK

11:50 Mycotoxins and beyond: the expanding role of the exposome concept in assessing exposure and effect of food contaminants
Dr Benedikt Warth, Department of Food Chemistry and Toxicology, University of Vienna, Austria

12:10 Is the horizon getting closer? Mycotoxins, economics and globalisation: look beyond the horizon!
Ronald Niemeijer, M.Sc., R-Biopharm AG, Germany

12:30 Lunch break
Exhibition and poster viewing

MONDAY 14 OCTOBER 2019

SESSION 1: CONTROLLING PLANT DISEASE AND MYCOTOXIN FORMATION
What is in the pipeline for obtaining plant disease resistance and reducing mycotoxin contamination?

Chair: Prof. Chiara Dall'Asta, University of Parma, Italy

13:45 Plant mechanisms counteracting *Fusarium* virulence factors, a way to reduce mycotoxin contamination?
Dr Gerhard Adam, Department of Applied Genetics and Cell Biology, BOKU Vienna, Austria

14:00 Is host resistance against *Aspergillus flavus* infection and aflatoxin contamination possible? Gene expression of resistant and susceptible maize lines provides insights!
Dr Matthew Gilbert, Agricultural Research Service, U.S. Department of Agriculture, USA

14:20 MS imaging reveals plant resistant mechanisms against mycotoxins – the location makes the difference
Dr Laura Righetti, Food and Drug Department, University of Parma, Italy and Institute of Inorganic and Analytical Chemistry, Justus Liebig University of Giessen, Germany

14:40 Use of endophytic fungi as biocontrol agents of cereal diseases
Dr Hans Jorgen Lyngs Jorgensen, Department of Plant and Environmental Sciences, University of Copenhagen, Denmark

15:00 Harnessing the microbiome to reduce *Fusarium* head blight
Dr Matthew Bakker, Department of Microbiology, University of Manitoba, Canada

15:15 Networking break & poster viewing

PLENARY SESSION
COMPANY PITCHES AND SPEED PRESENTATIONS

See page 10.

MONDAY 14 OCTOBER 2019

SESSION 2: PROCESS OPTIMISATION TO REDUCE MYCOTOXIN CONTAMINATION OF FOOD AND FEED

Where do we stand today? This session will introduce some options to optimise food processing to reduce mycotoxin contamination of food and feed under the motto: "from scientific findings to practical guidance: the ILSI Europe experience".

Chair: Dr Michele Suman, Barilla SpA, Italy

13:45 Mitigation of mycotoxins along food processing: from scientific findings to practical guidance: the ILSI Europe experience
Dr Michele Suman, Barilla SpA, Italy

14:00 Occurrence and toxicological scenario of relevant mycotoxins at the industrial level – state of the art
Dr Neil Buck, General Mills, Inc., Switzerland

14:20 First in-depth analysis: how to manage the cereals food production chain mitigating mycotoxins
Dr Johan De Meester, Cargill, Belgium

14:40 Second in-depth analysis: how to manage the dairy food production chain mitigating mycotoxins
Dr Luca Dellafiora, Department of Food and Drug, University of Parma, Italy

15:00 Final wrap-up and Q&A time
Michele Suman, Neil Buck, Johan De Meester, and Luca Dellafiora

15:15 Networking break & poster viewing

PLENARY SESSION: COMPANY PITCHES AND SPEED PRESENTATIONS

Chair: Dr Awanwee Petchkongkaew, Thammasat University, Thailand

15:45 Company pitches
Short presentations (5-minutes) by sponsors to inspire the audience to visit their booths
R-Biopharm – Adisseo – Biomin/Romer Labs – Trouw Nutrition – Alltech – Phileo – Olmix – Patent Co. – Vicam – Pribolab – ProGnosis Biotech – Neogen Europe – EnviroLogix – Eurofins Technologies – Devenish – Tolsa – Charm Sciences
For details, see pages 36-43.

17:15 Speed presentations
Short presentations (6-minutes) by selected poster presenters to provide an overview of their research
Omer Barda (Israel) – Gunnar S. Eriksen (Norway) – Ixchel Gilbert-Sandoval (the Netherlands – Ting Zhou (Canada) – Maximilian Kuner (Germany) – Riikka Peltomaa (Spain)
For details, see page 44.

18:00 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 15 OCTOBER 2019

SESSION 3: MITIGATING THE NEGATIVE IMPACT OF MYCOTOXINS
A look into recent and promising strategies related to mitigating mycotoxin contamination in food and feed. What's up?

Chair: Dr Katrina Campbell, Queen's University Belfast, UK

08:30 An integrated approach to mitigate mycotoxins in feed
Dr Paul Bruinenberg, Trouw Nutrition, the Netherlands

08:45 Oxidative stress in piglets fed diets containing purified mycotoxins and mycotoxin deactivator
Prof. Bruno Silva, Institute of Agricultural Sciences, Federal University of Minas Gerais, Brazil

09:00 The role of yeast fractions in mitigating the negative impact of mycotoxins in animals
Dr Virginie Marquis, Phileo Lesaffre Animal Care, France

09:15 Mycotoxin mitigation in the context of multiple mycotoxin challenge
Dr Alexandros Yiannikouris, Alltech, USA

09:30 Comparative *in vitro* assessment of a range of commercial feed additives with multiple mycotoxins binding claims
Oluwatobi Kolawole, Institute for Global Food Security, Queen's University Belfast, UK

09:45 Development of a lactonase for mitigation of zearalenone exposure of farmed animals
Dr Wulf-Dieter Moll, Biomin Research Center, Austria

10:00 Yellow mealworms are highly resistant to aflatoxin B1 and zearalenone – a possible approach for grain 'detoxification'?
Prof. Ronald Maul, National Reference Laboratory for Mycotoxins, German Federal Institute for Risk Assessment, Germany

10:15 Cold plasmas: a potential approach to mitigate microbiological and toxicological risks in the food & feed chain
Prof. Brendan Gilmore, School of Pharmacy, Queen's University Belfast, UK

10:30 Networking break & poster viewing

SESSION 5: FATE OF FREE AND MODIFIED FORMS OF MYCOTOXINS
From exposure to metabolism and degradation: a collection of recent research.

Chair: Dr Franz Berthiller, BOKU Vienna, Austria

11:00 DONEXPO project: exposure to deoxynivalenol as free form and its main urinary metabolites found in Italy, UK and Norway
Dr Barbara De Santis, Department of Food Safety and Veterinary Public Health, Istituto Superiore di Sanità, Italy

11:15 Elucidation of the mycotoxin human toxicokinetics: the key for an adequate biomonitoring exposure programme
Dr Arnau Vidal, Centre of Excellence in Mycotoxicology and Public Health, Ghent University, Belgium

11:30 Ochratoxin A and 2'*R*-ochratoxin A: new insights into pharmacokinetics and metabolism
Dr Benedikt Cramer, Institute of Food Chemistry, University of Münster, Germany

11:50 Microbial hydrolysis and metabolism of mycotoxins by intestinal microbiota
Dr Silvia Gratz, The Rowett Institute, University of Aberdeen, UK

12:10 Fate of ergot alkaloids during laboratory scale durum processing and pasta production
Dr Sheryl Tittlemier, Grain Research Laboratory, Canadian Grain Commission, Canada

12:30 Lunchbreak
Exhibition & poster viewing
WORKSHOPS (for details, see pages 20-21)

TUESDAY 15 OCTOBER 2019

SESSION 4: SMART STRATEGIES FOR EFFECTIVE MYCOTOXIN MANAGEMENT ALONG THE CHAIN: TOWARD FOOD & FEED 4.0 – PART 1. MYTOOLBOX

The project MyToolBox funded by the European Commission aims 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.

Chair: Marlous Focker, Wageningen Food Safety Research, the Netherlands

08:30 Integrated multi-actor partnerships in the EU and China as the key to tackle mycotoxins along the food and feed chain
Prof. Rudolf Krska, Department IFA-Tulln, BOKU Vienna, Austria

08:45 Control of *Fusarium* head blight – alternatives to triazole fungicides
Prof. Simon Edwards, Crop and Environment Sciences, Harper Adams University, UK

09:00 Forecasting mycotoxins in grains at the European level
Dr Cheng Liu, Wageningen Food Safety Research, the Netherlands

09:15 Cost-effective monitoring of aflatoxins in maize
Marlous Focker, M.Sc., Wageningen Food Safety Research, the Netherlands

09:30 Dynamic real-time decision support system for minimising mycotoxin risks in grain
Dr Angel Medina, Applied Mycology Group, Cranfield University, UK

09:45 Synergistic potential of pre-milling and milling strategies to minimise mycotoxins and increase fibre content of wheat-based products
Dr Michele Suman, Barilla SpA, Italy

10:00 Mitigation of deoxynivalenol during industrial baking: is it possible?
Dr Franz Berthiller, Department IFA-Tulln, BOKU Vienna, Austria

10:15 Mycotoxins during the processes of nixtamalization and tortilla production
Dr Sara Schaarschmidt, Department Safety in the Food Chain, German Federal Institute for Risk Assessment, Germany

10:30 Networking break & poster viewing

11:00 Safe use options of contaminated batches through detoxification of mycotoxins during bioethanol production using enzymes
Dr Gerd Schatzmayr, Biomin Research Center, Austria

11:15 Biological detoxification of mycotoxins in maize and its products
Prof. Liu Yang, Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences, China

11:30 *In vivo* evaluation of the efficacy of aflatoxin B1 and fumonisin B1 detoxifying feed additives in China
Dr Jinquan Wang, Feed Research Institute, Chinese Academy of Agricultural Sciences, China

11:45 Intention of European wheat farmers to use decision support systems for *Fusarium* spp. management
Prof. Ine van der Fels-Klerx, Business Economics Group, Wageningen University & Research and Wageningen Food Safety Research, the Netherlands

12:00 E-platform for mycotoxin prevention and control along the chain
Ignacio Montero Castro, IRIS Technology Solutions, Spain and Prof. Ine van der Fels-Klerx, Wageningen Food Safety Research, the Netherlands

12:30 Lunchbreak
Exhibition & poster viewing
WORKSHOPS (for details, see pages 20-21)

TUESDAY 15 OCTOBER 2019

SESSION 6: MYCOTOXINS – OCCURRENCE, EXPOSURE AND EFFECTS
The latest developments and new challenges in relation to the impact of mycotoxins on human and animal health will be presented.

Chairs: Dr Isabelle Oswald, INRA, France and Dr Michael Routledge, University of Leeds, UK

13:45 Chair's introduction

13:50 Cadmium and deoxynivalenol in durum wheat grains: physiological and biological basis of the co-contamination
Sylvain Chéreau, Mycologie et Sécurité des Aliments, INRA, France

14:10 Household-level aflatoxin contamination in rural village food systems: toward a participatory action research approach
Anthony Wenndt, Plant Pathology and Plant-Microbe Biology, Cornell University, USA

14:30 Human biomonitoring to estimate exposure to deoxynivalenol and zearalenone: a combined 24-hour duplicate diet – 24-hour urine study
Dr Hans Mol, Wageningen Food Safety Research, the Netherlands

14:50 Complementarity of internal and external dietary mycotoxin exposure: a comprehensive study in five European populations
Dr Marthe De Boevre, Centre of Excellence in Mycotoxicology and Public Healht, Ghent University, Belgium

15:10 Mycotoxin mixtures in food and feed: a holistic, innovative, flexible modelling approach for risk assessment – MYCHIF
Prof. Paola Battilani, Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Italy

15:30 Networking break & poster viewing

16:00 The role of mycotoxins in bacterial and viral disease outbreaks – a review
Julia Laurain, Olmix Group, France

16:15 Immunosuppressive activity of *Alternaria* toxins
Dr Giorgia Del Favero, Department of Food Chemistry and Toxicology, University of Vienna, Austria

16:35 Aflatoxins in food: EFSA's comprehensive risk assessment
Dr Kathleen Baert, Biological Hazards and Contaminants Unit, European Food Safety Authority, Italy

16:50 Assessing the combined toxicity of natural toxins by high content analysis
Dr Julie Meneely, Institute for Global Food Security, Queen's University Belfast, UK

17:10 Impact of chronic multi-mycotoxin dietary exposure on colorectal and liver cancer risk in Europe
Dr Inge Huybrechts, Nutritional Epidemiology Group, International Agency for Research on Cancer, France

18:30 – 22:30
Titanic experience & conference dinner (reservations only)
Join us for a very special event with an unforgettable experience in Belfast!
For details, see page 5.

TUESDAY 15 OCTOBER 2019

SESSION 7: SMART STRATEGIES FOR EFFECTIVE MYCOTOXIN MANAGEMENTALONG THE CHAIN: TOWARD FOOD & FEED 4.0 – PART 2. MYCOKEY

The project MycoKey funded by the European Commission aims at developing smart, integrated, sustainable solutions and innovative tool kits to reduce the major mycotoxins in economically important food and feed chains.

Chair: Dr Antonio F. Logrieco, Institute of Sciences of Food Production, Italy

13:45 MycoKey: a success story of EU-China cooperation for minimising mycotoxins along chains
Dr Antonio F. Logrieco, Institute of Sciences of Food Production, National Research Council, Italy

14:15 Prevention of *Fusarium graminearum* and mycotoxins in wheat by application of microbial antagonists on infected crop residues
Dr Susanne Vogelgsang, Research Division Plant Protection, Agroscope, Switzerland

14:30 A joint model for fumonisin and aflatoxin prediction in maize
Dr Marco Camardo Leggieri, Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Italy

14:45 A new antifungal device for the control of *Penicillium verrucosum* in barley during storage
Dr Giuseppe Meca, Department of Preventive Medicine and Public Health, University of Valencia, Spain

15:00 *In vitro* and *in vivo* efficacy assessment of a new bentonite-based material acting as a multi-mycotoxin binder
Dr Vito D'Ascanio, Institute of Sciences of Food Production, National Research Council, Italy

15:15 Fluorescence polarisation immunoassays for the determination of trichothecenes and their modified forms in wheat
Dr Vincenzo Lippolis, Institute of Sciences of Food production, National Research Council, Italy

15:30 Networking break & poster viewing

SESSION 8: MYCOTOXIN MITIGATION AT THE FRONT LINES – FEED THE FUTURE INNOVATION LABS AND GLOBAL COLLABORATIONS

See page 15.

TUESDAY 15 OCTOBER 2019

SESSION 8: MYCOTOXIN MITIGATION AT THE FRONT LINES – FEED THE FUTURE INNOVATION LABS AND GLOBAL COLLABORATIONS

The Feed the Future Innovation Labs draw on the expertise of top U.S. universities and developing country research institutions to tackle some of the world's greatest challenges in agriculture and food security. In this session, the focus is on mycotoxins.

Chair: Dr Andreia Bianchini, University of Nebraska-Lincoln, USA

16:00 When prevention fails: the need, use and estimated market for aflatoxin sequestering agents in three African countries
Prof. Adegbola Adesogan (Director, Feed the Future Innovation Lab for Livestock Systems), Department of Animal Sciences, University of Florida, USA

16:20 Aflatoxin exposure and health outcomes in infants and young children: findings from Nepal and Uganda
Dr Shibani Ghosh (Associate Director, Feed the Future Innovation Lab for Nutrition), Friedman School of Nutrition Science and Policy, Tufts University, USA

16:40 Integrated approaches to mycotoxin reduction in Africa, Asia and Central America: Feed the Future Innovation Lab for the Reduction of Post-Harvest Loss case studies
Dr Andreia Bianchini, Food Science and Technology Department, University of Nebraska-Lincoln, USA

16:55 Improved drying and storage practices that reduce aflatoxins in stored maize: experimental, evidence from smallholders in Senegal
Dr Jacob Ricker-Gilbert, Department of Agricultural Economics, Purdue University, USA

17:15 Overview of aflatoxin contamination challenge in food and feed and potential use of cold plasma to mitigate aflatoxins
Prof. Kevin Keener, School of Engineering, University of Guelph, Canada

18:30 – 22:30
Titanic experience & conference dinner (reservations only)
Join us for a very special event with an unforgettable experience in Belfast!
For details, see page 5.

WEDNESDAY 16 OCTOBER 2019

SESSION 9: UPDATE ON (MULTI-)MYCOTOXIN ANALYSIS
Recent developments – from sampling to multi-mycotoxin analysis and more – will get through here.

Chair: Dr Sheryl Tittlemier, Canadian Grain Commission, Canada

08:45 Maize meal slurry mixing: an economical recipe for precise aflatoxin quantitation
Joseph Kumphanda, Department of Applied Studies, Malawi University of Science and Technology, Malawi

09:00 Quantitation of ochratoxin A, 4-deoxynivalenol and zearalenone in wheat: production of certified reference materials and assessment of isotope dilution strategies
Dr Adilah Bahadoor, Metrology Research Centre, National Research Council of Canada, Canada

09:15 LC-MS/MS based quantitative multi-target approach for food and feed: crossing the limit of 1000 metabolites
David Steiner, M.Sc., Austrian Competence Center for Feed, Food, Quality, Safety and Innovation (FFoQSI), Austria

09:30 Benefits of collision cross section (CCS) data obtained by UPLC-ESI-IMS-QTOF MS for small molecules identification: application to mycotoxins screening
Prof. Juan V. Sancho, Research Institute for Pesticides and Water, Universitat Jaume I, Spain

09:45 Mycotoxin testing paradigm: challenges and opportunities for the future
Dr Mark Sumarah, London Research & Development Centre, Agriculture and Agri-Food Canada, Canada

10:00 Mycotoxin rapid testing – why the most innovative technologies fail on their way to the market
Dr Kurt Brunner, Romer Labs Division Holding GmbH, Austria

10:15 Ultrafast method for managing mycotoxins in the feed industry
Prof. Carlos Mallmann, Laboratory for Mycotoxicological Analysis, Federal University of Santa Maria, Brazil

10:30 Networking break & poster viewing

FINAL PLENARY SESSION
LOOKING AT MYCOTOXINS FORM A DIFFERENT ANGLE

See page 18.

WEDNESDAY 16 OCTOBER 2019

SESSION 10: IMPROVING FOOD SECURITY AND SAFETY AT THE GLOBAL LEVEL – MYTOX-SOUTH

Mytox-South is a partnership to improve food security and safety through mitigation of mycotoxins at the global level with the following long-term goals: building human and infrastructural capacity, bridging the gap between research, academia and industry, and creating a sustainable network on mycotoxin research.

Chair: Prof. Sarah De Saeger, Ghent University, Belgium

08:45 Introduction to Mytox-South
Prof. Sarah De Saeger (Mytox-South coordinator), Department of Bioanalysis, Ghent University, Belgium

09:00 Keeping mycotoxins away from the food: does the existence of mycotoxin regulations have any impact in Africa?
Dr Limbikani Matumba, Lilongwe University of Agriculture and Natural Resources, Malawi

09:15 Mycosafe-South, the EU LEAP-Agri project on mycotoxin mitigation in Africa
Prof. Siska Croubels (coordinator Mycosafe-South coordinator), Department of Pharmacology, Toxicology, and Biochemistry, Ghent University, Belgium

09:30 Mycotoxin challenge in food security and safety in Africa: role of the African Society for Mycotoxicology
Prof. Sheila Okoth (ASM-president), School of Biological Sciences, University of Nairobi, Kenya

09:45 The Africa Centre of Excellence for Mycotoxin and Food Safety, the World Bank project
Prof. Hussaini Makun (ACEMFS-coordinator), Department of Biochemistry, Federal University of Technology, Minna, Nigeria

10:00 Impact of mycotoxin research at grassroots level: understanding the plight of African subsistence farming
Dr Lindy J. Rose, Department of Plant Pathology, Stellenbosch University, South Africa

10:15 Beyond the lab bench: translating research into policy and action
Dr Melody Ndemera, Ministry of Higher and Tertiary Education, Science and Technology Development, Zimbabwe

10:30 Networking break & poster viewing

FINAL PLENARY SESSION
LOOKING AT MYCOTOXINS FORM A DIFFERENT ANGLE

See page 18.

WEDNESDAY 16 OCTOBER 2019

FINAL PLENARY SESSION: LOOKING AT MYCOTOXINS FORM A DIFFERENT ANGLE
Take a step back, take a deep breath and actually look at mycotoxins with a different perspective.

Chairs: Prof. Rudolf Krska, BOKU Vienna, Austria and Prof. Chris Elliott, Queen's University Belfast, UK

11:00 Relative importance/priority of mycotoxins compared to other public health risks
Kim Petersen, Risk Assessment and Management Unit, World Health Organization, Switzerland

11:20 Worldwide occurrence – where does the decades-old FAO figure of 25% contamination stand today?
Dr Gregor Kos, Department of Chemistry and Biology, Concordia University, Canada

11:40 Are very low doses of mycotoxins predisposing factors for other pathologies?
Dr Isabelle Oswald, Toxalim Research Centre in Food Toxicology, INRA, France

12:00 The toxic side to food fraud – what about mycotoxins?
Prof. Chris Elliott, Institute for Global Food Security, Queen's University Belfast, UK

12:20 Holistic approach to mould and mycotoxin risk management
Dr Guangtao Zhang, Mars Global Food Safety Center, China

12:40 **BEST POSTER AWARD PRESENTATION**

12:50 Looking beyond horizons – Top Five Answers learned at **WMF*meets*IUPAC**
Prof. Rudolf Krska and Prof. Chris Elliott

13:10 Looking forward to **WMF*meets*ASIA** and **WMF*meets*ITALY**

13:15 Closing of **WMF*meets*IUPAC**
Take your packed lunch to eat along the way!

MYCOTOXIN MANAGEMENT: DISCOVER HOW WE ADD MORE

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WORKSHOPS

WORKSHOP PROGRAMME

TUESDAY 15 OCTOBER 2019

12:30 – 13:30

ASSESSING GRAIN CONTAMINATION: THE FUTURE OF QUANTIFIABLE DECISION-POINT DIAGNOSTICS

SPONSORED BY ENVIROLOGIX

With the awareness of mono and co-occurring mycotoxin contamination increasing, the need for easy, accurate, and comprehensive decision-point testing has never been more critical to assessing grain quality and maximising operational efficiency. Effective, flexible solutions must be created to keep pace with evolving market needs. EnviroLogix, Inc. is a leading producer of mycotoxin and GMO tests serving the food and feed safety market. The company was the first to offer LFD technology for mycotoxin screening and remains committed to delivering innovative solutions to its markets. This workshop will discuss the innovative diagnostic solutions offered by EnviroLogix that improve operational efficiency in this evolving market.

TUESDAY 13 MARCH 2018

12:30 – 13:30

BIOCHIP ARRAY TECHNOLOGY: TOWARDS ACCURATE MULTI-ANALYTE AND MULTI-MATRIX MULTIPLEX TESTING

SPONSORED BY RANDOX FOOD DIAGNOSTICS

New horizons for mycotoxin testing have been established by means of the biochip array technology (BAT). This technology encompasses sensitivity, simplicity, rapidity, accuracy and ruggedness. Different matrices (cereals, cereal products, grains, grain products, feed) can be analysed for up to ten mycotoxins, quantitatively, simultaneously in one run. The technology has revolutionised mycotoxin routine testing in the food and feed industry, as well as generating detailed surveillance data for governmental food safety programmes.

TUESDAY 13 MARCH 2018

12:30 – 13:30

IMPROVING MYCOTOXIN ANALYSIS WITH NOVEL LC-MS/MS WORKFLOWS

SPONSORED BY SCIEX

Mycotoxin analysis continues to evolve with research and regulations requiring comprehensive coverage of ever more analytes. LC-MS/MS has become an essential tool in such analysis thanks to its accurate quantitation and confirmation of multiple target analytes in a wide variety of matrices. However, this diversity of complex matrices and challenging analytes often requires greater performance characteristics than many traditional instruments can deliver. In this workshop we will discuss how new MS technology is helping to stay ahead of the game, with the evolution of both nominal and accurate mass spectrometry offering greater compound coverage, more comprehensive confirmation and, most importantly, robust workflows which offer laboratories great confidence when reporting accurate quantitation results.

TUESDAY 13 MARCH 2018

12:30 – 13:30

A USER PERSPECTIVE (EUROFINS, UK) ON IMPROVING WORKFLOW FOR OCHRATOXIN ANALYSIS THROUGH AUTOMATION WITH RIDA®CREST AND IMMUNOPREP® ONLINE

SPONSORED BY R-BIOPHARM

R-Biopharm Rhône has worked in collaboration with Eurofins, UK to develop methods for a wide range of difficult commodities, such as spices and coffee for the automated determination of ochratoxin. During the workshop, Eurofins, UK will discuss the validation of the IMMUNOPREP® ONLINE ochratoxin cartridges, and the results and benefits they have found using this system for routine analysis of a wide range of samples.

EXCURSION (optional)

WEDNESDAY 16 OCTOBER 2019

14:00 – 16:00

VISIT TO THE HOME OF THE INSTITUTE FOR GLOBAL FOOD SECURITY, QUEEN'S UNIVERSITY, BELFAST

The excursion will include a visit to the newly opened Biological Sciences Building, which houses the Institute for Global Food Security. The 12,000 sqm building was constructed at a cost of € 50 M for over 300 researchers.

One of the centre pieces of the institute is its analytical facility which contains multiple mass spectrometry platforms, sensor technologies and Europe's first Cold Plasma Research Centre. A range of hands-on demonstration will be held to show how rapid, low cost decontamination of feeds and food can be performed using cold plasma technologies, as well as demonstrations across a range of technology platforms to undertake targeted and untargeted analysis for food safety and food fraud measurements.

The excursion is facilitated by the Institute for Global Food Security, Queen's University, Belfast, the top ranked research cluster in the UK in agriculture, food and veterinary sciences. WMFmeetsIUPAC would like to acknowledge the help from the staff within the institute for organising the excursion.

Please note: Participants who have registered for this event, shall gather at 13:30 sharp at the bus stop in Oxford Street, which is in front of ICC Belfast, the conference venue.


THE QUEEN'S UNIVERSITY OF BELFAST

LECTURE ABSTRACTS

MONDAY 14 OCTOBER 2019

PLENARY SESSION
LOOKING BEYOND HORIZONS
The horizon is visible. It represents the edge of what is known or manifest. It does not, however, define the limit of what exists or of what is possible. Think about it this way: the horizon is the beginning of a new challenge. Are you ready to take mycotoxin management and control to a new level?

THE GRAND CHALLENGE OF CLIMATE CHANGE: BEYOND MYCOTOXINS

Manfred Grasserbauer

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In the 'Anthropocene' we have encountered a 20-fold increase in energy consumption since 1850 and a dramatic shift of energy production by moving from 5% of the energy from fossil fuels (coal) to 80% of the energy from fossil fuels (coal, oil, gas). A major effect of fossil fuel use on the atmosphere has been an increase of the greenhouse gas (GHG) concentrations measured as CO_2eq (weighted sum of all GHGs) since preindustrial times from 280 to 450 ppmv (2016) and a rise of the global mean temperature by 0.89°C (0.69-1.08°C). The Fifth Assessment Report of the Intergovernmental Panel on Climate Change [1] developed scenarios for the future of fossil fuel emissions and associated temperature rise. For continuing with 'business as usual,' we are likely to encounter a doubling of annual CO_2 emissions till 2100 causing a climate forcing of 8,5 W/m2 (scenario RCP8.5) and a temperature increase from now by 3.7°C (range 2.6-4.8°C). On the other hand, an 'optimal stabilisation scenario' could be followed where the annual CO_2 emissions would have to approach zero by 2100 (scenario RCP2.6) limiting the temperature increase from now to 1°C (range 0.3-1.7°C).

For the conversion of GHG concentrations into a climate forcing effect many different models have been developed and very different results are obtained for the same scenario. There is a significant uncertainty in the 'prediction' of temperature increase. There is, however, widespread consensus that the GHG emissions are largely responsible for the global warming encountered.

Effects of climate change are observed everywhere, like warming of the water bodies or a strong retreat of Alpine glaciers. Massive further effects having severe impact on the environment are the reduction of Northern hemisphere snow cover, the sea ice extent and the near surface permafrost area, an increasing number of warm days and heat waves, a reduction of soil moisture, changes in precipitation, the reduction of water availability in arid regions, the expected substantial sea level rise, and a possible increase of number and strength of extreme weather events.

An effective climate change policy aiming at reducing climate change risks focusing on mitigation (reducing and avoiding GHG emissions) and on adaptation (reducing exposure and vulnerability) needs to be implemented. A climate and energy strategy of the European Union (EU) has already been established aiming to limit overall global warming to 2°C. So far, it consists of a series of legally binding actions by the EU Member States to achieve an emission reduction of 40% by 2030 and a strategic long-term vision for a 'prosperous, modern, competitive and climate neutral economy' with the goal of zero GHG emissions in the EU by 2050 [2]. At the global level, the Paris Agreement of 2015 signed by 196 countries sets a goal of limiting global warming to 'well below 2°C' compared to pre-industrial temperatures. The essential components of these measures, their consequences and the prospects for limiting global warming will be discussed briefly. In any case it is obvious that also extensive adaptation measures will be necessary since global warming will be progressing. Climate change is generally regarded as the biggest challenge for mankind today.

References
1. IPCC, 2013, Cambridge University Press, UK and New York, 1535 pp.
2. European Commission, 2018. COM(2018) 773.

AGRICULTURE 4.0 AND MYCOTOXIN MANAGEMENT

Prof. Josse De Baerdemaeker

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The Food Agriculture Organization of the United Nations (FAO) gives recommendations for Good Agricultural Practices (GAP) before/at the harvest that should reduce the risk for fungal growth and toxin development:

- plant resistant varieties and use certified seed that is free from fungal infections;
- sow the seed as early as possible, so that crop matures early;
- avoid nutrient stress and avoid drought stress;
- optimise the crop rotation and in case of minimum or zero tillage, remove crop residues;
- control weeds, insect and bird pests; and
- avoid grain damage during harvest, check moisture content before storage.

Implementation of these recommendations within the context of spatial and temporal variability and complexity of food production can be based on the interaction of the farmer, the available technology and the service- and consulting actors. The development of ever more advanced technologies that connect all the processes, the data analysis and the decision-making transforms agriculture, like other areas of economic and industrial activities, into what is becoming smart agriculture or agriculture 4.0.

An important concept in the technology component is the use of advanced electronic devices for sensing and communication on (autonomous) agricultural machines working on the field or during transportation. Weed and crop health detection lead to automatic control of field sprayers and fertilizer applicators to ensure the best treatment result without excessive use of inputs. Farmers and equipment manufacturers have become aware that while harvesting equipment has the basic task of collecting the grain, it can also harvest a lot of data about the yield and the quality of the grain that can be used for making decisions either on the short term for storage or processing, for food or feed use. Data collected by these sensors are not only available on the equipment for immediate use but can be communicated to other equipment and to on-farm or regional management systems for better information processing and decision making. In the longer term, this information can help in making decisions like crop scheduling, crop rotation and the optimisation of the soil preparation and cultivation practices. Grain quality, grain damage or fungal infection observed at harvest lead to on-line decisions about storage, drying or other treatments even before the grain arrives at the collection facilities. This information is also available as a field passport for future planning of field use, variety selection and crop rotation. Other management decisions can be, for example, the need for better irrigation systems. Crop yield, health and safety are important criteria in this decision making.

VERTICAL FARMING – SOLUTION TO OLD PROBLEMS?

Nigel D. Scollan[1] and G. Keeffe[2]

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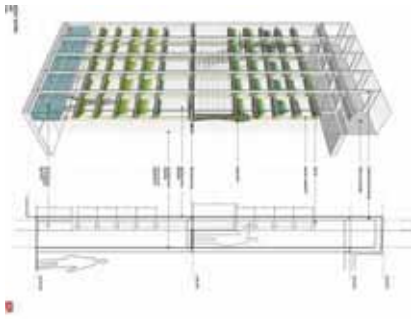
Interest in vertical farming (VF) systems is increasing influenced by the need to feed a growing world population, maximising use of natural resources, minimising environmental footprint and delivering food which is safe, authentic and of high nutritional value. VF (also referred to a controlled environment agriculture; CEA) is often associated with urban farm and food systems as they may be easily integrated into urban landscapes and positioned beside consumers, hence shortening supply-chains. This is rekindling connections between agriculture and architecture in the design of future cities and helping to extend 'green design' focused on minimising effects on construction projects on human health and environment to considering how food production may also be integrated. Systems are considered in shipping containers, disused warehouses, integrated into homes, businesses, supermarkets. In 2019, global VF was valued at $2.3 billion and projected to reach $13.3 in 2026.

VF allows for faster, more precision production using advanced nutrient delivery and sensor systems and LED lighting independent of need for soil, natural light and season. One acre of VF can provide produce equivalent to between 10-20 acres conventional land production and is much less subject to variation in climate, pests and pathogens. VF systems can be broadly divided in (i) those using multiple levels of traditional horizontal growing platforms and (ii) those where crop is grown on a vertical surface. Advanced systems grow crops based on hydroponics or aeroponics or aquaponics (combination of aquaculture and hydroponics, in which the plants use the fish waste as nutrients before the hydroponics filters the water before returning to the fish). Crops produced vary from lower value lettuce and herbs to high value plants for medicinal purposes. No emphasis has been placed on developing plants specifically for VF.

Aquaponic urban laboratory and farm



Integrated fresh food in future supermarket

VF systems are considered to offer a range of environmental benefits for example reduced land requirements, low water usage, closed system with no loss of nutrients to the environment, better control of waste, less impact to pests and disease, no variation due to weather etc with advanced lightening systems. High levels of control reduce the interaction between crops, pests or pathogens. However, use of life cycle analysis (LCA) illustrates that carbon-footprint of VF systems may be higher than conventional land-based systems, largely associated with energy to support advanced lightening systems. Hence, co-locating and integrating VF with renewable energy technologies or systems which produce a surplus of heat and energy such as anaerobic digestion will benefit both carbon-footprint and economic efficiency.

High technology VF systems represent a paradigm shift in farming and food production and ideally suited to urban environment. VF is in its infancy, and lack robust scientific studies to evidence the economic and environmental impact and nutritional density of the food produced. However, this will change, and VF is likely to be a major contributor to food production over the next 10-20 years.

MYCOTOXINS AND BEYOND: THE EXPANDING ROLE OF THE EXPOSOME CONCEPT IN ASSESSING EXPOSURE AND EFFECT OF FOOD CONTAMINANTS

Benedikt Warth

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During our lifetime, we are exposed to an assembly of food and environmental contaminants. These exposures broadly impact the aetiology of a large share of human disease, but occurrence and mechanisms often remain elusive and toxicological interactions are poorly understood. To address this key issue in public health research, the concept of the exposome, i.e., investigating the sum of lifespan exposures and their biological effect, was proposed but analytical technology remains a major limitation to the field [1]. Mycotoxin research is a well-suited playground for developing and optimizing assays suitable for omics-scale investigations as this class of natural food toxins is extremely broad in terms of chemical properties, concentrations found *in vivo*, and toxicological impact/mode of actions.

This contribution will introduce the concept of the exposome and the role of mycotoxins in the field. Current analytical and biological issues in exposomics will be discussed and specific methodological

advances presented. This includes targeted and untargeted workflows that are based on liquid chromatography coupled to mass spectrometry [2,3]. Moreover, metabolomics-guided pathway analysis for deciphering the toxicological impact of a specific toxin or toxin mixture in cell-based models will be a focus. Drug-exposome interactions will be discussed using a new combined breast cancer therapy and the myco-oestrogen zearalenone as examples [4,5].

References

1. Dennis, K.K. *et al.*, 2016. Environmental Health Perspectives 125: 502-510.
2. Warth, B. *et al.*, 2017. Analytical Chemistry 89: 11505-11513.
3. Preindl, K. *et al.*, 2019. Analytical Chemistry, in press.
4. Forsberg, E. *et al.*, 2018. Nature Protocols 13: 633-651.
5. Warth, B. *et al.*, 2018). Cell Chemical Biology 25: 291-300.

IS THE HORIZON GETTING CLOSER? MYCOTOXINS, ECONOMICS AND GLOBALIZATION: LOOK BEYOND THE HORIZON

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Mycotoxins have plagued mankind since the beginning of agriculture. Mycotoxins are natural toxins, therefore unpredictable and difficult to manage. Climate change and globalisation of trade flows have an additional impact on the mycotoxin risks all around the world. In response mycotoxin testing has evolved rapidly too. 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. Mycotoxin testing has become faster, more comprehensive and more automated. The use of mobile devices in mycotoxin analysis and sharing the analytical data in the cloud has opened entirely new ways of mycotoxin data use. Analytical data about the quality of commodities can be available from all locations in real-time. 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. So, is the horizon getting closer? Probably not.

Mycotoxins still have a major economic impact. According to the World Health Organization (WHO) still more than 25% of the world's crops are contaminated with of mycotoxins. Mycotoxins cause serious health issues to a significant part of the human population. Maybe not in the western world, where mycotoxin management has been very effective, but mycotoxins in staple foods are a serious health risk for consumers in Africa. Mycotoxins still cause significant losses of efficiency in livestock farming and maybe a health risk for pets. Climate change causes unusual mycotoxins to appear in unusual commodities from unusual places. Consumers are getting more concerned about the quality of food and regulations are increasingly enforced globally. As a result, mycotoxin testing is growing rapidly and projected to grow the coming years with an annual growth rate of 5-7%, and these growth rates are expected to be even higher in, e.g., the Asia-Pacific region. Mycotoxin testing has of course impact of the production costs for food products. So, for not just an effective mycotoxin monitoring but also an efficient mycotoxin management it is important to look at the economic aspects of mycotoxins too.

Mycotoxins are here to stay and continue to be a health risk for humans and animals and a major agricultural economic impact factor. But we get a broader, better view on the horizon and, looking beyond the horizon, we have the chance to make mycotoxin management more efficient.

MONDAY 14 OCTOBER 2019

SESSION 1
CONTROLLING PLANT DISEASE AND MYCOTOXIN FORMATION
What is in the pipeline for obtaining plant disease resistance and reducing mycotoxin contamination?

PLANT MECHANISMS COUNTERACTING *FUSARIUM* VIRULENCE FACTORS, A WAY TO REDUCE MYCOTOXIN CONTAMINATION?

Gerhard Adam[1], T. Svoboda[1], H. Michlmayr[1], P. Spörhase[1], K. Twaruschek[1], G. Wiesenberger[1], A. Ceranic[2], M. Doppler[2], R. Krska[2], F. Berthiller[2] and R. Schuhmacher[2]

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A better understanding of the interaction of the fungal pathogen *Fusarium graminearum* with its host plants should be a key for knowledge-based breeding of *Fusarium*-resistant crop plants, with low mycotoxin contamination. We present evidence that *F. graminearum* is not just a necrotrophic pathogen (killing the host plant with toxins and living on the dead tissue) but employs plant hormone signalling in an early infection phase as a virulence factor.

Transcriptome and metabolome studies have revealed that in response to infection tryptophan biosynthesis is upregulated and tryptamine (TAM) is formed in cereal hosts. Several amines, including TAM, are used for biosynthesis of hydroxycinnamic acid amides. These compounds have antifungal activity and accumulate in the narrow battle zone, the rachis, which the fungus has to pass when spreading to the next spikelet. We found that *Fusarium* can adapt and hydrolyse defence compounds, such as coumaroyl-tryptamine, and release TAM. *In vitro*, *Fusarium* can convert TAM in high yield into the plant hormone auxin (indole-3-acetic acid). Highly elevated auxin levels occur in *Fusarium*-infected wheat. Auxin is required for embryogenesis and seed development, and in many pathosystems auxin signalling is antagonistic with defence signalling. To test whether fungal auxin production is relevant for virulence, we disrupted all Cu-amine oxidase genes. The resulting septuple mutant produces very little auxin *in vitro* (at much later time points), and infection assays revealed reduced virulence. Slower spread through the wheat ear leads to a lower overall deoxynivalenol (DON) content of ears.

The mycotoxin DON is a known virulence factor of *F. graminearum* and is required for fungal spread. Previously we have identified plant UDP-glucosyltransferases capable of inactivating DON by formation of DON-3-O-glucoside. It has been shown that overexpression of glucosyltransferases from barley or *Brachypodium* increases toxin and *Fusarium*-resistance. Here, we report a role for the *Fusarium* metabolite culmorin, which can inhibit DON-glucosyltransferases to a variable extent.
The relevance of these findings for resistance breeding and durability of resistance will be discussed.

IS HOST RESISTANCE AGAINST *ASPERGILLUS FLAVUS* INFECTION AND AFLATOXIN CONTAMINATION POSSIBLE? GENE EXPRESSION OF RESISTANT AND SUSCEPTIBLE MAIZE LINES PROVIDES INSIGHTS

Matthew K. Gilbert

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Contamination of food and feed crops by *Aspergillus flavus* and the carcinogenic metabolites it secretes, the aflatoxins, causes millions of dollars in crop losses annually. Identifying the molecular mechanisms in both maize and *A. flavus* to target in mitigation strategies is an ongoing and vital component towards addressing this agricultural problem. Using RNA-sequencing technologies combined with computational analysis can allow for the identification of these vital gene networks. Gene identification followed by the development of host-induced gene silencing technologies provides a promising approach in the effort to mitigate pre-harvest *A. flavus* crop contamination. A description of our ongoing efforts and successes towards this end will be presented, including functional evidence of a successful RNAi-mediated host resistance strategy.

MS IMAGING REVEALS PLANT RESISTANT MECHANISMS AGAINST MYCOTOXINS – THE LOCATION MAKES THE DIFFERENCE

Laura Righetti[1,2], D.R. Bhandari[2], B. Spengler[2] and C. Dall'Asta[1]

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The investigation of the plant response to infection is essential for developing possible strategies to counteract mycotoxin accumulation. To date, mass spectrometry-based metabolomic strategies have extended our knowledge, revealing the biological pathways by which the plant interacts against fungal attack. Nevertheless, the primary constraint is related to the complexity of the plant metabolome. On the one hand, these metabolites cannot be isolated by a single extraction procedure, and consequently, a single analytical procedure is not able to take the entire picture. On the other hand, metabolomics approaches miss spatial information due to the extraction process.

In recent years, visualisation has become an essential feature in plant science, enabling to study the metabolites distribution within a tissue. In this regard, mass spectrometry imaging (MSI) has become a powerful tool capable of achieving the spatial distributions and chemical specificity by enabling unprecedented details of metabolic biology to be uncovered. Therefore, this research aimed to investigate the spatial tissue distribution of defence metabolites, useful to assign the metabolites' functional role. To address this challenge, transversal cross-sections obtained from wheat samples were analysed using atmospheric-pressure (AP)-scanning microprobe matrix-assisted laser desorption/ionisation MSI ion source ((AP-SMALDI5 AF, TransMIT GmbH, Germany)) couple to a Q Exactive HF orbital trapping mass spectrometer (Thermo Fisher Scientific GmbH, Germany).

Our results demonstrated the analytical potential of innovative high-throughput technique for gaining insight into the plant resistance mechanism against mycotoxin accumulation.

Acknowledgments
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USE OF ENDOPHYTIC FUNGI AS BIOCONTROL AGENTS OF CEREAL DISEASES

Hans J.L. Jørgensen, D.B. Collinge, M.A.C. Latz, E.C. Rojas and B. Jensen

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Endophytes are microorganisms living inside plants without causing any apparent disease. All plants harbour a range of microorganisms with lifestyles ranging from pathogenic over commensalistic to beneficial. These microorganisms, which mainly comprise fungi and bacteria, have recently gained increased interest due to their potential positive impacts on the host plant, among others for biological control of diseases. Biocontrol have gained momentum because issues with traditional fungicides in relation to environmental impact and fungicide resistance. Endophytes could represent ideal candidates in biological control since they may reside inside plants, thus being protected from adverse environmental effects.

In the Marie Skłodowska-Curie Action CerealPath (https://cerealpath.eu/), we have recently been searching for endophytic fungi with the potential to control diseases in wheat. Two of the most important diseases in wheat are Septoria tritici blotch (STB) caused by *Zymoseptoria tritici* and Fusarium head blight (FHB) caused by species in the genus *Fusarium*. Whereas STB causes significant yield losses in most wheat growing regions where the climate is humid, FHB, in addition to yield losses, also contaminate the grains with mycotoxins, rendering them and their products unhealthy for the consumers.

We isolated several endophytic fungi and tested their ability to control STB and FHB (*Fusarium graminearum*) in a plant-based screening approach. Potential endophyte candidates were isolated from apparently healthy plants among otherwise diseased individuals, the hypothesis being that their health could be attributed to colonisation by endophytic fungi, which protected them. The isolated strains were directly tested for disease-reducing ability on wheat leaves (STB) and detached spikelets and whole wheat heads (FHB). By using this approach, we were certain that the selected isolates had an effect when applied to plants.

It is important to understand how a biological control agent reduces disease in order to optimise efficacy, product stability and be able to register a product. Two isolates significantly reducing STB and FHB in wheat were selected for detailed studies of the mechanisms, one for each disease. Both isolates had limited effect on *in vitro* growth of the pathogens, showing that there is often a lack of correlation between *in vitro* and *in planta* effects. RNAseq analysis coupled with microscopy, enzyme assays and expression analysis of specific defence-related genes with a known effect against the pathogens revealed that induced resistance was a prominent mechanism involved in the disease reductions. Thus, application of the two isolates stimulates the plant to protect itself against the two diseases. Interestingly, for plants infected by *F. graminearum*, the pathogen showed reduced metabolism in endophyte-protected plants and genes involved in the pathway for the biosynthesis of trichothecene mycotoxins showed low expression levels. This could indicate that not only was the pathogen colonisation reduced, but also the production of some mycotoxins might be reduced. However, it is known that other biocontrol agents, such as *Clonostachys rosea*, can degrade the toxin zearalenone, showing a potential in future crop protection strategies.

Acknowledgements
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HARNESSING THE MICROBIOME TO REDUCE FUSARIUM HEAD BLIGHT

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Considering plant pathogens within their microbial community context may provide new insights into the development of plant diseases. To illustrate connections between *Fusarium graminearum* and the broader microbiome inhabiting wheat kernels, we assessed *Fusarium* load, deoxynivalenol content, and microbiome profiles for individual wheat kernels collected over two years from a disease-conducive environment. We find that the microbiomes of individual, hulled wheat kernels consist of dozens to hundreds of bacterial taxa and up to several dozen fungal taxa, and that year-to-year variation in microbiome structure was large. Several bacterial taxa, notably *Sphingomonas*, demonstrated relationships with pathogen load. Microbiome characteristics, such as richness and diversity, made small but significant contributions to explaining disease components within individual kernels.

Additionally, we screened a number of fungal endophytes for ability to colonise wheat plants. We have identified a particularly promising strain that colonises wheat extensively, slows Fusarium head blight progression, and significantly reduces accumulation of deoxynivalenol.

MONDAY 14 OCTOBER 2019

SESSION 2
PROCESS OPTIMISATION TO REDUCE MYCOTOXIN CONTAMINATION OF FOOD AND FEED

Where do we stand today? This session will introduce some options to optimise food processing to reduce mycotoxin contamination of food and feed under the motto: "from scientific findings to practical guidance: the ILSI Europe experience".

MITIGATION OF MYCOTOXINS ALONG FOOD PROCESSING: FROM SCIENTIFIC FINDINGS TO PRACTICAL GUIDANCE – THE ILSI EUROPE EXPERIENCE

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Addressing the mycotoxin issue is becoming a key challenge for food actors, as evidenced by the recent report of the International Agency for Research on Cancer (IARC) on the impact of climate change on mycotoxin proliferation; for instance they estimate that every day around 500 million of low income people basing their diet on maize and cereals and living in sub-Saharan Africa, Latin America, and Asia are exposed to aflatoxins and fumonisins. To prevent losses of otherwise suitable foodstuffs due to mycotoxins, decontamination processes have been developed and applied over the years. Food processing can impact mycotoxins in raw material in different ways: (i) physical elimination (e.g., cleaning, sieving); (ii) chemical transformation or interaction with food matrix which can result in metabolites of either lower or higher toxicity than the parent compound; (iii) release from masked or entrapped forms which may increase bioavailability; (iv) enzymatic detoxification; and (v) adsorption to bacterial cell walls which may be reversed during further processing or digestion. The various food processing that may have mitigating effects on mycotoxins include cleaning, milling, brewing, fermentation, cooking, baking, frying, roasting, flaking, alkaline cooking, nixtamalization, and extrusion.

In 2014-2016, the International Life Sciences Institute Europe (ILSI Europe) Process-Related Compounds and Natural Toxins Task Force dedicated a project to understand the possibility in mitigating mycotoxins, correspondently improving the safety of the food commodities. The main task defined was to critically review the state of the art about mycotoxin reduction by food processing summarising the impact of the different decontamination/detoxifying processes on various food commodities; and finally, the impact of transformed mycotoxins leading to a lower mycotoxin concentration and a lower toxicity was illustrated and discussed. This work conduced to a final open access manuscript published in Mycotoxin Research already downloaded more than 14,000 times [1].

The genuine interest of the scientific community around this work stimulated then a correspondent new effort in 2017-2018 as a follow-up activity to translate the scientific findings of the Expert Group on Reactions and Potential Mitigation of Mycotoxins during Food Processing to a concrete guidance for industry. The current Black & White Report Series is directly targeted to industry to actively participate in the global mycotoxin mitigation strategy. It will now help international food producers (related to various commodities, such as cereals and derived products, cocoa, fruit juices, dairy products…) with clear, easy to implement, practical suggestions and guidelines for process adaptation aiming to mitigate mycotoxins. It could also be useful for official body representatives who could then suggest global mitigation strategies to the all food chain actors and adapt regulation accordingly. This publication will be regularly updated with the most recent scientific developments and periodically extended to other commodities or enriched with innovative processes every 3-4 years.

References

1. Karlovsky, P. *et al.*, 2016. Mycotoxin Research 32: 179-205. (https://link.springer.com/article/10.1007%2Fs12550-016-0257-7)

OCCURRENCE AND TOXICOLOGICAL SCENARIO OF RELEVANT MYCOTOXINS AT THE INDUSTRIAL LEVEL – STATE OF THE ART

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The European Union maintains the most comprehensive regulatory controls in the world on mycotoxins. In 1993, it was agreed to create a list of food contaminants with associated regulatory limits, and from its inception this list contained a number of maximum limits for aflatoxins. In 2006, this list was transferred into its current form as Commission Regulation (EC) No 1881/2006, and between 2007 and 2015 a large number of requirements for mycotoxins were added including for patulin, deoxynivalenol, ochratoxin A, zearalenone, fumonisins, citrinin, and ergot sclerotia and alkaloids. Due mainly to their regulation, these mycotoxins and the commodities in which they occur became well known and there was an increase in knowledge including on their mitigation.

However, since 2015 there has been no further amendment to the regulatory requirements for mycotoxins. That situation is about to change. The European Commission is currently considering amended measures for citrinin, ergot alkaloids, ochratoxin A, and new measures for modified forms of deoxynivalenol, *Alternaria* toxins, and T-2 HT-2 toxins. In addition to the regulatory measures being finalised, the EFSA continues to generate substantial information, including for example on the risks to consumer health presented by contamination by sterigmatocystin, beauvericin, enniatins, and modified forms of zearalenone.

This presentation will summarise the toxicology of regulated mycotoxins, and explore the link between safety science, regulation and mycotoxin occurrence in the food chain.

FIRST IN-DEPTH ANALYSIS: HOW TO MANAGE THE CEREALS FOOD PRODUCTION CHAIN MITIGATING MYCOTOXINS

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Mycotoxins are toxic secondary metabolic compounds commonly occurring in cereals which pose a health risk to the final consumer. Worldwide legislation is in place setting maximum limits for mycotoxins in cereals and processed cereal products to ensure that they are not harmful for human consumption. Good agricultural practices, plant diseases management and adequate storage conditions reduce mycotoxins levels yet do not eliminate mycotoxins completely.

Food processing can further reduce exposure by removing mycotoxins from unprocessed cereals by physical treatment, chemical decontamination, and chemical or enzymatic transformation into less toxic products. Physical removal of mycotoxins is very efficient: large-scale automatic sorting used by grain industry significantly reduces food-borne mycotoxin exposure. Various food processing of cereals that may have mitigating effects on mycotoxins include cleaning, milling, brewing, fermentation, cooking, baking, frying, roasting, flaking, nixtamalization (alkaline cooking) and extrusion. Detoxification can be achieved enzymatically. Some enzymes able to detoxify mycotoxins naturally occur in food commodities or are produced by fermentation but more detoxification can be achieved by deliberate introduction of purified enzymes.

The objective of this presentation is to give an overview about the aspects indicated above and the correspondent work carried on by the ILSI dedicated expert group. Recent published results highlighting that the levels of modified forms of deoxynivalenol and other mycotoxins are changing depending on factors as temperature, fermentation and the presence of enzymes during the baking process will be presented in the view of ILSI Europe's Black &White Report.

SECOND IN-DEPTH ANALYSIS: HOW TO MANAGE THE DAIRY FOOD PRODUCTION CHAIN MITIGATING MYCOTOXINS

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Nowadays, the dairy chain globally serves over 7 billion consumers and provides livelihoods for nearly 1 billion people living on dairy farms. According to the FAO, approximately 150 million households are engaged in milk production around the globe, with a substantial increase expected taking place in the next two decades. Recent estimates reported that, based on milk equivalent, the average global milk consumption per capita amounts to about 100 kg milk/year, with the highest yearly consumption in the Western countries. On this basis, milk and dairy production chain appears prominent in terms of international trade, food supply and nutritional intake for specific population categories and geographical areas. In addition, milk is prone to manifold chemical contamination issues with possible carryover phenomena along the dairy production chain.

Specifically, mycotoxins contamination is among those of most concern due to the consequences in terms of food waste and detriments to consumers health. In this respect, a recent multi-country dietary exposure assessment reported that mycotoxin levels in milk often approach the maximum limits. Therefore, keeping in mind that mitigation actions have to be applied on compliant commodities, the development and implementation of strategies to reduce as much as possible the mycotoxins contamination of milk are strongly encouraged to ensure a more and more safe supply of milk and dairy products. Nevertheless, the scientific data currently available provide a limited ground facing only the contamination of aflatoxin M1 and ochratoxin A, which are the mainstream milk-related mycotoxins, while the wealth of other mycotoxins possibly occurring in milk (such as fumonisins, trichothecenes and zearalenone analogues) is still completely uncovered. In addition, the few methods identified so far cannot be applied regularly to the dairy production as they either affect the properties of milk products or they are characterised by a limited applicability that strictly depends on specific products. As a general remark, the current scientific literature describes a gap between the mitigation strategies currently proposed and the number of mycotoxins that may simultaneously contaminate milk. Therefore, a change of paradigm toward the implementation and adoption of novel and more effective multi-mycotoxin mitigation strategies is encouraged to keep up with the times, wherein both the occurrence analysis and toxicity assessment is moving from the individual substance to mixtures of mycotoxins.

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PLENARY SESSION: 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? Is automation an option? 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'. 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 as well as production sites in Glasgow, Washington (MO) and Berlin. 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. Today, the German parent company R-Biopharm is represented globally by local subsidiaries and an extensive network of more than 80 distributors.

ADISSEO
https://nutriad.com

Adisseo, the global player in mycotoxin management via acquisition of Nutriad. 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 all over the world, Adisseo 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, Adisseo's mycotoxin deactivators are produced in Belgium and sold worldwide through a network of sales offices and distributors, supported by several application laboratories and manufacturing facilities located on 3 continents. In addition to improving its product offering, Adisseo 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 Adisseo, 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 numerous 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.

BIOMIN
https://www.biomin.net

At BIOMIN, we harness the power of science to support animal health and performance. By applying state-of-the-art and proprietary technology we deliver natural, sustainable and profitable solutions to the livestock industry. For over 30 years, we have pioneered innovative solutions for mycotoxin risk management and gut performance. Naturally ahead. Our in-house R&D program at the BIOMIN Research Center is staffed by over 100 scientific researchers and supported by eight Centers for Applied Animal Nutrition and a research network of 200 academic and research institutions globally. Our clients in the poultry, swine, cattle and aquaculture sectors are located in more than 100 countries worldwide.

ROMER LABS
https://www.romerlabs.com

Romer Labs is a leading global supplier of diagnostic solutions for food and feed safety. We offer a broad range of innovative tests and services covering mycotoxins, food pathogens, food allergens, gluten, GMO, veterinary drug residues, and other food contaminants. Furthermore, we operate four accredited, full-service laboratories on three continents.

TROUW NUTRITION
https://www.trouwnutrition.com

Trouw Nutrition, a Nutreco company, is a global leader in innovative feed specialties, premixes, feed additives and nutritional services for the animal nutrition industry. It provides products, models and services to boost productivity and support animal health through all life stages. With unique, species-specific solutions, Trouw Nutrition has been meeting the needs of farmers and home-mixers, feed producers, integrators and distributors since 1931. Headquartered in the Netherlands, the company has locations in 28 countries and employs approximately 8,000 people. Innovation is one of Trouw Nutrition's core values. Over 100 experts conduct research in nutrition and its application in animal production for poultry, ruminant and swine. To do this research, Trouw Nutrition R&D has five major research centres in Canada, Spain and the Netherlands. Its in-house research is complemented with over 50 long-term research collaborations with scientific institutes across the globe. Trouw Nutrition R&D activities are closely aligned to the needs of the market. Application and Solution Centres in Europe and North America coordinate the interface between R&D and the operating companies of Nutreco to ensure an excellent fit between local needs and global nutritional solutions. Trouw Nutrition offers feed additive solutions as a complete package consisting of products, models and services. Focus areas are gut health, feed safety, and nutritional solutions. The company's Mycotoxin Risk Management programme enables feed producers to mitigate mycotoxins effectively and cost-effective, using state-of-the-art strategies and comprehensive diagnostics. This allows customers to actively monitor mycotoxin risk, raising confidence in their products. This integrated approach offers a three-step programme, involving the identification of which mycotoxins may impair animal performance and the measurement of contamination levels, implementation of risk control measures and a thorough evaluation of the effect of the measures taken. The Mycotoxin Risk Management programme combines knowledge, services and products into a customised solution, focussed on supporting animal health and productivity.

ALLTECH
https://www.alltech.com

Founded in 1980 by Irish entrepreneur and scientist Dr Pearse Lyons, Alltech is a cutting-edge technology company in a traditional industry, agriculture. Our products improve the health and nutrition of plants and animals, resulting in more nutritious products for people as well as less impact on the environment. With expertise in yeast fermentation, solid state fermentation and the sciences of nutrigenomics and metabolomics, Alltech is a leading producer of yeast additives, organic trace minerals, feed ingredients, premix and feed. We work with producers across the globe to address the issues most important to them, including the management of mycotoxins, which has been a core focus at Alltech for more than 25 years. The Alltech® Mycotoxin management programme helps mitigate the threat to animal health from these toxic metabolites consumed in contaminated feedstuffs. The program's line of tools helps identify mycotoxin contamination on-farm and in the feed mill, and it offers a picture of the physical and financial impact of mycotoxins on an operation. Additionally, the program highlights recommendations on the best solutions to protect animal health and profitability. These solutions are backed by science, and Alltech's commitment to product research and development has resulted in more than 150 peer-reviewed publications in conjunction with numerous academic institutions around the world, addressing the impact of mycotoxins on a variety of species and the role of organic adsorbent materials in their mitigation. Together, with our more than 5,000 talented team members worldwide, we believe in 'Working Together for a Planet of Plenty™'. With the adoption of new technologies, the adaptation of better farm management practices and the ingenuity inherent in the human spirit, we believe a world of abundance could be ours. Alltech is a private, family-owned company, which allows us to adapt quickly to our customers' needs and stay focused on advanced innovation. Headquartered just outside of Lexington, Kentucky, USA, Alltech has a strong presence in all regions of the world. For further information, visit www.alltech.com/news. Join us in conversation on Facebook, Twitter and LinkedIn.

PHILEO BY LESAFFRE
https://phileo-lesaffre.com

Nothing is more precious than life, and that's the philosophy that drives Phileo. Taken from the Greek verb 'to love' and associated with a spiral that illustrates the new momentum and openness to the future, Phileo by Lesaffre 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 by Lesaffre 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 150 people, Phileo by Lesaffre 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 by Lesaffre 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. Phileo by Lesaffre provides nutritional solutions based on live yeasts, bacteria and yeast products. 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 by Lesaffre 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. In every country, our progress is led by the most advanced science as well as practical on-farm experience.

OLMIX
https://www.olmix.com

Olmix Group: algae-based natural solutions. A desire to provide natural alternatives to agricultural additives led to the creation of Olmix Group in Bréhan, at the heart of Brittany (France), in 1995. In 20 years, the company has become one of the major global specialists in marine biotechnology and green chemistry. From the start, Olmix Group has innovated in trace elements, transforming by-products into high-value ingredients. 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. "The secret to Olmix Group's success has been our visionary approach, identifying marine algae as a renewable raw material with an unexpected potential to help feed 9 billion people sustainably by 2050." (Hervé Balusson, Olmix Group founder and CEO). As a specialist in marine biotechnology, Olmix Group provides natural sources of nutrition and health to plants, animals and people, for a complete, consistent food and health chain, thanks to algae! Olmix Group employs 910 people and has a turnover of EUR 170 million in 2018, 80% of them of sales exported. Olmix commercial structure is based on a network of 29 establishments covering more than 100 countries on the five continents. The company has 12 production sites in Europe, one in Asia and its innovative character, in tune with the change of environmental regulations in the world, constitutes an important reference in sustainable development.

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: (i) leader in mycotoxin control (Minazel, 1st generation adsorbent; Minazel Plus, premium mycotoxin adsorbent); (ii) 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 (iii) 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 at all pHs (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.

VICAM, A WATERS BUSINESS
http://vicam.com

VICAM, A Waters Business is a global leader in rapid monitoring solutions for mycotoxins and bisphenol A. Global food and agricultural safety depends upon early mycotoxin detection in order to segregate raw materials, monitor storage and processing operations – and to verify the suitability of finished products for release to market. Our complete family of simple, safe antibody-based detection technologies places actionable, precise data into the hands of global agricultural and food operations and service laboratories worldwide. VICAM launched our flagship AflaTest immunoaffinity column sample prep column in 1987. USDA-FGIS and AOAC-approved, AflaTest continues to be utilised in more than 100 countries to verify regulatory compliance with import/export shipments and internal quality requirements using on-site fluorometric quantification or laboratory-based LC and LC-MS/MS detection. VICAM's immunoaffinity columns may be used for single or multiple mycotoxin detection, including Myco 6-in-1+, a sample prep solution capable of isolating and purifying aflatoxins B1, B2, G1 and G2, fumonisins B1 and B2, ochratoxin A, T-2, and HT-2 toxin, deoxynivalenol, nivalenol and zearalenone for quantification by LC-MS/MS. The global shift toward preventive management inspired our scientific team to design and develop fit-for-purpose, field-based lateral flow strip tests. Qualitative screening and fully quantitative, USDA-FGIS approved methods are available which are fully equipped to detect aflatoxin, fumonisin, deoxynivalenol, ochratoxin A and zearalenone in inbound grains and other food and agricultural commodities. Simple enough for non-scientists to use on-site, VICAM's Vertu quantitative strip tests deliver sensitive, precise data which enables true quality management and control in order to prevent contaminated raw materials from entering the food and production value chain. VICAM re-affirms our commitment to global food and agricultural stakeholders – to provide scientifically and environmentally sound solutions for field, process and laboratory mycotoxin detection.

PRIBOLAB
http://www.pribolab.com

Very glad to introduce Pribolab to you here. Pribolab is a leading supplier focusing on the food safety area (especially biotoxins) with an independent research & development laboratory, manufacture centre, technical support department, after-sales service department and marketing team. Our company was established in Singapore in 2008 and moved to Qingdao city, China in 2014. Until now, Pribolab has expanded its product line to mycotoxins, marine toxins, food allergens, GMOs, veterinary drug residues, etc., servicing the food, feed, pharmaceutical, agricultural and environmental industries all over the world. We have built very good relationship with universities, third-party laboratories, research institutes, government laboratories and food factories. Pribolab testing products include Immunoaffinity columns, reference standards, ^{13}C isotope internal labelled standards, Elisa kits, rapid strips and CRM materials. Our instruments include automatic homogeniser; mycotoxin evaporator, automatic standard solution preparation system, immunoaffinity column operating rack, automatic immunoaffinity column purification system, regrinding and subsampling mill, derivatisation instruments and analytical readers (lateral flow reader, ELISA plate reader). Pribolab devotes all its passion to the food safety career by constantly product upgrading and technical innovation! If you are interested in our products, welcome to visit our booth and discuss more.

PROGNOSIS BIOTECH
https://www.prognosis-biotech.com

ProGnosis Biotech is an innovative biotechnology company, specialised in developing and manufacturing next-generation immuno-assays for the dairy, food and feed industries. Our company produces high-quality ELISA kits and lateral-flow tests, detecting and quantifying aflatoxin M1 in milk as well as all the major mycotoxins in grains, cereals, nuts and many other commodities including animal feed. ProGnosis Biotech offers a wide range of products that provide mycotoxin testing solutions according to any legislation globally. We regularly expand our product list as we always follow market's needs developing highly accurate and innovative products. Based in Greece and having also representative offices in Spain and China, ProGnosis is an export-oriented enterprise which through a large network of distributors, exports ELISA kits and Lateral-flow tests to more than 30 countries worldwide, in 4 continents. Our main goal for the forthcoming years is to expand our activities into new markets offering reliable and competitive products. In order to consistently provide top-quality products, ProGnosis participates on a monthly basis in proficiency tests in the UK, France, Germany, Italy, and the USA. Additionally, it offers validated products from the most highly esteemed organisations worldwide while all R&D and production procedures are certified with ISO 9001. Our Mission serves as the standard against which we weigh our actions and decisions: to provide innovative, unique and reliable solutions with our products and to create value and make a difference in the food and laboratories' sector.

NEOGEN EUROPE
https://foodsafety.neogen.com/uk

Neogen Europe has been developing and supplying diagnostic kits and expert services to determine the quality and safety of food and agricultural products since 1998. With wide ranging expertise, Neogen offers onsite diagnostic kits and laboratory testing services to ensure food safety throughout the entire supply chain, from farm to fork. Neogen offers solutions to detect mycotoxins, marine and other natural toxins, speciation and allergens as well as a wide range of products for traditional and rapid microbiology, pathogens and spoilage organisms. Neogen also offers a complete hygiene monitoring system. In addition, our forensic toxicology and life science kits are being used by many leading organisations across the globe. Neogen also offers world beating genomic testing programmes for the improvement of livestock and crops.

ENVIROLOGIX INC.
https://www.envirologix.com

EnviroLogix Inc. pushes diagnostic boundaries forward in the life sciences, grain markets and the food supply chain. Since our founding in 1996, our driving inspiration has been to create breakthrough diagnostic technologies for agriculture, seeking innovative ways to help customers in a broad array of verticals solve their problems and improve their operational efficiency. That drive to pioneer advancements continues today, as we develop smart, easy-to-use GMO and mycotoxin detection instruments, test kits, and software solutions. The data our decision-point solutions generate feeds into a growing spectrum of invaluable agricultural meta data used to make business decisions and ultimately to meet consumer demand for transparency. Headquartered in Portland, Maine, with a second location in Jaguariúna, Brazil, we take pride in our fast-paced and innovative technology. We value the diverse knowledge and experience of each contributor working at every level of the company.

TECNA, PART OF EUROFINS TECHNOLOGIES
https://www.eurofins.com/food-and-feed-testing/food-testing-services/technologies

Tecna has been developing, producing and selling screening test kits for the analysis of chemical contaminants in foods and feed since 1994. Tecna, though, is more than a list of kits. Since the beginning, with a small group of 4 biologists experienced in immunodiagnostics, the mission was to supply customers with comprehensive analytical solutions, including accessories, training, installation services and customer care. We have been developing immunoassays in a number of formats for almost thirty years. Along the way, we have deeply changed the aspect of microplate ELISAs, deleting the calibration curve and introducing the concept of master curve. Thanks to the stability of our reagents, quantitative results are obtained with no need to spend any determination for controls or calibrators, thanks to the availability of batch-related mean master curves. This options dramatically cuts the costs of the analysis and enables also small companies to afford the analysis. We do believe that food and feed industries need a reliable partner for a proper risk management of chemical contaminants monitoring. We encourage and assist small and medium companies in starting their own laboratory through our experience. We offer our science for the quality of your results. In 2016, Tecna joined an ambitious project in Eurofins and became part of the global provider of screening solutions Eurofins Technologies, together with Abraxis, Ingenasa, Gold Standard Diagnostics, Immunolab, GeneScan, and Amar. Eurofins Technologies product portfolio includes the best technologies available for the analysis of allergens, foodborne pathogens, GMOs, mycotoxins, pesticides, acrylamide, veterinary drug residues, animal species and food fraud, veterinary diagnostics, and environmental and water testing.

DEVENISH
https://www.devenishnutrition.com

Devenish is a farming and food company, delivering sustainable and innovative nutritional products and solutions for the feed industry, the food industry and for human health. With a growing world population to reach 9 billion people by 2050, sustainable food production is imperative. As such, the Devenish strategy, 'One Health, From Soil to Society', focuses on the importance of optimising nutrient utilisation in soil, plant, animal, environmental and human health, as key and interlinked components of the value chain. Devenish Nutrition aim to be a solution provider through knowledge and products. We invest heavily in research to help provide these solutions. Solutions to current and future problems can only be developed based on robust science. To achieve this Devenish Nutrition work with partners who are world renowned for their excellence in specific areas.

TOLSA
http://www.tolsa.com/industrial/en

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MONDAY 14 OCTOBER 2019

PLENARY SESSION: 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 105-172).

P10
The role of pH signalling transcription factor PacC in pathogenicity and ochratoxin A biosynthesis by *Aspergillus carbonarius*
Omer Barda
Institute of Postharvest and Food Sciences, Agricultural Research Organization, Volcani Center, Israel

P21
Urinary DON concentrations as biomarker of exposure in different age groups in Norway
Gunnar S. Eriksen
Norwegian Veterinary Institute, Norway

P27
Physiologically based kinetic (PBK) modelling-based reverse dosimetry of *in vitro* toxicity data to predict acute liver toxicity induced by aflatoxin B1 in rats and humans
Ixchel Gilbert-Sandoval
Department of Agrotechnology and Food Sciences, Wageningen University & Research the Netherlands

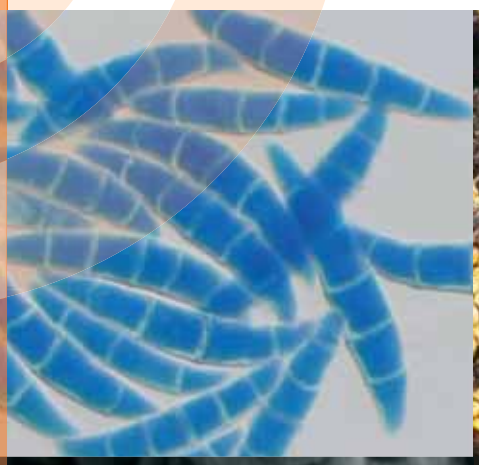
P45
Insights of the *in planta* toxicity of some bacterial metabolites of the mycotoxin deoxynivalenol
Ting Zhou
Guelph Research and Development Centre, Agriculture and Agri-Food Canada, Canada

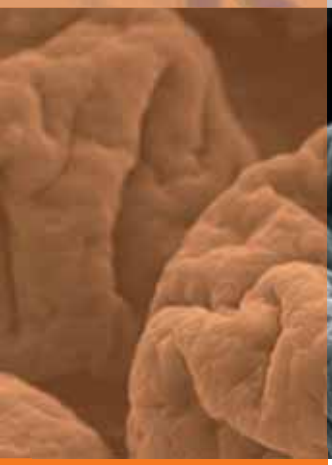
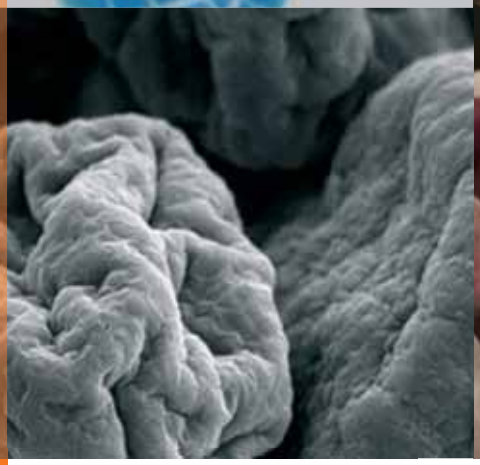
P113
Measuring the sum – a novel screening method for ergot alkaloids in food
Maximilian Kuner
Federal Institute for Materials Research and Testing, Germany

P124
Mimotope-based immunoassays for mycotoxin detection
Riikka Peltomaa
Department of Analytical Chemistry, Complutense University, Spain

SafWall

Integrated mycotoxin strategy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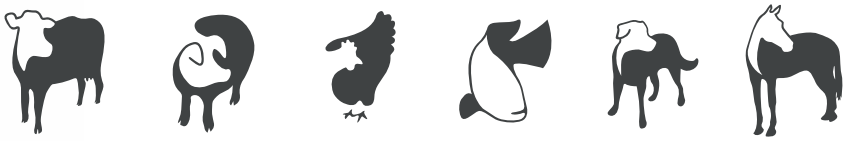
- **BARRIER INTEGRITY**
- **BODY PROTECTION**



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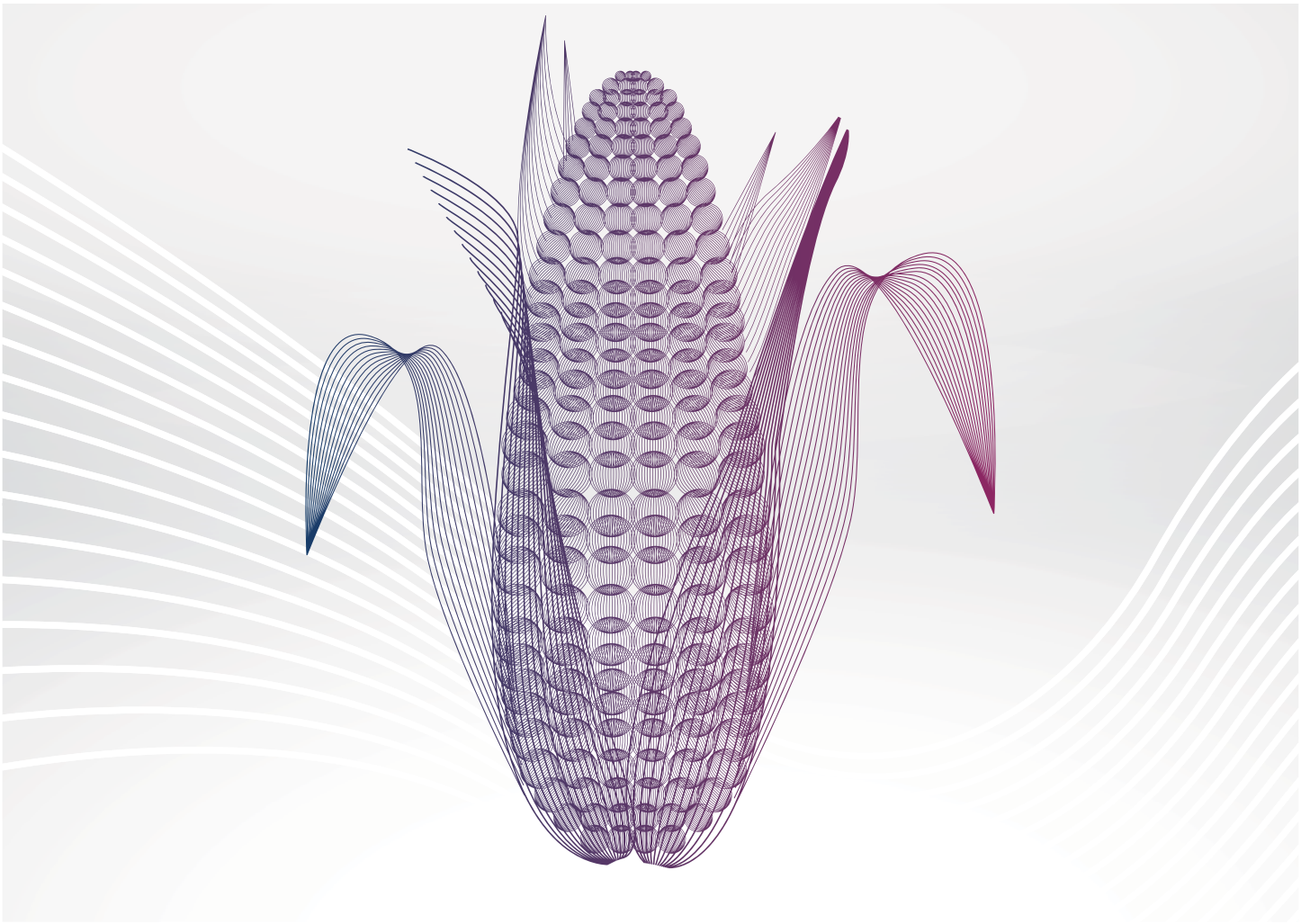

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TUESDAY 15 OCTOBER 2010

SESSION 3
MITIGATING THE NEGATIVE IMPACT OF MYCOTOXINS
A look into recent and promising strategies related to mitigating mycotoxin contamination in food and feed. What's up?

AN INTEGRATED APPROACH TO MITIGATE MYCOTOXINS IN FEED

Paul G. Bruinenberg, G. Wang and P. Caramona

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Feed safety is a pre-requisite to secure efficient animal production and food safety. A variety of fungal species can grow in grains, by-products and complete feed, decreasing its nutritional value, resulting in off-flavours and secondary metabolites, such as mycotoxins, which are recognised to affect animal health and performance. Feed safety and quality is a fundamental pillar of our research program and much attention is given to prevention of fungal spoilage in feed and mitigation of the adverse effects of mycotoxins on animal health and transfer through the feed-food chain. Propionic acid is an effective preservative and its mode of action, in preventing mould growth in feed ingredients, has been evaluated. Mould growth inhibition by propionic acid is due to damage of the cell membrane and the internal integrity of the germ tubes but also to partial inactivation of the conidia. Furthermore, our research has demonstrated a synergistic effect between propionic acid and medium chain fatty acids in growth inhibition of fungi isolated from spoiled poultry feed samples. The outcomes from these studies resulted in the development of an effective novel antifungal solution for application in feed.

Simultaneous exposure to mycotoxins may result in synergistic or additive adverse effects on several biological end-points such as the immune system. Several strategies can be applied to reduce the bioavailability of mycotoxins and mitigate their effects. Due to the molecular structure and chemical proprieties of mycotoxins, combining several strategies is most effective under a co-contamination mycotoxin challenge. Our research is focusing on three main strategies: adsorption affinity and capacity, intestinal integrity and immune modulation. Through extensive screening a bentonite, confirmed by the EU authority, was selected on its efficacy of adsorbing mycotoxins (especially aflatoxins), with intensive proofs both *in vitro* and *in vivo*. Besides mycotoxins, this bentonite also showed adsorption of endotoxins produced by gram-negative bacteria, such as *Escherichia coli*. Secondly, specific glucose biopolymers were selected reinforcing the tight junction complex in gut epithelial cells, which can be damaged due to mycotoxin exposure. This mechanism is crucial to reduce the bioavailability of mycotoxins (e.g., deoxynivalenol), which cannot be effectively adsorbed by a bentonite. Thirdly, 1,3-1,6-β-glucans extracted and purified from the cell wall of a specific yeast strain, were selected to stimulate macrophage activity and lymphocyte proliferation in the gastrointestinal tract, improving the immune modulation effects and enhanced vaccination response. *In vivo* studies showed that solutions combining the three above strategies, effectively reduced the negative effects of mycotoxins on the growth performance of farm animals.

OXIDATIVE STRESS IN PIGLETS FED DIETS CONTAINING PURIFIED MYCOTOXINS AND MYCOTOXIN DEACTIVATOR

E.S. Vivian[1], **Bruno A.N. Silva**[1], D.O. Fontes[2], M.S. Benfato[3], F.S. Hackenhaar[3], D.V. Jacob[4], R. Borutova[5], O. Averkieva[5], W.A.G. Araujo[6], L.L.M. Guedes[1], I.M.G. Lopes[1], M. Gonçalves[1], V.R. Lima[1] and L.A. Cardoso[1]

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Ingestion of mycotoxins by pigs can result in many problems, including decreased growth rates, liver damage, fertility reduction and immune suppression. The present study aimed to evaluate the impact of the supplementation of a mycotoxin deactivator in diets containing purified mycotoxins for piglets during nursery phase on their performance and oxidative status. A total of 90 piglets were used. Animals entered the experiment after weaning with 24 days of age (approximately 7 kg of body weight) and remained in the trial until 67 days of age. The trial consisted of five dietary treatments and six replications per treatment. The treatments consisted in feeding the animals with: a standard control diet considered a negative control (CN – mycotoxin levels at accepted regulatory Brazilian Ministry of Agriculture standards (deoxynivalenol (DON) <100 ppb, zearalenone (ZEN) <20 ppb, fumonisin (FB) <1 ppm aflatoxin <1 ppb); the standard diet with purified mycotoxins added to reach a low contamination level considered as positive low (CPL – DON 900 ppb, ZEN 100 ppb, FB 5 ppm) without deactivator; a positive low added deactivator (UNIKE Plus® 1 kg/ton); the standard diet with purified mycotoxins added to reach a high contamination level considered as positive high (CPH – DON 4,500 ppb, ZEN 500 ppb, FB 25 ppm) without deactivator; and a positive high added deactivator (UNIKE Plus® 5 kg/ton). Diets were formulated according to the growing stage of the animals. Pigs were individually evaluated at the beginning and at the end of each stage of the experiment. On day 7, 19, 34 and 43, from a subsample of 6 piglets/treatment, blood samples of approximately 10 ml were collected from the jugular vein into heparinised tubes for biochemical analyses for total superoxide dismutase (TSOD), glutathione peroxidase (GSHPx), malondialdehyde (MDA), vitamin C, vitamin E and vitamin A. Data were analysed according to a general linear procedure analysis of variance (GLM procedure of SAS, version 9.2). The treatments affected significantly (P<0.01) the performance and anti-oxidant metabolism. Pigs challenged with mycotoxins presented lower performance traits and lower TSOD, GSHPx, vitamin C, E and A levels, and higher MDA levels compared to the control and deactivator-associated treatments. Our findings clearly indicate that the use of a mycotoxin deactivator is of most importance, even under low contamination levels, to prevent productive losses and metabolic disorders in piglets during the nursery phase.

THE ROLE OF YEAST FRACTIONS IN MITIGATING THE NEGATIVE IMPACT OF MYCOTOXINS IN ANIMALS

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Despite many years of research, mycotoxin contamination of food and feed remains a topic of global concern. Many countries regulate for or suggest permitted levels of mycotoxins in foods and feed because of their public health significance and commercial impact. Indeed, mycotoxin contamination is considered an unavoidable and unpredictable problem, even where good agricultural, storage, and processing practices are implemented, leading to negative impact on animal health and significant economic losses [1]. In animals, left unchecked, mycotoxins to which cattle, sheep, horses, pigs and poultry are exposed, can cause serious health issues. There are many examples of depressed growth, loss of appetite, poor productive performance, poor reproductive efficiency and increased susceptibility to pathogens and diseases. Because of their adverse effects, several strategies have been developed for (i) reducing the growth of mycotoxigenic fungi and mycotoxin production, (ii) detoxifying contaminated

feed, (iii) decreasing the systemic availability once mycotoxins are ingested by the animal, and (iv) protecting against detrimental effects of the mycotoxins in animal cells.

This presentation aims to shed light on yeast-based solution for mycotoxin mitigation. Considerable research has been conducted to evaluate the potential animal growth performance and health benefits of adding yeast, yeast-derivatives, and yeast-containing ingredients into animal feeds [2]. In particular, supplementing animals with feed containing yeast cell wall has shown positive results at elimination of the mycotoxins and inhibition of their toxic effect. Beta-glucans are the most abundant poly-saccharides of the fungal cell wall. They are intermingled with chitins located near the plasma membrane, and mannans that are anchored to the outer cell wall, all of which are interspersed with glycoproteins. This specific composition and the resulting three-dimensional structure allow the yeast cell wall to bind mycotoxins and reduce the toxin bioavailability for the animals. The most important precondition for mycotoxin binding is the compatibility between the characteristics of a given mycotoxin (in terms of polarity, solubility, shape and charge distribution), and the surface properties and cell physiology of the yeast. In addition, yeast and yeast cell wall are immunomodulatory compounds that interact directly and indirectly with pathogens and components of the immune system. Yeast-based products, thanks to their immunomodulatory properties as well as their ability to maintain a favourable and healthy intestinal environment, contribute to reduce the toxic effects of mycotoxins.

References

1. Alshannaq, A. and Yu, J.-H., 2017. International Journal of Environmental Research and Public Health 14: 632.
2. Shurson, G.C., 2018. Animal Feed Science and Technology 235: 60-76.

MYCOTOXIN MITIGATION USING YEAST CELL WALL EXTRACTS IN THE CONTEXT OF MULTIPLE MYCOTOXIN CHALLENGE.

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ayiannikouris@alltech.com

The spread of the mycotoxin contamination has been demonstrated in many scientific works, owing to the advent of sampling plans (availability, precision and accuracy of simplex or multiplex detection methodologies) and fine characterisation and identification of mycotoxins, their metabolites or conjugated forms. Surveys applied to feedstuffs have shown that more than 95% of the samples returned positive results for one or more mycotoxins with at least 5 mycotoxins co-occurring on average based on an evaluation of 25,000 samples collected over 6 years and each evaluated for more than 37 toxins. The majority of the contamination involved chronic levels. Nevertheless, the multiplicity of mycotoxin types influenced by the complexity of feed matrices, the often-uncontrolled storage conditions, the blending of traded feed ingredients as well as the impact of unpredictable environmental weather patterns, has had an impact in animal production, economics, if not health.

In the same fashion that LC-MS/MS has enabled fast pace progression in the detection of mycotoxin in feed and food matrices, this technology is also essential for the understanding of the effectiveness of mitigation strategies. Using this approach, we have undertaken the evaluation of sorptive properties of yeast cell wall materials using realistic scenarios of multi-contamination profiles, and by readapting *in vitro* evaluation strategies. In complement, *in silico* characterisation of the docking energy involved in the interactions between beta-D-glucans chains, as major actor in the adsorption process, and 37 different mycotoxins showed potential differences in stability among toxins enabling a ranking of affinities according to electrostatic energies recorded from -3.6 down to -14.2kcal/mol for the most stable interaction. Number of docking sites, spread of affinity within each position, and degree of liberty of mycotoxins molecular structures were considered.

Finally, *in vivo* assessments of the mitigation efficacy of yeast cell wall extracts were undertaken in animal research trials, by evaluating the becoming of key mycotoxins, such as aflatoxin B1, zearalenone, or ochratoxin A ingested at chronic levels individually in a long-term exposure setting in pigs and poultry broilers. Evaluation of the degree of excretion in faeces of the ingested mycotoxin and its metabolites in pigs as well as distribution of the mycotoxin in liver and reproductive organs indicated that yeast cell

wall extracts could reduce the mycotoxin uptake and metabolism in pigs and poultry and increase its passage through the digestive tract.

COMPARATIVE *IN VITRO* ASSESSMENT OF A RANGE OF COMMERCIAL FEED ADDITIVES WITH MULTIPLE MYCOTOXINS BINDING CLAIMS

Oluwatobi Kolawole[1], O. Chevallier[1], D. Jones[2], L. Connolly[1] and C. Elliott[1]

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Contamination of animal feed with multiple mycotoxins is an ongoing and growing issue as more than 60% of crops worldwide have been suggested to be contaminated with more than one mycotoxin. The present study was carried out to assess the efficacy of commercial feed additives which carry multi-mycotoxin binding claims. Ten of these were obtained and categorised into three groups based on their main composition. Their capacity to simultaneously adsorb deoxynivalenol (DON), zearalenone (ZEN), fumonisin B1 (FB1), ochratoxin A (OTA), T-2 and aflatoxin B1 (AFB1) was assessed and compared using an *in vitro* model simulating a monogastric gastrointestinal tract. Results showed that only one product (a modified yeast cell wall) effectively adsorbed more than 50% of DON, ZEN, FB1, OTA, T-2 and AFB1, in the following order: AFB1 < ZEN < T-2 < DON < OTA < FB1. The remaining products were able to moderately bind AFB1 (44-58%) but had less or in some cases no effect on ZEN, FB1, OTA and T-2 binding (<35%). It is important to access the performance of binders under as real life situations as possible to fully understand their performance characteristics.

Acknowledgements
This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 722634.

DEVELOPMENT OF A LACTONASE FOR MITIGATION OF ZEARALENONE EXPOSURE OF FARMED ANIMALS

S. Fruhauf[1], M. Thamhesl[1], E. Kunz-Vekiru[2], H. Schwartz-Zimmermann[2], M. Aleschko[1], A. Höbartner[1], M. Killinger[1], F. Berthiller[2], G. Schatzmayr[1] and **Wulf-Dieter Moll**[1]

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Zearalenone was detected in 53% of more than 15 000 samples of wheat, maize and finished feed from all over the world, which were analysed as part of BIOMIN's annual Mycotoxin Survey in 2018. The average zearalenone concentration in contaminated samples was 122 µg/kg. This result illustrates why technology for mitigation of zearalenone contamination of feed would be desirable, despite good agricultural practice and mycotoxin concentration guidance values (e.g., 100 µg/kg in 'complementary and complete feedingstuffs for piglets and gilts' in the European Union).

We set out to investigate microbial conversion of zearalenone with the goal of finding an enzyme for use as feed additive for zearalenone degradation. We incubated mixed microbial cultures from soil samples and measured concentrations of added zearalenone with an LC-MS/MS method. Active cultures were enriched by repeated additions of zearalenone, and isolated strains derived from such cultures were tested individually. One such strain, PFA D8-1, was classified as *Rhodococcus erythropolis*. Its primary reaction product was hydrolysed zearalenone, which was non-oestrogenic in a bioassay with MCF-7 breast cancer cells. Clarified cell lysate also catalysed hydrolysis of zearalenone. Other strains of *R. erythropolis* were tested but none of them hydrolysed zearalenone. We cloned a genomic library of PFA D8-1 in an *E. coli*/*R. erythropolis* shuttle vector, transformed it in *R. erythropolis* PR4, and screened transformation clones for zearalenone hydrolysing activity. An active clone harboured a gene for an alpha/beta hydrolase in the cloning vector. We expressed this gene in *E. coli*, confirmed that it conveyed

zearalenone lactonase activity, and called it zenA, following conventions of bacterial gene nomenclature. The zenA gene mapped to a linear megaplasmid of *R. erythropolis* PFA D8-1, designated pSFRL1.

We produced the enzyme ZenA in *E. coli* and purified and characterised it. A database search revealed enzymes with related sequences, and we produced and characterised those, too. X-ray crystal structures were determined, and enzyme activity and stability were enhanced significantly by enzyme engineering. In a feeding trial with piglets, gastrointestinal activity of sequence variants of ZenA was confirmed by quantification of intact and hydrolysed zearalenone concentrations in urine samples. Subsequently, activity in the gastrointestinal tract of chickens and in bovine rumen was also confirmed. Further engineering of ZenA and optimisation of production are in progress to make enzymatic hydrolysis of zearalenone sufficiently robust and affordable for large scale application for mitigation of zearalenone contamination of animal feed.

YELLOW MEALWORMS ARE HIGHLY RESISTANT TO AFLATOXIN B1 AND ZEARALENONE – A POSSIBLE APPROACH FOR GRAIN 'DETOXIFICATION'?

Ronald Maul[1,2], N. Kroencke[1], K. Niermans[2], J. Woyzichovski[1] and R. Benning[1]

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Conventional livestock will not be able to cover the growing demand of the human population for protein of animal origin. Thus, there is an increasing need for protein from alternative sources for food and feed in the future. Insects could be an efficient source of animal protein incorporated in the food and/or feed production chain. On the global perspective, insects already have a long history as in many regions, e.g., Southeast Asia, China and Africa, insects have been consumed since a long time. In the USA, use of edible insects is still in in its infancy. Within the EU, the fat fraction and processed proteins of seven insect species are legally allowed in animal feed and increasing numbers are registered as novel food. Besides ecological and nutritional advantages compared to other protein sources, the high tolerance of some insect larvae for mycotoxin-containing feed is remarkable. However, studies on the mycotoxins aflatoxin B1 (AFB1), deoxynivalenol or zearalenone (ZEN) could often only show that the toxins are no longer detectable in the insects or residues, missing to reveal what happened to the biggest part of the ingested toxin amount. Before considering, e.g., yellow mealworm (*Tenebrio molitor*) larvae as suitable for human consumption, the possible accumulation of contaminants as well as their modified forms, must be evaluated.

The presentation focusses on two feeding studies in *T. molitor* larvae which were nourished on grain flour containing different levels of natural mycotoxin contamination as well as spiked ZEN and AFB1 levels. Biological parameters (survival rate and weight gain) were evaluated and parent mycotoxins along with their known metabolites were measured using LC-MS/MS technique. An average survival rate of almost 100% was found up to highest tested toxin amounts (2 mg/kg ZEN and 20 mg/kg AFB1) documenting the extraordinary tolerance of *T. molitor* larvae towards mycotoxins. No ZEN or ZEN metabolites were detected in the larvae after harvest. However, ZEN, α- and β-zearalenol (ZEL) were found in the residue samples consisting of faeces and small amounts of non-consumed feed. α- and β-ZEL were formed in the insects in equal amounts and accounted for up to 60% of the total mycotoxin recovery. Overall recovery of ZEN and its metabolites was high in the residue, indicating that for unknown metabolites only small amounts have to be expected. For AFB1 the amount of toxin that could be detected in the larvae accounted for 1-2% of the amount that was present in the feed the insects were reared on. Additionally, low amounts of AFM1 and presumably AFP1 and AFQ1 were detectable in the larvae. Larger quantities of AFB1 and its metabolites were detected in the residue. Apparently, *T. molitor* larvae are able to tolerate, efficiently excrete and also to transform the mycotoxins ZEN and AFB1. However, the intense formation of the more potent ZEN metabolite α-ZEL as well as the detailed metabolic fate of AFB1 should be addressed in further studies.

COLD PLASMAS: A POTENTIAL APPROACH TO MITIGATE MICROBIOLOGICAL AND TOXICOLOGICAL RISKS IN THE FOOD & FEED CHAIN

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Cold atmospheric pressure plasmas (CAP), generated at or near ambient temperatures, have received increasing attention for their potential application in a wide range of biological scenarios from treatment of biofilm infections, cancer therapy, stimulation of wound healing and, more recently, controlling both microbiological and chemical risks in the food and feed chain. Cold plasmas generate a rich and diverse chemistry at the site of application, comprised primarily of reactive oxygen and nitrogen species, which provides a safe, convenient and controllable antimicrobial approach to treatment of complex microbial consortia and their by-products.

This presentation will introduce the potential of cold plasma technology as an emerging technology in the agri-food sector, and its capacity to applied in a wide range of settings to control contamination caused by microorganisms, their microbial metabolites, such as signalling molecules and (bacterial and fungal) toxins, and exogenous chemical residues, including antibiotics.

SESSION 4
SMART STRATEGIES FOR EFFECTIVE MYCOTOXIN MANAGEMENT ALONG THE CHAIN: TOWARD FOOD & FEED 4.0 – PART 1. MYTOOLBOX

The project MyToolBox funded by the European Commission aims 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.

INTEGRATED MULTI-ACTOR PARTNERSHIPS IN THE EU AND CHINA AS THE KEY TO TACKLE MYCOTOXINS ALONG THE FOOD AND FEED CHAIN

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To tackle the mycotoxin issue, existing knowledge shall be combined with novel findings to bridge gaps on mycotoxin reduction along the food and feed chain. Hence, the MyToolBox project (www.mytoolbox.eu) has been launched in 2016 which is funded by the European Commission (EC) under the Horizon 2020 grant agreement No. 678012. The project applies a multi-actor and multi-disciplinary approach throughout the food and feed chain with 40% industry participation including five end-users and three well-known institutions from China, who collaborate closely with farmers and stakeholders from the industry. MyToolBox does not only pursue a field-to-fork approach to reduce mycotoxins in wheat, oats, maize, peanuts and dried figs, but also considers safe use options of mycotoxin contaminated batches. This increases the availability of high-quality food and feed in the EU as well as in China. Various agricultural strategies used so far have not been able to efficiently reduce the impact of fungal infection, let alone taking into account climate change related issues or other analytical challenges. The current situation pursues a novel and integrated multi-actor approach involving all actors in the food and feed chain, including the end-users. Consequently, the MyToolBox approach combines a series of integrated pre- and post-harvest measures, which enable a 20 to 90% reduction in losses of crops due to fungal and mycotoxin contamination, depending on the type of commodity and intervention. By using mainstream information and communication technology, losses and waste along the food and feed chain can be prevented, and traceable information to the supply chain and consumers can be provided. The end-user engagement, extending to food and feed industry, farming communities, agronomists, manufacturers, SMEs and academia from the EU and beyond, ensures the usability and applicability of the MyToolBox decision support system. This paper provides an update on the recent achievements of MyToolBox to control and reduce mycotoxins along the entire food and feed chain.

CONTROL OF FUSARIUM HEAD BLIGHT – ALTERNATIVES TO TRIAZOLE FUNGICIDES

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Chemical control of Fusarium head blight (FHB) is currently limited to a single chemical group, the triazoles. This creates a large selective pressure for fungicide resistance to develop. 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. Consequently there is a growing interest in the use of alternative products to control FHB. 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 old and new chemistry fungicides and biopesticides to control FHB and consequently reduce concentrations of deoxynivalenol (DON) in harvested grain. Products were compared to untreated control plots and plots treated with a standard triazole fungicide, prothioconazole. 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 general, old chemistry fungicides and biopesticides failed to adequately control FHB and DON. A new SDHI fungicide, Adepidyn™ developed by Syngenta was found to be highly effective at reducing FHB and DON both alone and in combination with prothioconazole. Once registered, this product will prove an important addition to the chemical control of FHB and DON contamination of cereals.

Acknowledgements
The MyToolBox project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 678012.

FORECASTING MYCOTOXINS IN GRAINS AT THE EUROPEAN LEVEL

H.J. van der Fels-Klerx, **Cheng Liu**, N. Liu, V. Manstretta and V. Rossi

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The use of forecasting models for mycotoxin occurrence enables better informed decision making on mycotoxin control along the cereal supply chain. Forecasting models can be used for short term on-farm decisions, like use of fungicides around the critical period of cultivation, or for mid-term on-farm decisions like the cultivar species used. In addition to the field or farm level, forecasting models can also be used at the regional level, for instance, by buyers for optimal routing and processing in the chain, and by food safety agencies for risk-based monitoring of mycotoxins.

To date, a variety of forecasting models are already available in Europe, but their aims, temporal and spatial scale, and modelling methods vary per country. As part of the H2020 MyToolBox project, available forecasting models were brought together and integrated as a model ensemble to be able to make predictions for European regions. New models were also developed under the MyToolBox project to enrich the model ensemble with different mycotoxin and crop combinations. A range of modelling techniques were used, including empirical modelling, mechanistic modelling, and Bayesian network modelling, as well as several of their combinations. Depending on the available data, the best performing technique was chosen. The models focused on forecasting deoxynivalenol (DON) in wheat and barley, zearalenole (ZEN) in wheat, and aflatoxins (AFs) in maize in the relevant growing areas in Europe. The developed models, together with the model ensemble, are integrated as part of the web-based decision-making platform for various actors in the cereal supply chain. During the conference, the available models and the model ensemble will be presented.

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COST-EFFECTIVE MONITORING OF AFLATOXINS IN MAIZE

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Monitoring of cereal batches for the presence of mycotoxins reduces the probability that a contaminated batch will be processed into feed or food. The objective of this study was to get insights into the most critical control points for aflatoxins along the maize supply chain, from the farmers to the feed producers, and into the number of batches and samples that needs to be collected per batch. A model was developed that optimises the number of batches sampled at each control point and the number of samples collected from each batch, with as constraint that the average aflatoxin concentration at the end of our chain did not exceed a pre-defined limit. As a case study, the focus was on a typical Dutch maize chain with maize grown in the Black Sea region, transported by ship to the Netherlands where the maize was used as ingredient in compound feed for dairy cattle. Four control points to monitor aflatoxins were chosen following current practices. The study shows that, if the aflatoxin concentration does not change along the supply chain, it is most cost-effective to collect samples and replace contaminated batches right after harvest since the replacement costs were the lowest. If aflatoxins are being produced during storage, and consequently their concentrations increase along the supply chain, it is most cost-effective to collect samples from all ship compartments and replace contaminated batches at the end of the chain to avoid having double costs for monitoring and replacing before and after transport. The further down the supply chain, the more stakeholders involved and the higher the replacement costs and/or recall costs. The optimal number of samples collected from the batches depends on the concentration. If the batch has a concentration far away (lower or higher) from the pre-set limit, a low number of samples is sufficient. If the batch has a concentration close to the pre-set limit, a high number of samples is needed and sometimes, even with a high number of samples, the probability to falsely classify the batch remains large.

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DYNAMIC REAL-TIME DECISION SUPPORT SYSTEM FOR MINIMISING MYCOTOXIN RISKS IN GRAIN

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Raw commodities are alive and respire at a basal level if stored under safe moisture content (m.c.) and temperature (T) conditions. The important safe m.c. for peanuts, wheat and maize are 8, 14 and 15% respectively, all equivalent to 0.70 water activity (a_w) at which no mould spoilage will be initiated. We examined the relationship between CO_2 production of stored wheat, maize and peanuts + associated mycobiota with/without mycotoxigenic mould inoculation to examine whether CO_2 production under different T and a_w levels to reflect good, intermediate and poor storage conditions can be used as a management tool. Overall, the respiration rate (R) increased with changes in interacting storage environmental conditions (a_w vs. T) impacting on dry matter losses. A_w and thus m.c. was a key parameter, as higher R occurred in the wettest conditions (0.95 a_w) examined. Interestingly, peanuts had a higher R rate base level when compared to cereals. Also, increases in T implied an increase in R at the same a_w level. Fungal spoilage could be detected very rapidly based on the increase in respiration relative to the baseline for safe storage.

Pilot scale studies using integrated sensors for CO_2, T and relative humidity (RH) for real time measurement of the changes in three dimensions was subsequently conducted at Barilla SpA (Italy). These studies supported the fact that CO_2 was a sensitive indicator of changes in the grain condition when a wet pocket was introduced. This showed that CO_2 changes occurred more rapidly than T. This suggests that biological activity can be detected very sensitively using CO_2, while T is a reflection of this activity and occurs much later. Indeed, mycotoxins were detected in the wet pocket samples, but not in the areas which remained at a safe m.c. Additionally, T and a_w models for *Aspergillus flavus* and *Fusarium graminearum* to identify specific risk combinations of these two parameters on fungal growth/mycotoxin production based on probabilistic models. This will help in the development of a real time post-harvest decision support system for improving commodity management during storage. This approach can be used to evaluate whether the risks of mould spoilage/mycotoxin contamination are low, intermediate or high. This will allow remedial actions to be implemented including aeration regimes or more rapid processing of such batches to minimise rejection based on quality criteria and mycotoxins.

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SYNERGIC POTENTIAL OF PRE-MILLING AND MILLING STRATEGIES TO MINIMISE MYCOTOXINS AND INCREASE FIBER CONTENT OF WHEAT-BASED PRODUCTS

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Whole grains (WGs) are one of the main examples of the minimally processed foods that are recognised as healthy by consumers. WGs contain many bioactive compounds, which are concentrated in the outer layers of the kernels [1]. Fibre certainly represents the most highly investigated bioactive compounds in cereals. Besides fibre, phenolic acids (PAs) and other phenolic compounds in general are receiving great attention, both for their relative abundance in cereal products and for their strong antioxidant properties. Several clinical studies from around the world show that a daily consumption of whole grain components can reduce the risk of cardiovascular disease and the development of diabetes, in addition to a reduction in the risk of cancer and mortality [2-4]. Producers must commit to guarantee a high level of safety of WGs food products, while developing creative recipes and food palatable proposals that encourage the public to use them. In fact, at the same time food based on grains (e.g., pasta, bread, bakery products) account for the largest contribution to mycotoxin exposure in all age classes, in particular due to the mycotoxins generated by Fusarium spp.

Within post-harvest interventions devoted to minimising mycotoxins impact with respect to diet intake, one of the first effective actions is to integrate novel down-stream processing approaches. Cleaning, debranning, peeling, soaking, dry- or wet- fragmentation, air separation etc. are efficient, proven and versatile, cost effective methods for companies to achieve high quality particle size reduction results and to allow more accurate separation of grain tissues with characterised different mycotoxin contamination levels. This presentation will illustrate how the synergic potential of these pre-milling and milling strategies permit to achieve an accurate separation of grain tissues with different mycotoxin levels, maximising the benefits of WGs foods and its components (mainly fibre and PAs) while mitigating the contamination risks (mainly DON but also pesticides & heavy metals) and the technological-organoleptic negative consequences.

Trials has been successfully conduced moving progressively from pilot plant to industrial scale level; then, focusing the attention on the main commodity derived from durum wheat chain, a novel pasta product concept was produced exploiting the optimised industrial raw materials thus obtained.

Acknowledgements
This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 678012 for the MyToolBox project.

References
1. Ciccoritti, R. *et al.*, 2017. Food Chemistry 225: 77-86.
2. Wu, H. *et al.*, 2015. JAMA Internal Medicine 175: 373-384.
3. Benisi-Kohansal, S. *et al.*, 2016. Advances in Nutrition 7: 1052-1065.
4. Zong, G. *et al.*, 2016. Circulation 133: 2370-2380.

MITIGATION OF DEOXYNIVALENOL DURING INDUSTRIAL BAKING: IS IT POSSIBLE?

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Deoxynivalenol (DON) is the most prevalent mycotoxin in cereal commodities. Although the population of industrial nations is exposed to DON mainly due to the consumption of bread and other bakery wares, it is not clear whether the toxicological impact of DON can be mitigated during industrial baking. After 30 years of research, the knowledge of degradation products that are formed from DON during baking and their toxicity is still incomplete. Furthermore, the extent of possible DON reduction is highly controversial. Although most studies found a reduction of DON, some even up to 50%, increases of up to 40% were also reported.

To determine whether the toxicological impact of flour that is contaminated with DON can be mitigated during the industrial production of bakery wares we:

- elucidated the full spectrum of DON degradation products by an untargeted liquid chromatography high resolution mass spectrometry approach using 13C labelling;
- compared the cytotoxicity of an important degradation product to DON;
- determined the amount of DON that is degraded during the production of crackers, biscuits and bread by targeted LC-tandem mass spectrometry (MS/MS) analysis; and
- evaluated the influence of different process parameter (e.g., baking temperature and time) on DON mitigation.

Our holistic approach in combination with the currently most accurate analytical methodology to determine DON degradation enabled us to clarify (i) how much DON is degraded during the production of crackers, biscuits and bread, (ii) whether DON degradation actually causes less toxicity, and (iii) how the choice of process parameter can maximise DON mitigation.

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MYCOTOXINS DURING THE PROCESSES OF NIXTAMALIZATION AND TORTILLA PRODUCTION

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Tortillas are a staple food in Mesoamerican and Hispanic cuisine and obtain also increasing popularity on a global level. Production of maize tortillas covers an alkaline-cooking process, called nixtamalization. Here, whole kernels are cooked and steeped in lime water. During this procedure, which improves also the nutritional value of the grain, the maize endosperm is softened and the pericarp loosened. During washing of the alkaline-cooked kernels (nixtamal), the kernel pericarp, the tip cap, and the germ are mostly removed. After grinding of the nixtamal to a maize dough (masa), tortillas are produced from that dough involving a short baking step. The entire process of tortilla production can affect mycotoxins in various ways. First of all, mycotoxins can be physically fractionated during nixtamalization. This takes place by leaching of water-soluble mycotoxins into the liquid fractions and/or by removing kernel material (pericarp, cap tip, germ) with potentially higher mycotoxin concentrations. Furthermore, processing comprises alkaline conditions and elevated temperatures. Those can result in partial degradation and/or modifications of the chemical structure of mycotoxins and might also affect binding processes in the matrix.

Several studies analysed the impact of alkaline cooking and tortilla making on aflatoxins and fumonisins, and show a high potential to reduce mycotoxin concentrations. Aflatoxin levels of raw maize were in tortillas often found to be lowered by around 50-100%. For fumonisin B1, the reduction described in the literature mainly amounted to approx. 75-100%. However, part of the initial mycotoxins can remain in the final product as undetectable or modified forms, which might also harbour some toxicity or might be reconverted into the parent form during digestion. The presentation will give an overview on the fate of aflatoxins and fumonisins during nixtamalization and tortilla production by taking modified and matrix-associated forms, as far as investigated, into account.

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SAFE USE OPTION – DETOXIFICATION OF MYCOTOXINS DURING BIOETHANOL PRODUCTION BY USING ENZYMES

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Maize is frequently used for the production of bioethanol. In the USA, more than 40% of the maize crop is used for production of ethanol [1]. In this process, carbon dioxide and distillers dried grains with solubles (DDGS) are produced besides bioethanol. As mycotoxins are very stable compounds, they can also withstand the conditions during ethanol production and hence accumulate in DDGS by a factor of 3 [2]. This poses a risk to livestock animals that consume the protein rich DDGS as part of their diet.

The rationale of the work package ‘Safe use options of contaminated batches‘ of the EU funded MyToolBox project was to reduce the concentration of certain mycotoxins (fumonisins and zearalenone) during the bioethanol process and hence to minimise the impact of fungal toxins on animal health. Additionally, such a process could offer an alternative and safe use option for highly contaminated batches, which otherwise could not be used elsewhere due to the regulatory limits for mycotoxins in food and feed. The approach in this project was to leverage the technology of mycotoxin degrading enzymes from the animal feed industry to the bioethanol process. Lab scale experiments were carried out to evaluate the efficacy of mycotoxin detoxifying enzymes (FUMzyme® and ZENzyme®) to degrade fumonisin B1 (FB1) and zearalenone (ZEN) in the bioethanol production process. Lab-scale bioethanol

process simulations were performed in 450 ml-scale using naturally contaminated maize (2,324 µg/kg FB1, 1,486 µg/kg ZEN). FUMzyme® and ZENzyme® were either added before liquefaction or before fermentation. FB1, its non-toxic metabolite hydrolysed FB1 (HFB1), ZEN and its not oestrogenic metabolite hydrolysed ZEN (HZEN) were quantified by LC-MS. The inclusion of FUMzyme® (60 U/kg maize) led to a 97% reduction of FB1 in the mash. Similarly, the addition of ZENzyme® (40 U/kg maize) during fermentation led to an 89% reduction of ZEN.

After the successful process simulation in lab scale an experiment in pilot scale (60 L) was carried out. For this, naturally contaminated maize (7,160 µg/kg FB1, 4,670 µg/kg ZEN) was used. The mycotoxin degrading enzymes were only spiked during ethanol fermentation. At the beginning and after 72 h of fermentation, the mycotoxins and their non-toxic metabolites were measured in the supernatant and the pellet. No FB1 could be detected in the supernatant and the pellet after 72 h. The recovery rates for ZEN after fermentation were 19.5 µg/kg and 177µg/kg in the supernatant and the pellet, respectively. The detoxification rates therefore could be calculated 100% for FB1 and greater than 90% for ZEN. These experiments revealed that specific mycotoxin degrading enzymes can be used in the bioethanol process to significantly reduce the content of certain mycotoxins leading to high quality DDGS for the benefit of both, bioethanol and livestock producers.

Acknowledgements
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References
1. Niphadkar, S. *et al.*, 2018. Biofuels 9: 229-238.
2. Schatzmayr, G. and Streit, E., 2013. World Mycotoxin Journal 6: 213-222.

BIOLOGICAL DETOXIFICATION OF MYCOTOXINS IN MAIZE AND ITS PRODUCTS

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Maize and its processed products (steep liquor, hulls, germ meal, distillers dried grains with solubles, etc.) are the main feedstuffs that are seriously contaminated by mycotoxins, including deoxynivalenol, zearalenone, aflatoxin B1, ochratoxin A, fumonixin B1, T-2 toxin, etc.), endangering the health of farmed animals and causing huge economic losses. A large number of microorganisms that could efficiently degrade mycotoxins, were screened from soil, hot springs and fermented food and so on. The microbial species were determined and the toxicity of the degraded products was studied and identified. The screened probiotics and the strains in the Catalogue of Strains for Feed Additives were directly obtained as microorganism preparations that could degrade mycotoxins. What is more, the detoxifying enzyme preparations could be made through the following sequential technics: detoxifying genes were identified via comparative transcriptome analysis, and protein isolation and purification technics, and then highly expressed enzymes were generated by engineering recombinant *E. coli* and yeast. It was found that detoxifying microorganisms and detoxifying enzymes could effectively alleviate the amount of mycotoxin in maize and its related products. Besides, a small and middle scale of fermentation of detoxifying microorganisms and detoxifying enzymes was carried out. In order to realise the extensive application in food and feed processing, we also conducted a screen for the acidity- and heat-resistant strains and engineered the detoxifying enzymes that were tolerant to acid and hot environment.

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IN VIVO EVALUATION OF THE EFFICACY OF AFLATOXIN B1 AND FUMONISIN B1 DETOXIFYING FEED ADDITIVES IN CHINA

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In vivo experiments in dairy cows and pigs have been conducted at the research facilities of the Feed Research Institute (Chinese Academy of Agricultural Sciences) in order to assess the efficacy of mycotoxin detoxifying feed additives under local conditions in China. Feeding trials have been performed according to (i) Commission Regulation (EC) No 429/2008 of 25 April 2008 on the preparation and the presentation of applications and the assessment and the authorisation of feed additive, and (ii) the scientific opinion of the European Food Safety Authority (EFSA) on guidance for the preparation of dossiers for technological additives. The aim is to compare the methods for evaluation of the efficacy of mycotoxin-detoxifying feed additives, which will contribute to the standard setting for authorisation of mycotoxin-detoxifying feed additives in China. In the animal trial of cows, 600 kg body weight Holstein dairy cows with 2-3 parities, close lactation days were selected for the experiment. Forty Holstein cows received four different treatments, control diet, aflatoxin B1 (AFB1) contaminated with 8μg AFB1/kg DM diet, AFB1 contaminated diet supplemented with an EU authorised feed additive and AFB1 contaminated diet supplemented with a local feed additive. The experimental animals were divided into four groups, and 10 cows were randomly allocated to each group. The results showed that there was no significant difference between the different treatment groups concerning milk yield, milk composition, and some blood biochemical indexes (P>0.05). The metabolite aflatoxin M1 (AFM1) could also be detected in the milk even at the pretty low concentration of AFB1 diet. The transfer rate of AFB1 from feed to milk (AFM1) showed no significant difference between the different treatment groups. In another animal trial of pigs, thirty-two healthy three-way crossbred weaned piglets of 50-days-old weighing about 20 kg were randomly divided into four groups, received four different treatments, control diet, contaminated diet with 5 mg FB1/kg, contaminated diet supplement with an EU authorised feed additive, and contaminated diet supplemented with a local feed additive. The results showed that there was no significant difference between the different treatment groups concerning growth performance (P>0.05). However, the 'biomarker' Sa/So ratio in serum of contaminated diet group was significantly increased compared with the control diet group (P<0.05). The addition of the EU authorised feed additive could significantly decrease the Sa/So ratio in serum(P<0.05), while there was no significant difference on the Sa/So ratio in serum between contaminated diet and contaminated diet with the local feed additive group (P>0.05), which indicated that the EU authorised feed additive is effective in the detoxification of FB1 *in vivo*.

INTENTION OF EUROPEAN WHEAT FARMERS TO USE DECISION SUPPORT SYSTEMS FOR *FUSARIUM* SPP. MANAGEMENT

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Because of the inherent difficulty to remove mycotoxins down the cereal supply chain, mycotoxin management mainly focusses on reducing initial fungal infection and production of mycotoxins in the field and during storage. At the moment, two Horizon 2020 projects, MyToolBox (www.mytoolbox.eu) and MycoKey (www.mycokey.eu), are working to improve mycotoxin management by developing tools to support mycotoxin management along the chain. It has been shown that the use of a decision support system reduces external inputs (i.e., seeds, fungicides, and fertilizers) and costs, maintains or increases crop yield and quality, and keeps mycotoxin contamination below the legal limit. Farmers play a key role in the prevention and control of mycotoxin contamination by applying various pre-harvest measures to reduce *Fusarium* infection and mycotoxins in wheat. It is therefore important to understand farmers' characteristics to get a better insight in how to stimulate a future change in their mycotoxin management and to encourage the uptake of decision support systems.

Within MyToolBox, data on mycotoxin management and farm(er) characteristics were collected from wheat farmers in the Netherlands, Serbia, Italy, UK and Austria by means of an online questionnaire. Data collected included amongst others: use of a decision support system, risk perception, age, gender, education, risk aversion, farm size, and mycotoxin knowledge. The aim of this study is to explore the use of decision support systems by European wheat farmers in relation to their farm(er) characteristics. Outcomes of the analysis can be used by processing industries, government agencies and farmer cooperatives to design a targeted approach for farmers to implement the designed decision support systems and further reduce mycotoxin contamination. The analysis is in progress: the final results will be ready for presentation and discussion during the conference.

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E-PLATFORM FOR MYCOTOXIN PREVENTION AND CONTROL ALONG THE CHAIN

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The occurrence of mycotoxins in various crops, mainly grain, fruits and nuts, produced in Europe is of major concern since it has large implications for food and feed safety, food security and international trade. Over the past three decades, a wide variety of knowledge has been gathered on prevention and control of mycotoxins, at the various stages of the feed and food production chain. This knowledge is, however, not easily retrievable and available for the intended end-users at the private level (feed and food companies) and the public level (governmental agencies).

A user-friendly and interactive web-based platform was developed as part of the H2020 project MyToolBox, which brings together all available information on prevention and control of mycotoxins, ready to use by the end-user. This platform focuses on the most important mycotoxins in small grain cereals and maize, dried figs and nuts. The platform has three main components, including: the pre-harvest forecasting system for mycotoxin contamination in the field; the post-harvest forecast systems for mycotoxin presence in silos; and the static guidelines for prevention and control over the entire chain. The forecasting module includes several mathematical models that are combined by using a model ensemble approach, which makes early predictions of mycotoxins occurrence at harvest possible for entire Europe. It uses weather data and agronomic information as inputs and provides either specific field predictions or European wide maps with predicted toxin risks as outputs. The post-harvest module makes use of CO_2, T and RH sensors in the silos, and fungal growth models to predict conditions suitable for toxin production in the silo. The static guidelines cover the various stages of the supply chain: prevention and control prior to harvest, during storage and transports, during processing for feed production, during processing for food production, and legal issues.

The MyToolBox platform will serve the needs of the various actors along the feed and food chain, including farmers, processors, feed suppliers or manufacturers from the food and feed industry, as well as governmental bodies. The system will be launched mid-2019 and will be demonstrated during the conference.

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TUESDAY 15 OCTOBER 2019

SESSION 5
FATE OF FREE AND MODIFIED MYCOTOXINS
From exposure to metabolism and degradation: a collection of recent research.

DONEXPO PROJECT: EXPOSURE TO DEOXYNIVALENOL AS FREE FORM AND ITS MAIN URINARY METABOLITES FOUND IN ITALY, UK AND NORWAY

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Deoxynivalenol (DON) is one of the most commonly occurring trichothecenes, produced mainly by *Fusarium graminearum*. Levels of total DON and de-epoxy deoxynivalenol (DOM-1) in human urine samples collected from different population groups (children, adolescents, adults, elderly, vegetarians, pregnant women) in Italy, Norway and the UK were analysed by liquid chromatography-mass spectrometry (LC-MS). Morning urine samples were collected over two consecutive days from 635 volunteers and associated food consumption was recorded on the same days. Levels of DON did not significantly differ between day 1 and day 2 urine samples. DON was detected in 99, 93 and 76 % of the urine samples from Norway, UK and Italy, respectively. The median total DON concentrations were similar between population groups in Italy and Norway but were approximately 3-fold higher in the sampled UK population. In Norway and the UK, levels of DON were roughly 2.5-fold higher in children compared with adults. For DOM-1, 12% of Norwegian and 1.5% of Italian urine samples were positive but DOM-1 was not detected in any sample from the UK. This difference may be explained by differences across analytical sites in the limit of quantification (LOQ). Associations between food consumption and urinary DON levels were assessed by ordered logistic regression models. In Italy, intakes of pasta and pasta-like products were significantly associated with higher levels of total DON after correction for creatinine on both days. In Norway, intakes of breakfast cereals and snacks (day 1) and bread and bread-like foods (day 1 and 2) were significantly associated with a higher level of total DON adjusted for creatinine. In the UK, biscuit intakes on day 1 were significantly associated with a higher level of the toxin.

ELUCIDATION OF THE MYCOTOXIN HUMAN TOXICOKINETICS: THE KEY FOR AN ADEQUATE BIOMONITORING EXPOSURE PROGRAMME

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Biomarker analysis has been proposed to assess human exposure to xenobiotics by using concentrations of the parent compounds and/or metabolites in biological matrices such as urine or blood. However, the gap of information on human mycotoxin absorption and excretion profiles generates many discrepancies to link mycotoxin (biomarkers) concentrations found in biological fluids with the mycotoxin dietary intake. Toxicokinetic studies permit to elucidate the process of the uptake of dietary mycotoxins by the body, their biotransformation, the distribution and the elimination of the mycotoxins and metabolites. Although mycotoxin toxicokinetic models have been developed in animals, the differences in metabolism among animals and humans are substantial.

At Ghent University, various human intervention studies were carried out with International Agency for Research on Cancer (IARC) Group 3-classified mycotoxins, namely deoxynivalenol, patulin, citrinin and

nivalenol (not classifiable as to its carcinogenicity to humans). Adult volunteers received a mycotoxin bolus at tolerable daily intake level (TDI, µg/kg body weight/day) after following a restricted mycotoxin-free diet for 4 days. After mycotoxin intake, all urine and faecal discharges for 24-48 h and blood samples at indicated time points were collected and analysed by LC-MS/MS. Common mycotoxin biomarkers of exposure were verified and quantified (for example, deoxynivalenol-3-glucuronide, deoxynivalenol-15-glucuronide and dihydrocitrinone). The preliminary results achieved after deoxynivalenol and deoxynivalenol-3-glucoside showed that 64% of deoxynivalenol and 58% of deoxynivalenol-3-glucoside were recovered in urine after a fast excretion of these toxins in urine (<16 h). Moreover, it was confirmed that glucuronides compounds, mainly deoxynivalenol-15-glucuronide (>50%), were formed and could attribute as a biomarker of exposure [1]. The biological samples' analyses permit to exemplify the adsorption rate, distribution level, metabolization rate and excretion of the dietary mycotoxins. From all these data, modelling and simulation approaches are described for each mycotoxin, and can further be used to determine: (i) the preferred (set of) urinary/blood biomarker(s) of exposure; (ii) the preferred urinary/blood collection period; (iii) a method to estimate the dietary exposure to these mycotoxins; and (iv) set-up adequate biomonitoring programmes.

References

1. Vidal, A. *et al.*, 2018. Scientific Reports 8: 5255.

OCHRATOXIN A AND 2'*R*-OCHRATOXIN A: NEW INSIGHTS INTO PHARMACOKINETICS AND METABOLISM

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Ochratoxin A (OTA, Figure 1) is a toxic secondary metabolite produced by several fungal species of the genera *Penicillium* and *Aspergillus* that can contaminate food and feed. During coffee roasting, OTA isomerises up to 26% to 2'*R*-ochratoxin A (2'*R*-OTA, Figure 1), resulting in regular exposure to 2'R-OTA through coffee consumption [1].

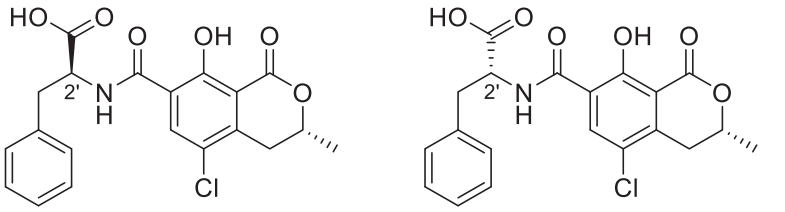

Figure 1. Molecular structures of OTA and its thermal isomer 2'*R*-OTA.

In plasma samples of coffee drinkers from Germany, the detected *2'R*-OTA levels were in average half of those found for OTA but in certain cases even exceeded the concentration of the parent compound [2]. These observations are surprising as OTA from coffee consumption only contributes to approx. 12% of average OTA uptake and 2'*R*-OTA to only 3% of total ochratoxin exposure. Thus, to understand the high levels of 2'R-OTA in the blood stream, a human intervention study with coffee drinkers was carried out [3]. The obtained data show an extremely stable 2'*R*-OTA level and an extraordinary long biological half-life of approx. 7 months. Additional binding studies with human serum albumin (HSA) surprisingly showed that this effect cannot be attributed to higher binding affinities of 2'*R*-OTA towards HSA compared to OTA [4].

To investigate the differences between the metabolism of OTA and 2'*R*-OTA, urine samples from coffee drinkers were screened for the presence of further OTA metabolites, especially phase-2 metabolites related to the reaction with glutathione. Using synthesized references of an ochratoxin-glutathione adduct (OTB-GSH), the corresponding urinary metabolite ochratoxin-*N*-acetyl-L-cysteine (OTB-NAC), and their stable isotope labelled analogues, it was possible to detect OTB-NAC in 11 out of 18 analysed urine samples. OTB-NAC was quantified in 5 of the urine samples in a range between 0.039 and

0.176 ng/mg creatinine which is in the same range as the determined OTA concentration. 2'*R*-OTA was only detected below the LOQ, while OTB-GSH and a possible 2'*R*-OTB-NAC were not found in the urine samples.

Acknowledgements
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References
1. Cramer, B. *et al.*, 2008.Journal of Agricultural and Food Chemistry 56: 5673-5681.
2. Cramer, B. *et al.*, 2015. Molecular Nutrition & Food Research 59: 1837-1843.
3. Sueck, F. *et al.*, 2019. Molecular Nutrition & Food Research 63: e1801026.
4. Sueck, F. *et al.*, 2018. Toxins 10: 256.

MICROBIAL HYDROLYSIS AND METABOLISM OF MYCOTOXINS BY INTESTINAL MICROBIOTA

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Numerous plant-bound mycotoxins have been identified and detected in various cereal-based commodities. These bound mycotoxins have been found in animal feed and human food, and the current work investigates the microbial hydrolysis and metabolism of a range of plant-bound *Fusarium* mycotoxins by intestinal microbiota. Mixed microbial incubations were performed by spiking individual mycotoxins (deoxynivalenol, DON; nivalenol, NIV; T2 and HT2 toxins; diacetoxyscripenol, DAS; zearalenone, ZEN; fumonisin B1, FB1) into microbial slurries and incubating anaerobically up to 72 h. Similarly, overnight cultures of single bacterial strain were spiked individual mycotoxins and bacterial growth and mycotoxin metabolism were assessed after 48 h of anaerobic incubation. Mycotoxins were analysed using LC-MS/MS.

We have demonstrated efficient hydrolysis of sugar moieties from glycosylated trichothecenes (B DON-Glc, NIV-Glc and A T2-Glc, HT2-Glc, DAS-Glc) as well as ZEN-Glc [1] and NDFrc-FB1 by mixed human faecal microbiota. The rates and efficiencies of hydrolysis varied between different groups with type B trichothecenes being hydrolysed similarly whereas type A trichothecenes contained the mycotoxins with the fastest (HT2-Glc) and slowest (DAS-Glc) metabolism. Glycosylated ZEN derivatives were hydrolysed most rapidly and NDFrc-FB1 was hydrolysed the slowest of all compounds. Deepoxydation of DON to DOM-1 was observed in faeces from 1 out of 5 volunteers and DOM-1 absorption from the large intestine was confirmed *in vivo* through urinary analysis [2]. Deacetylation of trichothecenes was also observed with DAS-Glc and DAS resulting in 15-MAS production while T2-Glc and T2 led to HT2 formation. Using porcine intestinal microbiota, we demonstrated that mycotoxin hydrolysis will occur in the terminal small intestine and throughout the large intestine with microbiota from the caecum, colon and faeces being equally efficient in hydrolysing DON-Glc [3]. In an attempt to identify the main microbial groups responsible for mycotoxin metabolism, single strain isolates representing the major bacterial groups of the human gut were used. *Bifidobacterium adolescentis* and *Lactobacillus plantarum* were confirmed as hydrolysing DON-Glc, but only *B. adolescentis* also hydrolysed HT2-Glc. Several strains belonging to *Lachnospiraceae*, *Ruminococcacea* and *Bacteroides* groups were tested and found to vary in their ability to hydrolyse glycosylated mycotoxins. Taken together, our results clearly show that all glycosylated mycotoxins tested are efficiently hydrolysed by human gut microbiota, although large differences were observed between mycotoxins in their speed of release. *B. adolescentis* remains the most efficient single strain of human gut microbiota to hydrolyse glycosylated mycotoxins, but further work will investigate other microbial contributors.

References
1. Gratz, S. *et al.*, 2017. Molecular Nutrition & Food Research 61: 1-10.
2. Gratz, S. *et al.*, 2013. Applied and Environmental Microbiology 79: 1821-1825.
3. Gratz, S. *et al.*, 2018. Applied and Environmental Microbiology 84: e02106-17.

FATE OF ERGOT ALKALOIDS DURING LABORATORY SCALE DURUM PROCESSING AND PASTA PRODUCTION

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Samples of Canadian Western Amber Durum (CWAD) containing varying amounts of ergot sclerotia were milled and used to prepare spaghetti. Milling products, fresh pasta, raw dried pasta, and cooked pasta were analysed for ergot alkaloids in order to investigate the fate of these mycotoxins. A sample of No. 1 CWAD was cleaned and hand-picked to remove sclerotia and any discoloured kernels. Sub-samples of this cleaned CWAD were fortified with ergot sclerotia (0.01–0.1% by mass) hand-picked from other naturally-infected CWAD samples. Along with an unfortified portion of the cleaned CWAD, all fortified samples were milled on a laboratory mill combined with a small-scale semolina purifier. Milling fractions were collected for ergot alkaloid analysis; semolina was sub-sampled and used to prepare spaghetti. Dried spaghetti was cooked, and portions of cooked pasta and cooking water were collected for ergot alkaloid analysis. All samples were analysed for 10 ergot alkaloids using liquid chromatography with tandem mass spectrometry. Approximately 84% of the ergot alkaloids were present in the bran, shorts, and feeds. These milling product fractions are associated with the outer kernel layers. Ergocristine, ergocristinine, and ergotamine were the predominant ergot alkaloids observed in the milling fractions and spaghetti. Consistent loss of ergot alkaloids was not observed during production nor cooking of spaghetti. However, differences in the ratio of *R*- to *S*-enantiomers were noted amongst milling fractions and were observed during cooking of spaghetti. Milling fractions containing bran, shorts, and feeds, as well as cooked spaghetti, contained a higher proportion of *S*-enantiomers.

TUESDAY 15 OCTOBER 2019

SESSION 6
MYCOTOXINS: OCCURRENCE, EXPOSURE AND EFFECTS
The latest developments and new challenges in relation to the impact of mycotoxins on human and animal health will be presented.

CADMIUM AND DEOXYNIVALENOL IN DURUM WHEAT GRAINS: PHYSIOLOGICAL AND BIOLOGICAL BASIS OF THE CO-CONTAMINATION

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Cadmium (Cd) and mycotoxins are among the most worrying contaminants that threaten the safety of food products derived from cereal kernels. Indeed, as recently supported by the last French total diet survey [1], deoxynivalenol (DON) and Cd human exposure through food mainly results from the consumption of cereal-derived products. Durum wheat is the most sensitive cereal culture for both DON and Cd accumulation in kernels, leading to a high frequency of co-contaminated harvests. This frequent co-occurrence (even though each toxic substance is in concentration within the EU regulatory limits) combined with the fact that Cd and DON are likely to be distributed in the same milling fractions raises the concern of consumer exposure to the cocktail Cd+DON.

To address the issue of DON+Cd co-contamination, the CaDON initiative (funded by the French National Agency for Research, 2015-2019) investigates the relations between Cd and DON occurrence in durum wheat, from crops co-contamination in the field to the milling end products, as well as the toxicity of Cd and DON mixtures upon ingestion. One of the objectives pursued by the CaDON project aims to elucidate the physiological bases of Cd+DON contamination of durum wheat kernels. The effect of soil Cd contamination on *Fusarium graminearum* infection and DON accumulation in kernels and conversely of *F. graminearum* infection on Cd accumulation were investigated through the implementation of greenhouse experiments. This presentation will focus on the results coming from this experiment.

References
1. Anses, 2011. https://www.anses.fr/fr/content/les-etudes-de-lalimentation-totale-eat

HOUSEHOLD-LEVEL AFLATOXIN CONTAMINATION IN RURAL VILLAGE FOOD SYSTEMS: TOWARD A PARTICIPATORY ACTION RESEARCH APPROACH

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Aflatoxin contamination threatens food value chains around the world. The bulk of exposure risk is shouldered by resource-poor farming communities in developing countries. In northern India, the food system includes a broad range of seasonal commodities that are moderately vulnerable to contamination. An understanding of the spatiotemporal landscape of risk in these communities would support the implementation of farmer-friendly diagnostics and interventions.

Here, we present the initial findings of an ongoing investigation on the utility of participatory action research (PAR) as a food system-wide surveillance and capacity-building tool in rural Indian communities. Since November 2017, we have engaged ~200 households across 6 small farming communities (population <1,000) in Unnao District, Uttar Pradesh, in a participatory longitudinal survey

of aflatoxin accumulation in household grain storage systems. We have established a farmer research network (FRN) in these communities, which aims to empower farmers to diagnose and develop solutions to food spoilage concerns. Our ongoing investigations seek to elucidate the complex seasonal dynamics of mycotoxin accumulation in Indian village food systems, and to test the efficacy of our participatory approach as a scalable, sustainable, locally-sensitive tool for bolstering resilience against mycotoxigenic moulds and other food safety threats. Our year-long survey of maize, groundnut, pearl millet, paddy (unmilled rice), and milled rice samples collected from participating households had rates of detection (>1 ppb AFB1) of 70, 57, 62, 51, and 40%, respectively. As expected, maize and groundnut samples exhibited the greatest magnitude of contamination, with 57 and 31% of samples exceeding the Indian regulated limit for aflatoxins (15 ppb), respectively. Paddy and milled rice, typically considered low-risk commodities, exhibited high rates of contamination in excess of the regulated limit (23 and 29%).

PAR was used to identify post-harvest issues in the target communities, with storage pests and excessive moisture found to be the highest priorities across the study area. We therefore deployed hermetic grain storage bags in a network-wide trial intended to familiarise communities with FRN concepts. After 5-7 months of storage trial, the technology prevented insect infestation and grain spoilage in 91% of surveyed households. Pending market availability, 100% of farmers reported that they would like to continue using the technology in future seasons. Among the respondents, 90% felt 'very confident' in their understanding of the technology. Cost was the most important barrier to widespread adoption. Presently, we are scaling up FRN programming and developing a model for participatory management of mycotoxins and related concerns.

HUMAN BIOMONITORING TO ESTIMATE EXPOSURE TO DEOXYNIVALENOL AND ZEARALENONE: A COMBINED 24-HOUR DUPLICATE DIET – 24-HOUR URINE STUDY

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Human exposure to mycotoxins is mostly based on food analysis data combined with food consumption data [1]. Human biomonitoring is an alternative way to assess exposure [2,3]. Mycotoxins can exist in several modified forms which are often not covered in food monitoring. Biomonitoring might be beneficial to assess the overall internal exposure. However, for use in risk assessment in food safety, it is necessary to link internal exposure data from human biomonitoring with external exposure through food.

To gain insight in such relationship, a pilot study was conducted in the Netherlands in 2018 in which duplicate diet samples and 24-hour urine samples* (until next morning's first void) were collected from the same 35 persons on the same day. In addition, food items consumed were recorded. Duplicate diets were analysed for deoxynivalenol (DON) and related compounds (3- and 15-acetyl-DON, DON-3G), and for zearalenone (ZEN) and related compounds (ZEN, a/b-ZEL, a/b-ZAL, ZAN), using LC-MS/MS based methods. Urine was analysed using dedicated methods involving enzymatic deconjugation and immunoaffinity clean-up in order to reach appropriate LOQs (<0.25 ng/ml for DON biomarkers, <0.02 ng/ml for ZEN biomarkers). In this presentation, first the outline of the study design and the analytical methods used will be presented. Then the data will be presented and discussed. DONs and biomarkers were found in all duplicate diet samples (2-16 ng/g) and urine samples (3-32 ng total-DON/ml). ZENs could also be detected in most of the samples, but at much lower concentrations (0.2-10 ng/g, and 0.02-0.5 ng/ml, respectively). For each individual, the ratio urinary excretion of the biomarker vs dietary intake was determined. The median ratio for DON was 84% which corresponded reasonably well with that reported in literature [3]. For ZEN the median was 67%. Especially for ZEN much higher and lower ratios were observed at an individual level, indicating that excretion may take longer than 24 h. The data indicate that human biomonitoring can be a valuable alternative to food monitoring, but also that more data on toxicokinetics (especially for ZEN) are required for adequate establishment of urinary biomonitoring equivalents related to health-based guidance values.

* Ethical approval was obtained through the METC of Wageningen University.

References
1. De Nijs, M. *et al.*, 2016. World Mycotoxin Journal 9: 831-845.
2. Choi, J. *et al.*, 2014. EFSA supporting publications 2015:EN-724, 321 pp.
3. Brera, C. *et al.*, EFSA supporting publication 2015:EN-818, 136 pp.
4. Vidal, A. *et al.*, 2018. Scientific Reports 8: 5255.

COMPLEMENTARITY OF INTERNAL AND EXTERNAL DIETARY MYCOTOXIN EXPOSURE: A COMPREHENSIVE STUDY IN FIVE EUROPEAN POPULATIONS

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Over the course of a human lifetime, dietary exposure to mycotoxins may be considered unavoidable. Even in the highly regulated food supply and sanitary living conditions of the developed world, nutritious and energy-dense agricultural products attract fungal colonisation throughout their production cycle. Mycotoxin contaminants in the food supply have the opportunity to be absorbed through the gastrointestinal tract, into the circulatory system. Since the initial drainage from intestinal vasculature passes first through the liver, hepatic metabolism of these toxins is able to transform some of them for rapid clearance from the body, via the urinary system. The broad variation in chemical structures among mycotoxins leads predictably to a broad variation in rates of metabolism. Therefore, in case mass equivalence is expected between dietary and biological analyses, it would be necessary to quantify all initial and metabolised forms over time, from the plate, through endogenous fluids, to excreta.

This presentation will highlight the relative utilities of different internal exposure measurements and external exposure estimates, particularly at the individual level. This comparison of cross-sectional survey methods includes human populations from five European nations, namely Belgium, the Czech Republic, France, the Netherlands and Norway (n=600). The population characterisation, dietary data, and biological samples were obtained from the European Food Consumption Validation (EFCOVAL) project, which was completed in 2011. The repeatability of short-term mycotoxin exposure measurements was investigated via repeated urine and dietary intake mycotoxin measurements, while long-term exposure assessments using the mean of the repeated measurements were compared with circulating mycotoxin (metabolite) levels measured in serum. Based on the acquired results, a thorough discussion will be held on the complementarity of internal and external dietary mycotoxin exposures. These pioneering results are imperative tools for further risk assessments and will be presented for the first time.

MYCOTOXIN MIXTURES IN FOOD AND FEED: A HOLISTIC, INNOVATIVE, FLEXIBLE MODELLING APPROACH FOR RISK ASSESSMENT – MYCHIF

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Mycotoxin contamination can occur in many agricultural products used in food and feed. Mycotoxins are produced as the result of fungal metabolism and plant-pathogen interaction. Therefore, many structurally-related congeners, defined as modified mycotoxins are generated by plant and/or fungi metabolism, or food processing, and coexist with their native forms. Studies investigating the co-occurrence of multiple mycotoxins reported 75-100% of samples containing more than one mycotoxin, referring to native compounds and 100% reported multiple modified forms. A major challenge in a risk assessment is to depict the biosynthesis of mycotoxin mixtures and their realistic occurrence. Over the last two years, the MYCHIF research project has been developing integrated and innovative modelling methodologies for the risk assessment of mycotoxin mixtures in food and feed. Particular efforts have been put into the investigation and understanding of complex systems and the identification of knowledge and data gaps.

Extensive literature searches were conducted to identify and collect relevant scientific data on (i) interaction of fungi with crops, (ii) variables influencing the synthesis of mycotoxins, (iii) their co-occurrence in major crops of interest, and (iv) toxicokinetics (TD) and toxicodynamics (TD) including biomarkers of exposure and effects in farm animals and humans. Differences on the toxicokinetic and toxicodynamic parameters of single mycotoxins vs. multiple mycotoxins were considered. Data gaps have been identified for each area of data collection and included lack of consistent data for (i) the co-occurrence of multiple mycotoxins in major crops, and (ii) toxicity of multiple mycotoxins in farm animals and humans. Nevertheless, modelling approaches were considered using TK and TD data for single mycotoxins and assumptions of dose addition for combined toxicity. In order to validate the modelling approaches, case studies were developed from an exposure and a hazard perspective using dose addition for combined toxicity as the default model. A whole food chain risk assessment workflow is proposed for the risk assessment of mycotoxin mixtures in food and feed as well as future work to fill major data gaps identified in the MYCHIF project at the level of co-occurrence and combined TK and toxicity dimensions.

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THE ROLE OF MYCOTOXINS IN BACTERIAL AND VIRAL DISEASE OUTBREAKS – A REVIEW

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The most frequent type of mould developing on plants in the field is *Fusarium*, producing harmful mycotoxins, such as trichothecenes (mainly deoxynivalenol and T-2/HT-2 toxins), zearalenone and fumonisins. Both deoxynivalenol and fumonisins have demonstrated deleterious effects on the health and performance of animals. Several studies highlight the individual and synergistic impact of deoxynivalenol and fumonisins on intestinal health and also their role in bacterial and viral disease outbreaks by through 3 mechanisms: (i) increasing gut colonisation, (ii) reducing gut barrier efficacy and (iii) altering immune defence. In addition to increasing the risk of bacterial or viral outbreaks, it seems that mycotoxins also modify vaccine response or even drug efficacy.

This presentation reviews the role of mycotoxins in bacterial and viral outbreaks and explain how mycotoxins leads to higher sensitivity to infections. The effects of mycotoxins on bacterial and viral outbreaks will have a huge impact on performance and profitability at farm level as it will decrease the level and quality of production, increasing also the cost of production.

IMMUNOSUPPRESSIVE ACTIVITY OF *ALTERNARIA* TOXINS

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Filamentous fungi of the *Alternaria* genus possess the ability to generate a spectrum of secondary metabolites with potential toxicological properties. Among these, the dibenzopyrone derivative alternariol (AOH) represents one of the major *Alternaria* toxins with respect to quantity but was found to provide only limited contribution to the overall genotoxic potential of complex *Alternaria* toxin mixtures. However, recent studies argue for immunomodulatory activity of AOH, whereby the expression of several cell surface markers during the phorbol 12-myristate 13-acetate (PMA) induced differentiation of THP-1 monocytes to macrophages was modified. Moreover, in human primary macrophages, RAW 264.7 and differentiated THP-1 macrophages AOH was found to reduce the lipopolysaccharide (LPS) induced immune response. Mechanistic studies revealed a suppression of lipopolysaccharide-induced NF-κB pathway activation by AOH. Subsequently, the secretion of the proinflammatory cytokines IL-8, IL-6, TNF-α was decreased whereas the protein levels of the anti-inflammatory cytokine IL-10 was enhanced. A distinct pattern of cytokine mRNA levels was monitored, varying between short- and long-term exposures. The impact of AOH on the immune response of macrophages was associated with modulation of crucial miRNAs. Actual studies demonstrate that the immunosuppressive activity of AOH is not limited to macrophages. In differentiated Caco-2 cells (human colon carcinoma) an immune response can be provoked by incubation with IL-1ß, resulting in enhanced expression of proinflammatory cytokines like e.g. IL-8. The presence of low micromolar concentrations of AOH was sufficient to suppress the immune response. Comparable to the observed effects in macrophages, AOH was found to modulate the expression of inflammation-related miRNAs also in differentiated colon cells.

Taken together, AOH itself does not induce a proinflammatory immune response, however, in an inflamed environment it possesses the ability to repress the appropriate immune response by targeting the NF-κB signalling pathway and regulatory miRNAs. Of note, ongoing studies indicate that the immunomodulatory activity is not limited to AOH but might also be of relevance for other Alternaria toxins out of different structural classes. Due to limited data with respect to occurrence and hazard characterisation, *Alternaria* toxins are still rated as ‘emerging’ mycotoxins. With respect to food safety, the presented results underline the necessity to consider potential immunomodulatory activity in future hazard characterisation of *Alternaria* toxins.

AFLATOXINS IN FOOD: EFSA’S COMPREHENSIVE RISK ASSESSMENT

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The European Food Safety Authority (EFSA) was established in January 2002 as an independent body providing scientific advice and communication on risks associated with the food chain (Regulation (EC) No 178/2002). As a risk assessor, EFSA produces scientific opinions and advice to provide a sound foundation for European policies and legislation and to support the European Commission (EC), European Parliament and EU Member States in taking effective and timely risk management decisions. In 2007, EFSA carried out a comprehensive risk assessment on aflatoxins in almonds, hazelnuts and pistachios. Since then, specific assessments were done to evaluate possible changes of MLs for specific

food commodities. Considering that the last full risk assessment by the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) was carried out in 2007 and that elevated aflatoxin levels were observed in some food commodities originating from European countries, the CONTAM Panel concluded in 2018 that a full, updated risk assessment would be appropriate. In view of this recommendation and the future discussions at the Codex Committee on Contaminants in Food (CCCF) on maximum levels for aflatoxins in food, the EC requested EFSA to assess the risks to human health related to the presence of aflatoxins in food. The scientific opinion will be published for public consultation in the autumn of 2019. The assessment of the human health risk is done following the risk assessment paradigm: hazard identification, exposure assessment, hazard characterisation and risk characterisation. For the hazard identification and characterisation, available toxicological, epidemiological and toxicokinetic studies in the open literature were considered. The exposure assessment combined the data on human consumption for the different food categories with the occurrence data on aflatoxins in the respective food categories. A range of intake/exposure scenarios were estimated covering all age groups from the dietary surveys covered by the EFSA Comprehensive European Food Consumption Database. In this presentation, the main conclusions of the EFSA risk on aflatoxins in food will be discussed.

Acknowledgements
EFSA wishes to thank the members of the (CONTAM Panel) and the members of the WG on aflatoxins in food. EFSA would also like to thank all European Competent Authorities and other stakeholders that provided occurrence data in food and supported the consumption data collection for the Comprehensive European Food Consumption Database.

ASSESSING THE COMBINED TOXICITY OF NATURAL TOXINS BY HIGH CONTENT ANALYSIS

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As human co-exposure to natural toxins through food and water is inevitable, risk assessments to safeguard health are necessary. Aflatoxin B1 and fumonisin B1, frequent co-contaminants of maize and microcystin-LR, produced in freshwater by cyanobacteria are all naturally occurring potent toxins that threaten human health. Populations in the poorest regions of the world may suffer repeated simultaneous exposure to these contaminants.

Using High Content Analysis, multiple cytotoxicity endpoints, namely, cell number (CN), nuclear area (NA), nuclear intensity (NI), mitochondrial mass (MM) and mitochondrial membrane potential (MMP) were measured for the individual toxins and mixtures in a number of cell lines. Results highlighted that significant cytotoxic effects were observed for aflatoxin B1 in all cell lines while no cytotoxic effects were observed for fumonisin B1 or microcystin-LR at the concentrations tested. Aflatoxin B1/microcystin-LR was cytotoxic in the order HepG2 > Caco-2 > MDBK. Fumonisin B1/microcystin-LR affected MDBK cells. The ternary mixture was cytotoxic to all cell lines. Most combinations were additive, however antagonism was observed for binary and ternary mixtures in HepG2 and MDBK cell lines at low and high concentrations. Synergy was observed in all cell lines, including at low concentrations. The combination of these natural toxins may pose a significant risk to populations in less developed countries. Furthermore, the study highlights the complexity around trying to regulate for human exposure to multiple contaminants.

IMPACT OF CHRONIC MULTI-MYCOTOXIN DIETARY EXPOSURE ON COLORECTAL AND LIVER CANCER RISK IN EUROPE

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Mycotoxins have been suggested to contribute to a diversity of adverse health effects in humans, even at low concentrations. Certain mycotoxins are established carcinogens, whereas for others research suggests potential carcinogenic effects. The recognition of multiple mycotoxins being carcinogenic, over singularly present mycotoxins, was echoed in recent research and reviews.

The aim of this study was to assess potential effects of single and multiple mycotoxin exposures in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. Questionnaire data from the EPIC study were matched to mycotoxin food occurrence data compiled by EFSA from EU Member States to assess long-term dietary mycotoxins exposures and relate them to the risk of colorectal (CRC, n=6,291 cases) and liver (LC, n=834) cancers and their different sub-sites. Analyses were conducted using Cox proportional hazards regression models to compute hazard ratios (HR) and 95% confidence intervals (95% CI) and with mycotoxin exposures expressed as µg/kg body weight/day. The most important food groups contributing to mycotoxin exposures were cereals and cereal-based products, vegetables, non-alcoholic beverages (including fruit juices) and fruits. A significantly increased risk for hepatocellular carcinoma (HCC) was found with multi-mycotoxin exposures ($HR_{T3\ vs\ T1}$ (95%CI)=1.68 (1.04-2.70), P_{trend}=0.03), and a borderline increased risk for colon cancer ($HR_{T3\ vs\ T1}$ (95%CI)=1.14 (1.00-1.28), P_{trend}=0.06). The sum of all *Fusarium* toxins was also significantly related to an increased colon cancer risk and more in particular for proximal colon cancer ($HR_{T3\ vs\ T1}$ (95%CI)=1.20 (1.01-1.42), P_{trend}=0.04). For individual mycotoxin groups, deoxynivalenol (DON) showed the strongest association with CRC risk ($HR_{T3\ vs\ T1}$ (95%CI)=1.14 (1.04-1.25), P_{trend} <0.01), and in particular with proximal colon cancer risk ($HR_{T3\ vs\ T1}$ (95%CI)=1.19 (1.00-1.41), P_{trend}=0.04). DON exposure was also positively associated with liver cancer risk ($HR_{T3\ vs\ T1}$ (95%CI)=1.48 (1.13-1.94), P_{trend} <0.01) and in particular with HCC risk ($HR_{T3\ vs\ T1}$ (95%CI)=2.16 (1.38-3.41), P_{trend} <0.001). Patulin (PAT) was strongly positively associated with rectum cancer risk ($HR_{T3\ vs\ T1}$ (95%CI)=1.18 (1.05-1.32), P_{trend} <0.01) and with HCC risk ($HR_{T3\ vs\ T1}$ (95%CI)=1.48 (1.04-2.12), P_{trend}=0.02). These results showed an increased cancer risk associated with long-term dietary mycotoxin exposures and will possibly help to raise awareness of these hazardous contaminants in the general public, as well as among food business operators and regulatory bodies. However, further research investigating potential mechanisms underlying these putative associations is warranted. Even though DON has not been classified as to its carcinogenicity to humans, this mycotoxin presents a potential threat to human health, mainly when co-occurring with other mycotoxins in the consumers' diet.

TUESDAY 15 OCTOBER 2019

SESSION 7
SMART STRATEGIES FOR EFFECTIVE MYCOTOXIN MANAGEMENTALONG THE CHAIN: TOWARD FOOD & FEED 4.0 – PART 2. MYCOKEY

The project MycoKey funded by the European Commission aims at developing smart, integrated, sustainable solutions and innovative tool kits to reduce the major mycotoxins in economically important food and feed chains.

MYCOKEY: A SUCCESS STORY OF EU-CHINA COOPERATION FOR MINIMISING MYCOTOXINS ALONG CHAINS

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A sound and interactive community strategy may play an important role in shaping agriculture and industry in China and Europe. There is a great interest of cooperation between EU China on food safety issues, and especially on mycotoxins. EU-China project MycoKey (http://www.mycokey.eu) was approved in Horizon 2020 and it is contributing to reduce mycotoxin contamination at global level with particular attention in Europe and China, where frequent and severe mycotoxin contaminations occur in crops, and where international trade of commodities and contaminated batches are increasing. An update review of an integrated management of pre-and post- harvest practices aiming at minimising the risk of mycotoxin contamination of the main crops of agro-food importance and main effective solutions proposed by MycoKey will be provided in the presentation. The project is significantly contributing to enhance the cooperation between EU-China in this strategic sector by exchanging knowledge and genetic material, sharing and optimising standard protocols and common strategies for prevention, intervention and remediation along food and feed chains (maize, wheat, barley, grape, dried fruits, milk, etc.), including industrial actions. The project is finally integrating key information and practical solutions for mycotoxin management into a smart ICT tool (MycoKey app). Tools and methodologies developed in MycoKey are strategically targeted for cost-effective application not only in the field and during storage, but also in processing and transportation in order to increase food safety along the food chains in the EU and China.

After the successful MycoKey events organised in Ghent, Belgium (2017) and Wuhan, China (2018), the International MycoKey-2020 conference entitled 'Integrated and innovative key actions for mycotoxin management in the food and feed chain' will be held in Bari, Italy, 9-12 March 2020. During the conference, experts and international scientists will present the latest knowledge and researches on mycotoxin management, including the outcomes of the four years EU projects, with special focus on the EU-China dialogue. Recently, the project was awarded as a success story by the European Commission with the following explanation: "By sharing advanced methodologies and practical solutions for growers, traders, food and feed manufacturers, and policy makers, the EU-funded MycoKey project is strengthening the partnership between China and the EU" (http://ec.europa.eu/research/ infocentre/ article_en.cfm?artid=50375).

PREVENTION OF *FUSARIUM GRAMINEARUM* AND MYCOTOXINS IN WHEAT BY APPLICATION OF MICROBIAL ANTAGONISTS ON INFECTED CROP RESIDUES

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Sustainable agriculture requires innovative and integrated methods to control Fusarium Head Blight (FHB) in wheat to reduce the risk of mycotoxins in food and feed. Preventive action against the dominating pathogen *Fusarium graminearum* (Fg) using biological control agents (BCA) on crop residues could contribute to reduced applications of synthetic fungicides while promoting conservation tillage practices.

Within the Horizon 2020 project MycoKey, we conducted field experiments with the known BCA *Clonostachys rosea* 016 (016) and *Trichoderma atrobrunneum* ITEM908 (ITEM908) to suppress Fg on maize residues and thus to reduce accumulation of deoxynivalenol (DON) and zearalenone (ZEN). At first, we confirmed the antagonistic activity of 016, originally tested on wheat haulms by Schöneberg *et al.* [1], on infected maize stalk pieces *in vitro*. The BCA 016 completely inhibited the formation of perithecia and consequently the discharge of ascospores. Subsequently, two field experiments were carried out to compare the effect of formulations of 016 and ITEM908 on artificially infected maize residues. The BCA 016 was formulated as wettable powder and both, 016 and ITEM908, were tested in combination with an oil soluble UV-protectant that showed significant protection against UVB radiation *in vitro*. The monitoring of deposited Fg spores with spore traps during the infection period revealed significant effects of the treatments and, compared with the control, 016 reduced Fg colony counts by up to 79% in the first (2016/17) and 85% in the second (2017/18) year. This reduction of airborne inoculum resulted in less disease development, grain infection and reduced mycotoxin accumulation in two varieties with different resistance to FHB. In 2016/17, 016 reduced the DON content in variety Levis significantly down to 1.1 mg/kg compared with 7.3 mg/kg in the control (-85%). ZEN was significantly reduced from 160 to 20 µg/kg (-85%). Significant reductions of DON by up to 93% (=4.1 mg/kg) and ZEN by up to 98% (=10 µg/kg) were also found in 2017/18 under increased disease pressure. In contrast, ITEM908 had no significant effect in the first year but showed strong effects in the second year where both DON and ZEN contents were reduced by up to 90 and 87%, respectively. In conclusion, the results confirm the great potential of formulated 016 to reduce FHB infections, which should be further investigated in on-farm experiments.

Acknowledgments
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References
1. Schöneberg, A. *et al.*, 2015. Journal of Applied Microbiology 118: 1165-1179.

A JOINT MODEL FOR FUMONISIN AND AFLATOXIN PREDICTION IN MAIZE

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The co-occurrence of different mycotoxins in food and feed, as well as the significant variability of toxin content between years, and in the same year between close geographic areas, has become increasingly important. A crucial role in this trend is played by climate change that increased uncertainty, with great variability of weather conditions between years but also during the growing season. In this context, different fungi with diverse ecological needs can co-occur in crops and find optimal ecological conditions

for growth and toxin production. For example, with mild temperatures and rainfall, *Fusarium* will be the favoured genus, while in warm and dry periods *Aspergillus* will dominate. This can happen also during the same growing season, making fungi co-occurrence highly probable and mycotoxin prediction very difficult. Therefore, it is essential to acquire knowledge on the impact of fungi co-occurrence on growth and mycotoxin production in different ecological conditions. For this purpose, trials were carried-out both *in vitro* and in the field; in particular, *Fusarium verticillioides* (Fv) and *Aspergillus flavus* (Af), fungi that commonly co-occur in maize, have been considered. *In vitro* trials were organised with single and co-inoculum of the cited fungi on maize base medium and incubated at different temperature regimes (5-35°C, step 5°C) for 21 days. Fungal growth was weekly measured; aflatoxin B1 (AFs) and fumonisin B1 and B2 (FBs) were quantified at the end of the incubation period. For the field trial, maize ears in a field crop were artificially inoculated after silk emergence with the 2 mentioned fungi, with single and co-inoculum. Inoculated ears were collected with a 14-day schedule from the inoculum up to harvest time; fungi incidence, AFs and FBs content were quantified.

Fungal interaction resulted as playing a role for fungal growth and mycotoxins production, both *in vitro* and in the field. *In vitro*, Af and Fv showed a decrease in colony diameter of 10 and 44% respectively, in case of co-inoculum, compared to single inoculum; the dynamic of toxin production followed a comparable trend with single or co-inoculum and resulted well described by a Beta function. In the field, Af resulted significantly dominant on Fv when co-inoculated and regarding mycotoxins production, Af always produced more AFs when Fv co-occurred; on the contrary, FB production was faster or higher when Fv occurred alone. These results contributed to develop a joint model starting from AFLA-maize [1] and FER-maize [2], two existing predictive models, obtaining an improvement in their predictive performances. The preliminary validation with field data suggests further improvements in predictive model performances are possible. Therefore, additional work will be managed to merge information coming from *in vitro* and field data.

Acknowledgments
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References
1. Battilani, P. *et al.*, 2013. Computers and Electronics in Agriculture 94: 38-46.
2. Battilani, P. *et al.*, 2004. Aspects of Applied Biology 68: 91-100.

A NEW ANTIFUNGAL DEVICE FOR THE CONTROL OF *PENICILLIUM VERRUCOSUM* IN BARLEY DURING STORAGE

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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 react easily with nucleophiles, such as amines, amino acids, alcohols, water, and sulphites during food treatment, under physiological conditions and also with several functional groups of many mycotoxins. The aims of this study were to evaluate the antifungal properties of the bioactive compound allyl isothiocyanate (AITC) against *Penicillium verrucosum* (D-01847 VTT), ochratoxin A (OTA) producer on barley. The experiments were carried out initially in a simulated silo system for laboratory scale composed of glass jars (1 l) containing 300 g of barley contaminated with 1×10^4 spores/g of *P. verrucosum*. The barley was treated with a disk of 12% hydroxyethylcellulose gel to which 500 µl of AITC was added and closed and incubated for 30 days at 21°C. The control group received no treatment. Next, simulated silos of 100 l capacity containing 30 kg barley were used. Barley was contaminated under the same conditions as the previous trial. They were treated with a disc of 12% hydroxyethylcellulose gel to which 5 ml of AITC were added, they were closed and incubated for 90 days at 21°C. The control group received no treatment. The fungal growth of the inoculated fungi and the reduction in the formation of OTA were determined. The best results were obtained in the 1 l jars,

where there was complete inhibition of fungal growth at 30 days. The amount of OTA present in the controls and the treated samples was 0.28 and 0.09 ppb, respectively.

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This work has been supported by the MycoKey project 'Integrated and innovative key actions for mycotoxin management in the food and feed chain" (H2020, grant agreement No 678781).

IN VITRO AND *IN VIVO* EFFICACY ASSESSMENT OF A NEW BENTONITE BASED MATERIAL ACTING AS A MULTI-MYCOTOXIN BINDER

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Bentonite-based products are selective in adsorbing mycotoxins, being quite effective for aflatoxin B1 (AFB1) and fumonisin B1 (FB1), but ineffective for other mycotoxins, namely zearalenone (ZEA), deoxynivalenol (DON), T-2 toxin and ochratoxin A (OTA). Within the framework of the European Union Project MycoKey (grant agreement No. 678781), an innovative bentonite-based material (bio-organoclay) was developed to act as a multi-mycotoxin binder. A bentonite containing Na-smectite as major mineral was modified by an acid-activation process followed by a functionalization with an organic, non-toxic modifier. The process was optimised at lab level, and optimal conditions (geological origin and physico-chemical properties of smectites, type and concentration of chemical agents, temperature, time of contact) were identified. Under optimal conditions, the new bio-organoclay sequestered *in vitro* more than 95% of AFB1, FB1, OTA, and ZEA in a large range of pH values (3-9). The adsorptions of mycotoxins occurred simultaneously with high capacity and affinity as determined by isothermal adsorption studies.

Thereafter, the bio-organoclay was tested for its efficacy in reducing the urinary excretion of mycotoxins in rats using the biomarker approach. AFB1, ZEA and its metabolites of phase I biotransformation (α-ZOL, β-ZOL and β-ZAL), OTA and FB1 were analysed in urine by in-house validated UPLC methods. For each toxin, two groups of rats (0.3 kg, initial body weight) were considered: the first group was used as control (C) and received a single intragastric bolus containing the mycotoxin; the second group (T) received the mycotoxin supplemented with the bio-organoclay (0.5% w/w feed consumption). Rats were housed individually in metabolic cages to collect urine at different time points (4-72 h for AFB1, ZEA and FB1; 4-320 h for OTA). Normalised urinary excretion data of mycotoxins/metabolites were expressed as nmol mycotoxin/nmol creatinine and presented as mean±SD. Area under the curve ($AUC_{0\rightarrow t}$) over 72/320 h and maximal urine concentration (C_{max}) were calculated for target mycotoxins/metabolites and were used to compare control and treated groups. Results of toxicokinetic excretion of mycotoxins showed that the bio-organoclay significantly reduced urinary excretion of AFB1, ZEA, FB1 and OTA (*P* values <0.05). The use of this bio-organoclay as feed additive can be considered a valid approach to reduce mycotoxins bioavailability in animals exposed to the main mycotoxins. This product can be considered safe, as it has been obtained using reagents that are listed in the European Union Register of Feed Additives (Regulation (EC) No 1831/2003).

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FLUORESCENCE POLARISATION IMMUNOASSAYS FOR THE DETERMINATION OF TRICHOTHECENES AND THEIR MODIFIED FORMS IN WHEAT

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The trichothecenes of major concern for cereals and cereal-based products, due to their incidence and toxicity, are deoxynivalenol (DON), T-2 toxin (T-2) and HT-2 toxin (HT-2). These toxins can be harmful to both human and animal health. Moreover, a large number of modified forms of DON, T-2 and HT-2 generated by fungi, plants and mammals have been isolated and characterised. To date, natural occurrence of modified forms of DON, such as 3-acetyl-deoxynivalenol (3A-DON), 15-acetyl-deoxynivalenol (15A-DON), and deoxynivalenol-3-glucoside (DON-3G), and of T-2 and HT-2, such as T-2 glucoside (T-2G) and HT-2 glucoside (HT-2G), have been reported by several authors. The development of analytical methods able to simultaneously detect mycotoxins and their modified forms, also expressed as the sum, has high impact because it could meet possible future requirements of European or international regulations.

For this reason, within the MycoKey project, two fluorescence polarisation immunoassays (FPIAs) have been developed and validated for the rapid (<15 min) and simultaneous determination, expressed as sum, of: (i) DON, 3A-DON, 15A-DON and DON-3G in wheat and (ii) T-2, HT-2, T-2G and HT-2G in wheat. Different extraction protocols, using organic and non-organic solvents, were tested for the developed FPIAs. All the developed FPIAs showed analytical performances, in terms of recovery (89-112%) and precision (≤13%) that fulfilled the criteria for acceptability of an analytical method for the determination of relevant native forms established by the European Union. Furthermore, in line with harmonised guidelines for the validation of screening methods, an experimental protocol for single-laboratory validation has been applied to the determination of these toxins by FPIAs according to Commission Regulation (EU) No 519/2014. The satisfactory analytical performances, in terms of precision (repeatability ≤9%, within laboratory reproducibility ≤13%), cut-off levels and rate of false positive results (<0.1%) confirmed the applicability of the proposed FPIAs as screening methods for assessing the content of DON, 3A-DON, 15A-DON and DON-3G (expressed as sum) and T-2, HT-2, T-2G and HT-2G (expressed as sum) in wheat at regulatory levels. Moreover, the developed FPIAs are low-cost, portable, can be automated, and do not require a high level of technical skills. These findings indicate that the proposed FPIAs are appropriate for high-throughput screening of these trichothecenes and their modified forms in wheat.

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TUESDAY 15 OCTOBER 2019

SESSION 8
MYCOTOXIN MITIGATION AT THE FRONT LINES – FEED THE FUTURE INNOVATION LABS AND GLOBAL COLLABORATIONS
The Feed the Future Innovation Labs draw on the expertise of top U.S. universities and developing country research institutions to tackle some of the world's greatest challenges in agriculture and food security. In this session, the focus is on mycotoxins.

WHEN PREVENTION FAILS: THE NEED, USE AND ESTIMATED MARKET FOR AFLATOXIN SEQUESTERING AGENTS IN THREE AFRICAN COUNTRIES

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Aflatoxin poses a significant threat to the safety of food and feed in several developing countries. The B1 form is carcinogenic and though the M1 form is less toxic, recent studies suggest that, like the B1 form, it can contribute to childhood stunting and the associated cognitive, growth and health problems. Aflatoxin contamination has resulted in rejection of vast quantities of agricultural commodity exports from several developing countries causing millions of dollars in lost revenue. Most aflatoxin mitigation strategies focus on prevention of food or feed contamination however, when such interventions fail, aflatoxin-sequestering agents can be used to bind the ingested toxin and prevent it from being absorbed. While some developing countries routinely use aflatoxin binders to ensure feed safety, many others do not. This study examined the level of awareness about aflatoxin and the associated food safety and health risks and the market for binders for aflatoxin in three African countries. Three country-specific consultants conducted desk studies followed by one to two-week field assessments consisting of key informant interviews in Rwanda, Nigeria and Ethiopia. The three countries were chosen because they vary greatly in factors like the size and growth of the economy and livestock population, demand for livestock products, and livestock imports and exports. The countries also differ in issues that more directly affect the potential markets for aflatoxin binders, such as the growth rate of feed markets, information and awareness of the incidence and levels of aflatoxin contamination of feeds, and the existence of a policy framework on animal feed and regulation of its contamination by aflatoxin. The study showed that Rwanda's market for aflatoxin binders is the least developed, Ethiopia's is just beginning to develop, and Nigeria's is much more mature. This was evident from the fact that only one binder, used by one feed company, was found in Rwanda, whereas, in Nigeria, 31 brands of binders are available from several feed processors and vendors. The Ethiopia's situation is intermediate with 9 binder products found in the marketplace. Interviews with large- to small-scale processors and farmers in the three countries showed high awareness about the problem and risks of aflatoxin contamination by large to medium scale processors in Nigeria and by large-scale processors in Rwanda and Ethiopia. Awareness among small-scale farmers and processors was low in all countries as was awareness among medium-scale farmers in Rwanda and Ethiopia. This study highlights the need to increase awareness about aflatoxin and the associated food and feed safety risks among small to medium scale farmers in the three countries as well as medium-scale farmers in Rwanda and Ethiopia. It also illustrates the need to increase the use of aflatoxin binders in Rwanda and Ethiopia to reduce the risks to animal and human health posed by the toxin.

AFLATOXIN EXPOSURE AND HEALTH OUTCOMES IN INFANTS AND YOUNG CHILDREN: FINDINGS FROM NEPAL AND UGANDA

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Serum aflatoxin levels in pregnancy have been associated with birth outcomes and linear growth in Ghana, Benin, Togo [1,2]. There is however conflicting evidence of the relationship in Nepal with the speculation that the relationship might be stronger in younger children [3,4]. Little is known about the relationship of maternal aflatoxin in pregnancy and birth outcomes in different populations. This presentation synopsises findings of three such studies, two in Uganda and one in Nepal.

All studies utilised a longitudinal approach of recruiting women in pregnancy and following up with birth visits. Two of the studies (one in Uganda and one in Nepal) followed the new-born infants through 12 months of age with the Nepal study subsequently conducting follow ups through 24 months of age. All data were collected using pretested standardised questionnaires and serum samples collected analysed for aflatoxin using HPLC. Anthropometric measurements included weight and length at birth which were transformed into small for gestational age (SGA), weight for age (WAZ), weight for length (WLZ) and length for age (LAZ) Z-scores. Prevalence of SGA, low birth weight and low birth LAZ were computed. Analyses for each study included bi-variable and multi-variable regressions (linear or logistic) adjusted for confounding factors. The first study conducted in the Mukono, Uganda, followed 220 pregnant women through birth. The geometric mean aflatoxin levels in pregnancy at 5.89 pg/mg albumin (95% CI: 5.25–6.60 pg/mg albumin) were significantly associated with lower weight, lower weight for age z-score, smaller head circumference and lower head circumference for age z-scores [5]. In Nepal, the Aflacohort study, conducted in Banke, Nepal, recruited 1675 pregnant women and were followed through birth and through the infant turning 2 years of age [6]. The geometric mean was 1.37 pg/mg albumin (95% CI: 1.3, 1.44 pg/mg albumin). Mean serum aflatoxin levels were significantly associated with SGA (OR: 1.13; 95% CI: 1:00, 1.27; P<0.05). The second study in Uganda was conducted in 8 districts (4 in the North and 4 in the South West of Uganda) with a total of 5000 women followed through pregnancy, birth and through infant 12 months of age. A sub-sample (n=3352) from the large study was randomly selected for assessing the relationship of aflatoxin levels in women at birth and birth outcomes. Multi-variable analysis is currently underway. Using a longitudinal design, we were able to assess the variability in relationship in maternal aflatoxin and birth outcomes in Uganda and Nepal. Findings from Uganda show higher levels of aflatoxin and stronger associations with several birth outcome indicators while in Nepal, levels are lower and smaller but significant associations are being observed only with one indicator. There is a variability in the relationship of aflatoxin and birth outcomes based on the population and the level of exposure.

References

1. Gong, Y.Y. *et al.*, 2002. BMJ 325: 20-21.
2. Shuaib, F.M. *et al.*, 2010. Tropical Medicine & International Health 15: 160-167.
3. Mitchell, N.J. *et al.*, 2017. PLoS One 12: e0172124.
4. Hoffmann, V. *et al.*, 2018. BMJ Global Health 3: e000983.
5. Lauer, J.M. *et al.*, 2019. Maternal & Child Nutrition 15: e12701.
6. Andrews-Trevino, J.Y. *et al.*, 2019. The Journal of Nutrition nxz122.

INTEGRATED APPROACHES TO MYCOTOXIN REDUCTION IN AFRICA, ASIA AND CENTRAL AMERICA: FEED THE FUTURE INNOVATION LAB FOR THE REDUCTION OF POST-HARVEST LOSS CASE STUDIES

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Feed the Future Innovation Labs and other global collaborations have been essential to mycotoxin mitigation in Africa, Asia and Central America. Among these, the Kansas State University-led Feed the Future Innovation Lab for the Reduction of Post-Harvest Loss (PHLIL) is working to build national research capacity and address post-harvest loss issues in durable stored product crops, across the value chain. Focus countries currently include Bangladesh, Ethiopia, Ghana, Guatemala, Honduras and Nepal. The PHLIL program currently includes eight US and seven in-country universities, as well as government agencies, non-governmental organisations and private sector partners in the US and internationally. The core programme conducts research focused on developing and validating locally appropriate, scalable drying and storage innovations. In addition to biophysical scientists, the interdisciplinary research team also includes agricultural economists, social scientists and agricultural education/extension experts, enabling the creation of robust extension materials for inclusive and sustainable scaling of PHLIL innovation packages. Highlights of our mycotoxin projects from two countries, Honduras and Nepal, will be showcased. Mycotoxin assessment is the first step in better understanding the current situation associated with the food supply chain of countries of interest. These assessments provide essential information on potential exposure of populations to mycotoxins, can help as part of a larger capacity building process in the country, and serve as the baseline to inform nationally-appropriate intervention strategies. Progress toward identifying and deploying post-harvest loss interventions across PHLIL countries will also be highlighted, as an example of how empowering national systems can help secure a safe harvest for all.

IMPROVED DRYING AND STORAGE PRACTICES THAT REDUCE AFLATOXINS IN STORED MAIZE: EXPERIMENTAL EVIDENCE FROM SMALLHOLDERS IN SENEGAL

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Consuming food contaminated with aflatoxins has large negative consequences on the health and economic productivity of millions of people in developing countries. We conducted a randomised control trial with nearly 2,000 small-scale maize-producing households in Senegal during the 2016/17 harvest and post-harvest season. Our goal was to test which constraint(s), (i) low awareness of aflatoxins, (ii) lack of effective drying technologies and/or (iii) lack of effective storage technology were the greatest barrier to storing safe maize. A novel feature of our intervention is that we offered both drying and storage technologies to farmers and evaluate their combined impact. We varied four inputs provided to each household: (i) training on recommended post-harvest practices; (ii) low-cost moisture meters to detect if maize is dried to a safe level before storage; (iii) tarps to reduce ground drying; and (iv) hermetic (airtight) bags to store maize after it has been dried. Only hermetic bags caused a statistically significant reduction in total aflatoxin levels after 3-4 months of storage. The reduction was relatively meaningful (8.4 ppb, or 34% of the control group average). All maize stored by households that received the bags, even that stored in other containers, showed lower total aflatoxin levels, suggesting that effective storage technologies were the main binding constraint. Our results provide practical guidance on ways to lower aflatoxins for populations that mainly grow maize for home consumption and suggest that strategies to reduce aflatoxins should address issues from harvest to storage in a comprehensive manner.

OVERVIEW OF AFLATOXIN CONTAMINATION CHALLENGE IN FOOD AND FEED AND POTENTIAL USE OF COLD PLASMA TO MITIGATE AFLATOXINS

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Aflatoxins are secondary metabolites produced by aflatoxigenic *Aspergillus* spp., i.e., *A. flavus* and *A. parasiticus* producing aflatoxin (AF) B1, B2, G1 and G2. Aflatoxins are very toxic to humans and animals; they cause liver cancer, associated with immunosuppression, stunting, and at high doses will lead to death. Human exposure to aflatoxins is the result of ingestion of contaminated foods, such as cereal grains or milk (M1 toxin) produced by animals previously exposed to aflatoxins in feeds. Cold plasma is a physical, novel non-thermal method, which is currently used in food industry. Cold plasma can refer to 'gas-derived mix of atoms in their quasi-neutral ionised forms mainly composed of photons, ions, and free electrons as well as atoms in their fundamental or excited state with a net neutral charge'. The plasma is generated using only atmospheric air and electricity.

In this study, plasma was generated using high voltage atmospheric cold plasma (HVACP) operated at 85 kV and 60 Hz and generated 180 W. Pure aflatoxin (B1, B2, G1 and G2) was dissolved into chloroform, and an equivalent of 200 µM poured onto watch glasses until complete dry. Watch glasses containing aflatoxin were placed into boxes (27.31 cm x 17.78 cm) with 4.44 cm of gap distance, sealed into hermetic plastic bag, and directly treated in duplicate for 2, 5, 10, and 20 min with HVACP system described above. After the treatment, boxes were left over night to allow generated reactive species recovering their fundamental state into air. Aflatoxin residuals were extracted with chloroform and analysed with LC-MS/MS. Results showed that aflatoxin B1 and G1 ("1") were sensitive to HVACP system; the aflatoxin B1 showed a reduction 90% after 2 min of HVACP treatment and aflatoxin G1 74% decrease after 2 min. After 10 min, the aflatoxin G1 residuals were below the limit of detection. Aflatoxin B2 and G2 ("2") were reduced for 38 and 79%, respectively after 20 min HVACP treatment. The large variability seen with B2 and G2 reduction likely results from the primary difference between the "1" and "2" toxins, which is the presence of a double bond at C8-C9 position, and readily degraded by reactive gas species. This double bond is absent in B2 and G2. The reduction of this C8-C9 double bond significantly lowers the aflatoxin toxicity. Chemical analyses are on-going to confirm these hypotheses.

Future studies will correlate the aflatoxin degradation to toxicity. Toxicity assessment will be performed using *in vitro* methods using HepG2 cell and test for cytotoxicity, DNA fragmentation, and apoptosis.

WEDNESDAY 16 OCTOBER 2019

SESSION 9
UPDATE ON (MULTI-)MYCOTOXIN ANALYSIS
Recent developments – from sampling to multi-mycotoxin analysis and more – will get through here.

MAIZE MEAL SLURRY MIXING: AN ECONOMICAL RECIPE FOR PRECISE AFLATOXIN QUANTITATION

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Aflatoxin contamination in grains, particularly maize, remains a major challenge globally. Owing to the toxicity of aflatoxins to humans, most countries worldwide have set regulatory limit mostly at 10 µg/kg to minimise dietary exposure. However, such low level of contamination is associated with high variation of measurement which may lead to misclassification of acceptable or rejectable lots. While great strides have been made in improving sampling plans and analytical methods to reduce variability, sample preparation remains neglected. Test variability can be reduced by increasing the test portion, however this makes the analysis unreasonably expensive due to excessive organic solvent demand. This, therefore, necessitates innovative ways of reducing measurement variation without increasing solvent cost.

In this study, the precision of aflatoxin analysis in comminuted maize samples using 25 g slurry (prepared from 250 g test portion of comminuted maize, water/matrix (1+1, v/w)) and 12.5 g dry grind test portion were compared against the conventional 50 g dry grind test portion through replicated (10) Aflatest® immunoaffinity fluorometric tests of naturally contaminated samples with aflatoxin concentration ranging from 4.9 to 81.7 µg/kg. The results showed an overall mean aflatoxin concentration obtained from the 10 different samples tested using 12.5 g and 50.0 g dry grind procedures being 12% significantly (P<0.05) lower (poorer) compared to 25 g slurry. The sample preparation plus analytical variance associated with testing 25.0 g slurry, 50.0 g dry grind and 12.5 g dry grind test portions were in the ratio of 1:5:15, respectively. Additionally, the current innovation, practically cut the cost associated with methanol usage by a factor of 4.

Acknowledgements
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QUANTITATION OF OCHRATOXIN A, 4-DEOXYNIVALENOL AND ZEARALENONE IN WHEAT: PRODUCTION OF CERTIFIED REFERENCE MATERIALS AND ASSESSMENT OF ISOTOPE DILUTION STRATEGIES

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Ochratoxin A, 4-deoxynivalenol and zearalenone are mycotoxin contaminants commonly encountered on various agricultural products, including cereal grains and present a significant threat to consumer health and safety. To safeguard global food supplies, these mycotoxins are regularly monitored in susceptible food commodities. Screening of mycotoxins to ensure regulatory limits are met requires

adherence to quality assurance guidelines, which recommend the use of certified reference materials (CRMs). The National Research Council of Canada (NRC) has produced ochratoxin A (OTAN-1), synthetic [$^{13}C_6$]-ochratoxin A (OTAL-1), 4-deoxynivalenol (DONN-1) and zearalenone (ZERA-1) calibration solution CRMs via quantitative proton NMR, that are traceable to the Système Internationale (SI) of units. The OTAN-1 and OTAL-1 calibration solutions were used to certify the mass fractions of ochratoxin A in a mycotoxin contaminated rye flour CRM (MYCO-1). The performances of OTAN-1, OTAL-1 and MYCO-1 were evaluated on commercial wheat samples via external calibration, single, double and multi-point isotope dilution methods with MYCO-1 serving as a quality control sample. Our results show that due to matrix effects, far less accurate results for ochratoxin A were obtained via external calibration. Single point isotope dilution method, whereby quantitation is achieved directly with OTAL-1 acting as an internal standard provided lower than expected levels of ochratoxin A in the wheat samples. The double or multi-point isotope dilution, where OTAL-1 is spiked into both the sample and primary standard provided the most accurate quantitation of ochratoxin A, as it eliminated the nearly 5% isotopic enrichment bias when using the single-point approach. The correlation of this bias with increasing degrees of ^{13}C labelling will be highlighted.

LC-MS/MS BASED QUANTITATIVE MULTI-TARGET APPROACH FOR FOOD AND FEED: CROSSING THE LIMIT OF 1000 METABOLITES

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Risk assessment, control, monitoring and prevention of residues and contaminants in food and feed are crucial subjects in order to ensure public health, agricultural production, food processing and trade. The natural occurrence of contaminants like secondary fungal metabolites or plant toxins as well as anthropogenic agricultural inputs as pesticides or veterinary drugs are a major public concern nowadays. Therefore, there has been an increasing trend towards multi-target analysis based on LC-MS/MS to cover most of the relevant food and feed contaminants. In this work, a liquid chromatography-electrospray ionisation tandem mass spectrometric method was developed, to allow a simultaneous quantification of around 800 natural biotoxins (secondary fungal metabolites, plant- and bacterial toxins), 500 pesticides and 100 veterinary drugs. The aim of this presentation is to demonstrate the limitations of tandem mass spectrometry in relation to the number of analytes that can be detected within a single method. Special focus will be on the issue of dwell time, the consistency of matrix effects and the variation of extraction efficiency. Finally, the applicability of the method in terms of precision and sensitivity will be highlighted.

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BENEFITS OF COLLISION CROSS SECTION (CCS) DATA OBTAINED BY UPLC-ESI-IMS-QTOF MS FOR SMALL MOLECULES IDENTIFICATION: APPLICATION TO MYCOTOXINS SCREENING

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Ion mobility mass spectrometry (IMS) separates ions on the basis of their shape, mass, charge as well as their interactions with a buffer gas, providing a powerful analytical tool for investigating complex samples. Collisions occur between ions and a neutral buffer gas under an electric field, resulting in different drift times in the range of milliseconds. The collision cross-section (CCS) of an ion is a value unique to IMS and is derived from the drift time. The chemical structure and three-dimensional conformation of a molecule influences the CCS, with smaller (more spherical) molecules having smaller CCS values than the more extended ones (planar structures, extended chains, helices) with bigger CCS values. With all this chemical information within one value, CCS can thus be used as an additional parameter in the identification of a compound.

Traditionally, detection and identification of compounds was based on the comparison of retention time and accurate mass spectrum with a reference standard. However, with conventional liquid chromatography (LC)-based approaches an additional problem arises, isobaric components from the sample matrix can interfere with the signal from a residue present in the sample or be mistaken for residues not present in the sample, giving rise to false negatives or positives, respectively. CCS values, meanwhile, are undertaken in the gas phase, remotely from the ion source, meaning they are unaffected by the sample matrix and are consistent between instruments and across a range of experimental conditions. For these reasons, the use of CCS values as a fourth dimension (alongside retention time, exact mass and intensity) to identify compounds presents a great avenue for analytical chemists. Among the wide range of compounds of interest for analytical chemists, mycotoxins are a challenging group that has been evolving from detection and quantification to metabolism and biomonitoring and recently participating in the new concept of exposomics. Mycotoxins are secondary metabolites of fungi that contaminate food in several stages and their increasing presence in food chain demand further control. Human exposure to mycotoxins occurs mostly through dietary intake, thus, understanding the biotransformation of mycotoxins and identification of reliable biomarkers in the human body is important for accurate risk assessment of mycotoxin exposure.

In this work, the benefits and limitations of implementing IMS powered LC-ESI-QTOF MS systems, in particular travelling wave IMS (TWIMS) separators in the mycotoxin screening workflow will be addressed; from the screening and confirmation of the mycotoxins present in a food product to metabolite ID in urine and plasma for discovery of biomarkers of exposure. The main benefits of adding IMS separation to UPLC-ESI-QTOFMS involve:

- Cleaner mass spectra both at low and high collision energy when IMS aligned, similar to product ion scan (MS/MS) without the need for re-injecting the samples and preselecting the precursor ion of interest. The data can be interrogated retrospectively in the future, with good quality data for parent and fragment ions.
- Possibility of resolving coeluting isobaric/isomeric compounds based on marginal differential ion mobilities. Interesting when many potential isomers might be expected, as well as for discriminating isomeric conjugated metabolites during metabolite identification discovery.
- CCS values are less prone to be affected by matrix like chromatographic retention time. Therefore, CCS values are comparable across the same family of instruments (same ion mobility separator), which promotes the building of empirical CCS library for mycotoxin and metabolites identification.
- CCS values are related to the different shape of the ionised molecules. Thus, prediction of CCS for suspect mycotoxins or metabolites lacking standards could be a good alternative for tentative identification together with accurate mass and fragments annotation.

MYCOTOXIN TESTING PARADIGM: CHALLENGES AND OPPORTUNITIES FOR THE FUTURE

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Mycotoxin contamination of food and animal feed is an important food and feed safety problem. In order to manage this issue, fast, reliable and inexpensive testing methods are needed. Fungal populations and their toxins are changing as a result of climate and new agricultural practices. This has led to the discovery of mycotoxins in new geographic regions and the emergence of NX toxin and 15-ADON-3G. LC-MS/MS is the gold standard for laboratory analysis but it is expensive, requires highly qualified personnel and dedicated facilities. Existing rapid tests use antibody-based or spectrophotometric methods the pros and cons of which are discussed in our 'Mycotoxin Testing Paradigm'. The development of miniaturised mass spectrometry could allow for more accurate data and information on mixtures of toxins present in a sample. This is critical for feed products, which are often blends of multiple commodities and as such are potentially contaminated with a number of mycotoxins and their related derivatives.

MYCOTOXIN RAPID TESTING – WHY MANY INNOVATIVE TECHNOLOGIES FAIL ON THEIR WAY TO THE MARKET

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Within recent years, highly sophisticated on-site techniques for analysing food and feed have emerged all over the world. Particularly in the academic field, innovative concepts and methods for the analysis of samples were presented and promoted, ranging from novel analytical detection methods to modern chip-based microfluidic technologies. Various analytical targets are of great interest for the implementation of those cutting-edge technologies, among them are the mycotoxins. However, while science keeps pushing the envelope, industry does not seem to care. Despite all the newly developed high-tech methods, simple ELISA plates and strip tests such as lateral flow devices (LFDs) are still in use, or even more dominant than ever. Such tests have gained more and more acceptance within recent years and are now an integral part of sample monitoring in the food and feed industry. But why are ELISA and strip tests still the champions? What are the reasons behind this and how can it be explained? What would have to happen to cause industry to change and be willing to pay for novel – more sophisticated – approaches? This presentation will contrast the industrial point of view with what we have learned from the scientific community.

ULTRAFAST METHOD FOR MANAGING MYCOTOXINS IN THE FEED INDUSTRY

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Mycotoxins management practices have been adopted in agribusiness to maximise profitability of agricultural production along with animal performance. It is not feasible to completely eliminate the formation of mycotoxins, especially when climatic conditions favour their development, which can occur in the three stages of production (field, harvest and storage). Thus, its correct monitoring is essential. One of the hitherto limiting factors in the monitoring process was agility in decision making; nonetheless, access to rapid diagnostic tools has made it possible. The near-infrared spectroscopy (NIRS) technology

allows ultra-fast, easy and inexpensive diagnosis of mycotoxins. It uses the energy absorption which results from the organic compounds present in the sample and may be employed to obtain a direct or indirect estimation of the concentration of a substance. The technique comprehends the integration of the spectral evaluations with a database obtained from analyses made by conventional methodologies, which are based on the accuracy of the reference methods; those are necessarily performed by accredited laboratories which comply with ISO 17.025. This information is subjected to chemometric methods, resulting in the prediction equations.

The benefits of using NIRS for control and monitoring of mycotoxins are the ease of sample preparation and analysis. The traditional extractions and dilutions required in other methodologies are not used with NIRS; it is only necessary to grind the sample and read the spectra, a process which takes approximately 10 min. This is what makes this technology unique; its ultimate goal is to calculate the mycotoxins risk, thus allowing for real-time decision making. This calculation is based on an algorithm which considers not only the analytical result, but the average contamination, prevalence, combination with other mycotoxins, exposure time to toxins, and inclusion rate of raw materials within a defined time frame, thus generating a history. Other parameters, such as animal species, rearing phase, age, health factors and environmental management conditions, are also inserted in this algorithm, leading to the creation of management information. All in all, the NIRS tool for the analyses of mycotoxins has multiple advantages, besides enabling real-time management and control of mycotoxins at a cost benefit which is unsurpassed by any other technology. The presentation of the mycotoxins risk in its current form is surely the state of art in the complex system of mycotoxin management.

WEDNESDAY 16 OCTOBER 2019

SESSION 10
IMPROVING FOOD SECURITY AND SAFETY AT THE GLOBAL LEVEL – MYTOX-SOUTH

Mytox-South is a partnership to improve food security and safety through mitigation of mycotoxins at the global level with the following long-term goals: building human and infrastructural capacity, bridging the gap between research, academia and industry, and creating a sustainable network on mycotoxin research.

INTRODUCTION TO MYTOX-SOUTH

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Food safety is key to address global food security and improve human health. Mycotoxins, toxic fungal secondary metabolites, are a significant food safety threat in low- and middle-income countries. The mycotoxin problem has become more challenging partly due to knowledge on co-occurrence of multiple mycotoxins and climate change. Addressing mycotoxins effectively requires multi-disciplinary approaches that focus on prevention and remediation measures, and assessment for monitoring and control purposes. Building capacity in low- and middle-income countries to assess local risk timely and develop interventions and policies is key. To mitigate mycotoxins in the food system requires concerted action leveraging efforts from researchers from different fields of mycotoxicology, as well as stakeholders from food industry, civil societies and governments.

MYTOX-SOUTH (http://mytoxsouth.org) is an intercontinental, multi-disciplinary partnership that strives to improve food security and food safety through mitigation of mycotoxins at global level. It has the following long-term goals: (i) building human and infrastructural capacity through training of South partners; (ii) bridging the gap between research and the development; and (iii) stimulate the environment for a fruitful public-private partnership to create a sustainable network. This presentation will give a general introduction to the MYTOX-SOUTH International Thematic Network, as a starting point for the Session 'Improving food security and safety at the global level – MYTOX-SOUTH'. The short introduction will give an overview of the MYTOX-SOUTH partners and will highlight recent achievements (research grants, traineeships, research results, seminars, conferences).

KEEPING MYCOTOXINS AWAY FROM THE FOOD: DOES THE EXISTENCE OF REGULATIONS HAVE ANY IMPACT IN AFRICA?

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Following the discovery of aflatoxins in the early 1960s, there have been many studies leading to the uncovering of many mycotoxins and the understanding of associated health effects in animals and humans. Consequently, there has been a global increase in the number of countries with mycotoxin regulations in foods. However, many African countries have only regulations for aflatoxins (or a few other mycotoxins) in specific foods, or no regulations at all. This paper critically reviews the challenges thwarting the establishment of mycotoxin regulations and their impacts on human dietary mycotoxin exposure in Africa. Mycotoxin regulatory limits for different countries are compared with mycotoxin tolerable daily intakes established by international food safety bodies taking into account consumption

patterns. The agrarian setup, food insecurity, and mycotoxin analytical challenges in African countries are discussed; and more feasible mycotoxin dietary exposure reduction strategies are proposed.

MYCOSAFE-SOUTH, THE EU LEAP-AGRI PROJECT ON MYCOTOXIN MITIGATION IN AFRICA

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MycoSafe-South, the 'European-African partnership for safe and efficient use of mycotoxin-mitigation strategies in sub-Saharan Africa', intends to harness the expertise and infrastructure available in Europe by strengthening the capacity of the Southern partners to mitigate the mycotoxin problem and food safety issues. The project started in 2018 and proposes safe and efficient post-harvest mitigation strategies to reduce aflatoxins (AFs) and fumonisins (FBs) exposure in Africa, with focus on children. Food security remains a major challenge in developing countries, particularly in Sub-Saharan Africa, with the highest prevalence of undernourishment. Worldwide, cereal-based crops are spoiled by toxigenic moulds and their mycotoxins. Food spoilage by moulds and mycotoxins not only reduces the amount of the available food and can have deleterious effects for animal and human health, it also adversely affects the ability of Africa to trade with the rest of the world [1,2]. Acute high-level exposure to AFs leads to human death, which has repeatedly occurred in Sub-Saharan Africa, while chronic exposure to AFs or FBs may cause liver or oesophageal cancer, respectively. AFs and FBs are involved as important causal factors of stunting in African children [2]. AFs are critical for young children since exposure may already occur in utero through transplacental passage, and through breastfeeding. Also, weaning foods are cereal- and tuber-based, both of which are susceptible to AFs and FBs [3,4]. Furthermore, the immunosuppressive effects of mycotoxins enhance the susceptibility of humans and animals to infectious diseases [5] and worsen the current HIV pandemic [6]. Moreover, mouldy food and feed is frequently fed to food-producing animals, impairing food safety by carry-over of AFs to animal products such as milk, meat and eggs [7].

Therefore, there is an urgent research priority for the development of safe, efficient and sustainable post-harvest intervention strategies to reduce animal and human exposure to AFs and FBs, and applicable to both rural small-scale subsistence and commercial farming in Sub-Saharan Africa. This project aims (i) to provide safe-use options for AFs and/or FBs-contaminated food for children and adults through means of safe and efficient post-harvest intervention strategies, including nixtamalization, dehulling, fermentation and the usage of mycotoxin binders and/or modifiers investigated via *in vitro* and *in vivo* studies, (ii) to develop intervention strategies to reduce human exposure to AFs through animal products, i.e., poultry food products and milk, and (iii) to improve impact of the acquired results by organising education programmes and awareness campaigns that will facilitate best practices, transfer the acquired knowledge and help stakeholders to understand and effectively manage mycotoxin-associated health risks. First results will be presented at the conference.

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References

1. Wu, F., 2015. World Mycotoxin Journal 8: 137-142.
2. Wild, C.P. *et al.*, 2010. Carcinogenesis 31, 71-82.
3. Magoha, H. *et al.*, 2014. Maternal & Child Nutrition 12: 516-527.
4. Magoha, H., *et al.*, 2014. World Mycotoxin Journal 7: 277-284.
5. Antonissen, G. *et al.*, 2014. Toxins 6: 430-452.
6. Williams, J.H. *et al.*, 2010. The American Journal of Clinical Nutrition 92: 154-160.
7. Völkel, I. *et al.*, 2011. Food and Nutrition Sciences 2: 852.

MYCOTOXIN CHALLENGE IN FOOD SECURITY AND SAFETY IN AFRICA: ROLE OF THE AFRICAN SOCIETY FOR MYCOTOXICOLOGY

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In Africa, mycotoxin contamination is considered to be a major problem with implications that affect human and animal health and the economy. Crops that are most susceptible to mycotoxin accumulation are staple foods. Maize, groundnuts, sorghum, millet and tubers are reported to be heavily contaminated with several mycotoxins produced by diverse fungi. Of particular economic and toxicological importance are aflatoxins and fumonisins. Aflatoxin-related hepatic diseases, ochratoxin and fumonisin toxicity in humans and animals is widespread in Africa. In East and West Africa, cases of aflatoxicosis or confiscation of aflatoxin-contaminated grains, are recorded yearly with levels over 48,000 µg/kg. Exposure to multiple mycotoxins is pronounced in Africa due to the wide range of agro-ecological conditions, temperature and humidity which favour growth and toxin production by the fungi; methods of pre- and post-harvest practices; a low level of awareness of mycotoxins contamination, the health risks and potential mitigation measures; weak governance and legislative framework, nationally and regionally; limited human resource capacity and access to up-to-date laboratory infrastructure for monitoring and evaluating levels of contamination and exposure; numerous non-synergistic interventions driven by multiple interest groups. No single technology or intervention emerges as a standalone strategy for wide-scale adoption in Africa. Each has its unique benefits and drawbacks.

Though significant investments have been made, especially by the research, academic and donor community, in mitigating the mycotoxin challenge and exploring possible solutions to control contamination, varying measures of success is observed. Despite the vast knowledge base, the challenge of controlling mycotoxin contamination persists. The African Society of Mycotoxicology was formed in 2015 with a vision to promote and coordinate mycotoxin research carried out by African researchers and their international collaborators to improve synergy and solve the mycotoxin issues in Africa using practical solutions along the entire food value chain. The society promotes sharing of research outcomes (or recommendations) engaging stakeholders including industry, other private sector, governments in discussions on managing African mycotoxin threat and providing a platform for scientists to engage with them on needs assessment and new technologies available in the market to jointly reduce the African mycotoxin threat. Joint effort of solving mycotoxin problem is seen in the society's affiliation with the Africa Center of Excellence for Mycotoxin and Food Safety (Nigeria), Partnership of Aflatoxin Control in Africa, International Society for Mycotoxicology and Mytox-South (Ghent University, Belgium) to work together to reduce the African mycotoxin threat.

THE AFRICA CENTRE OF EXCELLENCE FOR MYCOTOXIN AND FOOD SAFETY, THE WORLD BANK PROJECT

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The creation of the Africa Center of Excellence for Mycotoxin and Food Safety (ACEMFS) will create learning opportunities and research results to address Africa's shortage of expertise and applicable solutions to ensure a safe, controlled and sufficient food supply that will support economic growth and public health. The education objective of the centre of excellence (CoE) is to create an interdisciplinary, experiential education model that will prepare a cadre of future leaders focused on the rapidly emerging need for innovations at the nexus of food security, food safety, agricultural productivity and economics from local to global scales. The CoE will be established at the Federal University of Technology, Minna to leverage the research infrastructure available in the National Centre for Genetic Engineering and Biotechnology and its strategic plan to train a skilled and innovative work-force that would transform Africa's natural resources into goods and services, driven by entrepreneurship and information and communication technology (ICT), to positively affect the economy and thus the quality of life of her people. The Research objectives of the CoE are (i) to foster impactful interdisciplinary research and (ii)

to implement solutions that improve the quality of life of Africans through fit-for-purpose interventions fostering economic growth and access to sufficient safe food for all. These objectives address the five sustainable development goals of poverty reduction, zero hunger, good health and wellbeing, and quality education, and gender equality by 2030.

The intervention strategies will include developing early warning systems, fit-for-purpose good agricultural and food processing codes of practice, drought, pest and mycotoxin resistant cultivars, phytofungicides, bio-competitively eliminating mycotoxin producing fungi at the farm, nano-based mycotoxin feed binder and detoxifiers, and portable detection systems. Activities will also entail educating value chain actors (crop and livestock producers, food and beverages industries, food regulators) and graduate students. The activities will be realised with a view to establishing an integrated prevention and control scheme through the entire value chain from farm to fork of the most consumed and economically valuable crops and their food and feed products (i.e. maize, rice, sorghum, millet, wheat, soybean, cassava, sesame, groundnut, animal feed, livestock products including milk, fish and egg, fruits and vegetables). The activities of the CoE will be accomplished with 46 industry/sectoral and academic partners, and international scientific advisory board members from across six continents. The research component will focus mainly on surveillance and intervention of mycotoxins. The CoE will also only conduct regional monitoring of heavy metals, veterinary drug and pesticide residues as dictated by the needs of national food control systems and Codex Alimentarius Commission for Development of Standards. The production of world class graduates and non-graduates in food safety in the course of this project is in tandem with the strategic plan of the Federal University of Technology, Minna seeking to train skilled and innovative work-force that would transform Nigeria's natural resources into goods and services, driven by entrepreneurship and ICT to positively affect the economy and thus the quality of life of her people.

IMPACT OF MYCOTOXIN RESEARCH AT GRASSROOTS LEVEL – UNDERSTANDING AFRICAN SUBSISTENCE FARMING

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Subsistence farmers predominantly produce the major grain crops, including maize and groundnut, which serve as staple foods to Africans. Mycotoxigenic fungi, associated with these grain crops, produce mycotoxins that reduce the yield and quality of these crops; and further compound grain production in subsistence systems. Several agricultural practises may influence mycotoxin accumulation although subsistence farmers often employ poor or inadequate agricultural practises due to limited resources. Subsistence farmers cannot readily access quality seed. Local landraces with poor agronomic traits or seed retained from the previous season is often used. Additionally, the lack of resources including fertiliser and insecticides to support optimal plant growth significantly contributes to infection by mycotoxigenic fungi and subsequent mycotoxin contamination. Most African subsistence farmers sort damaged, mouldy grain prior to storage, but are not aware of mycotoxins and their consequences to animal and human health. Discarded grain is, however, used as livestock feed or to produce traditional beer, thereby increasing their risk of mycotoxicosis and in animals that represent an alternative food or income source. Some farmers practise crop rotation but use crops that serve as hosts of mycotoxigenic fungi, thereby increasing inoculum for infection. Metal tanks and wooden structures are most commonly used for grain storage though largely inefficient in preventing postharvest mycotoxin contamination. Low-cost storage solutions that limit moisture and humidity would effectively limit further mycotoxin contamination. Moreover, mycotoxin awareness campaigns are vital amongst African subsistence farmers with a clear need for the surveillance of mycotoxin levels in subsistence farmed food crops.

BEYOND THE LAB BENCH: TRANSLATING RESEARCH INTO POLICY AND ACTION

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What began as research into emerging food hazards has become a key food safety issue worldwide. Mycotoxin research has evolved from just studying occurrence in commodities to the determination of state-of-the-art analytical methodologies and determination of health risks. The existence of human health risk as evidence by toxicological data has resulted in the development of control measures to minimize the overall exposure to the ubiquitously present mycotoxins in the food chain. Control measures include the implementation of various legislation and policies at intercontinental level, the existence and invoking of sanitary and phytosanitary (SPS) measures to minimise the introduction of mycotoxins to unexposed regions through trade, and the development and implementation of local standards to ensure an appropriate level of protection for citizens. As alluded to, research into mycotoxins and data on mycotoxins health risk is available and improving including research from the African continent where exposure to mycotoxins is quite high. However, there is a disparity regarding the level of research as compared to the magnitude of measures applied to reduce human health risk from mycotoxin exposure.

This talk will seek to elucidate the extent of the problem of translating mycotoxin research in Africa into workable policy and action to reduce the human health menace currently affecting the continent and its population particularly when it comes to issues of health and trade. It is recommended that more effort be put to ensure compliance to health-based guidelines and to base these on the scientific evidence available in order to achieve an appropriate level of protection from mycotoxins for the continent.

WEDNESDAY 16 OCTOBER 2019

FINAL PLENARY SESSION
LOOKING AT MYCOTOXINS FORM A DIFFERENT ANGLE
Take a step back, take a deep breath and actually look at mycotoxins with a different perspective.

RELATIVE IMPORTANCE/PRIORITY OF MYCOTOXINS COMPARED TO OTHER PUBLIC HEALTH RISKS

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Kim Petersen kindly agreed to replace Dr Peter Ben Embarek. Unfortunately, the abstract could not be submitted in time anymore for publication in the book of abstracts.

WORLDWIDE OCCURRENCE – WHERE DOES THE DECADES-OLD FAO FIGURE OF 25% CONTAMINATION STAND TODAY?

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Mycotoxin contamination of agricultural commodities such as cereals poses a significant global health risk and results in multi-million dollars economic losses each year. With trade of agricultural products on a global scale an assessment of mycotoxin contamination in food and feed products is highly desirable. For several decades now, an estimate by the Food and Agriculture Organization (FAO) of the United Nations states that 25% of global food crops are contaminated with mycotoxins, a number that has been widely cited in the scientific and general literature.

However, little is known, how this number was calculated, and which thresholds were employed. To review the FAO estimate, recent studies on mycotoxin occurrence in the scientific literature were summarised. Data from the European Food Safety Authority and from a large global survey for six mycotoxins with existing legal limits and guidance values were evaluated. Employing thresholds established by the European Union and Codex Alimentarius limits the FAO 25% estimate was confirmed. However, using detectable levels as a threshold, up to 80% of samples were found to be contaminated with one of more mycotoxins, likely to increase in sensitivity of analytical methodology and changes related to climate change. This must not be ignored since exposure to co-occurring mycotoxins in food and feed is potentially responsible for adverse health effects.

ARE VERY LOW DOSES OF MYCOTOXINS PREDISPOSING FACTORS FOR OTHER PATHOLOGIES?

P. Pinton, D. Payros and **Isabelle P. Oswald**

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The toxicity of mycotoxins is known from ancestral periods including the 10 plagues of Egypt in the Old Testament or ergotism in the Middle Ages. More recently, aflatoxin was identified as the causative agent of 'Turkey X' disease in England in the 60s and fumonisin triggering leukoencephalomalacia, a neurologic disease of horses identified in the 70s. Toxicological researches on mycotoxins first focused on their acute effects. Regulations reduce the overall levels of mycotoxin contamination and limit outbreaks of acute toxicosis. Even rare, acute intoxication can still occur, such as aflatoxicosis reported in Kenya, poisoning with deoxynivalenol (DON) and fumonisins in India or outbreak of ergotism in Ethiopia. Chronic exposure to low doses of mycotoxins can lead to several metabolic disturbances with effects depending on exposure conditions. Chronic exposure to mycotoxins is nonetheless specific to each of them. For example, deoxynivalenol mainly targets the intestine and the immune response, zearalenone the reproductive function and fumonisins the metabolism of lipids.

There are also exposure scenarios in which, by themselves low doses of mycotoxins have no adverse effect. It is now important to focus our research on this type of exposure and to determine if, even at very low concentration, exposure to mycotoxins cannot be a predisposing factor for certain pathologies. In our laboratory, we have recently shown that the presence of DON in the diet of rats, at a dose without any apparent effect, exacerbates the genotoxicity of an *E. coli* strain producing the colibactin. This is of importance as these bacterial strains are increasingly present in the microbiota of asymptomatic humans. Similarly, using a dextran sodium sulfate-induced model of colitis in rats, we have observed that food contaminated with DON exacerbates the colitis whereas the same dose of DON alone has no effect on the intestine. These results suggest that DON, even present at very low dose, is a risk factor for other pathologies. It exacerbates the genotoxicity of a naturally present intestinal genotoxin and is a co-factor contributing to the development of intestinal bowel disease.

In conclusion, the deleterious effects of mycotoxins were first evaluated in the context of their acute and chronic toxicities. It seems now important to focus on the effect of very low doses of mycotoxins and to analyse their interactions with other contributing factors in pathological situations.

THE TOXIC SIDE TO FOOD FRAUD – WHAT ABOUT MYCOTOXINS?

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Fraud in the feed and food industries have been occurring for a very long time. Reports dating back to ancient Greek and Roman times highlight this! However, it is widely believed that the level of criminal activities in our feed and food systems are increasing. This is due to the ever-growing complexity of supply chains, the growing role of organised crime in feed and food fraud, and major supply and demand issues caused by issues such as climate change. The ways in which fraud is perpetrated are numerous and can be fairly straight forward or highly complex. The fraudsters set out to evade detection by feed and food companies, auditors and government agencies. While their business model is not to harm anyone as this means the fraud becomes known it can happen and when it does the consequences can be catastrophic.

During the presentation the various types of fraud will be explained and who the victims of each can be. In addition, a number of case studies will be presented where the presence of natural toxins in feed and food were linked to fraud incidents and caused major economic and health consequences.

HOLISTIC APPROACH TO MOULD AND MYCOTOXIN RISK MANAGEMENT

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Aflatoxins are estimated to cause 25% or more of the world's food crops to be destroyed annually. Around 4.5 billion people globally are chronically exposed to aflatoxins through their food. Aflatoxin consumption has been linked to liver cancers and stunting in children. Facing this challenge, Mars Incorporated has developed a system approach to mitigate the risk of mycotoxins in many different raw materials. There are many factors that influence mycotoxin production and it is essential to understand the risks at each step and evaluate each year. How to manage mycotoxin risk in the field, at harvest, during storage, and during manufacturing is very important. Mars has created a comprehensive material quality management system, which includes material risk assessment, specification development and approval, supplier risk assessment and approval, and supplier management. This iterative cycle continuously improves quality of high-risk materials, such as maize and peanuts. With simple measures, such as fines removal, gravity sorting, and ammoniation, risk of aflatoxin contamination can be significantly reduced. Mars Global Food Safety Center in Beijing is actively working on innovative ways to decontaminate the waste stream after sorting contaminated raw materials.

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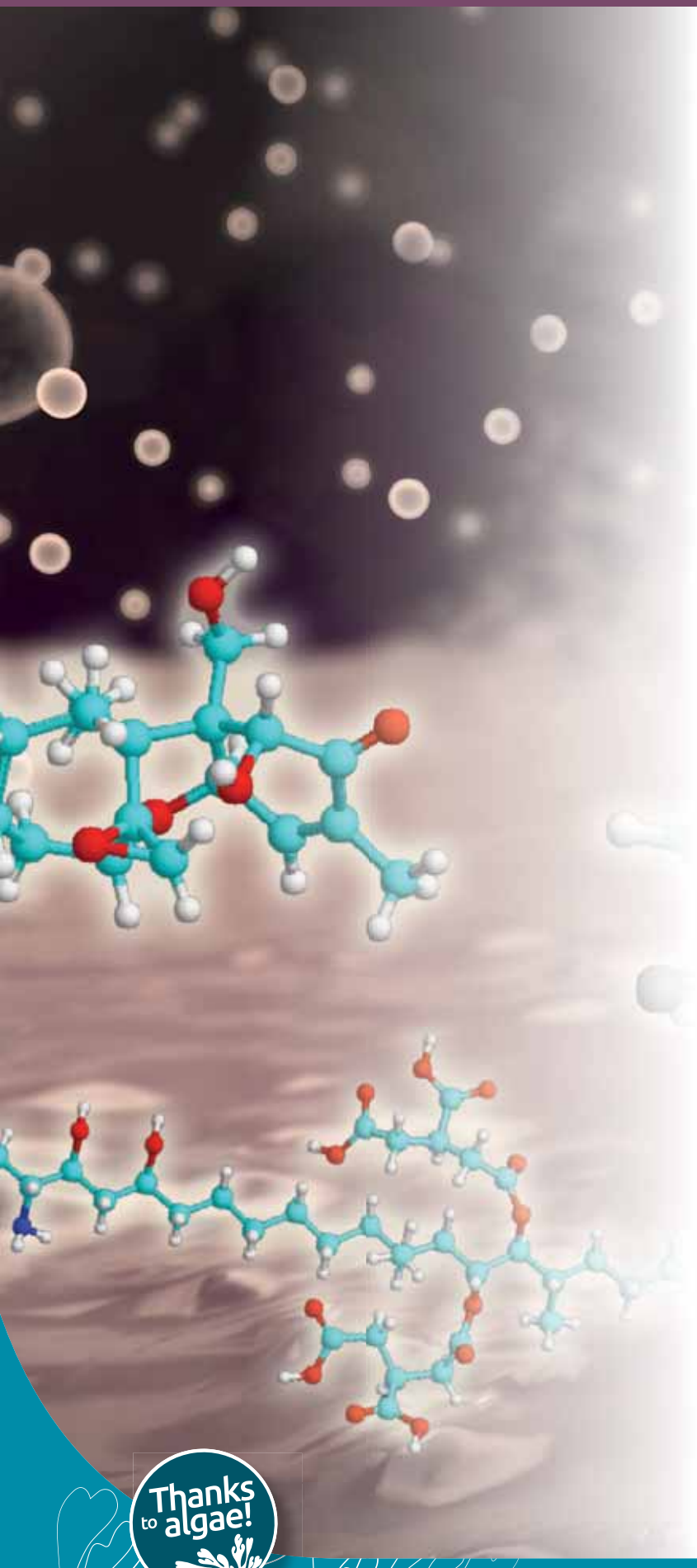

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POSTER ABSTRACTS

P1 – P11
CONTROLLING PLANT DISEASE AND MYCOTOXIN FORMATION

P1 Identification of *Chlamydomonas reinhardtii* genes involved in the toxicity of trichothecene mycotoxins
Matthew G. Bakker[1], M. Kafri[2], W. Patena[2], M.M. Vaughan[3], S. McCormick[3] and M.C. Jonikas[2]
[1]Department of Microbiology, University of Manitoba, Canada, [2]Department of Molecular Biology, Princeton University, USA and [3]Mycotoxin Prevention and Applied Microbiology Research Unit, U.S. Department of Agriculture, USA

P2 Characterisation of species composition, chemotype, and *in vivo* and *in vitro* fungicide sensitivity of *Fusarium* from wheat and maize in Michigan, USA
Mikaela Breunig[1], A.M. Byrne[1], J.L. Jacobs[1], T.J. Ward[2] and M.I. Chilvers[1]
[1]Department of Plant, Soil, and Microbial Sciences, Michigan State University, USA and [2]Mycotoxin Prevention and Applied Microbiology Research Unit, Agricultural Research Service, U.S. Department of Agriculture, USA

P3 Relationship between susceptibility to aflatoxin contamination and yield in maize hybrids
Dragana Budakov, T. Barosevic, Z. Savic, V. Stojsin, M. Grahovac, T. Dudas and F. Bagi
Faculty of Agriculture, University of Novi Sad, Serbia

P4 Deciphering the effect of ambient pH on the enniatins production by *Fusarium avenaceum*
Charlotte Gautier, N. Ferrer, S. Chéreau1, E. Zehraoui, N. Ponts and F. Richard-Forget
UR1264 MycSA, Centre de Recherche INRA Bordeaux-Aquitaine, France

P5 Application of a droplet digital PCR method (ddPCR) for molecular monitoring of citrinin and ochratoxin biosynthesis by *Penicillium verrucosum*
J. Politis, M. Schmidt-Heydt and **Rolf Geisen**
Max Rubner-Institut, Germany

P6 Loss of ochratoxin A biosynthetic cluster in *Aspergillus ochraceus* and other section *Circumdati* species
Jéssica Gil-Serna, C. Vázquez and B. Patiño
Department of Genetics, Physiology and Microbiology, University Complutense of Madrid, Spain

P7 Trichothecene genotypes of toxigenic *Fusarium* species associated with wheat head blight in western Canada
Mohamed Hafez[1], M. Telfer[1], N. Schatz[1], R. Gourlie[1], K. Turkington[2] and R. Aboukhaddour[1]
[1]Lethbridge Research and Development Center and [2]Lacombe Research and Development Center, Agriculture and Agri-Food Canada, Canada

P8 Resistance to *Fusarium langsethiae* in Norwegian oats – SafeOats
Ingerd Skow Hofgaard[1], H.U. Aamot[1], M. Lillemo[2], G. Brodal[1], E. Lysøe[1], M. Almvik[1], A.-G.R. Hjelkrem[1], M. Åssveen[1], A.L Russenes[1], E. Strand[1], Å. Bjørnstad[2], H. Skinnes[2], S. Gobena[2], E.S. Sørensen[3], T. Buraas[3], A. Ceplitis[4], B. Henriksen[5], B. Rodemann[6], and S. Edwards[7]
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P9 Genetic and genomic analysis of *Fusarium* resistance in different maize populations
Alessandra Lanubile and A. Marocco
Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Italy

P10 The role of pH signalling transcription factor PacC in pathogenicity and ochratoxin A biosynthesis by *Aspergillus carbonarius*
Omer Barda[1,2], D. Prusky[1] and E. Sionov[1]
[1]Institute of Postharvest and Food Sciences, Agricultural Research Organization, Volcani Center, Israel and [2]Department of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Israel

P11 Requirement of velvet complex proteins for development, ochratoxin A biosynthesis and fungal virulence in *Aspergillus ochraceus*
Gang Wang, H. Zhang, J. Ma, B. Yang, C. Zhang and Y. Liu
Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences / Key Laboratory of Agro-products Quality and Safety Control in Storage and Transport Process, Ministry of Agriculture and Rural Affairs, China

P12 – P48
OCCURRENCE, EXPOSURE AND EFFECTS

P12 Occurrence of ochratoxin A in feed, blood and milk samples collected from jennies and blood from their foals
Shafaq Asif[1,2], V. Lippolis[2], M. Pascale[2], S. Cervelleri[2], E. Mancini[2], A. Carluccio[1], D. Robbe[1] and F. Minervini[2]
[1]Faculty of Veterinary Medicine, University of Teramo, Italy and [2]Institute of Sciences of Food Production, National Research Council, Italy

P13 Co-occurrence of mycotoxins in dairy cow feed collected from Kanjanaburi and Ratchaburi provinces in Thailand
Darika Awapak[1], A. Petchkongkaew[1], M. Sulyok[2] and R. Krska[2]
[1]School of Food Science and Technology, Thammasat University, Thailand, [2]Department IFA-Tulln, BOKU Vienna, Austria

P14 What impact will climate change scenarios and acclimatisation of *Aspergillus flavus* strains have on colonisation and aflatoxin B1contamination of pistachio nuts
Alaa Baazeem[1,2], A. Rodriguez[1], A. Medina[1] and N. Magan[1]
[1]Applied Mycology Group, Cranfield University, UK and [2]Department of Biology, Taif University, Saudi Arabia

P15 Aflatoxins reduction and their bioaccessibility in extruded maizemeal products: a contribution for risk assessment
K.C. Massarolo[1], T. Verma[2], J.R. Mendoza[2], L. Kupski[3], E.B..Furlong[1] and **Andreia Bianchini**[2]
[1]School of Chemistry and Food, Federal University of Rio Grande, Brazil, [2]Food Science and Technology Department, University of Nebraska-Lincoln, USA and [3]Technology Center, State University of Maringá, Brazil

P16 Understanding the potential risk of aflatoxin exposure through consumption of contaminated ugali, a popular Rwandan maize-based food
R.F. Bayimenye[1], J.R. Mendoza[1,2], K.C. Massarolo[3], J. Stratton[1,2] and **Andreia Bianchini**[1,2]
[1]Department of Food Science and Technology and [2]The Food Processing Center, University of Nebraska-Lincoln, USA and [3]School of Chemistry and Food, Federal University of Rio Grande, Brazil

P17 Bioaccessibility of fumonisin B1 in extruded maize-based foods
K.C. Massarolo[1], T. Verma[2], J.R. Mendoza[2], L. Kupski[3], E.B..Furlong[1] and **Andreia Bianchini**[2]
[1]School of Chemistry and Food, Federal University of Rio Grande, Brazil, [2]Department of Food Science and Technology, University of Nebraska-Lincoln, USA and [3]Technology Center, State University of Maringá, Brazil

P18 Occurrence of mycotoxins in Brazilian maize and soybean meal in 2018
Tiago Birro and L. Sá
BIOMIN do Brasil, Brazil

P19 Age-related differences in exposure to deoxynivalenol and deoxynivalenol-3-glucoside unravelled: toxicokinetic study in the piglet as a human paediatric surrogate model
Amelie Catteuw[1], S. De Baere[1], G. Antonissen[1], L. Ivanova[4], S. Uhlig[4], A. Martens[2], S. De Saeger[3], M. De Boevre[4], M. Devreese[1] and S. Croubels[1]
[1]Department of Pharmacology, Toxicology and Biochemistry, [2]Department of Surgery and Anaesthesiology of Domestic Animals and [3]Department of Bioanalysis, Ghent University, Belgium, [4]Chemistry Section, Norwegian Veterinary Institute, Norway

P20 An *in silico* 'structural toxicology' approach to move the toxicological investigation of mycotoxins at an individual level – a case study on zearalenone oestrogenicity
Luca Dellafiora, G. Galaverna and C. Dall'Asta
Department of Food and Drug, University of Parma, Italy

P21 Urinary DON concentrations as biomarker of exposure in different age groups in Norway
Gunnar S. Eriksen[1], M. Sandvik[1], H.K. Knutsen[2], C. Brera[3], B. De Santis[3], F. Debegnach[3] and A.L. Brantsæter[2]
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P22 European mycotoxin survey January - June 2019
Thomas Ertelthaler, U. Hofstetter and A. Müller
BIOMIN Holding GmbH, Austria

P23 Deoxynivalenol-3-glucoside production in Canadian barley and wheat varieties in response to infection by *Fusarium graminearum*
J. Tucker[1,2], C. Amarasinghe[1], A. Badea[2] and **Dilantha Fernando**[1]
[1]Department of Plant Science, University of Manitoba and [2]Agriculture and Agri-Food Canada, Canada

P24 Risk estimation for ochratoxin A and aflatoxins due to capsicum consumption in a Chilean rural area
Claudia Foerster[1], L. Delgado- Rivera[2], A. Rivera3, S. Cortés[4], C. Ferreccio[4] and G. Rios[5]
[1]Institute of Agricultural and Veterinary Sciences, Universidad de O'Higgins, Chile, [2]Food Chemistry Section, Public Health Institute of Chile, Chile, [3]Food and Nutrition Department, Chile Ministry of Health, Chile, [4] Advanced Center for Chronic Diseases, Pontificia Universidad Católica de Chile, Chile and [5]Department of Food Science and Technology, University of Concepción, Chile

P25 Mycotoxin survey of straw across the UK
Robert Furmage, H.l. Ho, M. Steele and D. Parfitt
Micron Bio-Systems, UK

P26 The genotoxicity of caecal water in gilts exposed to low doses of zearalenone
K. Cieplińska[1], **Magdalena Gajęcka**[2], A. Nowak[3], M. Dąbrowski[2], Ł. Zielonka[2], M.T. Gajęcki[2]
[1]Microbiology Laboratory, Non-Public Health Care Centre, Poland, [2]Department of Veterinary Prevention and Feed Hygiene, University of Warmia and Mazury in Olsztyn, Poland and [3]Institute of Fermentation Technology and Microbiology, Lodz University of Technology, Poland

P27 Physiologically based kinetic (PBK) modelling-based reverse dosimetry of *in vitro* toxicity data to predict acute liver toxicity induced by aflatoxin B1 in rats and humans.
Ixchel Gilbert-Sandoval, S. Wesseling and I.M.C.M. Rietjens
Department of Agrotechnology and Food Sciences, Wageningen University & Research the Netherlands

P28 Patulin contamination in home-made vinegar and its importance on public health
Ece Günalan İnci and Z.D. Heperkan
Department of Food Engineering, Istanbul Aydın University, Turkey

P29 Co-contamination of mycotoxin in grain inoculated with *Fusarium graminearum* MTCC1893 on maize and rice and *F. sporotrichoides* MTCC1894 on wheat – evaluated for normal ambient temperature and moisture simulating rainy condition
Manoj B. Kudupoje[1], A. Yiannikouris[1] K.A. Dawson[1], M.U.Ahmed[2] and V. Malathi[2]
Center for animal Nutrigenomics & Applied Animal Nutrition, Alltech Inc., USA and [2]Department of Livestock Production and Management, Karnataka Veterinary, Animal and Fisheries Sciences University, India

P30 Mycotoxin occurrence in raw material and finished feedstuffs in Ireland
Anna Lavery[1], M. Stevenson[1], M. Little[1], D. Vega-Sampedro[2] and P. Ramos Caramona[2]
[1]Trouw Nutrition Ireland, Ireland and [2]Trouw Nutrition, the Netherlands

P31 Survey of major ergot and tropane alkaloids in bread in the Netherlands using LC-MS/MS
A. Veršilovskis, P. Mulder, M. de Nijs and **Hans Mol**
Wageningen Food Safety Research and EURL for mycotoxins & plant toxins in food and feed, the Netherlands

P32 The negative effects of ZEN and DON on proliferation, phenotype and immunoglobulin production of porcine B cells *in vitro* are abrogated in their derivatives HZEN and DOM-1.
Alix Pierron[1], E. Vatzia[1], A. Saalmüller[1], E. Mayer[2] and W. Gerner[1]
[1]Institute of Immunology, Department of Pathobiology, University of Veterinary Medicine, Austria and [2]Biomin Research Center, Austria

P33 A multi-year survey of mycotoxins in Canadian barley harvest samples
Kerri Pleskach, R. Blagden and S.A. Tittlemier
Grain Research Laboratory, Canadian Grain Commission, Canada

P34 Mycotoxins in poultry feed: 2018 survey and nutritional solution
Damien P. Prévéraud, R. Borutova and O. Averkieva
Adisseo France SAS, France

P35 Characterisation of free and modified forms of *Fusarium* and *Alternaria* mycotoxins within malt production
Nela Průšová, Z. Džuman, P. Jonátová, J. Hajšlova and M. Stránská-Zachariášová
Department of Food Analysis and Nutrition, University of Chemistry and Technology Prague, Czech Republic

P36 Multiple myxotoxins detected in maize samples received from five continents between October 2018 and March 2019
Jog Raj, H. Farkaš, Z. Jakovčević, J. Bošnjak-Neumüller and M. Vasiljević
PATENT CO, DOO., Serbia

P37 Presence of *Fusarium* mycotoxins in total mixed rations for dairy cows as affected by their composition
M. Rodríguez-Blanco, S. Marín, M. Prim, V. Sanchis and **Antonio J. Ramos**
Applied Mycology Unit, Food Technology Department, University of Lleida, Spain

P38 Screening studies and exposure estimation of mycotoxins presented in different varieties of *Camellia sinensis* collected in Latvia
Ingars Reinholds[1,2], E. Bogdanova[1], I. Pugajeva[1], and V. Bartkevics[1,2]
[1]Institute of Food Safety, Animal Health and Environment 'BIOR', Latvia and [2]Faculty of Chemistry, University of Latvia, Latvia

P39 Effects of deoxynivalenol-contaminated feed on health of broiler chickens
Insaf Riahi[1], A.M. Pérez -Vendrell[1], V. Marquis[2], A.J, Ramos[3] and J. Brufau[1]
[1]IRTA Animal Nutrition, Spain, [2]Phileo Lesaffre Animal Care, France and [3]Food Technology Department, University of Lleida, Spain

P40 Subsistence farmer perceptions and practises that contribute to increased risk of mycotoxin exposure in humans and animals
Lindy J. Rose[1], S. Phokane[1,2], B.C. Flett[2], E. Ncube[2] and J.P. Rheeder[3]
[1]Department of Plant Pathology, Stellenbosch University, South Africa, [2]Agricultural Research Council – Grain Crops, South Africa and [3]Institute of Biomedical and Microbial Biotechnology, Cape Peninsula University of Technology, South Africa

P41 Additions to the membrane disruptor effect of fumonisin B1 – *in vivo* test in rats in the kidney and liver
András Szabó[1,2], H. Fébel[3], O. Ali[2] and M. Kovács[1,2]
[1]Mycotoxins in the Food Chain Research Group and [2]Faculty of Agricultural and Environmental Sciences, Kaposvár University, Hungary, and [3]Research Institute for Animal Breeding, Nutrition and Meat Science, National Agricultural Research and Innovation Centre, Hungary

P42 Field survey on mycotoxins in wheat in the Netherlands: results of one decade
Ine (H.J.) van der Fels-Klerx, M. Focker, C. Liu, ED van Asselt
Wageningen Food Safety Research, the Netherlands

P43 Multi-annual mycotoxin occurrence in animal commodities and feedstuffs
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P44 Mycotoxins in oats: new insights into the impact of climate change factors on the *Fusarium*:oat pathosystem
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P45 Insights of the *in planta* toxicity of some bacterial metabolites of the mycotoxin deoxynivalenol
X.-Z. Li, Y.I. Hassan, D. Lepp and **Ting Zhou**
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P46 Protection of curcumin on OTA induced liver oxidative damage in duck is mediated by modulating lipid metabolism and intestinal microbiota
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P47 Exposure assessment of aflatoxin B1 in Pakistan using urinary aflatoxin M1 biomarker
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P48 High levels of aflatoxin exposure biomarkers in populations from three sub-Saharan Africa countries
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MITIGATING THE NEGATIVE IMPACT OF MYCOTOXINS

P49 Assessment of zearalenone reduction ability of plant-derived *Lactobacillus plantarum* BCC47723
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P50 *In vitro* activity of *Brassica*-based isothiocyanates against mycotoxin-producer *Fusarium graminearum*
Samina Ashiq, A. Watson, S. Edwards and M. Back
Harper Adams University, UK

P51 Lactic acid bacteria and their metabolites for the control of ochratoxin A
J.A. Feijó Correa[1,2] A.C. Schoch Marques Pinto[2,3], A. Gonçalves Evangelista[1] and **Fernando Bittencourt Luciano**[1]
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P52 Effect of the wheat milling type on the distribution of *Fusarium* mycotoxins
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P53 The preservative propionic acid differentially affects survival of conidia and damages germ tubes of feed spoilage fungi
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P54 Biocontrol agents of cereals mycotoxigenic fungi: elucidating mechanisms of action
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P55 Ability of soil actinobacteria to avoid and biodegrade aflatoxin B1 and ochratoxin A
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P56 Mycotoxin producing on different matrices
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P57 Mycotoxin biodetoxification potential of the *Cupriavidus* genus
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P58 Prevention of Fusarium head blight and mycotoxins in wheat with antifungal mulch layers and botanicals
Dimitrios Drakopoulos[1,2], A. Kägi[1], E. Jenny[1], H.-R. Forrer[1], A. Gimeno[1], G. Meca[3], T. Musa[1], J. Six[2] and S. Vogelgsang[1]
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P59 Knowledge Centre for global food and nutrition security (KC-FNS): scope, structure, future prospective.
Monica Ermolli[1], J. Stroka[2] and F. Rembold[1]
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P60 Evaluation of the effectiveness of an antimycotoxin additive to reduce toxic effects in weaned pigs consuming deoxynivalenol-contaminated feed
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P61 Assessment of the spread and respiration of *Fusarium graminearum* in wheat grains: a three-dimensional in situ study
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P62 Effectiveness of essential oils to prevent mycotoxin production by *Aspergillus* species using turbidimetric measurements with the Bioscreen C
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P63 The accumulation of *Fusarium graminearum* mycotoxins in wheat in response to the biological control agent *Clonostachys rosea* formulated in oil
Alejandro Gimeno[1], I. Bänziger[1], A. Kägi[1], E. Jenny[1], M. Leimgruber[1], D. Drakopoulos[1], B. Keller[2] and S. Vogelgsang[1]
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P64 Sodium sulfite interferes with fermentation during bioethanol production
D. Kotz, S. Rose, **Christiane Gruber-Dorninger**, G. Schatzmayr and D. Schatzmayr
BIOMIN Research Center, Austria

P65 *Saccharomyces cerevisiae* as a mycotoxin remediator
Hsueh lui Ho, R. Furmage, M. Steele and D. Parfitt
Micron Bio-Systems, UK

P66 Spatial analysis of mycotoxin development in stored grain to reduce waste
R. Kerry[1], **Ben Ingram**[2], E. Garcia-Cela[3] and N. Magan[3]
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P67 Determining future aflatoxin contamination risk scenarios for maize in southern Georgia, USA using spatio-temporal modelling and future climate simulations
Ben Ingram[1], R. Kerry[3], E. Garcia-Cela[2] and N. Magan[2]
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P68 The role of modification treatment of bentonites on the adsorption of mycotoxins
Stamatina Karakitsiou, C. Kapralou and C. Dedeloudis
Imerys Greece Industrial Minerals S.A, Greece

P69 Cold plasma interactions with mycotoxins and their fungal producers
C. Sarangapani[1], A. Los[1], D. Ziuzina[1], S. Chaple[1], P. Bourke[1,2], H. Shi[3] and **Kevin Keener**[1,3,4]
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P70 Sustainable pest control system for eliminating fungi and weeds using benzo derivatives
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P71 Efficacy of an algo-clay complex on decreasing aflatoxins toxicity on piglets
R. Pereira, **Julia Laurain** and M.A. Rodriguez
Olmix Group, France

P72 A forecasting model for aflatoxins contamination in maize: a case study in Serbia
Ningjing Liu[1], C. Liu[1], F. Bagi[2], M. Sulyok[3], T. Dudaš[2], Z. Savić[2], A. Stankov[2], M. Loc[2] and H.J. van der Fels-Klerx[1]
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P73 Mycotoxins – an increasing problem? costs of mycotoxin management
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P74 Application of lactic acid bacteria as biopreservation agents
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P75 Antifungal activity of allyl isothiocyanate: from raw materials to finished product
Giuseppe Meca, J.M. Quiles, R. Torrijos, C. Luz, T. Nazareth and J. Mañes
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P76 Climate change factors: transcriptomic and metabolomic shifts on *Aspergillus flavus* during maize storage
Angel Medina[1], M.K. Gilbert[2], M. Sulyok[3], C. Verheecke-Vaessen[1], E. Garcia-Cela[1], B.M. Mack[2], D. Bhatnagar[2] and N. Magan[1]
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P77 The role of resistance in reducing toxin contamination in wheat and maize
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P78 Barley production status in the northwestern of Uruguay regarding food safety
Lucia Pareja[1], C. Palladino[2], A. Muela[1], B. Taborda[1], F. Puigveri[1], N. Benitez[3] and D. Peloche[3]
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P79 Treatment with *Origanum virens* essential oil affects cell viability and alters morphological ultrastructure of *Aspergillus steynii*
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P80 Mycotoxin-sequestering feed additives reduce aflatoxin M1 levels in milk of lactating dairy cows fed aflatoxin
Damien P. Prévéraud[1], R.O. Rodrigues[2], R.O. Rodrigues[2], D.R. Ledoux[2], G.E. Rottinghaus[3], R. Borutova[1], O. Averkieva[1] and T.B. McFadden[2]
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P81 Efficacy of mycotoxin deactivators on health and growth of swine: a case-challenge study on weaned pigs and meta-analysis
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P82 Fate of *Alternaria* mycotoxins during apple concentrate production
María Agustina Pavicich[1], M. De Boevre[2], A. Vidal[2], S. De Saeger[2] and A. Patriarca[1]
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P83 Transformation of aflatoxin B1 and zearalenone using an evolved high-redox potential laccase.
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P84 Development of a multi-mycotoxins based method for studying adsorption of mycotoxins
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P85 ZenA renders ZEN non-oestrogenic in ruminants
J. Faas[1], B. Doupovec[1], M. Aleschko[1], C. Stoiber[1], M. Killinger[1], A. Höbartner[1], Q. Zebeli[2] and **Dian Schatzmayr**[1]
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P86 Advanced grain cleaning solutions for mycotoxin reduction
Katarina Slettengren[1], M. Pascale[2], M. Reichel[3], A. Vega[1], V. Lippolis[2], S. Cervellieri[2], B. Conde[1] and A.F. Logrieco[2]
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P87 *In vitro* efficacy assessment of a bentonite-based material acting as a multi-mycotoxin binder
Clarisse Techer, C. Messant, M. Doret-Aubertot and Q. Bruey.
MiXscience, France

P88 Remediation of deoxynivalenol (DON): developing an *in vitro* model to screen potential detoxifying agents on DON degradation and detoxification in a simulated gastro-intestinal tract of a pig
Ilse Vanhoutte[1], R. Desmet[4], A. Vidal[3], M. De Boevre3, S. Verstringe[4], J. Vande Ginste[4], K. Audenaert[2] and L. De Gelder[1]
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P89 Impacts of climate change on aflatoxins in maize and the dairy production chain
Ine (H.J.) van der Fels-Klerx, A.K. Gavai, L.C. Vermeulen and C. Liu
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P90 Isolation and characterisation of soil-borne bacteria for biocontrol of *Aspergillus flavus*
Cui Wang, F. Xu, J. Wang, A. Stevenson and G. Zhang
Mars Global Food Safety Center, China

P91 *In vitro* lipooligosaccharide adsorption by bentonite at different biological pH levels
Guanlin Wang
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P92 Essential tools to manage grain and flour quality and food safety
Paul Wehling[1] and A.R. Bridges[2]
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P93 Utilisation of compost for the degradation of deoxynivalenol
C. Cai[1,2], X.-Z. Li[1] and **Ting Zhou**[1]
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P94 – P136
SAMPLING AND ANALYSIS

P94 Microscale thermophoresis for non-competitive measurement of HT-2 toxin in beer
Henri Arola[1], J. Nowak[2] and T. Nevanen[1]
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P95 Extraction of mycotoxins from vegetal oils using natural deep eutectic solvents: a green alternative to conventional methods
F. Pradanas-González, R. Aragoneses-Cazorla, E. Andrade-Bartolomé, F. Navarro-Villoslada, **Elena Benito-Peña** and M.C. Moreno-Bondi
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P96 Lateral flow test to detect ergot alkaloids
Kirsten Berry and J. Wallace
Neogen Europe Ltd., UK

P97 The effect of grind and extraction size on deoxynivalenol result variability
V. Brunkhorst[1], C. Maune[1], J. Brunkhorst[1] and **Jordan Bierbaum**[1] and R. Niemeijer[2]
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P98 The effect of grind and extraction size on zearalenone result variability
V. Brunkhorst[1], C. Maune[1], J. Brunkhorst[1] and **Jordan Bierbaum**[1] and R. Niemeijer[2]
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P99 Looking beyond the horizon
Jacqueline M. Cea
Technological Laboratory of Uruguay, Uruguay

P100 Simplifying multi-toxin testing using a single extract for five common mycotoxins
John Davenport
VICAM, A Waters Business, USA

P101 Development of an UPLC-MS/MS method for the quantitative determination of six aflatoxins in different food matrices of animal origin
Siegrid De Baere[1], G. Antonissen[1,2], P.E. Ochieng[1,3,4], D.C. Kemboi[3,5], J. Lindahl[3], E. Kang'ethe[3], M.-L. Scippo[4], J. K. Gathumbi[5] and S. Croubels[1]
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P102 Beyond mycotoxins: pyrrolizidine alkaloids, where are we and where are we going?
Emiliano De Dominicis, P. Molon, A. Beghetto and S. Saner
Mérieux NutriSciences Research, Italy

P103 European Union Reference Laboratory for mycotoxins & plant toxins in food and feed: uniform testing for safe food
Monique de Nijs, D. Pereboom, A. Veršilovskis, L. van Raamsdonk, T. de Rijk, P. Mulder and H. Mol
Wageningen Food Safety Research, the Netherlands

P104 EURL proficiency test for DON, acetyl-DONs, and DON-3G in cereals
D. Pereboom, R. van Dam, T. de Rijk, H. Mol and **Monique de Nijs**
EURL for mycotoxins & plant toxins in food and feed, Wageningen Food Safety Research, the Netherlands

P105 Influence of capsule shells on the analysis of citrinin in red yeast rice food supplements using LC-MS/MS
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P106 Analysis of spices using IMMUNOPREP® online ochratoxin cartridges
R. Rhemrev, M. Pazdanska, N. MacKay, J. Wilcox, E. Manning and **Carol Donnelly**
R-Biopharm Rhône, UK

P107 Verification of multi-toxin immunoaffinity columns in determination of mycotoxins in animal feed and swine urine by UHPLC-MS/MS
P. Jedziniak[1], A. Tkaczyk[1], L. Panasiuk[1], J. Wilcox[2], N. Mackay[2], C. Milligan[2] and **Carol Donnelly**[2]
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P108 Analysis of mycotoxins in cannabis and related products using multi-toxin immunoaffinity clean-up in conjunction with HPLC-FLD
J. Wilcox, T. Nazeer, P. Brown, E. Marley, C. Milligan and **Carol Donnelly**
R-Biopharm Rhône Ltd., UK

P109 Efficient automated analysis of mycotoxins in cannabis and related products using online immunoaffinity clean-up conjunction with HPLC-FLD
C. Milligan, J. Wilcox, **Carol Donnelly** and E. Manning
R-Biopharm Rhône Ltd., UK

P110 A rapid and simple LC-MS/MS method for the quantification of the EU regulated mycotoxins in cereal-based products
Nicola Dreolin and S. Stead
Waters Corporation, UK

P111 ST: SniffTox – system for the detection of mycotoxins with electronic nose technology
Silvia Gastaldello, M. Grillo, L. Tassoni, F. Scurria and C. Maran
ATPr&d S.r.l., Italy

P112 Validation of the Reveal® Q+ for DON method for quantitative determination of deoxynivalenol in grains and grain products
R. Lucas Gray, C. Kronemeyer, A. Kostin, G. Anderson, B. Roman, F. Klein and R. Donofrio
Neogen Corporation, USA

P113 Measuring the sum – a novel screening method for ergot alkaloids in food
Maximilian Kuner[1], S. Kühn[2], H. Haase[3] and M. Koch[1]
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P114 Investigation into the performance of a NIR equipment on the prediction of mycotoxins in silo-stored lots of maize
J.K. Vidal, A.O. Mallmann, D. Tyska, L.T. Gressler and **Carlos A. Mallmann**
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P115 Development of antibodies for the mycotoxin citreoviridin
Chris M. Maragos[1], N. Kobayashi[2], Y. Uchiyama[2] and Y. Sugita-Konishi[2]
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P116 Near infrared hyperspectral imaging for deoxynivalenol detection in wheat grain
A. Femenias, A.J. Ramos, V. Sanchis and **Sonia Marín**
Applied Mycology Unit, Food Technology Department, University of Lleida, Spain

P117 A rapid SERS detection of ochratoxin A in wine
Lourdes Martinez, Y. Qu and L. He
Department of Food Science, University of Massachusetts, USA

P118 Novel strategies to ochratoxin A antibody generation and immunochemical analysis in wine and grape juice samples
D. López-Puertollano[1], **Josep V. Mercader**[2], C. Agulló[1], A. Abad-Somovilla[1] and A. Abad-Fuentes[2]
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P119 Interlaboratory variability in quantitative determination of mycotoxins
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P120 A five-minute ELISA, using mycotoxin-free one standard and precalibrated curve for the quantification of mycotoxins
E. Angeli, K. Badra, D. Foulos, C. Tsaridou, **Georgios Papageorgiou**, A. Ntantasios, S. Drakouli and S.D. Athanasiou
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P121 Validation of an innovative 5-minute lateral flow test for the quantification of ochratoxin A in wine samples
S. Iliopoulou, C. Tsaridou, A. Skliris, N. Natsaridis, **Georgios Papageorgiou**, A. Ntantasios, S.D. Athanasiou and S. Drakouli
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P122 Quantification of all mycotoxins using symmetric lateral flow technology and one step multi-toxin aqueous extraction
S. Drakouli, A. Skliris, M. Tziortziou, S. Iliopoulou, N. Natsaridis, **Georgios Papageorgiou**, A. Ntantasios and S.D. Athanasiou
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P123 Validation of an innovative ultra-fast 5-minute ELISA for the quantification of mycotoxins
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P124 Mimotope-based immunoassays for mycotoxin detection
Riikka Peltomaa[1], E. Benito-Peña[1], T. Head[2], S. Deo[2], S. Daunert[2], R. Barderas[3], F. Amaro[4], G. Orellana[4] and M.C. Moreno-Bondi[1]
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P125 Nanoparticle-based mycotoxin detection in food and agricultural products
Jan Pietschmann[1], H. Spiegel[1], H.J. Krause[2] and F. Schröper[1]
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P126 Application of an enzyme-linked immunosorbent assay to the detection of all prevalent twelve ergot alkaloids in cereals and cereal based animal feed proposed for European Commission regulation
Monika Plotan, J. Porter, L. Sibanda, M.L. Rodríguez, R.I. McConnell and S.P. FitzGerald
Randox Food Diagnostics, UK

P127 Innovative multiplex technology, challenges and the future of mycotoxin determination for predominant, masked and emerging mycotoxins
Monika Plotan, J. Porter, L. Sibanda, M.L. Rodríguez, R.I. McConnell and S.P. FitzGerald
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E. Bianco, **Federica Tamburlini** and F. Diana
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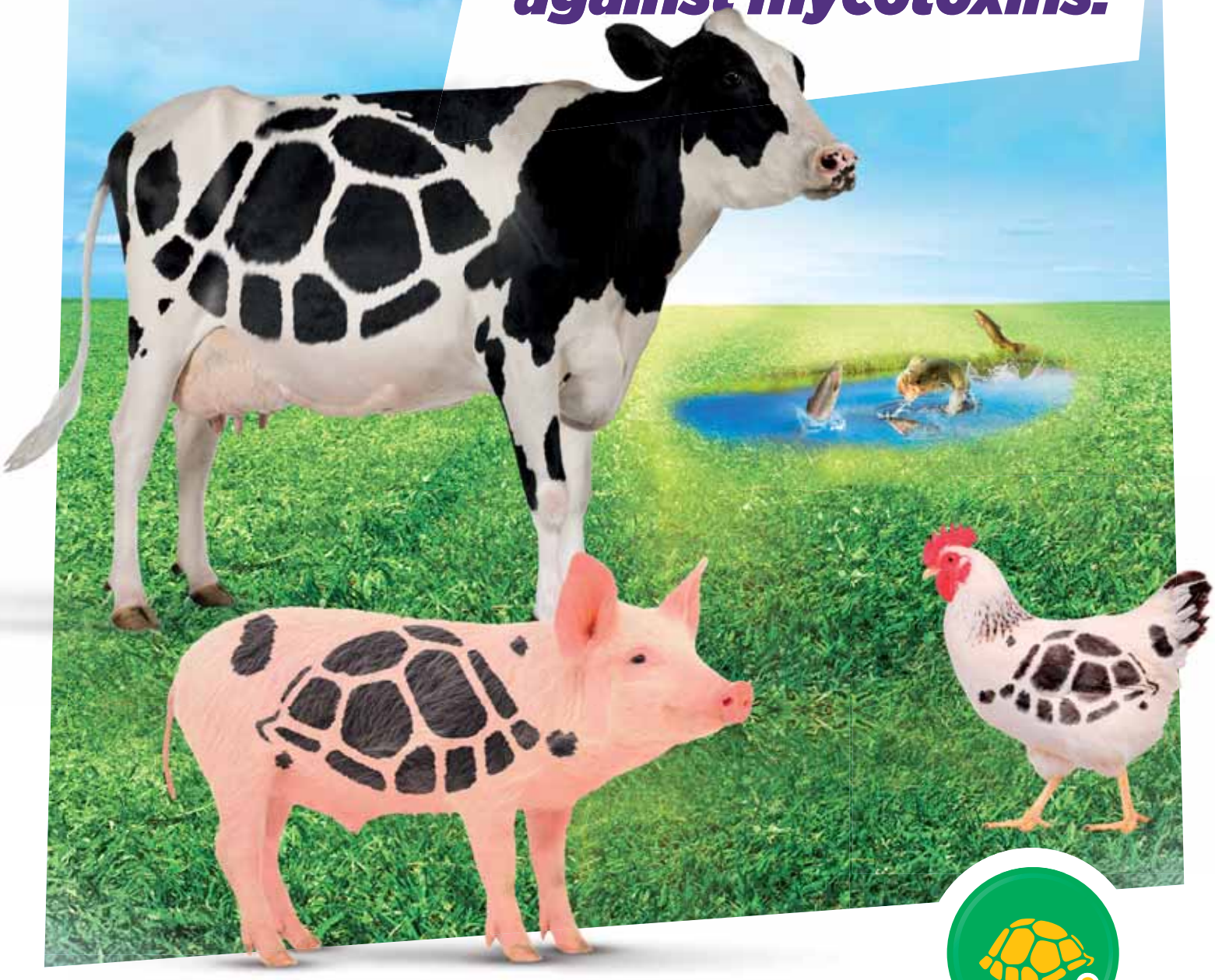

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CONTROLLING PLANT DISEASE AND MYCOTOXIN FORMATION
P1 – P11

P1
Identification of *Chlamydomonas reinhardtii* genes involved in the toxicity of trichothecene mycotoxins
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Trichothecenes are a family of small molecules of fungal origin that are toxic to many eukaryotes and that create a safety risk when contaminating food and feed. In the case of fusarium head blight of wheat, trichothecenes also function as a virulence factor that promotes disease progression. This latter activity suggests that a detailed understanding of how these toxins impact plants may provide insights that are useful for improving disease management and plant health. We identified plant genes that may be related to the toxicity of trichothecenes, using a library of mutants of the model plant *Chlamydomonas reinhardtii*. Mutant strains were pooled and the mixed community was grown in the presence of 15-acetyldeoxynivalenol, in the presence of trichodermin, or in the absence of trichothecenes (reference condition). After incubation, the relative abundance of the *C. reinhardtii* strains was determined by sequencing the internal barcodes within the cassettes that had been used to generate mutants. Genes linked to differential abundance in the presence of toxin were identified by subjecting normalised read count ratios (abundance in the presence of toxin : abundance in the control) to Fisher's exact tests with false discovery rate multiple testing correction. It was more common for gene knockouts to enhance susceptibility than to enhance resistance toward trichothecenes. Growth of *C. reinhardtii* in the presence of trichothecenes was significantly impacted by genes with putative functions that included protein kinases, transporters, pleiotropic drug resistance, RNA helicase, cell signalling, and regulation of the plant cell death pathway. This work provides candidate plant genes for further testing to determine the mechanism by which disrupting gene function alters susceptibility to trichothecene toxins.

P2
Characterisation of species composition, chemotype, and *in vivo* and *in vitro* fungicide sensitivity of *Fusarium* from wheat and maize in Michigan, USA
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Fusarium species are a major concern due to mycotoxin contamination of wheat and maize grains in North America. To characterise the population of *Fusarium* in Michigan, over 500 isolates were collected and species composition, chemotype (15-ADON, 3-ADON, NIV, NX) and fungicide sensitivity are being determined. Thus far, *F. graminearum* was the major species associated with wheat, but members of the *Fusarium tricinctum* complex, *F. culmorum*, *F. cerealis*, and *F. poae* were found as well. Greater species diversity was found in maize, with a smaller proportion identified as *F. graminearum* and more identified from the *Fusarium fujikuroi* complex. *In vitro* sensitivity to triazole chemistries registered in the United States (metconazole, tebuconazole, and prothioconazole) were assessed with mycelial growth assays. Isolates were most sensitive to metconazole, and less sensitive to prothioconazole and tebuconazole. A small portion of isolates within *F. graminearum* had EC_{50} values 10-100 fold greater than sensitive isolates. In order to determine if this reduced sensitivity *in vitro* would lead to practical resistance, a field trial was established in 2019. A subset of *F. graminearum* isolates were chosen for investigation, four identified as sensitive *in vitro* (EC_{50} 0.01-0.1 ppm), and four with reduced sensitivity *in vitro* (at least 10-fold greater). Plots were inoculated with spore suspensions of each isolate 48 h prior to fungicide applications in a factorial manner in a randomised complete block design. No differences in the relative fungicide efficacy were found, signalling no practical resistance currently exists despite differences *in vitro* and widespread use in wheat throughout Michigan for the last 10 years. If isolates with EC_{50} values greater than 10 ppm are found in the future, these would warrant further field testing. Interestingly, the isolates classified as sensitive *in vitro* were actually more pathogenic than those classified as resistant with an average 10% greater incidence, but only 5% on average greater severity. DON production was quantified and was slightly higher for sensitive isolates as well (mean 1.3 ppm greater) but was not statistically significant. The efficacy of DON reduction by the fungicide was not significantly impacted by the sensitivity of the isolates. We have yet to explore *in vivo* sensitivity of

species besides *F. graminearum* and hope to further explore toxin production in these different species and isolates.

P3

Relationship between susceptibility to aflatoxin contamination and yield in maize hybrids

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Aspergillus flavus causes ear rot, one of the most important diseases of maize. Significance of this fungus rises from its ability to produce aflatoxins, which are the best known and the most intensively researched mycotoxins in the world, with proven toxic and carcinogenic effects on human and livestock health. In order to control Aspergillus ear rot, a number of measures are employed, however, none of them alone sufficiently controls this disease. There are currently no commercial maize hybrids completely resistant *to A. flavus* infection, because high level of genetic resistance is difficult to achieve. Additionally, resistance to *A. flavus* infection is different from maize resistance to aflatoxin contamination, while both of these are complex traits that are influenced by environmental factors. The aim of this study was to evaluate resistance of maize hybrids to aflatoxin contamination and to test if the resistance trait coincides with the yield. For this purpose, twenty commercial maize hybrids were included in the field trials during 2017 and 2018. Plants were inoculated with *A. flavus* 10-14 days after plants entered the flowering stage using toothpick method. During harvest, together with measuring yield, samples of each hybrid were collected for aflatoxin B1 analysis. Significant differences were noted in level of contamination between hybrids, as well as between yield of tested hybrids. **Acknowledgements**. This study was conducted within project tasks of MyToolBox project. This project received funding from European Union's Horizon 2020 research and innovation programme under grant agreement No 678012.

P4

Deciphering the effect of ambient pH on the enniatins production by *Fusarium avenaceum*

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Enniatins belong to the group of so-called 'emerging mycotoxins' produced mainly by *Fusarium avenaceum* and *F. tricinctum*. Little is known about the mechanisms by which the biosynthesis of these mycotoxins can be modulated, increased or repressed. This lack of knowledge hinders the development of control tools to ensure minimum levels of contamination in cereal harvests. The aim of this study was to decipher the factors and events that can affect the biosynthesis of enniatins, with a specific focus on the effect of ambient pH. Using a set of 12 *F. avenaceum* strains cultivated in an enniatin-inducing FDM medium [Madry *et al.*, 1983. Eur.J. Appl. Microbiol. Biotechnol. 17:75], we demonstrated that this *Fusarium* species increased the environmental pH, in contrast to the acidification reported for the deoxynivalenol-producing species. First, the growth and mycotoxins production of the 12 strains were tested in FDM media buffered at various pH values. Whatever the *F. avenaceum* strain considered, the highest mycelium biomass was obtained at pH 7, supporting that alkaline media are suitable for *F. avenaceum* development. Concerning enniatins production, our results showed that these mycotoxins can be produced at a wide range of pH values, between 4 and 7. However, the optimal pH value for their yield was strain-dependent: while in most strains, the highest amounts of toxins were quantified at pH 4-5, pH values higher than 6 were shown to promote the production of enniatins in a sub-set of four strains. Second, we investigated the role of the PacC homologue from *F. avenaceum*, FavPac1, in the regulation of enniatins production. FavΔPac1 deletion mutants were constructed in three *F. avenaceum* strains characterised by contrasted responses to variations in environmental pH. Fungal development, expression of the peptide synthetase gene (*esyn*) coding for the biosynthesis of enniatins and the accumulation of toxin at different pH were tested in the mutants and in their corresponding wild strains. The resulting data revealed the mechanisms by which FavPac1, a transcription factor that regulates pH homeostasis, is involved in the modulation of the production of enniatins by *F. avenaceum*.

P5

Application of a droplet digital PCR method (ddPCR) for molecular monitoring of citrinin and ochratoxin biosynthesis by *Penicillium verrucosum*

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Penicillium verrucosum is an important contaminant of cereals, especially in regions with a slightly humid climate and moderate temperatures. It can also be found on certain fermented vegetables like salted olives. This species is able to produce the two important mycotoxins ochratoxin and citrinin. Both are polyketides with very similar structures. The most important difference between both is the attachment of phenylalanine and chlorine to the polyketide structure of ochratoxin. According to literature data [O´Callaghan *et al.*, 2013, Int. Food Microbiol. 161:172], the *otapks*PV gene is involved in the biosynthesis of ochratoxin. This statement is based on gene inactivation data. However, subsequent genome analysis showed that this gene is obviously identical to the *pks*CT gene, which is the pks gene for citrinin biosynthesis. The obtained transformants of *P. verrucosum*, however, were not able to produce ochratoxin. So, the authors concluded that this gene is also responsible for the biosynthesis of ochratoxin. To monitor citrinin and ochratoxin biosynthesis in cereals, wheat was inoculated with spores of *P. verrucosum* and incubated at 15, 20 and 25°C, for 7, 12 or 18 days. At these time points, samples were withdrawn and subjected to DNA and RNA quantification by ddPCR, with the *pks*CT gene as the target gene. Furthermore, the toxins produced were quantified by HPLC. The ddPCR analysis of the samples revealed an increase in fungal DNA content over time, indicating the increase in biomass. Especially at day 12, the DNA copy number was at its highest. The highest expression of the *pks*CT gene (several orders of magnitude, compared to the other conditions) was found at day 18, at 20 and 25°C. These results indicate that the *pks*CT transcript copy number is not directly correlated to the DNA copy number. The HPLC demonstrated that citrinin production is favoured at lower temperature ranges (15 and 20°C), whereas higher amounts of ochratoxin are produced at higher temperature ranges (20 and 25°C). The highest amounts of both toxins were produced after 18 days of incubation. Interestingly, the *pks*CT gene showed its highest expression at conditions were also the biosynthesis of ochratoxin was at its highest level (18 days, 20 and 25 °C), which, in agreement with O'Callaghan *et al.* (2013), also suggests the involvement of this gene to ochratoxin biosynthesis. Indeed, an inactivation of the *pks*CT gene of *P. verrucosum* during the current analysis revealed a transformant strain which was neither able to produce citrinin nor ochratoxin.

P6

Loss of ochratoxin A biosynthetic cluster in *Aspergillus ochraceus* and other section *Circumdati* species

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Ochratoxin A (OTA) is one of the most important mycotoxins due to its high toxicity and frequent occurrence in foodstuffs. It is produced by some *Aspergillus* section *Circumdati* species, among others. This toxin was firstly detected in a culture of *Aspergillus ochraceus* and this fungus was considered for a long time the most important OTA-producing species. However, several taxonomic revisions supposed the description of new species that are able to produce higher levels of the toxin, mainly *A. steynii* and *A. westerdijkiae*. Moreover, recent studies demonstrated that the first OTA-producing isolate described was indeed *A. westerdijkiae*. The five genes involved in OTA biosynthesis are clustered in the same 20 kb-long genomic region presenting a conserved syntheny in all producing species. The location of this cluster in *A. westerdijkiae* and *A. steynii* genome is conserved and it is flanked by two genes encoding a hydrolase and an oxidorreductase. The sequence of these genes was used to design degenerate primers to amplify this region in *A. ochraceus*. These primers amplified a region of approximately 3,300 bp in all fourteen isolates analysed and was sequenced in five *A. ochraceus* strains from different origins. The results showed that *A. ochraceus* isolates have a non-functional OTA biosynthetic cluster which only presents a few fragments of the halogenase and the polyketide synthase encoding genes. The results obtained here indicated that *A. ochraceus* cannot be an OTA producer and, therefore, its presence in food products might not pose a risk for food safety. The loss of the OTA biosynthetic cluster seems to be widespread in other *Aspergillus* section *Circumdati* species. The analysis of the complete genome of *A. sclerotiorum* revealed the presence of a truncated OTA cluster but different from the version detected in *A. ochraceus*. In this case, the cluster maintains a short fragment of the genes encoding the halogenase, bZIP transcription factor, cytochrome p450 monooxygenase, and polyketide synthase together with small parts of the intergenic regions of the biosynthetic cluster. **Acknowledgements**. This work was supported by AGL 2014-53928-C2-2R.

P7

Trichothecene genotypes of toxigenic *Fusarium* species associated with wheat head blight in western Canada

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The *Fusarium* trichothecenes contaminate cereal-based foods and feeds globally and have generated extensive quality-control problems in the grain industry. Contamination of grains by *Fusarium* mycotoxins, especially trichothecenes (DON, nivalenol and T2) is a worldwide problem. In Canada, the most common trichothecene in grain is DON, the first evidence of wide spread contamination of wheat in western Canada by *F. graminearum* and DON was in 1985. Fusarium head blight (FHB) is the most damaging fungal disease of wheat and cereals and is caused by a numerous species of *Fusarium*. In this study, infected wheat stem and grain samples were collected from three field plots in Alberta (Lethbridge, Lacombe and Beaverlodge) and one field plot in Saskatchewan (Scott) to characterise the major *Fusarium* species associated with FHB in western Canada as well as the associated trichothecene genotype. A *Fusarium*-selective medium was used to isolate *Fusarium* species and the translation elongation factor 1 alpha (*TEF1α*) gene sequences were used for molecular identification of the isolates. A total of 263 *Fusarium* isolates belonging to nine *Fusarium* species were recovered. PCR assay based on the *Tri5* gene (encoding trichodiene synthase) was used to screen for trichothecene-producing species, and the results showed that, 132 *Fusarium* isolates (46.4%) were found to amplify *Tri5* gene (*Tri* +). Trichothecene genotyping using two multiplex PCR assays based on both *Tri3* and *Tri12* genes encoding for trichothecene 15-O-acetyltransferase and trichothecene efflux pump, respectively, showed that the 3-ADON trichothecene is the most dominant genotype in all trichothecene producing *Fusarium* species tested during this study followed by type-A trichothecene (29.5%), while 15-ADON was found to be the least trichothecene genotype observed (2.5%). A phylogenetic analysis of selected *Fusarium* isolates based on the *TEF1α* and the *Tri5* gene sequences showed that, trichothecene genotype differences are not well correlated with the species evolutionary relationships of FHB-associated *Fusarium* species. The results presented here extend the previous knowledge about the adaptive evolution within trichothecene genes.

P8

Resistance to *Fusarium langsethiae* in Norwegian oats – SafeOats

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Occasionally, high mycotoxin levels are observed in Norwegian oat grain lots. The development of moderate resistant oat cultivars is therefore highly valued in order to increase the share of high-quality grain into the food and feed industry. The Norwegian SafeOats project (2016-2020) aims to develop resistance screening methods to facilitate the phase-out of *Fusarium*-susceptible oat germplasm. Furthermore, SafeOats will give new insight into the biology of *Fusarium langsethiae* and HT2+T2 accumulation in oats. The relative ranking of oat varieties according to *F. graminearum*/DON versus *F. langsethiae*/HT2+T2 content has been explored in naturally infested as well as in inoculated field trials. Routine testing of the resistance to *F. graminearum* in oat cultivars and breeding lines has been conducted in Norway since 2007. We are currently working on ways to scale up the inoculum production and fine tune the methodology of *F. langsethiae* inoculation of field trials to be routinely applied in breeding programs. Through greenhouse studies, we have analysed the content of *Fusarium* DNA and mycotoxins in grains of selected oat varieties inoculated at different development stages. Furthermore, we are studying the transcriptome during *F. langsethiae* and *F. graminearum* infestation of oats. The project also focusses on the occurrence of *F. langsethiae* in oat seeds and possible influence of the fungus on seedling development in a selection of oat varieties. On average, the fungus was observed on 5% of the kernels in 168 seed lots tested during 2016-2018. No indication of transmission of *F. langsethiae* from germinating seed to seedlings was found in a study with germination of naturally infected seeds. So far, the studies have shown that the ranking of oat varieties according to HT2+T2 content in non-inoculated field trials resembles the ranking observed in inoculated field trials. The ranking of oat varieties according to DON content is similar in non-inoculated and *F. graminearum* inoculated field trials. However, the ranking of oat varieties according to DON content does not resemble

the ranking for HT2+T2. The results from SafeOats will benefit consumers nationally and internationally by providing tools to increase the share of high-quality grain into the food and feed industry. Acknowledgements. The project is financed by The Foundation for Research Levy on Agricultural Products/Agricultural Agreement Research Fund/Research Council of Norway with support from the industry partners Graminor, Lantmännen, Felleskjøpet Agri, Felleskjøpet Rogaland Agder, Fiskå Mølle Moss, Norgesmøllene, Strand Unikorn/Norgesfôr and Kimen Seed Laboratory.

P9

Genetic and genomic analysis of *Fusarium* resistance in different maize populations

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Fungal infection by *Fusarium verticillioides* is cause of substantial reductions in maize yield and grain quality worldwide. Developing natural resistance in maize genotypes is an effective way to achieve sustainable control of *F. verticillioides* in the field, and breeding for resistance may be accelerated by identifying 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. Several quantitative trait loci (QTL) and single-nucleotide polymorphism markers for resistance to *Fusarium* deriving from QTL mapping and genome-wide association studies have been described in three different maize populations: (i) bi-parental population; (ii) association mapping panel; and (iii) multi-parent advanced generation inter crosses (MAGIC). To guide the identification of candidate genes within the identified QTL, transcriptomic and sequencing information have been exploited. Promising candidate genes associated with disease resistance and pathogen related-mechanisms at the *Fusarium* resistant loci have been identified on maize chromosomes 4, 5 and 7. Many of the identified candidates genes offer hints to key metabolic pathways that may have a significant effect on reducing Fusarium infection. Measuring Fusarium resistance in open field could confirm and support their direct use in maize breeding either through crosses or genome editing approaches. Acknowledgements: This work was funded by the European Union's Horizon 2020 research and innovation programme under Grant Agreement No. 678781 (MycoKey).

P10

The role of pH signalling transcription factor PacC in pathogenicity and ochratoxin A biosynthesis by *Aspergillus carbonarius*

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Pathogenic fungi must respond effectively to changes in environmental pH for successful host colonisation, virulence and toxigenicity. *Aspergillus carbonarius* is a mycotoxigenic pathogen with the ability to colonise many plant hosts and secrete ochratoxin A (OTA). In this study we characterized the functions and addressed the role of PacC-mediated pH signalling in *A. carbonarius* virulence using designed *pacC* gene knockout mutant. *pacC* mutant displayed an acidity-mimicking phenotype resulting in poor growth at neutral/alkaline pH, accompanied by reduced sporulation and conidial germination compared to the wild-type. The *ΔacpacC* mutant was unable to effectively acidify the growth media as a direct result of poor gluconic and citric acid production. Furthermore, loss of *AcpacC* resulted in significant reduction of OTA production at acidic pH. Additionally, *ΔacpacC* mutant was less virulent than the wild-type strain in grapes and nectarine fruits. Reintroduction of *pacC* gene into *ΔacpacC* mutant restored the wild-type phenotype. Our results demonstrate important roles of PacC in OTA biosynthesis through regulation of the genes in the cluster, and in pathogenicity of *A. carbonarius*, possibly by controlling transcription of acid-expressed genes important for fungal infection.

P11

Requirement of velvet complex proteins for development, ochratoxin A biosynthesis and fungal virulence in *Aspergillus ochraceus*

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Ochratoxin A (OTA) is the secondary metabolite of *Aspergillus* and *Penicillium* species, classified as a possible human carcinogen (group 2B) by the International Agency for Research on Cancer. OTA was first isolated from *A. ochraceus* in 1965 and *A. ochraceus* was reported to be the major contributor of OTA in cereal, maize, coffee, fruits and beverage. The heterotrimeric velvet complex, LaeA/VeA/VelB, has been most studied in fungi to clarify the relation between light dependent morphology and secondary metabolism. In this study, we examined the role of velvet complex proteins in development, OTA biosynthesis and fungal virulence in *A. ochraceus*. To this aim, *laeA*, *veA* and *velB* genes were deleted in an ochratoxigenic *A. ochraceus* strain by target gene replacement. Loss of *laeA*, *veA* and *velB* yielded mutants with differences in vegetative growth and conidial production. Especially, *ΔlaeA* almost lost the ability to generate conidiophore under dark condition. The deletion of *laeA*, *veA* and *velB* drastically reduced the production of OTA. The wild type *A. ochraceus* produced about 1 and 7 μg/cm^2 OTA under light and dark condition on media, while the three gene deletion mutants produced less than 20 ng/cm2 OTA, which was correlated with a downregulation of OTA biosynthetic genes. Furthermore, virulence studies of *ΔlaeA*, *ΔveA* and *ΔvelB* showed differential reduction in disease severity in pears, ranging from 40 to 82%. Taking together, these results reveal that velvet complex proteins play crucial roles in morphological development, OTA biosynthesis and fungal virulence in *A. ochraceus*.

OCCURRENCE, EXPOSURE AND EFFECTS
P12 – P48

P12
Occurrence of ochratoxin A in feed, blood and milk samples collected from jennies and blood from their foals
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Natural dietary exposure to ochratoxin A (OTA) has been previously reported for horses and their foals, but no data are currently available for donkeys. The present study was aimed to assess the natural OTA exposure of seven Martina Franca jennies by analysing feed samples and blood and milk collected every 15 days from jennies during late pregnancy and after delivery. The transfer of OTA to foals was investigated by analysing their blood. Fifty-three feed samples were collected from January to September 2018 and analysed by the AOAC Official method No. 2000.03 based on immunoaffinity column (IAC) clean-up of extracts and HPLC/FLD detection (LOD 0.1 ng/g). Eighty-seven percent of samples showed low OTA levels up to 2.7 ng/g. A total of 67 and 34 blood samples were collected from jennies and foals, respectively, and analysed for OTA by ELISA (LOD 50 ng/l). Twenty-three percent of samples were confirmed by HPLC analysis. In jennies the OTA incidence rate of positive blood samples was 73%, with median value of 114 ng/l and concentrations ranging from 51 to 6,000 ng/l. A season effect on OTA levels in the blood samples was observed with increases in 46% of the positive ones collected from April to June. Concerning foals, the incidence rate of positive blood samples was 50% with median value of 136 ng/l and concentrations ranged from 79 to 4,030 ng/l. A total of 33 milk samples were collected from jennies and analysed by HPLC/FLD method based on IAC clean-up (LOD 15 ng/l). The incidence of milk positive samples was 36% with levels ranging from 17 to 82 ng/l. A positive relationship (r=0.77) between serum OTA level in jennies and the ratio serum/milk OTA was found. In conclusion, the occurrence of OTA in blood and milk samples showed a natural exposure of jennies and foals to this mycotoxin. In addition, the presence in jenny milk could pose a risk for human new-born considering its well-known nutritional and health properties. **Acknowledgements**. This study was supported by Rep-Eat – H2020 MSCA-COFUND 2015. Grant Agreement No.713714.

P13
Co-occurrence of mycotoxins in dairy cow feed collected from Kanjanaburi and Ratchaburi provinces in Thailand
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Mycotoxins can be found in milk due to transfer from contaminated feed. This study investigated the incidence and concentration of mycotoxins and co-contaminants in dairy cow feed samples collected from Kanjanaburi and Ratchaburi provinces between December 2018 and March 2019. Samples of concentrate (n=21), roughage (n=34), and mixed feed (n=18) were analysed using liquid chromatography coupled to tandem mass spectrometry. The study focused on mycotoxins regulated in the European Union and non-regulated mycotoxins: beauvericin, enniatins, moniliformin, deoxynivalenol-3-glucoside, alternariol monomethyl ether, alternariol, tenuazonic acid, and sterigmatocystin. All samples of concentrate were contaminated with at least four mycotoxins, in a range from four to fifteen. Zearalenone, beauvericin, and alternariol monomethyl ether were found in combination with other mycotoxins, most commonly with fumonisins, moniliformin, enniatin B, and alternariol. Similarly, all mixed feed samples contained between two and twelve mycotoxins. Beauvericin co-occurred most frequently with zearalenone and fumonisin B1, in both cases in 72.2% of the samples. In contrast, only 20.6% of the roughage samples contained more than one mycotoxin. Beauvericin was detected with sterigmatocystin in chopped whole maize and straw. The co-occurrence of enniatin A, A1, B, and B1, and of beauvericin with enniatin A1, B, and B1 were detected only in brewer's grain. Although the contamination levels of mycotoxins detected were low, the number of different fungal metabolites found in the sample set calls for systematic investigations on potential synergism.

P14

What impact will climate change scenarios and acclimatisation of *Aspergillus flavus* strains have on colonisation and aflatoxin B1contamination of pistachio nuts

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Pistachio nuts can become contaminated by *Aspergillus flavus* under warm and humid conditions. This can result in contamination with aflatoxin B1 (AFB1), classified as a class 1A carcinogen. There have been no studies on the impact that interacting climate change (CC) factors on colonisation by *A. flavus* and AFB1 contamination of pistachio nuts. In addition, no data are available on whether acclimatisation of *A. flavus* strains for several generations at elevated CO_2 will affect colonisation and AFB1 contamination. Thus, the objectives of this study were to examine the effect of interacting climate change (CC) factors of temperature x water activity x CO_2 (400 vs. 1000 ppm) on (i) growth of *A. flavus*, (ii) on relative gene expression of the *aflD* and *aflR* genes, (iii) on AFB1 production, and (iv) on whether acclimatization at 1000 ppm CO_2 of *A. flavus* strains AB3 and AB10 (5 generations) affected AFB1 production and mycelial growth under interacting CC conditions and compare this with non-acclimatised cultures. These studies showed that the relative expression of the biosynthetic genes (*aflD* and *aflR*), and AFB1 production were affected by interacting CC interaction factors. Growth of *A. flavus* was not significantly affected by the interactions. The expression of the structural gene *aflD* was generally related to AFB1 levels. CO_2 affected AFB1 production because there was an increase in toxin amounts at 35°C + 1000 ppm CO_2 at 0.98 a_w when compared to the control (400 ppm CO_2). This suggests that CC factors may have a differential effect depending on the interacting conditions of temperature (35 vs. 37°C) as in some cases AFB1 production was stimulated while in others remained the same. With regards to the impact of acclimatisation, the results of this study differed for the two strains examined. One strain showed faster growth and a clear stimulation of AFB1 production after 5 generations of acclimatisation. The other strain showed no difference from the control. This suggests that there may be intra-strain differences of effects of acclimatisation and this could influence mycotoxin contamination of such commodities as mixed populations of contaminant fungi often occurs. More studies are needed on the acclimatisation of fungal pathogens and their effect on food commodities under CC scenarios to obtain more accurate data on implications for mycotoxin contamination of these economically important commodities.

P15

Aflatoxins reduction and their bioaccessibility in extruded maizemeal products: a contribution for risk assessment

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One of the components of a risk assessment is the determination of hazard exposure. In the case of mycotoxins, that is a very complex question that has many implications. One point to consider is the amount of toxin that would be available for absorption due to food processing and preparation. Therefore, the objective of this study was to evaluate the effect of extrusion on the levels of aflatoxins B1, B2, G1 and G2 and their bioaccessibility in maizemeal after processing, in order to mitigate the risk of exposure to mycotoxins. For this, maizemeal samples spiked with aflatoxins (50 ng/g) and 22% of moisture were extruded in the absence and presence of high amylose maize starch (Hylon VII) (20%, w/w). A single-screw extruder was used with the temperatures set at 50, 140, and 160°C for feeding, transition, and metering zones, respectively, and screw speed of 210 rpm. Aflatoxins were quantified before and after the extrusion process. The extruded products were subjected to an *in vitro* digestion process and the toxin bioaccessibility was determined. Toxin quantification in all samples was done by HPLC-fluorescence detector. Extrusion of maizemeal samples led to a reduction of aflatoxins levels in the extruded product (B1 – 83.7%, B2 – 80.5%, G1 – 74.7% and G2 – 87.1%), and when Hylon VII was added to the formulation, higher reductions were observed (B1 – 89.9%, B2 – 88.6%, G1 – 75.0% and G2 – 89.9%). However, after the digestion process the aflatoxins levels detected in samples was increased, partially reverting the extrusion effect. When the formulation included Hylon VII, the bioaccessibility of aflatoxins was the highest (B1 – 51.3%, B2 – 69.4%, G1 – 66.7% and G2 – 99.8%). This *in vitro* study indicates that part of the aflatoxin reduction promoted by the thermal extrusion process may be caused by interactions between aflatoxins and the food matrix macromolecules. Once the digestion is completed, part of these toxins become available for absorption in the small intestine.

However, not all toxin reduction is converted back, indicating that some may be degraded or remain bounded to some non-digestible component. Therefore, extrusion cooking seems to be a promising food processing technology to mitigate the risk of exposure to aflatoxins in food products.

P16

Understanding the potential risk of aflatoxin exposure through consumption of contaminated ugali, a popular Rwandan maize-based food

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Mycotoxins are mould-produced secondary metabolites that cause diseases in humans and animals, also known as mycotoxicoses. While there are numerous types of mycotoxins, aflatoxins are known to be the most potent, the most frequently encountered in different grain commodities, and thermally stable. Further, this fungal toxin is typically monitored on foods based on regulatory limits established for different products. Food is a major source for human exposure to mycotoxins, and during digestion these compounds may be released from the food matrix becoming (bio)available for absorption. The bioaccessibility of mycotoxins is dependent on factors such as the composition of the food product and the methods used for food processing and/or preparation. The purpose of this study was to evaluate aflatoxin reduction in raw maizemeal and toxin bioaccessibility in the final product when ugali, a popular Rwandan food, was cooked following a traditional recipe. Samples of raw white maizemeal were spiked with 40 ppb of aflatoxin B1 (AFB1), and a portion of each sample was used to prepare ugali. Samples of both raw and cooked products were subjected to an *in vitro* digestion assay. Aflatoxin quantification was performed in the raw maizemeal, in the ugali, as well as in the liquid fraction of the digested product. All tests were done using immunoaffinity columns followed by fluorometry. The method used for toxin quantification was evaluated based on its ability to recover AFB1 from raw maizemeal, cooked product and the digested liquid fraction. Aflatoxin levels in raw maizemeal and ugali were compared to evaluate any apparent reduction due to the thermal process. Additionally, the bioavailability of AFB1 was determined in the raw product, cooked ugali and digested liquid fraction. Results showed that the methods used to detect AFB1 in the samples were adequate, with acceptable recoveries. The thermal process used for the preparation of ugali led to an AFB1 reduction when compared to the raw material. This was likely due to molecular degradation or structural changes that may have altered the aflatoxin epitopes. The cooked material, ugali product, showed a greater AFB1 bioaccessibility than the uncooked maize. This implies that the reduction observed during the thermal process could be offset by the higher bioaccessibility of the toxin upon cooking. Therefore, it is always recommended that the Rwandan population prepare ugali with maize of high quality and safety to minimise their exposure to aflatoxins.

P17

Bioaccessibility of fumonisin B1 in extruded maize-based foods

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Food processing has several advantages, such as the increase in nutritional bioavailability of foods and the reduction of toxic components. However, when mycotoxins are considered, a lack of understanding exists regarding the true effect of food processing. Previous reports have indicated a potential reduction of mycotoxins by several food processes and the effect of specific food ingredients, but few have considered the role of digestion in reversing those reductions. Therefore, the objective of this research was to determine the effect of added amylose on fumonisin B1 levels in extruded maize-based products and toxin bioaccessibility upon processing. Maizemeal and maizemeal combined with high-amylose maize starch (20%, w/w) were extruded in the presence of fumonisin B1 (1.5 µg/g) and 22% moisture. The extrusion was achieved using a single-screw extruder (temperature of 50, 140 and 160°C in the feeding, transition, and metering zones, respectively) and screw speed of 210 rpm. Fumonisin B1 was quantified in unextruded and extruded products by HPLC-fluorescence detector with pre column derivatisation. An *in vitro* digestion model was then applied to the extruded products to evaluate the amount of fumonisin B1 that was free for absorption in the small intestine. Extrusion cooking resulted in significant (P<0.05) reductions of fumonisin B1 in all treatments relative to unextruded controls. The addition of high amylose maize starch further reduced fumonisin B1 (74.9%), when compared to the effect of the extrusion by itself (66.0%), indicating the possible association of fumonisin with this macromolecule. This interaction between fumonisin and matrix components may be beneficial

depending on the degree to which the mycotoxin will be released in the gastrointestinal tract. The presence of high-amylose maize starch in extruded products promoted a significant (P<0.05) lower bioaccessibility of fumonisin B1 (35.1%) than when products were formulated without this ingredient (43.4%). This indicates that adding amylose to thermally processed foods may contribute to both a greater reduction in fumonisin levels and to a lower toxin bioaccessibility. This research highlights that the food matrix in which mycotoxins occur, can have an effect on their bioavailability, as complex and diverse reactions occur during thermal processes for food production. These interactions between mycotoxin and the food matrix components, will result in different levels of free toxin available for absorption in the intestinal tract. The extrusion cooking, as shown, may contribute to mitigate fumonisin B1 hazards in foods with the addition of amylose, possibly, further minimising the risk of exposure.

P18

Occurrence of mycotoxins in Brazilian maize and soybean meal in 2018

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The challenge to detect and diagnose problems related to mycotoxins are not simple because its effects are diverse. Depending on different elements, the effects caused by mycotoxins on animals can vary from immunosuppression until death. A mycotoxin risk management needs to have accuracy and precision to support the application of the best solution in order to decrease the negative effects caused by mycotoxins present in the animal feed and with the best return of investment to the producers. In Latin America, the main ingredients in poultry and swine feed are maize and soybean meal (SBM). For this reason, maize and SBM samples were collected from several states of Brazil to be analysed for aflatoxins (Afs), zearalenone (ZEN), deoxynivalenol (DON), fumonisins (FBs) using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS, Spectrum 380®), and enzyme-linked immunosorbent assay (ELISA). It was considered all data collected during the year of 2018 to calculate monthly positivity, average of contamination and the median of each mycotoxin. It was observed that maize had a high positivity in FBs, ZEN and DON and the average levels of the contaminated samples were significantly high for FBs and DON. Even though the level of contamination of ZEN were not high, it still is a concern to sows. The positivity of SBM was high for ZEN and half of the year for DON. The average of the contaminated samples was significantly high for DON. Considering an average feed formulation in Brazil (60% maize and 30% SBM) for poultry and swine, the estimation of contamination for FBs would be 1,712 ppb, which would be a high risk of contamination for swine in general; for ZEN would be 66 ppb, considered medium risk of contamination for sows, piglets and breeders; and for DON would be 422 ppb, which would be medium risk of contamination for sows, grower and finisher swine, and poultry in general. At this level of DON, the risk of contamination would be considered high for piglets. DON, FBs and ZEN are highly prevalent in the main ingredients of poultry and swine Brazilian feed. The average levels of contamination observed for these mycotoxins is considered significantly and it can cause several problems in the poultry and swine production causing economical loss. The results emphasize the importance of mycotoxin risk management throughout the year in Brazil.

P19

Age-related differences in exposure to deoxynivalenol and deoxynivalenol-3-glucoside unravelled: toxicokinetic study in the piglet as a human paediatric surrogate model

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Besides free deoxynivalenol (DON), modified DON forms such as deoxynivalenol-3-glucoside (DON-3G) are frequently detected in food and feed. Based on the higher internal exposure due to metabolic and physiologic immaturity and based on their relatively higher food/feed intake, juveniles are amongst the vulnerable population groups. The EFSA CONTAM Panel recommends to perform toxicokinetic studies in pigs and recommends urinary excretion studies in all population groups in order to reduce the uncertainties associated with the current risk assessment in humans and animals [Knutsen *et al.*, 2017. EFSA Journal 15: 4718]. Consequently, the goal of this study was to unravel age-related differences in toxicokinetic processes of DON and DON-3G in weaned piglets as a human paediatric surrogate model. In particular, weaned piglets (2-4-week-old) are an appropriate model for human infants (1-month until 2-years-old) concerning absorption, distribution, metabolism and excretion [Gasthuys *et al.*, 2017. Curr.

Pharm. Des. 22: 4069]. The study was conducted on eight healthy piglets (4-week-old, ± 8 kg bodyweight (bw)), sexes equally divided, which were surgically provided with a double lumen central venous catheter, inserted via the vena jugularis. Additionally, four of the piglets received a single lumen portal catheter to study presystemic hydrolysis and biotransformation. A single bolus of DON-3G (55.7 µg/kg bw) and DON (36 µg/kg bw, equimolar dose) was administered to all piglets by intravenous and oral administration, following a double two-way cross-over design. Blood and urine were sampled at different time point pre- and post-administration and plasma/urine concentrations of DON, DON-3G and their metabolites were quantified using validated LC-MS/MS methods. Data were processed using tailor-made compartmental models in order to accurately estimate toxicokinetic parameters. Results were compared to toxicokinetic parameters obtained in a recent study using 11-week-old pigs in order to unravel age-related differences [Broekaert *et al.*, 2017. Arch. Toxicol. 91: 699]. Statistical comparison resulted in significant age-related differences concerning volume of distribution, elimination half-life and time of maximal plasma concentration after both DON-3G and DON administration. Moreover, in accordance with Broekaert *et al.*, a total presystemic hydrolysis of DON-3G to DON occurred after oral administration of DON-3G. Significant differences were noted for the absorption lag time and orally absorbed fraction of DON-3G between 4- and 11-week-old pigs (94 vs. 16%, respectively, $P<0.001$), reflecting higher absorption of DON-3G after hydrolysis to DON at the level of the distal intestines, probably caused by a higher transit time and higher intestinal permeability. Results may contribute to EFSA risk assessment concerning modified DON forms in food and feed. **Acknowledgements**. This work was supported by Horizon 2020 (H2020-MYCOKEY-GA 678781).

P20

An *in silico* 'structural toxicology' approach to move the toxicological investigation of mycotoxins at an individual level – a case study on zearalenone oestrogenicity

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Mycotoxins pose a sever threat for public health at a global scale. However, the overall impact on humans' wellbeing may vary on geographical basis (more severe short-terms outcomes usually occur in developing countries) and at an inter-individual level (making difficult the characterization of long-terms effects among individuals). Among the various mycotoxins of food origin, the Fusarium mycotoxin zearalenone is in the few regulated so far. From a mechanistic point of view, its oestrogen receptor-dependent estrogenic activity has been largely described and evidences collected over the years have suggested a role in developmental abruptions in humans, such as alteration of pubertal timing and the onset of early thelarche symptoms in children. However, more data are needed to consolidate an epidemiologically relevant relationship between zearalenone exposure and emergence of physiological disorders in humans. In this light, it must be kept in mind that in the human population several mutations on the oestrogen receptors with graded effects in altering the physiological response to steroids-dependent stimulation may occur. Therefore, possible relevant effects of such mutations on zearalenone sensitivity in humans cannot be excluded, and they deserve investigations to better support the study of zearalenone epidemiology. In this framework, an *in silico* 'structural toxicology' approach has been proposed as an early warning system analysis to identify mutations possibly causing altered sensitivity to zearalenone. In particular, a structure-based approach relying on pharmacophoric modelling, docking simulations and molecular dynamics was used to study the effects of zearalenone on the activation of oestrogen receptors when mutations with effects on the receptors' modulation occur. Once a knowledge-based retrospective procedural validation has been done, a set of mutations possibly causing enhanced sensitivity to zearalenone stimulation was mapped. Therefore, the presented workflow proved to be a valuable and cost-effective first-line tool to study the possible inter-individual differences of zearalenone toxicity providing rational basis for future investigations. In summary, this study described: (i) the likely existence of genetically-based inter-individual differences to mycotoxins stimulation; (ii) the relevance of collecting data on the 'personalised' toxicology of mycotoxins, leading the way toward a paradigm of investigation to tackle the risk assessment at the individual level.

P21

Urinary DON concentrations as biomarker of exposure in different age groups in Norway

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The dietary consumption of bread is high in Norway and previous intake exposure estimates indicate intakes exceeding the TDI in young age groups. We therefore collected spot samples of morning urine on two days from 255 individuals. Individuals belonging to different age groups (children, adolescents, adults and elderly as well as pregnant woman and vegetarians were recruited to participate. All samples were analysed for free and conjugated deoxynivalenol and for the metabolite DOM-1. The participants also registered the dietary consumption on the days prior to sample collection. DON and/or metabolites were detected in samples from all individuals but one. This individual had not registered intake of any grain product the days prior to sampling. The urinary concentrations of DON are in the same range as reported from other European countries. Children had the highest urinary concentrations of DON metabolites, followed by adolescents. The urinary concentrations of DON were not different in pregnant women and vegetarians compared to other adults. The dietary intake of DON was estimated using 3 different models; two models estimated the intake using urinary concentrations combined with different models of daily creatinine excretion and the last model used standard volume of urine secretion. The correlations between the models and between each model and urinary DON concentrations will be analysed. Intake estimations based on the use of standard urinary daily excretion volumes resulted in higher estimated intake of DON than estimations based on daily creatinine excretion. The estimated daily intakes were lower than previously estimated intakes in the Norwegian population based on food consumption and concentrations in flour. A possible explanation is that the urine samples were collected in a year with low DON contamination levels in Norwegian grain but may also be due to differences in the methodology. There is a need for evaluation of different methods to estimate exposures based on urinary DON excretion.

P22

European mycotoxin survey January – June 2019

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High temperatures in April and June 2019 caused huge concerns in the European agricultural industry, since last two year's harvest-output was already highly below the normal average due to the extreme weather conditions around harvest. Nevertheless, a rather cold and rainy May seems to have saved this year's harvest, with only small to moderate losses predicted. The unusual dry and hot conditions during April and June, and the unstable weather during May, however, still had an impact on the growth of fungi and in consequence the mycotoxin production. To locate these toxic secondary metabolites and discover at what level they occur, 1,918 samples of different commodities from whole Europe were analysed with ELISA, HPLC and partly with LC-MS/MS between January and June 2019 as part of the annual BIOMIN mycotoxin survey. The results indicate that 95% of the 1,918 investigated samples were contaminated with at least one mycotoxin. Deoxynivalenol (DON), zearalenone (ZEN) and fumonisins (FBs) were the most common toxins with 64, 62 and 59% prevalence, respectively. DON causes the highest potential threat as 31% of positive samples were above the risk threshold (risk threshold for DON, 150 µg/kg) with an average concentration of 340 µg/kg and a maximum concentration of 15,300 µg/kg. FBs and ZEN revealed moderate average concentrations of 604 and 83 µg/kg, respectively. The maximum concentrations of both mycotoxins on the other hand are of major concern, as they were as high as 100,000 µg/kg for FBs and 11,983 µg/kg for ZEN. ZEN was also detected to a high extent in sugar beet samples, a commodity, which is not routinely analysed for mycotoxins. Analysis of 55 samples revealed an average concentration of 549 µg/kg ZEN, with 100% of samples being contaminated with ZEN. Beside the high maximum concentration of 11,983 µg/kg ZEN, also 70% of samples were above the risk threshold (risk threshold for ZEN, 50 µg/kg), which may cause a potential threat to livestock. Extreme weather conditions are of major concern when it comes to mycotoxin contamination in the field. As the latest survey results reveal, temperature fluctuations, droughts, above or below average rainfall, soil quality and many other factors influence the resistance of plants and the risk of mycotoxin contamination.

P23

Deoxynivalenol-3-glucoside production in Canadian barley and wheat varieties in response to infection by *Fusarium graminearum*

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Fusarium head blight (FHB), incited primarily by *Fusarium graminearum*, is a devastating disease of barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) worldwide due to accompanying mycotoxins, such as deoxynivalenol (DON) which have negative quality impacts for either respective industry. Breeding FHB resistant cultivars has been a long-term objective for both barley and wheat breeders in Canada. UDP-glycosyltransferase (UGT) enzymes are commonly produced by either cereal. Such enzymes contribute to detoxification and subsequently FHB resistance, through glycosylating DON into DON-3-glucoside (DON-3G). While DON-3G is considered a less-toxic form, it is readily converted back to DON in the mammalian gut. As DON-3G is not detected by conventional chemistries, it can pose a masked threat to cereal industries. While UGTs are common in both cereal species, differential epidemiological patterns are apparent with respect to varietal response. Differential sets of Canadian barley and wheat varieties were evaluated in independent experiments in irrigated FHB nurseries in Manitoba, Canada. FHB resistance-status was associated with a higher DON-3G/DON ratio in wheat varieties, but not so for barley. DON-3G was found to constitute a significant portion of all DON-like compounds in grains of either cereal, which should be considered in limits set by industry.

P24

Risk estimation for ochratoxin A and aflatoxins due to capsicum consumption in a Chilean rural area

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Ochratoxin A (OTA) and aflatoxins (Afs) are carcinogenic toxins produced by fungi, which have been found in Chile especially in spices derived from capsicum. The aim was to estimate OTA and Afs exposure by capsicum consumption in a Chilean rural area by the probable daily intake (PDI) estimation and to assess the risk comparing de PDI to the tolerable daily intake (TDI) or the margin of exposure (MoE). PDI was estimated based on the reported consumption of capsicum and the OTA and Afs concentration in capsicum according to the Chilean Surveillance Programme of Mycotoxins from 2013 to 2018, and assuming a mean adult weight of 70 kg. Probabilistic models of each variable were sampled by the hypercubic Latin sampling method and variables were associated with a Monte Carlo simulation. Since mycotoxin levels and food consumption data were not normally distributed, they were adjusted by the best fitting model or the model with the lowest Akaike information criterion (AIC), assuming in <LOD a LOD/2. According to the food consumption survey, 26% of the subjects were capsicum consumers, with a mean of 1.93 (± 2.26) g/day. The reported prevalence (>LOD) of OTA and Afs were 60% (171/283) and 23% (67/291), with average levels of 13.72 (SD,42.89) and 1.36 (SD,10.55) ng/kg respectively. The estimated median (P25-P75) PDI was 0.016 (0.004-0.18) ng/kg weight/day for OTA and 0.005 (0.002-0.01) ng/kg weight/day for Afs. The PDI/TDI ratio was below 1 at P95 in the case of OTA, but the MoE was <10,000 in the P95 in case of aflatoxins, meaning that could be considered of high health concern. Both toxins are present in foods consumed in Chile, so it is urgent to study other sources and measure biomarkers in the population for a more accurate exposure and risk assessments.

P25

Mycotoxin survey of straw across the UK

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Fungal pathogens are responsible for millions of pounds of damage to cereal crops worldwide. Mycotoxins, secondary metabolites produced by fungi, pose a significant danger to the health and productivity of farm livestock, and may end up in animal products thereby posing a potential risk to human health [Gallo, A. *et al.*, 2015. Toxins 7: 3057]. Straw (produced from cereal crops) is used in farming as a constituent of animal feed and can be used as bedding in animals; where straw is used in bedding, the animals may consume the straw (Terre, M. *et al.*, 2007. Anim. Feed Sci. Tech. 137: 115]).

While monitoring and control of mycotoxin contamination is carried out, this is usually focused on grains and to a lesser extent on silage. Monitoring of mycotoxins in straw is generally a neglected area despite the potential risk of mycotoxins exposure to animals via this route. The aim of this survey was to provide data on the occurrence of mycotoxins in straw used for animal feed and bedding in the UK. Straw was sampled from farms across the UK and analysed for the presence of mycotoxins using a Waters LC/MS running a multi-mycotoxin detection method. Of the 71 straw samples tested, 80.3% of straw samples contained 1 or more mycotoxin, while 14.1% of samples had 3 or more mycotoxins. The most frequently detected mycotoxin was Deoxynivalenol (DON) and was found to be present in 63.4% of the samples. DON causes a range of undesirable health effects in animals including vomiting, feed refusal, gastrointestinal lesions and immune dysregulation. Furthermore, all animal species evaluated to date are susceptible to DON. From the current survey, it is clear that straw contributes to the exposure of livestock to mycotoxins. Good farm management practices should ideally include monitoring and control of mycotoxin contamination in straw to reduce the risk of exposure of livestock to mycotoxins via this route.

P26

The genotoxicity of caecal water in gilts exposed to low doses of zearalenone

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Mycotoxins are toxic low-molecular-weight molecules that are naturally produced by moulds on crops as secondary metabolites. The aim of this study was to determine the genotoxicity of caecal water (CW) collected successively from the caecal contents of gilts exposed to low doses (LOAEL, NOAEL and MABEL) of zearalenone during the experiment. The experiment was performed on 60 clinically healthy pre-pubertal gilts with average body weight of 14.5±2 kg, divided into three experimental groups (group ZEN5, ZEN10 and ZEN15; n=15) and a control group (group C; n=15) administered placebo. Group ZEN5 gilts were orally administered ZEN at 5 μg/kg bw, group ZEN10 pigs at 10 μg ZEN/kg bw and group ZEN15 pigs at 15 μg ZEN/kg bw. Five gilts from every group were euthanised on analytical date 1 (exposure day 7), 2 (exposure day 21) and 3 (exposure day 42). Caecal water samples for *in vitro* analysis were collected from a 10 cm long intestinal fragment resected from the ileocaecal region and the colon. The results can be summarised as follows: (i) significant differences ($P \leq 0.05$) are indicative of genotoxicity, in particular after analytical date 1; (ii) the results confirm the presence of positive correlations on date 1 in groups C and ZEN 5 (weak correlation) with a strong decreasing trend followed by the absence of a linear relationship in group ZEN5; (iii) in groups ZEN10 and ZEN15 (moderate correlation) with a minor decreasing trend on successive days of exposure; (iv) the electrophoresis of the exposed cells revealed numerous comets without tails in groups C and ZEN5, and less numerous comets with strongly and very strongly expressed tails in groups ZEN10 and ZEN15, respectively; and (v) the percentage of DNA in LLC-PK1 cells in the tail ranged from 15 to 20% in groups C and ZEN5, and from 30 to 60% in groups ZEN10 and ZEN15. The percentage of cells with damaged DNA was determined at up to 20% in groups C and ZEN5 and at 5-15% in the remaining groups. In conclusions, the analysis of CW genotoxicity during exposure to very low doses of ZEN (LOAEL, NOAEL and MABEL) revealed the presence of a counter response and a compensatory effect in gilts. The extent of DNA damage was proportional to the ingested mycotoxin dose. Therefore, the proposed MABEL dose could be considered as a preventive dose for pre-pubertal gilts.

P27

Physiologically based kinetic (PBK) modelling-based reverse dosimetry of *in vitro* toxicity data to predict acute liver toxicity induced by aflatoxin B1 in rats and humans.

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Aflatoxicosis is an acute poisoning by aflatoxins resulting in acute liver damage that can be lethal to animals and humans. In humans, oral doses resulting in fatal cases have been estimated based on aflatoxin B1 (AFB1) levels in contaminated foods and from associated biomarkers in serum or urine. In the present study the aim is to predict oral dose levels causing *in vivo* acute liver toxicity in rats and humans, by using a physiologically based kinetic (PBK) modelling-based reverse dosimetry approach enabling quantitative *in vitro* to *in vivo* extrapolation (QIVIVE). To this end, PBK models for AFB1 kinetics in rats and humans were developed. *In vitro* cytotoxicity was assessed in the hepatic stem cell line

(HepaRG), and also in rat and human primary hepatocytes using the MTT assay, from which concentration-response curves were derived and converted to *in vivo* dose-response curves. Median effective doses (ED50) were obtained from these curves and compared with estimated median lethal doses (LD50) from the literature data. The results show that the predicted ED50s fall within the range of LD50s derived from the available *in vivo* toxicity data. To conclude, this study shows a proof-of-principle for QIVIVE by integrating *in vitro* assays with in silico PBK modelling-facilitated reverse dosimetry to predict doses that may cause acute liver toxicity of aflatoxin B1 in rats and human.

P28

Patulin contamination in home-made vinegar and its importance on public health

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Vinegar is a product obtained by fermentation of fruits by yeast and bacteria in order to give flavour to salads, sauces and dishes. Commercially produced vinegar is mostly made from apples and grapes in large quantities by different methods. However, vinegar made at home is produced by natural fermentation from almost all kinds of fruits in order to meet the needs of people in their homes. Another important feature of household vinegar is its use as a beverage. In this study, the contamination of patulin was investigated in home-made vinegar collected from various regions of Anatolia. The determination of patulin in vinegar samples was carried out by HPLC (Shimadzu) system with DAD detector. Samples were extracted with acetic acid and then purified by immunoaffinity column (R-Biopharm P250-P250B). The recovery of patulin in vinegar was 75%. The limit of detection (LOD) was 3,97 µg/l and the limit of quantification (LOQ) was 4,70 µg/l. Out of 33 home-made vinegar samples, the amount of patulin was greater than LOQ value in 19 of them, and greater than LOD value in 1 of them, whereas the remaining 13 samples were lower than both LOD and LOQ. The highest patulin contamination determined in vinegar samples was over 1,450 µg/l. Although vinegar is not a principal foodstuff, the contamination of patulin is important, because vinegar is believed to have beneficial properties for health and is frequently consumed. The results found in this study showed that home-made vinegar contains high amounts of patulin, and it is important to re-evaluate the vinegar production method at home in order to protect public health.

P29

Co-contamination of mycotoxin in grain inoculated with *Fusarium graminearum* MTCC1893 on maize and rice and *F. sporotrichoides* MTCC1894 on wheat – evaluated for normal ambient temperature and moisture simulating rainy condition

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Associations between different mycotoxins and their toxic effects are complex and poorly characterised. Surveys of contaminated diet prove that co-occurrence of multiple mycotoxins is inevitable and should be considered as the rule rather than the exception. In this study, the co-contamination of fusarial toxins using different grains types exposed to *Fusarium* species under normal environmental conditions has been examined. The *Fusarium* species were cultured on potato-dextrose agar (25°C, 14 days) and conidial suspension was separated by centrifugation. The toxins subsequently produced on 50 g of autoclaved-sterilised grain (n=100; 35% moisture, 22°C) inoculated with 1 ml suspension containing 10^5 of fungus spores (*Fusarium graminearum* MTCC1893 on maize and rice; *F. sporotrichoides* MTCC1894 on wheat) were profiled. The samples were autoclaved (121°C, 15 min) and oven dried before LC-MS/MS analysis for mycotoxins (37+ Lµaboratory, Ireland). The data was analysed by one-way ANOVA (SPSS statistical software) to examine the overall differential impact of the substrate on the toxins produced. Overall, the total amount of toxin produced by *F. sporotrichoides* in wheat (480 µg/g) was greater than toxin produced by *F. graminearum* (362 µg/g in maize and 47 µg/g in rice, $P<0.01$). The different substrates examined diverged significantly in their ability to support toxin production. *F. graminearum* produced greater ($P<0.01$) levels of zearalenone (359 µg/g) in maize than in rice media (3-80 µg/g). On the other hand, beauvericin was produced in higher quantity (140-184 µg/g) by *F. sporotrichoides* in wheat followed by T-2 toxin (82-111 µg/g), HT-2 toxin (65-81 µg/g), neosolaniol (40-104 µg/g), zearalenone (35-60 µg/g) and diacetoxyscirpenol (<2 µg/g). The variation in overall toxin yields are probably due to differences in resistance offered by grains to fungal invasion. Even though *F. graminearum* is known to produce deoxynivalenol, it unexpectedly produced large quantity of zearalenone, irrespective of the substrate type. If we consider field production of mycotoxins in plants,

trichothecenes metabolites, due to phase 1 and 2 metabolization process including conjugation products of deoxynivalenol (DON) with glucose, DON-S-cysteinylglycine (DON-S-cys-gly), DON-S-cysteine (DON-S-cys) and biotransformation in acetylated metabolites of DON could occur. Because the herein work was performed on processed grain, none of those metabolites were found confirming that they are the result of the plant metabolism. In conclusion, mycotoxins co-occurrences may vary from commodity to commodity and will be affected by environmental conditions. Therefore, proper grain handling and storage of grain under conditions which prevent mould growth are essential for the control of mycotoxin contamination.

P30

Mycotoxin occurrence in raw material and finished feedstuffs in Ireland

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Mycotoxin contamination of raw materials and resulting feedstuffs not only presents a risk to animal health but also negatively impacts the nutritional quality of the feed, reducing its economic value. With the imports of grain into Ireland increasing year on year and variations between growing conditions in countries of origin, the mycotoxin prevalence of raw materials in the Irish market can vary greatly. The use of raw materials/cereals in feed rations can also differ between and within years depending on market availability and pricing, i.e., in 2019, high maize inclusion in monogastric diets. For that reason, regular monitoring of raw materials for mycotoxin occurrence is important to understand the challenge within the feed industry as preventing the growth of mycotoxin producing fungi is difficult. Trouw Nutrition Ireland analysed 687 samples, of raw materials and complete feed between May 2017 and May 2019. The samples were analysed using a lateral flow methodology (Mycomaster). Overall, 61% of samples tested contained detectable amounts of aflatoxins (Afs), deoxynivalenol (DON), zearalenone (ZEN) and fumonisins (FBs). The most prevalent mycotoxins were DON and FBs (88 and 83%, respectively). When reviewing the mycotoxin contamination profile from 2017 to 2019, there was a tendency for mycotoxin prevalence to increase year on year, from 55% in 2017 to 61% in 2018, and it is expected to continue to rise in 2019 (63% based on preliminary data). The raw material that was most frequently tested was maize, with 50% of maize samples tested positive for mycotoxin contamination. The most common mycotoxins detected in maize were DON and FBs (84 and 68%, respectively). With regards to monogastric final feed, including both poultry (broiler and layer) and pigs (fattening and sow), samples tested were positive for DON (91%) and FBs (85%). This was similar for ruminant feed, with the majority of samples tested contaminated with DON (95%) and FBs (94%). This study highlights the prevalence of mycotoxins in both raw materials and final feeds used in the Irish animal feed industry. Consequently, as a major importer of raw materials, regular mycotoxin monitoring of raw materials and end feed in Ireland is important to manage potential challenges. This knowledge can support the development of a robust mycotoxin management plan to improve feed quality.

P31

Survey of major ergot and tropane alkaloids in bread in the Netherlands using LC-MS/MS

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Ergot alkaloids are mycotoxins which can occur in food. EU regulation for 12 ergot alkaloids in processed cereal products is currently in preparation. Tropane alkaloids are plant toxins of which two, atropine and scopolamine, are regulated for cereal based foods for children in the EU (Commission Regulation (EU) 2016/239). Both ergot and tropane alkaloids can be quantified simultaneously by acidified water/method extraction from matrix, followed by LC-MS/MS separation and identification. In this work, an analytical method was developed and validated for twenty ergot alkaloids and six tropane alkaloids in the bread matrix. The method was applied in a survey on 40 samples of bread obtained from retail stores in the Netherlands. LOQs (expressed as LOQ for each individual alkaloid) ranged from 0.3 to 1.0 µg/kg for the tropane alkaloids and 0.3 to 1.2 µg/kg for the ergot alkaloids, respectively. Recoveries for alkaloids from both groups varied between 85 and 110%, with repeatability between 1.3 to 21%, fulfilling relevant performance criteria. All bread samples contained quantifiable amounts of at least two or more ergot alkaloids. A total of 18 of the 20 ergot alkaloids were detected at or above individual LOQ ranging from 0.3-71 µg/kg for each individual ergot alkaloid. Tropane alkaloids were not detected above the LOQ. This study shows that both tropane alkaloids and ergot alkaloids can be quantified using the validated LC-MS/MS method. Since 18 of the 20 alkaloids were detected at varying levels in bread, it is recommended to monitor the occurrence and variation in ergot alkaloid levels in bread more regularly.

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P32

The negative effects of ZEN and DON on proliferation, phenotype and immunoglobulin production of porcine B cells *in vitro* are abrogated in their derivatives HZEN and DOM-1.

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Zearalenone (ZEN) and deoxynivalenol (DON) are mycotoxins that frequently contaminate grains. Produced by *Fusarium* fungi, they are a health hazard for pigs which consume cereal-rich diets. Among other effects, it has been shown that ZEN and DON can modulate immune responses. For example, DON is described to reduce the antigen-specific antibody production *in vivo*, indicating a negative impact on the humoral immune response and antibody-producing B cells. Consequently, the influence of ZEN and DON on proliferation, immunoglobulin production (IgG, IgA, IgM) and phenotype of porcine B cells in *in vitro* assays was investigated. Additionally, the modified mycotoxins HZEN and DOM-1, obtained by biological detoxification strategies, were tested. The effects of these metabolites on immune cells are poorly documented so far. Peripheral blood mononuclear cells isolated from healthy pigs were stimulated with the Toll-like receptor (TLR) 1/2-agonist Pam3Cys-SK4 or the TLR7/8-agonist resiquimod together with increasing ZEN and DON concentrations (2.5-40 µM and 0.1-1.6 µM, respectively), or with 40 µM of HZEN and 1.6-16 µM of DOM-1. Proliferation assays combined with phenotyping by flow cytometry for the B-cell markers CD21, CD79α, IgG and IgM revealed a strong decrease in B-cell proliferation from 20 µM of ZEN and 0.8 µM of DON onwards. At higher concentrations (40 µM of ZEN and 1.6 µM of DON), nearly a complete loss of live CD79α+ B cells was observed. Moreover, CD21 expression of IgG and IgM expressing B-cell subsets was already decreased from 10 µM ZEN and 0.4 µM DON onwards. ELISpot assays showed a decrease of IgG-secreting B cells from 10 µM of ZEN and 0.4 µM of DON onwards for both TLR agonists. Analyses on secreted IgG, IgA, and IgM by ELISA assays showed mainly a decrease from 10 µM of ZEN and from 0.1 µM of DON onwards for all immunoglobulin classes and for both TLR agonists. Our results corroborate *in vivo* findings of a compromised humoral immune response in the presence of DON and provide additional insights on negative effects of ZEN on porcine B-cell function. In contrast, the modified mycotoxins HZEN and DOM-1 induced no changes on the investigated parameters of B-cell functionality, showing the efficiency of these detoxification strategies.

P33

A multi-year survey of mycotoxins in Canadian barley harvest samples

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Canadian barley samples from 2015-2017 grown in Manitoba, Saskatchewan and Alberta were analysed for a suite of mycotoxins. Samples were submitted through the Harvest Sample Program (HSP) and analysed by a multi-mycotoxin method on a UPLC-MS/MS. The HSP is a voluntary programme that is run annually by the Canadian Grain Commission (CGC) where producers can submit a grain sample to the CGC at the time of harvest. From 2015-2017 culmorin, enniatin B, beauvericin and deoxynivalenol had a frequency of occurrence of >50% in all samples, with culmorin being detected in 60% of the samples, followed by enniatin B at 56%, beauvericin at 54% and then DON at 52% in the samples. HT2, T2, altenuene, ochratoxin A, aflatoxins (B1, B2, G1, G2) and fumonisins (B1 and B2) were not detected in any samples. In 2016, higher precipitation in the growing season led to an increased incidence of Fusarium head blight and DON concentrations reflected this. Comparing median DON concentrations between the three years were found to be statistically different (P=0.008). In 2015, DON median concentration was less than the limit of quantitation (<0.03 ppm), in 2016 DON median was 0.501 ppm and in 2017 the median was 0.068 ppm. Our study shows that concentrations of culmorin are correlated to concentrations of DON (CUL=0.165 * DON + 2.417, r2= 0.974, P<0.001), suggesting that in barley under similar conditions, that the source of culmorin is the same as DON. The ratio of DON-3G/DON ranged from 0.248-2.511 over the three-year period of different varieties being grown within different growing conditions across the prairies.

P34

Mycotoxins in poultry feed: 2018 survey and nutritional solution

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Mycotoxins can negatively affect broilers health resulting in reduced productivity of the birds and mortality in extreme cases. To better adapt the right solutions that can be used to deactivate mycotoxins in feed, the broiler industry needs to anticipate the mycotoxins profile and prevalence.
A mycotoxin screening survey performed in 2018 included 147 wheat samples from France (30), Poland (67), and UK/Ireland (50), and 90 maize samples from Poland. This survey provided insight into the incidence of aflatoxin B1 (AFB1), zearalenone (ZEN), deoxynivalenol (DON), T-2 toxin/HT-2 toxin, fumonisin B1, fumonisin B2 and ochratoxin A (OTA), the 7 most frequently found in agricultural commodities intended for animal production. The samples were collected directly from farms or animal feed production sites almost immediately after harvesting. Mycotoxins were analysed by LC-MS/MS. Then, 15 trials were conducted to evaluate the efficacy of a dietary mycotoxin deactivator on broilers fed with naturally contaminated wheat and maize-based diets. The survey showed that 46.3% of the wheat samples were contaminated with DON with the highest concentration found in a French sample (4,260 µg/kg). 1.36% of wheat samples were contaminated with OTA and the highest concentration found in a single sample from UK was 76.7 µg/kg. The results also showed that 1.7% of samples were contaminated with T-2/HT-2 toxin (highest concentration in a sample from Poland was 56.6 µg/kg). The analysed maize samples showed that 80% of them were contaminated with DON and 14.4% with ZEN. The highest concentration of DON detected in one of the samples reached 857 µg/kg. The average concentration of ZEN was 67.7 µg/kg. 76.6 % of the samples contained fumonisins. The maximum concentration of fumonins found was 1,060 µg/kg. Only 4.4% of samples had low levels of AFB1. The meta-analysis performed on the 15 broilers trials aimed to conclude that birds treated with the mycotoxin deactivator had better final body weight (+6.4%), higher feed intake (+2.7%) and then, an improved feed conversion ratio (-3.7%). The 2018 wheat and maize crops should not automatically be considered safe for inclusion in finished feed rations for poultry species. Nevertheless, broad-spectrum mycotoxins contaminated diets can be controlled by feed additive solutions.

P35

Characterisation of free and modified forms of *Fusarium* and *Alternaria* mycotoxins within malt production

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Brewing barley is often contaminated with mycotoxins, especially by those of *Fusarium*, *Alternaria* and other fungal species. These contaminants include both free (parent) compounds, as well as modified (mainly glycosylated and/or sulfated) forms. While it is well known that some modified mycotoxins, especially deoxynivalenol glycosides, are being formed during malting and brewing, much remains to be understood about the fate of other Fusarium and Alternaria toxic secondary metabolites. To investigate this, we analysed nine raw barley samples (artificially inoculated by specific fungal species), their malting intermediates (barley after steeping and germination), final malts and waste sprouts. As regards analytes' isolation, QuEChERS-like method was used for quantitative analysis of 57 mycotoxins and aqueous acetonitrile extraction for the modified forms. Ultra-performance liquid chromatography coupled to high resolution tandem mass spectrometry (UHPLC-HRMS/MS) was utilised to detect both free and modified mycotoxins, and assess increases and decreases in their levels during the particular technological steps. Besides the 15 free Fusarium and Alternaria mycotoxins (HT-2 toxin, T-2 toxin, neosolaniol, diacetoxyscirpenol, nivalenol, deoxynivalenol, zearalenone, enniatins B, B1, A, A1, beauvericin, alternariol, alternariolmethylether and tentoxin), six modified metabolites (glucosides of diacetoxyscirpenol, neosolaniol, HT-2 and T-2 toxin, as well as alternariol and alternariolmethylether sulates) were detected. After the first malting technology step, barley steeping, decreases in mycotoxins contents were noticed, presumably because of their washing into water. However, significant increases were observed further, especially during barley germination, resulting in considerable contamination of the final malt and malt sprouts. Particular mass balances of free mycotoxins and relative ratios to their glycosylated and sulfated forms will be presented in detail on the poster.

P36

Multiple myxotoxins detected in maize samples received from five continents between October 2018 and March 2019

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The aim of the present study was to screen maize samples for mycotoxins received from different countries from five continents between October 2018 to March 2019. The samples were analysed by LC-MS/MS triple quad (Agilent 6460 series) based multi-mycotoxin method for quantitation of all mycotoxins (aflatoxin B1, B2, G1 and G2, ochratoxin A, zearalenone (ZEN), deoxynivalenol (DON), fumonisin B1 and B2 (FB1 and FB2), T-2 and HT-2 toxins) regulated in the EU in feed by EU Directive 2002/32/EC, 2006/576/EC and 2013/165/EU. The summary of results as per continent is as follows: (i) Asia – The maize samples were received from India, Pakistan, Vietnam, Taiwan and Thailand and after testing 98 % samples were found to be contaminated with one or more mycotoxins. FB1 (and FB2), AFB1, ZEN and DON were detected in 94, 72, 26 and 10% of the maize samples, respectively; (ii) Europe – The maize samples were received from Russia, France, Croatia and Balkan countries. 90% of these maize samples were found to be contaminated with one or more mycotoxins. FB1 in 67%, FB2 in 53%, DON in 33%, ZEN in 25%, OTA in 20%, and AFB1 in 4% of the samples were detected using LC-MS/MS; (iii) Africa – The maize samples were received from Uganda, Kenya, South Africa and Egypt. 98% of these maize samples were found to be contaminated with one or more mycotoxins. DON in 84%, FB1 in 46%, FB2 in 32%, AFB1 in 8%, ZEN in 30%, and OTA in 11% of the maize samples were detected using LC-MS/MS; (iv) North and South America – The maize samples were received from USA, Mexico, Chile, Brazil and Argentina. 99% of these maize samples were found to be contaminated with 1 or more mycotoxins. DON in 22%, FB1 in 80%, FB2 in 99%, AFB1 in 8%, ZEN in 21%, and T-2 in 6% of the samples were detected using LC-MS/MS. This survey concluded that maize harvested around the world in 2018 is mostly contaminated with fumonisins. In more than 400 samples analysed worldwide, fumonisins were present in most of the samples (78%) with a median of 668 ppb and a total of 95 % maize samples were found contaminated with one or more mycotoxins. Other than fumonisins, DON in 30%, AFB1 26%, ZEN in 21% and OTA in 11% of the samples were detected. Field mycotoxins, such as fumonisins, DON and ZEN, were the most frequently occurring mycotoxins in North and South America, Africa and Europe. Fumonisins and AFB1 were predominant in Asia.

P37

Presence of *Fusarium* mycotoxins in total mixed rations for dairy cows as affected by their composition

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Mycotoxins produced by certain fungal species of the *Fusarium* genus are frequently found contaminating cereals and feedstuffs. Fumonisins (FBs), deoxynivalenol (DON) and zearalenone (ZEN) are of especial concern with regard to animal health and productivity. The aim of this work was to analyse the levels of Fusarium mycotoxins contamination in samples of total mixed rations (TMR) for dairy cows. In order to be able to detect small amounts of these toxins as well as some modified mycotoxins in a single run, an HPLC-MS/MS multi-mycotoxin method was developed and validated. The relation between the formulation of TMR samples and the presence of mycotoxins was also studied. From February 2016 to January 2018, a total of 193 TMR samples for dairy cows collected from farms located in different areas of Spain, were analysed for the presence of FBs, ZEN, and DON and their metabolites. The analysis showed that 112 samples (58%) were contaminated with at least one mycotoxin and 38 (20%) presented more than one mycotoxin. FBs were the mycotoxins more frequently found, being detected in 66 samples (34%). DON was detected in 32 samples (17%), and 31 were positive for the presence of ZEN (16%). Although DON and ZEN usually co-occur in these kinds of materials, these compounds were simultaneously detected in only four samples. Among the metabolites analysed, deoxynivalenol-3-glucoside was found in three samples and 15-acetyl-deoxynivalenol in 18 samples. The level of the mycotoxins in the TMR samples did not exceeded the maximum guidance values recommended by the EU for feedstuffs. The complexity of the TMR samples and the wide variety of ingredients used in their formulation, made it difficult to reach definite conclusions, although it seemed that some cereal silages and concentrates, as cereals or compound feed used as ingredients of the TMR, may be related with the presence of mycotoxins. Hence, these materials should be strictly controlled in order to prevent health hazards in animals derived from the chronic exposure to low levels of various mycotoxins.

P38

Screening studies and exposure estimation of mycotoxins presented in different varieties of *Camellia sinensis* collected in Latvia

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The beverages of *Camellia sinensis* (black and green teas) are used in or daily practice. The specific varieties such as Pu-erh, oolong teas have also become also popular in Europe. The tea shrub leaves are rich in phenolic compounds, which contribute to potential antioxidant and cardiovascular health benefits of such beverages [Sun, H. *et al.*, 2018. J. Food Sci. Technol. 55: 399; Roda, G. *et al.*, 2019. Molecules 23: 473]. However, the differences of production conditions and environmental factors may affect potential risks of fungal growth and production of mycotoxins as reported by studies overviewed recently [Sedova, I. *et al.*, 2018. Toxins 10: 444]. While Pu-erh ripe teas may rise greater concerns due to potential fungi formation within pile-fermentation, the recent reports towards *Penicillium* and *Aspergillus* metabolites (ochratoxin A, aflatoxins), which have certain regulations at least in herbal teas and spices, determine issues of potential contamination [3]. Our recent studies based on the assessment of mycotoxins in medical herbs based sensitive time of flight mass spectrometry (TOF-MS) detection indicated a rather high frequency of different *Fusarium* mycotoxins [Wang, J. *et al.*, 2014/ Environ. Sci. Technol. 48: 4817]. The reported study provided an advanced screening of 70 mycotoxins (including different deoxynivalenol (DON) mycotoxins and certain *Alternaria* metabolites) in 135 *Camellia sinensis* samples of different varieties (Pu-erh, black, green, oolong, etc.), including blends with added fruit flavourings and /or herbal or fruit inclusions (bergamot, citrus, peppermint). The samples were purchased from super markets and tea houses serving those teas for consumers. An advanced method based on online-heart-cutting two dimensional-liquid chromatography with TOF-MS detection was used for the analysis in combination with simple sample preparation using amine-SPE clean-up column. The method was validated based on the Pu-erh tea matrix and showed good sensitivity (the level of detection based on Pu-Erh matrix (m-LOD) ranged between 0.01 µg/kg for roquefortine C to 7.17 µg/kg for fusaric acid, respectively, when 2D-LCxLC-TOF-MS method has been applied for multi-mycotoxin analysis as compared to data provided by other studies [3]. The results indicated to different factors influencing mycotoxins, including the packaging (weight and tea bag samples were compared). As expected, Pu-erh teas showed one of the most frequent distribution of contamination, whereas the total summary concentration of DON, 3-Ac-DON, and 15-Ac-DON ranged between 436 and 15,943 µg/kg in all the selected Pu-erh samples (n=20). The risk assessment of potential mycotoxin intake risks was based on the available tea exposure data and the probability density functions, which were provided for DON and its mycotoxins according to recently reported methods based on EFSA recommendations [Reinholds, I. *et al.*, 2019. Food Addit. Contam. Part B 12: 199]. **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.

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Effects of deoxynivalenol-contaminated feed on health of broiler chickens

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Deoxynivalenol (DON) is one of the most prevalent cereal contaminants. As a trichothecene, DON can induce both immunosuppression and immunostimulation depending on its concentration, duration and time of exposure. The aim of this study is to evaluate the effect of DON at different levels at 5 mg/kg of feed (European regulatory level) and at 15 mg/kg feed on blood haematological, biochemical and immunological parameters of broilers chickens. Forty-five, 1-day-old male broiler chicks (Ross 308) were divided randomly into 3 groups (15 chicks per group) and treated for 45 days. Chicks for each group received one of the following dietary treatments: (i) control non-contaminated, (ii) contaminated diet with 5 mg DON/kg of feed, and (iii) contaminated with 15 mg DON/kg of feed. Blood biochemistry and haematology and antibody titres against Newcastle disease virus (NDV) and against infectious bronchitis virus (IBV) were determined. The level of interleukin-8 (IL-8) in plasma was quantified by an ELISA test kit and the jejunal cytokines level (IL-1B, IFN-gamma, IL-6 and IL-10) were measured by qRT-PCR. Results revealed that DON at both levels did not affect antibody response to IBV and to NDV. Increasing the dietary DON concentration, decreased blood cholesterol level (P<0.05) and haemoglobin concentration (P<0.001) without changing the haematocrit percentage. The erythrocytes value only decreased with a content of 15 mg DON/kg feed. The IL-8 in plasma increased in response to dietary

DON (5 and 15 mg/kg of feed). DON at 5 mg/kg up regulated *(*P< 0.001) the mRNA relative expression of IL-1B and up regulated (*P*<0.05) the MRNA expression of IFN-gamma at 5 and 15 mg/kg. DON at 15 mg/kg of feed down-regulated (*P*<0.05) the mRNA relative expression of IL-10 in jejunal tissue in comparison with DON at 5 mg/kg. In conclusion, the immune response in broiler chickens was modulated by the presence of DON in feed even at the low dose of 5 mg/kg.

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Subsistence farmer perceptions and practises that contribute to increased risk of mycotoxin exposure in humans and animals

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Mycotoxigenic fungi are common pathogens of maize and groundnuts, producing mycotoxins that reduce the yield and quality of these grain crops. Several agricultural practises are known to impact on mycotoxin accumulation although subsistence farmers often employ poor or inadequate agricultural practises due to limited resources. The farming and storage practises in maize and groundnut subsistence farming systems in five districts of northern KwaZulu-Natal province of South Africa were determined. A questionnaire on production practises, grain storage and perceptions around mycotoxins was presented to 52 maize and 30 groundnut subsistence farmers. At least 90% of the farmers were not aware of mycotoxins and their consequences to animal and human health. The majority of the farmers did not practise crop rotation; however, farmers did sort damaged, mouldy grain (maize and groundnuts) prior to storage, thereby limiting human exposure. The damaged and mouldy grain was, however, largely used as animal feed, thereby increasing the risk of mycotoxicosis in animals that represent an alternative food or income source to subsistence farmers. Metal tanks and 'inqolobane' (wooden structure) were the most common storage structures. Maize grain was mostly used for household consumption but was also sold to the local community. The implementation of mycotoxin awareness campaigns is necessary particularly in these districts. The storage facilities used by the subsistence farmers allowed increased moisture and insect invasion. The need for the surveillance of mycotoxins in subsistence farmed food crops is vital.

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Additions to the membrane disruptor effect of fumonisin B1 – *in vivo* test in rats in the kidney and liver

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Male Wistar rats were treated intraperitoneally with fumonisin B1 (FB1; 0, 20, 50 and 100 mg/kg dietary dose equivalent) for 5 and 10 days (n=24-24 in each setting) to gain dose- and time-dependent effects on antioxidant traits and oxidative stress, clinical chemical endpoints, liver and kidney histopathology and to assess effects on renal and hepatic phosphatidylcholine (PC), phosphatidyl-inositol (PI) and phosphatidyl-ethanolamnine (PE) fatty acid (FA) profile. FB1 decreased feed intake, body weight gain and absolute liver weight, irrespective of the dose. Relative kidney weight increased in the 10-day setting. Linear dose response was found for plasma aspartate aminotransferase, alanine aminotransferase, total cholesterol, urea and creatinine, and exposure time dependence for plasma creatinine level. Latter was paired with renal histopathological findings, tubular degeneration and necrosis and the detachment of tubular epithelial cells. Pronounced antioxidant response referred to renal cortical response. Hepatic alterations were moderate, referring to initial phase lipid peroxidation (difference of conjugated diene and triene concentrations), and slight functional disturbance (↑ total cholesterol). In the lipids, renal PC provided increasing FA saturation (SAT) after 5 days; after 10 days polyunsaturation (PUFA) decreased markedly (Σ n3, Σ n6, PUFA, unsaturation index (UI) and average FA chain length (ACL)), mostly with linear dose response. In the PI Fas similar changes were observed, decreasing monounsaturated FA, PUFA, UI and ACL (5 and 10 days), while the PE fraction was responsive in Σ n6 (↓) and SAT (↑), but only after the 5-day (without dose response (PI and PE)). Liver PC provided increasing saturation (C16:0), decreasing polyunsaturation (C20:3 n6, DGLA; C20:3 n3); the PI FA profile showed similar alterations after 5 days. PC and PI FA failed to respond in a dose-dependent manner to FB1. In PE FA profile DGLA decreased. In conclusion, rat kidney has been found to be more reactive in terms of histopathological modifications and as well membrane lipid compositional modifications, induced by low dose and relatively short term FB1 exposure. The most responsive lipid

fraction in the rat renal cortex was the PC, providing perturbed n6, n3, and thus, PUFA balance (in a dose-dependent manner), which was partly proven as well for the PI. Renal tissue underwent detectable oxidative stress, but it still remains unclear, whether lipid profile alterations are direct or indirect consequences of this imbalance.

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Field survey on mycotoxins in wheat in the Netherlands: results of one decade

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In the Netherlands, about half of its territory is used for agriculture, with wheat being one of the major crops. Wheat is cultivated for both human and animal consumption, as well as for the production of by-products (e.g. starch and bio-ethanol). According to FAOSTAT, the Netherlands is ranked 4th in terms of wheat yield (hectogram per hectare). In order to keep having a high yield or to improve this yield, avoiding wheat diseases is crucial. One common disease in wheat is *Fusarium* spp. Infection, which reduces wheat yield and can lead to the presence of mycotoxins such as deoxynivalenol (DON).
The aim of this study was to investigate the relationships between the mycotoxin concentration, in particular deoxynivalenol (DON), in wheat at harvest and farm agronomics such as tillage method, crop rotation, cultivar used, and fungicides applied. If such relationships exist, results can be used to advise farmers in mycotoxin reduction. Annual field surveys were performed in the Netherlands in the period 2009-2018. In the spring of each year, farmers received questionnaires and were asked about their farm location, soil type of the field, the previous crop on the field, the tillage method, the cultivar, the resistance of this cultivar against *Fusarium* spp., the use of fungicides, the flowering date of the wheat and the harvest date. After having filled in the questionnaires, the farmers were asked to send a wheat sample collected at harvest from the combine. These wheat samples were then analysed for the concentrations of multiple mycotoxins using LC-MS/MS at Wageningen Food Safety Research. Eight years of data were collected, including in total more than 300 harvest samples and related field agronomics. Descriptive statistics as well as a regression analyses were used to investigate the relationships between the agronomic factors and the mycotoxin concentrations. Correlations between the mycotoxins DON, nivalenol and zearalenone were investigated as well. The results will be presented during the conference.

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Multi-annual mycotoxin occurrence in animal commodities and feedstuffs

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Mycotoxins have a recognised negative impact on health, productivity of farm animals and by reducing the nutritive value of feedstuffs. Since the presence of moulds and mycotoxins is ubiquitous in feed ingredients, the feed industry has risen the interest to establish monitoring and control methods to prevent contamination across the feed-food chain. Trouw Nutrition analysed 67,301 samples of raw material and complete feed during January 2015 to June 2019 from all over the world. The majority of the samples were analysed by using a lateral flow methodology (Mycomaster), and around 45% by enzyme-linked immunosorbent assay (ELISA). Results showed that overall, 56% of the samples contained detectable amounts of aflatoxins (Afs), deoxynivalenol (DON), zearalenone (ZEN), fumonisins (FBs), ochratoxin A (OTA) and T-2/HT-2 toxin (T2/HT2). Moreover, this data showed that within the analysed mycotoxins the most prevalent were produced by *Fusarium* fungi such as DON, FBs, ZEN (71, 64 and 57% respectively). In most cases, the concentrations were in accordance to the EU guidelines. However, the current data does not take into account co-contaminated samples, which might exert the tolerable daily intake due to synergistic and additive interactions between mycotoxins. Mycotoxin contamination shows a tendency to be increasing throughout the years. The uppermost mycotoxin prevalence occurred in 2018 where an increase of 30% was observed, and based on preliminary data, it is expected to continue to rise through 2019. Regarding the commodities, maize and wheat were the most widely tested. In maize, the higher registered levels were DON and FBs (74 and 71%, respectively). Meanwhile, in wheat, the most prevalent mycotoxin was DON, which was present in 86% of the tested samples. Regarding the final feed samples for poultry (layer and broiler feed), pig (sow, piglet and fattening feed) and ruminants, 63% were positive for the tested mycotoxins. Within the most prevalent mycotoxins in the final feed, FBs were present in 70% of the analysed samples, as well as ZEN and DON (62%). In conclusion, the present survey showed that mycotoxins were present in the majority of the samples. This prevalence represents a potential risk in the performance, welfare, and

health of the animals. The acknowledgment of mycotoxins present in both commodities and feed is essential to build holistic monitoring and control programmes in order to decrease the effects of mycotoxins in the feed to food chain.

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Mycotoxins in oats: new insights into the impact of climate change factors on the *Fusarium*:oat pathosystem

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Oats and oat-based product consumption in the UK and Ireland are increasing as they have beneficial health benefits. It has been shown that oats can be infected during the critical ripening phase with *Fusarium langsethiae*, which produces no visible symptoms. However, the grain can be contaminated with type A trichothecenes, T-2/HT-2 toxins, for which EU directives exist on maximum levels in oats destined for human consumption and feed. There is no information on how climate change (CC) scenarios could influence the infection of oats by this pathogen and the impacts on mycotoxin contamination and thus oat grain quality. Our objective was to examine the impact of CC on oats mycobiota, including *F. langsethiae*. First, we examined the ecophysiology of newly isolated *F. langsethiae* strains in comparison with the genome sequenced *F. langsethiae strain* (Fl201059). We then defined the temporal expression profile for key trichothecene biosynthetic genes (*Tri5*, *Tri6* and *Tri16*) and phenotypic toxin production. Based on these data, the impact of interacting CC environmental factors was examined in oat-based media and stored oats. This involved examination of the effect of three-way CC related interacting factors: water stress (0.98-0.95 a_w), CO_2 (400 vs. 1000 ppm) and temperature (20-25-30°C); on colonisation, biosynthetic gene expression and toxin contamination. We found that there was a significant impact on *F. langsethiae* growth, on gene expression and T-2/HT-2 toxin production *in vitro* and in stored oats. In stored oats, using multi-targeted LC-MS/MS, the effect on production of T-2, HT-toxins was identified. Moreover, for the first time we describe the presence of HT-2 toxin glucuronide and a new dehydro T-2 toxin *in vivo*. The ratio of T-2:HT-2 toxin was also changed by CC interacting abiotic factors. Studies are in progress to examine the effect of CC scenarios on interactions between *F. langsethiae* and other phyllosphere mycobiota (*F. graminearum*, *F. poae*, *Epicoccum nigrum*, *Cladosporium* and *Alternaria* species) on production of T-2/HT-2 toxin and related secondary metabolites. **Acknowledgements**. This research is supported by a BBSRC-SFI research grant (BB/P001432/1) to Cranfield University.

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Insights of the *in planta* toxicity of some bacterial metabolites of the mycotoxin deoxynivalenol

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The growing interest of identifying microbial and enzymatic approaches to address the accidental contamination of food and feed commodities with mycotoxins led to the isolation of tens (if not hundreds) of microbes and organisms that produce different metabolites of such mycotoxins. These metabolites generally come with altered physicochemical characteristics from parental mycotoxins, but their toxicological profile(s) are still considered closed boxes that need to be cautiously evaluated for more insights and in-depth understanding in order to develop better future empirical applications. In many cases a standardised toxicity profiling is initiated using pre-approved model organism (such as specific mammalian cell lines, mice, rats…) to show the reduced toxicity of such metabolites ignoring the unique relationship that exits between each target-host and each presented metabolite and making 'generalisation statements' in regard to toxicity invalid at best. In our quest of identifying feasible approaches to detoxify deoxynivalenol (DON) through a bacterial enzymatic system that we have deciphered and reported most recently, we got across two specific metabolites, namely 3-keto-DON and 3-epi-DON that are produced as an intermediate and as a final bio-transformation product, respectively. Our pervious results argue for the need of a complete detoxification process/pathway where DON is taken all the way to 3-epi-DON through the short-living intermediate 3-keto-DON despite the so many earlier reports found in the literature which show and actually argue in many situations the sufficiency of DON to 3-keto-DON step. Elaborating on the minimal reduction of toxicity (5-fold decrease) and chemical instability of 3-keto-DON, we present in this work a case where the host sensitivity towards such a metabolite plays a paramount influence on toxicity/exposure outcomes showing that 3-keto-DON possesses higher potency as a toxin *in planta*. This metabolite does not only retain its capacity to act as a potent eukaryotic toxin, but it also suppresses the growth and root-development of wheat seedlings

as well as other plant species. Mechanistic insights in regard the aforementioned plant toxicity are being gathered/obtained through a detailed RNA_seq approaches.

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Protection of curcumin on OTA induced liver oxidative damage in duck is mediated by modulating lipid metabolism and intestinal microbiota

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Curcumin has the functions of antioxidation, regulating intestinal microbial composition and alleviating mycotoxin toxicity. This study was conducted to explore whether curcumin alleviates OTA induced liver injury through intestional microbiota. A total of 720 one-day-old White Pekin ducklings with initial body weight (bw) (43.4±0.1 g) were randomly assigned into four groups: (i) CON (control group, without OTA); (ii) OTA (a group fed 2 mg/kg OTA-contaminated diet); (iii) CUR (ducks fed with 400 mg/kg curcumin in diet); (iv) and OTA+CUR (2 mg/kg OTA plus 400 mg/kg curcumin). Each treatment consisted of six replicates, each containing 30 ducklings and treatment lasted for 21 days. Our results demonstrated that OTA increase serum LDL-C level (P<0.05), decreased liver antioxidant capacity (P<0.05), and curcumin supplementation allievited these changes caused by OTA. 16S rRNA sequencing suggested that curcumin increased the richness indices (ACE index) and diversity indices (Shannon index) compared with OTA group (P<0.05), as well as recover the composition of intestional microbiota alterd by OTA. Curcumim supplementation relieved the decreased the relative abundance of butyric acid producing bacteria, including *Blautia*, *Butyricicoccus* and *Butyricimonas*, induced by OTA (P<0.05). Macrogenome sequencing indicated that curcumin decreased expression of genes related to oxidative stress pathways (oxidative phosphorylation and the tricarboxylic acid cycle). OTA also significantly influenced the metabolism of intestinal microbiota, such as ABC transporters, choline metabolism in cancer. Curcumin could alleviate the up-regulation of oxidative stress pathway induced by OTA. OTA treatment increased *SREBP-1c* expression (P<0.05). Curcumin group had the lowest expression of *FASN* mRNA and *PPARγ* mRNA (P<0.05) and the highest expression of *Nrf2* mRNA and *HO-1* mRNA. In summary, our results indicated that curcumin could alleviate the oxidative injury by modulating the caecum microbiota and lipid metabolism disruption induced by OTA.

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Exposure assessment of aflatoxin B1 in Pakistan using urinary aflatoxin M1 biomarker

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Aflatoxin, produced by *Aspergillus flavus* and *A. parasiticus* is one of the most toxic of all the mycotoxins and is known to induce hepatocellular carcinoma. Agricultural commodities such as maize, wheat, cereal, and rice are all susceptible to aflatoxin contamination. As naturally occurring aflatoxin mixture has been classified as a class 1 carcinogen, the regulations for aflatoxin levels in food are strict. Nevertheless, outbreaks of often fatal acute aflatoxicosis still occur in low income countries where enforcement of regulations is difficult. Only a few studies have been conducted in Pakistan to determine the mycotoxin level in different types of foods. Aflatoxin has been found with a relatively high prevalence in rice (56%), maize (27%), spices (62%) and chocolate (~90%) but the aflatoxin exposure in the Pakistan population has not been extensively assessed. Aflatoxin B1 is metabolised to aflatoxin M1 (AFM1) and excreted in urine of exposed individuals. In this study, urinary AFM1 was analysed as a biomarker of exposure in 301 urine samples collected from six different villages in the districts of Kasur, Sahiwal, Bahawalpur and Rahim Yar Khan in Pakistan. Sociodemographic factors and food consumption data was recorded. Urinary AFM1 was extracted using an immuno-affinity column and analysed by LC-MS/MS. AFM1 was detected in 69 % of all urine samples at a range of 1.0-393.4 pg/ml. The median and mean concentration of AFM1 in urine was 4.2 and 22.8 pg/ml, respectively. A significant difference (P<0.01) was found at the mean level of AFM1 between the six villages. The residents from a village in Sahiwal Chichawatni showed the highest mean level of AFM1 (38.8 pg/ml) and residents from a village in Ahmedpur Sharqia showed the lowest (3.4 pg/ml). Urinary AFM1 levels also showed significant correlations with gender (male 22.9 vs. female 14.5 pg/ml, P=0.02) and weight (P=0.02) but did not show significant correlations with age or with wheat or rice intake (food frequency). In conclusion, the high prevalence and biomarker levels of urinary AFM1 in Pakistan show there is a high aflatoxin B1 exposure in parts of Pakistan. Our research suggests that action needs to be taken to reduce the

aflatoxin exposure in the Pakistan population.

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High levels of aflatoxin exposure biomarkers in populations from three sub-Saharan Africa countries

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Aflatoxins are the secondary metabolites of *Aspergillus* species of fungi and frequently contaminate dietary staples, especially maize and groundnuts in sub-Saharan Africa. Aflatoxins are well recognised as a carcinogen, with chronic exposure to aflatoxins also associated with child growth impairment and immune dysfunction. Acute high-levels of exposure can result in severe liver damage and even death. Here we present the aflatoxin exposure levels in children from two longitudinal studies in Gambia and Malawi, and data from an aflatoxicosis outbreak in Tanzania. Aflatoxin-albumin adduct (AF-alb) were used as the indicator of aflatoxin exposure, measured using a competitive ELISA method. Children from Malawi were recruited at 6 months and followed up to 9 months of age. The geometric mean (GM) level of AF-alb was 14.7 (95%CI 10.5-20.6) and 52.4 (95%CI 39.9-68.8) pg/mg, respectively. The AF-alb level was also measured in children from Gambia at 6, 12 and 18 months of age, with the GM level of 3.5 (95%CI 3.2-3.9), 25.4 (95%CI 22.4-28.8) and 52.6 (95%CI 39.9-68.8) pg/mg, respectively. Children from Malawi were fed family food at an earlier age (less than 6 months) and showed higher AF-alb level than the same aged children from Gambia. These results, and others, show the high levels of chronic exposure to aflatoxin in children in sub-Saharan Africa. The severe aflatoxicosis outbreak in Tanzania caused 20 deaths in patients aged from 1.5 to 70 years old. The range of AF-alb level in non-patients was 10-7,152.1 pg/mg, while biomarker values were as high as 32,791 pg/mg in patients. This highlights the risk from acute outbreaks of aflatoxin contamination. Further interventions and approaches to reduce the exposure risk and establish surveillance and warning systems are urgently needed.

MITIGATING THE NEGATIVE IMPACT OF MYCOTOXINS
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Assessment of zearalenone reduction ability of plant-derived *Lactobacillus plantarum* BCC47723

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Zearalenone)ZEN(, a harmful secondary fungal metabolite, is produced by plant pathogenic fungi mostly belonging to genus *Fusarium*. This toxin is involved in reproductive disorders of animals due to its structure being similar to the oestrogen hormone that causes precocious pubertal changes, fertility problems and hyperoestrogeny. Lactic acid bacteria (LAB) are one of the alternative strategies for ZEN reduction. Our previous study indicated that *Lactobacillus plantarum* BCC47723 isolated from Thai fermented vegetables, exhibited the ZEN removal ability from liquid medium. However, the data demonstrating the role of bacterial cell components on ZEN reduction were limited. Therefore, the aim of this study was to assess the bacterial cell components of *L. plantarum* BCC47723 involved in ZEN reduction using chemical and enzymatical treatments. Scanning electron microscopy coupled with energy dispersive X-ray spectroscopy)SEM-EDS(was used to observe the alteration of cell morphology and elements on the bacterial cell surface. The results revealed that using chemical and enzymatical treatments differently affected ZEN reduction by *L. plantarum* BCC47723. Sodium dodecyl sulfate)SDS(, urea, polymyxin B, and pronase E treatment enhanced the capacity of bacterial cells for ZEN removal)$P<0.05$(. In contrast, *m*-periodate and lipase significantly decreased ZEN removal)$P<0.05$(. The SEM images illustrated that the bacterial cell was deformed, and surface damage was observed both after chemical and enzymatical treatments as compared with the control)viable cells(. These treatments also significantly altered the elements on the bacterial cell surface even though the main elements were still C, O, N, P, and K. These results suggest that chemical and enzymatical treatments affected the cell morphology and elements on the bacterial cell surface resulting in changes in the chemical structures, including proteins, polysaccharides, teichoic acid, and lipids. This means that the integrity of the bacteria cell was not involved in ZEN reduction, but the alteration of components on the bacterial cell surface was the main factor related to ZEN reduction.

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In vitro activity of *Brassica*-based isothiocyanates against mycotoxin-producer *Fusarium graminearum*

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Fusarium genus is a producer of the largest and most diverse group of mycotoxins which occur abundantly in cereals. The globally important cereal pathogen, and mycotoxin producer, *Fusarium graminearum* causes head blight in wheat, resulting in yield losses and mycotoxin contamination. *F. graminearum* is known to produce the mycotoxins, deoxynivalenol and zearalenone. Currently, triazole fungicides are used to suppress *F. graminearum*, however, limited effectiveness of triazoles and concerns over safety of pesticides have led to the pursuit of safe alternatives, such as biofumigation. Biofumigation, a low-cost approach, involves growing short term *Brassica* crops, followed by maceration of the plant tissue and rapid incorporation into the soil. Inhibitory substances, particularly isothiocyanates are released as a result of damage to *Brassica* plant tissue causing suppression of soil-borne pests and diseases. Isothiocyanates are formed by enzymatic hydrolysis of glucosinolates present in *Brassica* species. The application of biofumigant *Brassica* crops, as a safe replacement to pesticides for managing soil-borne pathogens and pests is increasingly gaining interest. However, little is known of the potential of biofumigation to reduce the inoculum of *Fusarium* species affecting cereals. The aim of this study was to evaluate the antifungal activity of five isothiocyanates, namely allyl, benzyl, ethyl, 2-phenylethyl and methyl isothiocyantes, against germination and growth of *F. graminearum* under *in vitro* conditions. Among the tested isothiocyanates, allyl and methyl isothiocyanates were more efficient, showing lower ED_{50} values (34-99 mg/l) for conidial germination and mycelial radial growth. The findings suggest that *Brassica* plants containing allyl and methyl glucosinolates could have a suppressive effect on reducing the inoculum of *Fusarium graminearum* in soil prior to cereal production.

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Lactic acid bacteria and their metabolites for the control of ochratoxin A

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Ochratoxin A (OTA) is a ubiquitous mycotoxin found in foods and feed produced by filamentous fungi such as *Aspergillus*. OTA is toxic to animals and humans, thus developing safe and low-cost methods for the control of this toxin are of great interest. Lactic acid bacteria (LAB) are reported to show antifungal and detoxifying effects towards mycotoxins, supporting their potential as biocontrol agents. The objectives of this study were to evaluate: the inhibitory potential of inactive fermented supernatants (IFSs) from 16 potentially bioprotective LAB strains against the ochratoxigenic fungi *Aspergillus sclerotioniger* CECT 20583 and *A. ochraceus* CMT 00336; and the detoxifying activity of these strains towards OTA. 7 ml tubes of De Man, Rogosa and Sharpe (MRS) broth were inoculated with 100 µl of LAB fresh cultures, incubated (37°C, 48 h, 110 rpm), autoclaved (121°C, 20 min), centrifuged (5000xg, 10 min), and the supernatants were stored (-20°C) until utilisation. Minimum inhibitory concentrations (MICs) of the 16 IFSs were determined by microdilution for the 2 fungal species in tryptic soy broth (TSB). For the detoxification assay, 1.5 ml microtubes received 0.5 ml of OTA solution (500 ppb in TSB), 0.4 ml of TSB and 0.1 ml of bacterial inoculum in mid-exponential phase (tested individually, 0.5 MacFarland Scale). Microtubes were incubated (24 h, 37°C, 110 rpm), syringe-filtered (0.22 µm) and directly injected to a HPLC-FLD system for OTA quantification. The 16 IFSs have shown very similar inhibitory effects. *A. sclerotioniger* was more resistant than *A. ochraceus* and was only inhibited in media containing 50% IFS. The latter was visually inhibited with doses as low as 1.56%. IFSs obtained from *Pediococcus pentosaceus* UM116 and *P. pentosaceus* CHR Hansen® PC-01 were more effective against *A. ochraceus* (inhibition at 0.78%). On OTA detoxification, all 16 strains have shown significant reductions of OTA concentration when compared to untreated controls (246.4±4.2 ppb). Lowest reduction level was 11.6±5.5% for *Lactobacillus fermentum* CCT 1629, while the highest was 53.1±3.6% for *L. reuteri* Protectis BioGaia® – reaching a concentration of 115.5±8.8 ppb. Biodegradation of OTA is a promising approach, since it may reduce the toxic effects of this toxin to animals and humans alike. IFSs application could be also an advantageous approach as a natural preservative to avoid mould growth and, consequently, to avoid mycotoxin production. Further assays shall be carried to verify the activity of IFSs and LABs in food and feed matrices. **Acknowledgments**. Financial support by Brazilian National Council for Scientific and Technological Development (CNPq), processes 437728/2018-8 and 304299/2015-4.

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Effect of the wheat milling type on the distribution of *Fusarium* mycotoxins

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There is an increasing trend of usage of traditional products which are perceived by the general public as a 'healthy' alternative to the industrial heavy processed foods. In order to verify those health claims that traditional flour producers are often using, this study compared three industrial and three traditional mills. In order to get comparable results, the wheat cleaning and conditioning (moisturising of wheat prior the milling) was performed by industrial mills and fraction of the wheat was separated for milling in traditional mills (two watermills and one traditional electrical mill). In this way, loss of water-soluble mycotoxins (e.g., nivalenol, deoxynivalenol) as an effect of pre-treatment was prevented, or difference due to lack of wheat cleaning before milling on traditional mills. For every sample, at least 10 kg sample was milled in traditional mills due to difficulties in the cleaning of those mills. After milling, only flour products were analysed and compared with the results of the analysis on the whole wheat. On average, there was a 64% reduction of mycotoxin concentration from wheat to flour in industrial mills, while in traditional mills there was on average 15% reduction. It is worth mentioning that all regulated mycotoxins were below their respective regulatory limits. Also, some mycotoxins that were not found in the wheat sample, were detected in the flour of traditional mills probably due to carryover from previous samples and difficulties in cleaning. Although there were somewhat higher concentrations of *Fusarium* mycotoxins in flour from traditional mills, they also have more nutritionally valuable parts of the outer

shell (fibres, vitamins, and microelements) that can improve human health. The health claims of traditional mills were not justified if mycotoxins are focal point. Overall, if the wheat does not have high mycotoxin concentrations, the flour produced by traditional mills should be safe for consumption, otherwise in this sense it is better to choose industrial mills.

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The preservative propionic acid differentially affects survival of conidia and damages germ tubes of feed spoilage fungi

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The weak organic acid propionate is an important preservative in food and feed and inhibits the growth of various spoilage bacteria, yeasts and fungi, including mycotoxigenic fungi. The mode of action of this compound on fungal survival structures (conidia) and germ tubes of xerophilic feed-spoiling fungi is scarcely studied. We have isolated and identified fungal strains from nine samples of poultry feed originating from different countries using a shelf-life test and molecular methods. Xerophilic *Aspergillus* were present very high predominance. We assessed the sensitivity of a panel of isolated fungi for propionic acid and evaluated the viability of treated conidia and germ tubes. MIC values were measured by means of a microtiter plate assay. Survival of conidia was tested after a 24 h exposure to 31 mM propionic acid. To evaluate if propionic acid damaged germ tubes, a novel method was developed in which young biofilms of the fungi were tested for 30 min with 31 mM propionic acid in Erlenmeyer flasks using the live-dead fluorescent dye TOTO-1. The MIC values of 4.6 to 32.1 mM of these poultry-feed-specific fungi were well in the range as described in the literature. Propionic acid prevents outgrowth of conidia (spores) in a species-dependent manner. Twenty percent of *Aspergillus chevalieri* and 71% of *Penicillium lanosocoeruleum* conidia germinated after exposure. Dependent on the species, cell damage was visible after incubation with propionic acid. Germ tubes of *P. lanosocoeruleum* in a biofilm showed extensive (85 %) cell death after a 30 min treatment with propionic acid and slightly lower sensitivity was observed with *A. proliferans* (62% cell death). Microscopic analysis of these fungal biofilms revealed extensive damage to the cell membrane and showed distorted intracellular structures. Fluorescent life-dead staining of the germ tubes showed a clear dose response of propionic acid indicating a fungicidal effect on these growing cells. These results show that conidia can be inactivated by propionic acid, but that germ tubes show a much higher sensitivity. These observations shed new light on the mode of action of this important preservative to prevent fungal contamination of feed.

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Biocontrol agents of cereals mycotoxigenic fungi: elucidating mechanisms of action

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One of best way to reduce the mycotoxin content in food and feed is the prevention of their formation in the field. With the decrease in the use of pesticides due to their toxicity, one alternative strategy to fight against mycotoxigenic fungi can be the use of antagonist microorganisms. They can inhibit the fungus growth and the accumulation of mycotoxins. However, the implementation of such measures requires a broad understanding of biological mechanisms that regulate the interaction between mycotoxigenic fungus and biocontrol agents (BCA). To this end, our investigation was focused on combining complementary methods including microbiology, biochemistry and meta-omics techniques. With these objectives in mind, three commercials BCAs were selected (contrasted use and microorganism type; *Trichoderma asperellum*, *Streptomyces griseoviridis*, *Pythium oligandrum*), studied with *in vitro* confrontation with *Fusarium graminearum* and *F. verticillioides*, respectively trichothecene and fumonisin producer, respectively. Growth kinetics and mycotoxin production of pathogens were studied in different experimental conditions. Variable levels of mycotoxinogenesis reduction have been observed depending on the microorganism type of BCAs or on the culture conditions (e.g, different nutritional sources), which suggest contrasted biocontrol mechanisms. *S. griseoviridis* reduces both pathogens growth and mycotoxins concentration by 50% after 7 days of confrontation. This BCA leads to a growth inhibition zone where pathogen mycelium structure is altered suggesting an antimicrobial compound diffusion. *T. asperellum* shows a pigment accumulation in the confrontation zone, which may involve particular metabolism activation. *P. oligandrum* progresses over the pathogen colony, which suggests a close interaction such as mycoparasitism. Both are able to reduce pathogens growth and mycotoxins concentration: *T. asperellum* by 70 and 80%, respectively, and *P. oligandrum* by 70 and 89%, respectively, for both pathogens. On this basis, a toolbox has been developed and used to

investigate the mechanisms of BCAs: microscopic imaging, competition, production of antimicrobial compounds, ability to inhibit pathogens spore germination or to degrade mycotoxins, confrontation on wheat spikelet. In addition, hypotheses on the mode of action of BCAs (biocompetiton, mycoparasitism, antibiosis) and how *Fusarium* physiology is impacted will be presented. In the next step, the transcriptome and metabolome analysis of different pathogen-BCA systems will allow to confirm and explore deeply these mechanisms by identifying differential expressed genes in confrontation. Overall, the results of this study will be used to promote the development of new antimycotoxigenic BCAs that may help to limit the accumulation of mycotoxins in cereals as well as the residues of plant protection chemical products, which is a double health safety objective.

P55

Ability of soil actinobacteria to avoid and biodegrade aflatoxin B1 and ochratoxin A

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Aflatoxin B1 (AFB1) and ochratoxin A (OTA) are secondary metabolites produced by *Aspergillus flavus* and *Penicillium verrucosum*, respectively, which develop mostly during seed storage. Both mycotoxins represent public health concerns due to their genotoxic and carcinogenic effects. Several treatments for mycotoxin reduction have been studied through application of diverse molecules, plasma, pulsed light and adsorption by various matrixes. Biodegradation of mycotoxins into less harmful molecules through enzymatic activity has been described for many bacteria, yeast and fungi. Among them, actinobacteria already demonstrated their ability to bind or degrade mycotoxins, as well as a strong capacity to inhibit fungal growth and mycotoxin production. The objective of this study is to evaluate the ability of actinobacteria and their metabolites to degrade AFB1 and OTA and/or to decrease their production. Sixty strains of actinobacteria have been tested for their ability to prevent AFB1 and OTA formation by *in vitro* direct confrontation or with cell free extracts (CFEs). The results showed that all sixty strains reduced AFB1 from 42 up to 100%. Seventeen strains inhibit OTA production from 27 to 94%. Thirty-tree actinobacteria CFEs diminished AFB1 from 10 to 90% while twenty-five lowered OTA amount from 20 to 40%. To investigate if the decrease of mycotoxin originated from an inhibition of their synthesis and/or a degradation mechanism, actinobacteria were cultured on solid and liquid medium containing pure AFB1 or OTA. This assay showed that most of the strains were able to reduce AFB1 from 10 to 96% on solid medium, while on liquid medium reduction percentage went from 7 to 80%. OTA was reduced almost completely by 33 strains in liquid medium whereas only 4 were able to decrease it from 60 to 100% on solid medium. Our first results allowed us to identify isocoumarin (Otα) in samples where OTA amount was decreased, which indicates that OTA was hydrolysed into at least one much less toxic compound by some strains. CFEs incubated with pure mycotoxins are currently being tested, in order to evaluate if mycotoxin decrease is related to their metabolization by actinobacteria, or as a result of the secretion of extracellular enzymes. Finally, animal models, such as nematodes or zebrafish embryos, will be used as they have recently proved to be useful for the evaluation of remaining toxicity. The identification of the degradation by-products and their potential harmful effects will improve the development of novel decontamination techniques of food and feed.

P56

Mycotoxin producing on different matrices

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Investigation of the effect, fate and behaviour of the different mycotoxins in the food chain requires feeding experiments. For this purpose, our group developed a production method for the six most common and important mycotoxins (aflatoxin B1, AFB1; ochratoxin A, OTA; T-2 toxin, T-2; deoxynivalenol, DON; zearalenone, ZEN; and fumonisin B1, FB1) on grain and cereal matrices. The first step was to build fungal strain collection containing mycotoxin producing filamentous fungi. Identification on molecular level and optimisation of growth parameters were the second and third steps in small scale (Petri dish level). The fourth step was to increase the amount of infected matrices (maize, rice). The last step was to increase the mycotoxin concentration on cereals. Finally, we were able to produce mycotoxin containing grains in the following concentrations till now: 8 ppm aflatoxin, 160 ppm OTA, 370 ppm T-2, 530 ppm DON, 70 ppm ZEN and 100 ppm fumonisin; these were the highest concentration levels. The produced mycotoxins are not 100% clean on the grain, usually among the main mycotoxin family, such as aflatoxins, 10% is aflatoxin B2, G1, G2 but 90% is AFB1. We are not

cleaning and separating the mycotoxins. Our aim is to provide grains for running and for further feeding experiments with known quality and mycotoxin concentration. **Acknowledgements**. This research was supported by Development and Innovation Fund (NKFIA); Grant Agreement: NVKP_16-1-2016-0009 and NVKP-16-1-2016-0035. Mátyás Cserháti was supported by the Bolyai János Postdoctoral Fellowship provided by the Hungarian Academy of Sciences.

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Mycotoxin biodetoxification potential of the *Cupriavidus* genus

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The occurrence of mycotoxins is intrinsic to various agricultural commodities food and feed products globally. Among mycotoxins, five are the most important, i.e., aflatoxin B1 (AFB1), ochratoxin A (OTA), zearalenone (ZEN), trichothecene (T-2) and deoxynivalenol (DON). The detoxification and degradation of mycotoxins is urgent objective due to their toxicity levels. There are different methods which have been developed in order to eliminate or reduce the effects of these toxic compounds such as chemical (ozonation), physical (absorbents) and biological (biodegradation via microorganisms). Biological methods are the most promising approach to detoxify and degrade or reduce the mycotoxins level. *Cupriavidus* genus is characterised as gram-negative bacteria that can inhibit several niches, such as root nodules and aquatic environments, as well as to its resistance to heavy metals; some of them are significant xenobiotics degraders. The genus has 16 type strains, which have been investigated for their biodegradation and detoxification potential of the main five mentioned mycotoxins. The biodetoxification was measured by different methods, according to the mycotoxins, SOS Chromo test for genotoxicity of AFB1, BLYAS test for oestrogenic activity of ZEN, zebra *Danio* embryo injection test for OTA, DON and T-2 effects. Till the present time, five strains are able to degrade OTA, three strains can degrade AFB1, and four strains degrade ZEN from the 16 type strains. **Acknowledgements**. This research was supported by Development and Innovation Fund (NKFIA); Grant Agreement: NVKP_16-1-2016-0009. Mátyás Cserháti was supported by the Bolyai János Postdoctoral Fellowship provided by the Hungarian Academy of Sciences.

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Prevention of Fusarium head blight and mycotoxins in wheat with antifungal mulch layers and botanicals

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Fusarium head blight (FHB) is one of the most important fungal diseases of wheat worldwide causing yield reductions and contaminations in grains with mycotoxins that jeopardise food and feed safety. During two consecutive years, field experiments were conducted to investigate prevention strategies to suppress FHB and mycotoxin contamination in wheat. We simulated a maize-wheat rotation under no-till, a system with high FHB disease pressure, by applying maize residues artificially inoculated with *Fusarium graminearum* in field plots with emerged wheat plants. For mulch layers, crops grown in separate fields were harvested, chopped and applied in autumn ('cut-and-carry'), whereas botanical water extracts were applied in either autumn or spring onto the infected maize residues. The mulch treatments included white mustard (WM; *Sinapis alba*), Indian mustard (IM; *Brassica juncea*) and berseem clover (Cl; *Trifolium alexandrinum*), while the used botanicals were mustard-based (Tillecur® and Pure Yellow Mustard) or Chinese galls. Overall, minimum cross-contamination among the experimental plots was detected, underlining that the experimental approach to assess preventative measures to control FHB in wheat was appropriate. All mulch layer treatments consistently suppressed FHB infection and decreased deoxynivalenol (DON) in wheat grains by 40-50% (WM), 37-58% (IM) and 53-56% (Cl) depending on the year. Zearalenone (ZEN) content in grains was also reduced by 76 and 87% with use of WM and Cl, respectively. The botanicals were more effective in the second year reducing DON and ZEN contents in grains by 22-42% and 60-78%, respectively. However, it was not clear whether the application time (autumn or spring) had an influence on the control of the disease. Cereal growers could benefit from the recommended prevention strategies by decreasing the risk of mycotoxin contamination in the harvested products and sustaining or even improving grain yield and quality.

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Knowledge Centre for global food and nutrition security (KC-FNS): scope, structure, future prospective.

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The European Commission (EC) though its scientific services is constantly monitoring agricultural resources, food and nutrition security and food crises with the final aim to provide advanced knowledge base to help achieve zero hunger in 2030. In the framework of the Commission Knowledge Centre for Global Food and Nutrition (KC-FNS) on food-insecure countries, one of the priority topics identified by the Steering Committee in 2019 has been mycotoxins and food security. Subsequently, one of the proposed tasks is to help communities of Sub-Saharan Africa, Latin America and Asia to prevent the consumption of crops highly contaminated by mycotoxins. As reported by WHO, evidences indicate that 500 million of poorest people are exposed at level of mycotoxins contamination that significantly increases mortality and morbidity. Additionally, in 2012, 162 million of children younger than 5 years worldwide were stunted; at the same time, it was showed that the large knowledge gap in how to prevent stunting, included the lack of systems to prevent exposure to mycotoxins. Furthermore, on the other hand, FAO estimates that crop fungal infestation causes the 5-10% of crop losses worldwide, with severe economic consequences for certain developing countries. In the coming years, particular attention will be devoted by the EC KC-FNS to observing the sub-Saharan African region where the monitoring of the weather condition changes and the introduction of preventive measurements and systems which should lead to help reducing the food and feed mycotoxins contamination. Appropriate regulation and governmental regulatory frameworks are envisaged as important drivers of innovation and effective implementation of standards. Information tools, early warning systems and trainings are also considered part of the preventive measures. Data from various sources as the results of the on-going EU founded project (e.g., MyToolBox and MycoKey), of the most relevant international studies will be summarised and structured for being uploaded on the KC-FNS portal. The ability to reach communities as well as individuals with relevant information such as dietary diversity, post-harvest losses measures, improved crop storage and other information is also part of the challenge. The aim of the KC-FNS is to serve as solid scientific informative tool to citizens in addition of acting as scientific service of the EC direct in support to the policy makers who have to shape intervention strategies aimed to reduce human exposure to mycotoxins.

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Evaluation of the effectiveness of an antimycotoxin additive to reduce toxic effects in weaned pigs consuming deoxynivalenol-contaminated feed

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Deoxynivalenol (DON) is a mycotoxin belonging to the group of trichothecenes. The main effects of dietary exposure of animals to DON are weight gain suppression, anorexia and altered nutritional efficiency. Therefore, the objective of the study was to evaluate the efficacy of an antimycotoxin additive to reduce the toxic effects of weaned pigs after consuming food contaminated with 3,200 µg/kg of DON (21 days). 48 weaned pigs were selected and divided in three treatments of 2 pigs with 8 repetitions and one of the diets was assigned: (i) T1 negative control group, without DON; (ii) T2 positive control group, contaminated with DON; and (iii) T3 challenge group, with 1.5 kg/t of the antimycotoxin additive and DON. The piglets were weighed at the beginning of the experiment (28 days of age) and later each week until the end of the experiment. Blood samples were obtained for hematic biometry, hepatic and renal profiles. The information obtained was analysed by the Tukey test where the significance value was based on 0.05 probability. From the 14 and 21 days of experimentation, there were statistically significant differences between treatments in weight gain, feed intake and concentration of the glutamyl transferase enzyme serum. Intestinal integrity was affected in the DON group. The results obtained show that DON present in the diet of the intoxication group affected the consumption of food and consequently the weight gain. The additive used at a rate of 1.5 kg/t feed for this experiment had a protective effect on the weight gain of 71%.

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Assessment of the spread and respiration of *Fusarium graminearum* in wheat grains: a three-dimensional in situ study

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Fungi spread through a complex three-dimensional (3D) matrix of grains in storage silos. However, most in situ studies consider fungal growth in two dimensions. The relevance of the 3D growth in fungal activity is not well understood. Previous studies point out the critical points sources of contamination in stored wheat are the top of the storage silo where there is the manhole opening and the high moisture areas, which in winter is at the top of the silo and in summer is at the bottom. Moreover, silo walls are also important contamination source in which fungal development was detected [Limay-Rios *et al.*, 2017. PloS One 12: 1]. In order to manage storage conditions, it is necessary to understand the fungal spread behaviour through different contamination points.

This study aims to assess the 3D growth of *Fusarium graminearum* in wheat at 25°C for ten days considering the fungal growth pattern in surface area (mm^2/day) and volume (mm^3/day). Also, biomass was indirectly quantified by carbon dioxide (CO_2) production and ergosterol content. Approximately 50 spores were inoculated on the same amount of dried irradiated wheat at water activities (a_w) 0.95 and 0.97. Three inoculation sites were considered: (i) top-centre, (ii) bottom-side, or (iii) bottom-centre of cubic jars in which fungal growth was measured daily. CO_2 measurements were done daily and ergosterol analysis every 48 h. Results showed significant differences (P>0.05) among inoculation positions, although no differences between a_w. Top-centre contamination results in faster fungal spreading whereas bottom-side inoculation has slower fungal growth than other contamination points. Indirect biomass measurements were in line with the fungal growth patterns observed. Moreover, it has been possible to correlate traditional methods of biomass measurements as ergosterol content, with new preventive strategies like CO_2 production. Overall, these results provide a better understanding of the 3D growth of *F. graminearum* in wheat grains. Current research is focused on analysed how the mycotoxin profile is affecting by the original position of the inoculum source. **Acknowledgements**. This project (MyToolBox) was funded from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 678012.

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Effectiveness of essential oils to prevent mycotoxin production by *Aspergillus* species using turbidimetric measurements with the Bioscreen C

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Aflatoxins (Afs) pose a serious risk to food security, contaminating crops in the field and during storage. They are produced by several *Aspergillus* section *Flavi* species, and the best strategy to prevent their presence in food products is to avoid fungal growth. The application of chemical products to control these fungi is effective, but more eco-friendly and safer methods are necessary, such as essential oils (Eos), which have fungicide properties and reduce mycotoxin synthesis. In this work, the efficiency of two Eos (*Satureja montana* and *Origanum virens*) against two *Aspergillus flavus* isolates (A7 and A10) under three different water activity conditions was examined *in vitro*. The tests were performed on YES semi-solid media (0.05% agar w/v), supplemented with glycerol to modify water activity levels up to a_w 0.94, 0.96 and 0.98. The medium was supplemented with Eos (0, 350, 700 and 1000 µg/ml) and was inoculated with *A. flavus* (final concentration of 10^5 spores/ml by well). For this trial, 100-well microtiter plates were used, with final volumes of 300 µl/well and ten replicates for each treatment. The plates were incubated at 25ºC using a Bioscreen-C Microbiological Growth Analyzer. The optical density was automatically recorded at 30 min intervals using the 600 nm filter over 7 days period. Data were recorded using the software Easy Bioscreen Experiment. Afs production was evaluated at the end of the experiment by HPLC-FLD. The results showed that both Eos affected to some extent fungal growth and mycotoxin production, being *S. montana* the most effective one. Eos concentration and aw had a significant effect on growth using both compounds and isolates, obtaining the best results at 1000 µg/ml and 0.94 a_w. In general, a similar effect of both factors were found regarding Afs production by both *A. flavus* strains, except for an increase in Afs concentration found at 0.96 a_w at 700 µg/ml. In conclusion, the Eos evaluated reduced significantly fungal growth and Afs production under different water activity levels. These compounds might be applied during storage as sustainable control method. The

combination of Eos with good storage practice could be a successful approach to control the Afs risk in stored grain. **Acknowledgements**. This work was supported by AGL 2014-53928-C2-2R.

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The accumulation of *Fusarium graminearum* mycotoxins in wheat in response to the biological control agent *Clonostachys rosea* formulated in oil

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Fusarium mycotoxins produced by fungal pathogens of the Fusarium head blight disease complex (FHB) as well as synthetic fungicides, which are widely used to combat the fungal pathogens, are jeopardising the quality and safety of food and feed. Since 2016, the European Horizon 2020 project MycoKey has made a united effort to investigate alternative strategies to mitigate the risk of mycotoxins as part of an integrated and sustainable approach. One of the goals is to develop control strategies against the predominant FHB causing species *Fusarium graminearum* (teleomorph *Gibberella zeae*) by using microbial biological control agents (BCA) in the field. Here, we present the findings of three consecutive years (2016-2018) of field, growth chamber and laboratory experiments to investigate the ability of the soil-borne fungal antagonist *Clonostachys rosea* to protect wheat from pathogen attack. We therefore established our own infection protocol to simulate the natural *F. graminearum* pressure of a maize-wheat crop rotation in the field, used a laboratory screening system to characterise the UV-resistance of local and foreign *C. rosea* strains and developed an oil-based formulation to protect the BCA from UV irradiation. The application of formulated *C. rosea* at the beginning of flowering significantly reduced the average contamination with the trichothecene deoxynivalenol by up to 42% in the field and by up to 80% in the growth chamber, which in case of field applications was on average five times higher, compared with an application of conidia without any UV protection. Interestingly, however, the same treatments with oil-formulated BCA showed contrasting results for the myco-oestrogen zearalenone, which is produced at later stages of the cereal development. In this case, the mycotoxin analysis revealed a mean increase by up to 120 % compared to the untreated control, depending on the *C. rosea* strain that was applied. In conjunction with data collected on disease symptoms in the field, yield parameters, as well as fungal incidence and *F. graminearum* DNA in wheat grains, the latest conclusions will be presented and discussed.

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Sodium sulfite interferes with fermentation during bioethanol production

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Maize used for bioethanol production is frequently contaminated with mycotoxins. Mycotoxins are known to be enriched in solids during the production process. Deoxynivalenol (DON) was shown to be enriched up to three times in dried distillers grains with solubles (DDGS) compared to the initial grain. As DDGS are used as animal feed, detoxification of DON during bioethanol production would be beneficial to feed safety. Sodium sulfite (Na_2SO_3) is known to react with DON yielding DON sulfonates (DONS). As DONS are less toxic than DON, treatment of DON-contaminated maize with sodium sulfite could be used as a detoxification strategy. In this study, we investigated the efficacy of sodium sulfite to detoxify DON in naturally contaminated maize during a lab-scale complete bioethanol process. In two experiments, sodium sulfite was added to DON-contaminated maize (1 mg DON/kg) during mash preparation before liquefaction at a concentration of 10 g/kg. In either experiment, the same bioethanol process was performed without sodium sulfite addition as negative control. DON and DONS concentrations were measured in solids and supernatant after fermentation to evaluate DON detoxification by sodium sulfite. Mass loss during fermentation was measured to determine fermentation efficiency. In both experiments, sodium sulfite addition reduced the DON concentration (≥ 97.9% reduction) and caused the formation of DONS in solids and supernatant. However, in both experiments, mass loss during fermentation was strongly reduced upon sodium sulfite addition compared to the negative control. Furthermore, the final ethanol concentration was decreased upon sodium sulfite addition. In conclusion, sodium sulfite added to naturally contaminated maize during the bioethanol production process detoxified DON but interfered with the fermentation process. Consequently, sodium sulfite treatment is not a suitable strategy for DON detoxification in bioethanol production.

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Saccharomyces cerevisiae as a mycotoxin remediator

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One of the major challenges in food sustainability is the spoilage and contamination of crops from secondary metabolites, mycotoxins, produced by fungi. Mycotoxins pose a significant danger to the health and performance of farm livestock and cause a variety of different symptoms including decreased feed intake, poor reproductive performance, reduced milk production and even death [Gallo, A. et al., 2015. Toxins 7: 3057]. The aim of this study was to investigate whether *Saccharomyces cerevisiae* could degrade mycotoxins. *S. cerevisiae* (R404) was inoculated in 50 ml of nutrient broth with or without 1 µg/ml zearalenone (ZEN), and incubated at 37°C, at 200 rpm for 48 h. Samples were taken at 0, 1, 2, 3, 4, 5, 6, 7, 8, 24, and 48 h for mycotoxin analysis. Samples were analysed using a Waters LC/MS for the presence of ZEN and its metabolites. *S. cerevisiae* was able to degrade ZEN to its metabolites α- and β-zearalenol (ZOL). The presence of β-ZOL was detected after 1 h, while the more toxic α-ZOL was detected after 3 h. The less toxic metabolite β-ZOL was detected at a higher concentration than the more toxic α- ZOL. *S. cerevisiae* is known to have a probiotic effect in animals and humans and helps to maintain the integrity of the intestinal epithelial lining [Shurson, G.C., 2018. Anim. Feed Sc. Tech. 235: 60]. In this study, we have shown that *S. cerevisiae* primarily degrades ZEN to its less toxic daughter metabolite β-ZOL. This suggests that *S. cerevisiae* can be used as a probiotic and mycotoxin remediator in the treatment of animals contaminated with ZEN.

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Spatial analysis of mycotoxin development in stored grain to reduce waste

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Measuring mycotoxins in stored grain to ensure legislative limits are met is done by collecting one large bulked sample composed of several smaller samples taken systematically from the stored grain to give an average contamination level. Previous studies [Rivas Casado, M. *et al.*, 2009. Food Addit. Contam. 26: 857] have used variograms to characterise the spatial variability of mycotoxins to inform how this sub-sampling for bulking should be conducted. However, to avoid waste, a better use of systematic sampling data from stored crops would be to characterise and improve the understanding of the spatial variation of toxins within stored grain and to identify the most heavily contaminated areas. Heavily contaminated grain could then be removed or remedial drying regimes implemented to reduce crop contamination, and minimise the risk of the crop being rejected. Real-time monitoring of grain respiration using CO_2 sensors placed in an informed way in a grain silo could allow early detection of the initiation of spoilage and mycotoxin contamination and the identification in 3D of pockets requiring remedial action. This would minimise waste and maximising profits. This study re-analysed a dataset used previously [Rivas-Casado, M. *et al.*, 2010. World Mycotoxin Journal 3: 95]. Fumonisins (FB1) and (FB2) were measured in 3D (50 points in each of three layers) in a pile of grain stored against a silo wall. No spatial structure was found in these data previously and using the normal score transformation to deal with the large skewness, 3D variograms and 2D variograms for individual layers showed some spatial structure suggesting patches of contamination with an average 3-4 m diameter and with the largest patches of contamination in the layer closest to the grain surface and smallest closest to the wall. 3D local Moran's I analysis also showed statistically significant clusters of low values at the top of the grain pile and more microaerophilic locations close to the wall while significant clusters of large values were at the base of the grain pile particularly in surface layers. This is consistent with humidity diffusing through the silo over time under the influence of gravity and FB1 and FB2 tending to develop in moister, more oxygen rich locations. Variogram and local Moran's I analysis also showed that patches of FB1 and FB2 contamination tended to be co-located and patches for the latter were smaller suggesting that FB2 was developing from foci of FB1 contamination.

P67

Determining future aflatoxin contamination risk scenarios for maize in southern Georgia, USA using spatio-temporal modelling and future climate simulations

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Climate change threatens future food security due to the probable increase in temperature and changes in precipitation patterns. These could affect the distribution of where different crops may be viably grown. When determining the future viable distribution of a crop, consideration should be made of the likelihood of build-up of contaminants, such as aflatoxin in crops. Kerry *et al.* [Crop Protection 2017, 94: 144] and Yoo *et al.* [Spatial Statistics 2018, 28: 84], through analysis of a 20-year county-level aflatoxin survey, developed spatio-temporal models for estimating aflatoxin risk in maize crops cultivated in southern Georgia, USA. Their aims were to: (i) inform local farming practices by determining the highest risk years and counties so that management strategies to reduce risk could be applied, and (ii) to reduce spending on expensive aflatoxin testing surveys in low risk years in this region. Drought conditions identified by June rainfall less than 30 year normals and June maximum temperatures greater than 30 year normals were identified as the two most important factors linked to aflatoxin contamination. The distribution of the maize crop and soil types prone to drought stress were also found to be important. From the 20 years studied, the 20 ppb total aflatoxin limit set by the FDA for feed was exceeded in most of the 53 counties studied in the three high-risk years. Also, the 100 ppb FDA limit for food was breached on average one year in five in the centrally located counties of southern Georgia. As drought conditions could increase in prevalence and their spatial patterns change with future climate changes it is important to determine future aflatoxin contamination risk scenarios. The current study identified future aflatoxin contamination risk scenarios, by using climate projections of June rainfall and maximum temperatures in the region, based on climate model simulations derived from different emissions scenarios. Based on these climate projections, spatio-temporal models that estimate aflatoxin risk in maize crops were developed. These can be used to generate risk maps to help farmers and agricultural decision makers plan future cultivation practices, such as the spatial extent of maize cultivation and planting of more resistant varieties and improved irrigation to mitigate aflatoxin risk due to climate change. This work demonstrates that as temperature rises, enhanced soil degradation and shorter, more intense rainfall patterns, aflatoxin risk estimates increase significantly in Georgia and that there will be a need to re-evaluate maize cultivation zones.

P68

The role of modification treatment of bentonites on the adsorption of mycotoxins

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The industrial and scientific interest has been focused in recent years on the elimination of mycotoxins from animal feed. In life sciences, mycotoxins have been identified as a major issue for the productivity and wellbeing of domestic animals, such as cows, pigs and poultry and even in fish farms. The prevailing types of mycotoxins worldwide are fumonisins, deoxynivalenol, ochratoxins, aflatoxins and zearalenone, while trichothenes are less usual. A big part of the researches conducted in recent years has been focused on the study of adsorbents, based on minerals, modified minerals and additives (i.e., yeast). The types of natural solutions, such as bentonite, zeolite, talc and diatomite, have been extensively studied for the adsorption of different types of mycotoxins, but among them none appeared to have a good mycotoxin adsorption performance except for bentonite for aflatoxin B1. In this study, the mycotoxin adsorption capacity of bentonite treated with various methods was evaluated. Through structural modification of smectite, bentonite's main ingredient, the mechanism of mycotoxin binding was elucidated in order to identify solutions with high mycotoxin efficiency. The structure of smectite was intentionally modified in terms of d-spacing, surface area, cation exchange capacity and hydrophobicity. The methods applied in order to achieve the modifications were organophiliation, acidification, thermal treatment and combinations thereof. The binding efficiency was evaluated with *in vitro* testing and was correlated to bentonite intrinsic or engineered characteristics. In conclusion, by using bentonite-based solutions all mycotoxins of interest can be efficiently eliminated except for deoxynivalenol that continues to be a challenge in the field. Aflatoxin B1 can be captured and retained efficiently by natural or treated bentonite, as long as there is enough available interlayer area and enough aflatoxin binding sites. Ochratoxins, zearalenone and fumonisins can be also bound and retained by more than 90% using engineered bentonite solutions that have specific interlayer space and

high contact angle values. Trichothecenes can be bound and retained by up to 60%, which is 3 times more that the current available mineral based solutions in the market.

P69

Cold plasma interactions with mycotoxins and their fungal producers

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Mycotoxins are secondary metabolites of fungi, which contaminate grains worldwide. The influence of cold plasma (CP) on pure mycotoxins in solution and mycotoxins inoculated onto cereal grains (wheat and maize) were investigated. *Aspergillus flavus*, as a main producer of aflatoxin B1, was used to study the potential of CP for controlling the growth of mycotoxic filamentous fungi and therefore to reduce the impact of mycotoxins in cereal grain production. Mycotoxins (AFM1, AFB1 and DON) were artificially inoculated onto the wheat and maize kernels and prepared in known concentrations in water solutions. The CP system used was a high voltage (HV) dielectric barrier discharge (DBD) contained system. The samples were subjected to direct cold plasma treatment at 80 kV voltage over a range of treatment times from 2 min up to 60 min for toxin solutions and grains. Toxin concentrations were analysed using UHPLC-MS/MS. For microbiological evaluations, organic wheat grains were sterilised and inoculated with *A. flavus* spore suspension to obtain a final concentration of spores on the grain surface of 6 log10 cfu/g. Inoculated grains (2 g) were treated for up to 20 min at 80 kV in air. Samples were exposed to either direct treatment (exposed to the plasma discharge) or indirect treatment (outside of plasma discharge) and subjected to 24 h of post-treatment storage time. The morphology changes of HV cold plasma treated *A. flavus* spores were observed using scanning electron microscopy. The results showed a reduction of 78, 71 and 25% of AFM1, AFB1 and DON, respectively, after 60 min of treatment at 80kV. Over 97% reduction in aqueous mycotoxin solutions was achieved CP treatment. Direct CP treatment reduced *A. flavus* on grains up to 1.95 log10 cfu/g after 20 min of treatment. Further optimisation of plasma process parameters is required to obtain efficient control of toxigenic fungi and aflatoxin accumulation in the food and feed chains.

P70

Sustainable pest control system for eliminating fungi and weeds using benzo derivatives

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Contamination of crops by fungi, especially those resistant to fungicide or producing mycotoxins, is problematic since effective fungicides or intervention methods are sometimes very limited. Certain azole fungicides such as propiconazole/tebuconazole that are applied to agricultural fields have the same mode of antifungal action as clinical azole drugs. Such long-term application of azole fungicides to fields provides selection pressure for the emergence of pan-azole-resistant agricultural/clinical strains [Bowyer P. and Denning, D.W., 2014. Pest Manag. Sci. 70: 173]. Recently, fungicide-potentiation of mycotoxin production in resistant strains has been reported in a number of aflatoxin-, trichothecene-, citrinin-, or patulin-producing fungi [Kim, J.H. *et al.*, Int. J. Mol. Sci. 16: 26850]. Of note, weeds also function as the reservoir of mycotoxin-producing fungi in orchards, and thus mycotoxin contamination increases near maximum thresholds if weeds are not controlled properly [Reboud, X. *et al.*, 2016. Agron. Sustain. Dev. 36: 43]. Therefore, there is a continuous need to enhance the effectiveness of conventional antifungals or herbicides or discover/develop new intervention strategies. We describe a new pathogen intervention method, which incorporates economic and ecologically benign benzo derivatives to crop production processes. The benzo derivatives identified by our group are listed as generally recognised as safe chemicals by US FDA, thus are environmentally friendly and/or emits volatiles with antifungal and herbicidal properties. Our preliminary studies also indicate that these compounds can help overcome fungicide resistance and reduce mycotoxin production. Therefore, the developed method is an ecosystem-based strategy, which can lower pesticide burden in orchards, and also minimises risks to human health and the environment, contributing to an integrated pest management programme. Altogether, our strategy can lead to the development of high efficiency pest management system, which enhances the control of fungi and weeds in orchards.

P71
Efficacy of an algo-clay complex on decreasing aflatoxins toxicity on piglets
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The aim of this study was to measure the efficacy of an algo-clay complex (ACC) on aflatoxins toxicity. The study was conducted by the Samitec Institute (Brazil). 30 male piglets with an average body weight of 12.56 kg were distributed into 5 treatments with 6 replicates each. The trial was run for 28 days. Treatments differed in aflatoxin contamination at 0 or 1 ppm, and inclusion rate of the ACC at 2.5 kg/ton or 5kg/ton. Performance and liver parameters were measured: feed intake, body weight, and individual relative liver weight (RWL). Results were analysed by ANOVA one-way and Bonferroni's test, $P \leq 0.05$. The aflatoxin exposure strongly impaired the feed intake compared to the negative control from day 1 to day 28 (-18%, $P \leq 0.001$. The inclusion of 5 kg/t of ACC in the contaminated diet significantly improved the feed consumption compared to the aflatoxin group (+16%, $P \leq 0.0001$). The exposure to 1 ppm of aflatoxin strongly decreased the piglets' weight compared to the negative control (-14%, $P = 0.0092$). The inclusion of 5 kg/t of ACC in the contaminated diet permits to avoid the loses in body weight to the level of the negative control group. The RWL of piglets exposed to 1 ppm of aflatoxin was higher than in the negative control (+28%, $P = 0.0027$). The inclusion of ACC in the contaminated diet significantly lowered the RWL compared to the aflatoxin group (-13%, $P \leq 0.05$). These results highlight the potential of algo-clay complex to protect animals towards aflatoxin animal toxicity.

P72
A forecasting model for aflatoxins contamination in maize: a case study in Serbia
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Maize is one of the major crops produced in Serbia. The produced maize is mainly used as ingredient for compound feed for animals, and for human consumption and starch production. Serbia is one of the largest maize producer and exporter in Europe in the recent years. The presence of aflatoxins, produced by *Aspergillus flavus*, in maize grain is an increasing problem since this secondary metabolite is very toxic to human and animal health. Predicting aflatoxin levels prior to crop harvest enables early indication of contaminated maize field and is important for risk management. This study aimed to develop a mathematical model to predict total aflatoxin levels (sum of aflatoxin B1, B2, G1 and G2) in maize up to harvest using Bayesian Network (BN) modelling with inputs from an existing weather-driven mechanistic model. A total of 869 samples were collected from 117 maize fields in Serbia between 2012 and 2018. The samples were analysed by LC-MS/MS to obtain the total aflatoxins levels. According to the EU regulations, the samples were classified into two contamination classes, being high (above 10 µg/kg) and low (below 10 µg/kg). Meteorological data of related fields were obtained from a public database, being the JRC Interpolated Meteorological Dataset. For BN modelling, the entire dataset was split into two independent datasets by harvest year as follows: (i) training set including 752 observations collected from 2012-2016; and (ii) validating set including 117 observations collected from 2017-2018. Results of the BN model showed a prediction accuracy of 91.0 and 83.8% with training set and the validating set, respectively. This study presents a novel approach to combine mechanistic modelling with machine learning techniques to predict aflatoxin contamination in maize with a very high accuracy.

P73
Mycotoxins – an increasing problem? Costs of mycotoxin management
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Mycotoxin contamination of the feed supply chain is considered as the 'hidden threat' in modern animal industry. The word 'mycotoxin' is derived from the Greek 'myke' and 'toxicum'. It can be literally translated as "the fungus' poison", which refers to the mycotoxin being a naturally occurring, toxic secondary metabolites produced by different array of fungi. Thus, they are enhanced by moist and warm conditions. As the presence of fungi occurs naturally on cereals and other raw materials, mycotoxins are found universally, currently existing more than 450 classes of mycotoxins identified and characterised. Moreover, mycotoxins are becoming an increasing problem due to globalised feed grain trade and climate change. Both circumstances help distribute mycotoxins outside of their natural

occurrence geographical areas, complicating the prediction of mycotoxin contamination in compound feed. The economic impact of mycotoxins to human society can be measured in two different ways: (i) economic losses derived from contaminated food or feed, and (ii) human health losses from adverse effects associated with mycotoxin consumption. Both ideas need to be internalised by farmers and other players in the feed industry and assessed with a correct mycotoxin management. Farmers are very cost sensitive, but the sooner we realise control measures are largely more economically efficient than potential losses from animal performance reduction in daily gain and feed conversion ratio, the sooner we will control the mycotoxins situation. To introduce a few data, an infection from mycotoxins may cause an increase in the production costs from 18 to 74% (mean from 75 references consulted (veterinaries, farmers, research, press publications, and bibliography)), whereas the cost of preventing these damages with an effective toxin binder only meant a 10% increase on the complete production cycle. The presence and importance of mycotoxins is becoming so relevant that the EU has recently adopted a new functional group, SRMC (Substances for the Reduction of Mycotoxin Contamination). These substances can suppress or reduce absorption, promote excretion of mycotoxins or modify their mode of action. Clays represent around 60% of these SRMC, although it must be highlighted that their mode of action and efficacy absolutely depends on its nature, chemical composition and isomorphism substitutions. Surface area, cation exchange capacity, active centres or charge density are some of the parameters to measure binder efficiency, and thus, they will help us focus in our search for the most cost-effective solution for mycotoxin management. The present study will analyse the performance and efficacy in either *in vitro* and *in vivo* trials of Atox Nature Silver, a Tolsa's product designed for the animal feed market, which acts as a toxin binder, promoting adsorption of mycotoxins without impacting in the nutrient's absorption.

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Application of lactic acid bacteria as biopreservation agents

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This work aimed to evaluate the antifungal activity of selected lactic acid bacteria (LAB) to be employed as novel biopreservation agents for food and feed and as to promote also the mycotoxins reduction in food. Twenty-seven strains of LAB obtained from the Spanish Type Culture Collection (CECT) were used to ferment different foodstuff and media, such as wheat sourdough, milk whey, MRS broth. After that, the antifungal activity of the fermented media in solid and liquid medium was performed against a group of thirty toxigenic fungi belonging to the genus *Fusarium*, *Penicillium* and *Aspergillus*. On the one hand, sourdough and milk whey fermented were employed to improve shelf life of loaf bread contaminated with *Penicillium* spp. Also, MRS broth medium was fermented by LAB in order to develop a new biocontrol product for maize and maize ears against *Aspergillus flavus* and *Fusarium graminearum* growth. The same LAB tested as biopreservatives and biocontrol agents were also tested for ochratoxin A (OTA) degradation in MRS broth and during human gastrointestinal digestion. The breads elaborated with sourdough fermented by *Lactobacillus plantarum* CECT 749 and *L. bulgaricus* CECT 4005 and milk whey fermented by *L. plantarum* CECT 220 evidenced an improvement of the shelf life of 2 and 8 days respectively. After maize and maize ears conservation, the treatment with MRS fermented by LAB showed an averaged reduction in the production of toxins compared to the control. The results of the OTA reduction study showed that the LAB reduced OTA in MRS medium and during the *in vitro* gastrointestinal digestion in a range variable from 33 to 99%, respectively. Therefore, LAB assessed in this study could be considered promising alternative in the inhibition of toxigenic fungi, and as substitute of synthetic compounds for food preservation.

P75

Antifungal activity of allyl isothiocyanate: from raw materials to finished product

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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 and they can react easily with nucleophiles, such as amines, amino acids, alcohols, water, and sulfites during food treatment and under physiological conditions and also with several functional groups of many mycotoxins. The objectives of this study were to evaluate the antifungal properties of allyl isothiocyanate (AITC) and *p*-hydroxybenzyl isothiocyanate (p-HBITC) against *Penicillium verrucosum* (D-01847 VTT) producing ochratoxin A (OTA) both in raw material (wheat) and in final product (bread). The first

experiment was carried out in a laboratory scale silo system composed of 1 l glass jars containing 300 g of wheat contaminated with $1x10^4$ spores/g of *P. verrucosum*. The cereal was treated with a 12% hydroxyethylcellulose gel disk containing 500 µl of AITC (500 ppm in the headspace) and incubated for 30 days at 21°C. The control group of cereals did not receive any treatment. At 1 and 30 days, the fungal population, the mycotoxin content and the AITC content in the samples and in the headspace were determined. In addition, the shelf life of loaf bread contaminated with *P. nordicum* CECT 2320 and treated with different amounts of yellow mustard flour (natural producer of p-HBITC) as natural ingredient was evaluated. The reduction of fungal growth on wheat after 1 day of treatment in the jars was 0.9 log, having practically disappeared in at 30 days. The mycotoxin analysis did not show any significant reduction in the presence of OTA. Finally, no growth of *P. nordicum* CECT 2320 was observed in the contaminated bread and treated with 6 and 8 g/kg of yellow mustard flour during the storage period of 10 days of the study.

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Climate change factors: transcriptomic and metabolomic shifts on *Aspergillus flavus* during maize storage

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There is a significant interest in the impact that climate change (CC) factors may have on mycotoxigenic fungi. In previous research, we examined the impact that three-way interactions between water availability, temperature and elevated CO_2 have on expression of all the genes in the aflatoxin biosynthetic gene cluster using RNAseq, and the impact on phenotypic aflatoxin B1 production by *Aspergillus flavus* (NRRL3352). However, to date, there has not been any research analysing temporal shifts in the secondary metabolite production pattern of *A. flavus* under current and predicted CC conditions (water activity 0.985, 0.93; temperature 30, 37°C; and CO_2 exposure 400, 1000 ppm). In this work, we highlight the impact of interacting abiotic CC factors on secondary metabolite gene clusters and the related metabolome data including aflatoxin B1 and up to 167 other fungal secondary metabolites in a kinetic study of maize after 4 and 8 days storage. Aflatoxin B1 production increased under elevated CO_2 conditions. Similarly, metabolomic production shifts were observed for other secondary metabolites related including aflatoxin B1 derivatives, metabolites from the aflatoxin pathway and some other metabolites during colonisation of the maize grain. This study provides in depth new knowledge using RNAseq and metabolomics analyses of the dynamics and impacts of CC scenarios may have on mycotoxin contamination of a staple cereal. Such data sets could be effectively utilised to improve the prediction and potential risk of such mycotoxin contamination of staple food crops. This would be particularly important for improving our understanding of sustainable food production, food safety and the food security agenda.

P77

The role of resistance in reducing toxin contamination in wheat and maize

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The toxin contamination of the maize and wheat is the largest food and feed safety challenge we know. As toxin contamination is the last decades has been connected to epidemics, the idea is not surprising to see the role of the susceptibility behind this and the increased resistance as a possible solution. The testing methodologies are own development. In wheat, the QTLs protect to all *Fusarium* spp. Tested (four independent isolates as a rule). Again, a large variability was found in commercial cultivars and lines. In breeding, highly resistant winter wheat lines were produced, and medium resistant cultivars are in commercial production. 80% of the genotypes tested showed close (r=0.80) correlation between resistance and deoxynivalenol contamination, and also here genotypes with toxin overproduction were identified. Relative resistance was also found. Toxin control is inevitable to discard the more resistant but high toxin producers. In maize, research found tenfold or higher resistance difference between hybrids indicating the possibility to select the most resistant ones for commercial production (kernel resistance, toothpick method). Resistance test with different hybrid populations showed that about 10-15% of the hybrids were superior to *Fusarium graminearum*, *F. verticillioides* and *Aspergillus flavus* ear rot, about 5-10% were highly susceptible to all, the rest gave diverging results in any possible combinations. As individual isolates of a given toxigenic species may give diverging results, all tests

were done with two isolates/fungal species. The toxin contamination correlated most cases significantly indicating the role of resistance to reduce toxin contamination, but in each set about 10-15% of the hybrids proved, in yearly replicated trials, very strong toxin overproduction, more seldom a relatively low toxin content. As genetically the background is mostly different, we need separate tests to al fungal species. From the test we develop a risk profile for each hybrid. Of course, highly preferred are hybrids that are lower infected and have lower toxin contamination than the controls or the experimental mean. In both crops, the low infected and low toxin producers can be identified with high probability. Therefore, toxin control starts with discarding disease and toxin sensitive genotypes and start a breeding programme for lower susceptibility and toxin contamination. This should be supported by fungicides and good agronomy. The multi-toxin approach is of high importance. A significant reduction in toxin contamination is possible in 2-3 years.

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Barley production status in the northwestern of Uruguay regarding food safety

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Barley represents the second most important winter crop in Uruguay. Yield and quality losses due to Ramularia leaf spot (RLS) and Fusarium head blight (FHB) are major constraints to barley production. In the case of FHB, the decrease in grain quality lies in the production of mycotoxins, such as zearalenone and deoxynivalenol, which are the most prevalent in Uruguay. FHB and RLS require different managements practices. For FHB, the most used is preventive chemical management; RLS gives place to foliar fungicide intervention when the first symptoms appear, and the weather conditions predispose it. Both scenarios can lead to unnecessary applications in cases where the conditions for the development of both diseases are non-existent, generating fungicides residues in the grain. In our country little information has been produced regarding the concentration levels of fungicides and mycotoxin in barley grain. The goal of this work was to evaluate which of the different chemical managements applied presented the best quality of grains in terms of mycotoxins and fungicide residues levels. Several surveys to farmers were carried out in order to understand their attitudes, uptake, chemical managements used and priorities regarding barley production. In addition, a sample of their grain harvested in the 2017/18 season was requested to determine mycotoxins and fungicides residues with a previously validated methodology. Also, a field study under controlled conditions was conducted to evaluate the effects of the most used approaches for the chemical control of FHB and RLS, yield, mycotoxins and fungicide residues concentration in grain. The developed method consisted of a QuEChERS template without clean-up and analysis by LC-QQQ-MS/MS for two mycotoxins and ten fungicides. The final method presented recoveries in the 75-109% range, and relative standard deviations below 17% in accordance with guidance document SANTE/11813/2017 at 0.01, 0.1 and 1 mg/kg. Regarding fungicides residues, six compounds were detected in some of the analysed samples; carbendazim, trifloxystrobin and isopyrazam were below the LOQ whereas fluxapyroxad, pyraclostrobin and azoxystrobin were in a concentration between 0.01 and 0.05 mg/kg. None of the samples presented zearalenone but deoxynivalenol was detected in all of them (<LOQ-1.67 mg/kg). The results confirm that the chemical management used by barley farmers and the studied ones under controlled conditions for the control of FHB and RLS in barley crops are effective and do not exceed the MRLs, showing that the barley sector is prepared to attend the demand of international markets.

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Treatment with *Origanum virens* essential oil affects cell viability and alters morphological ultrastructure of *Aspergillus steynii*

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Aspergillus steynii is one of the main ochratoxin A (OTA) producing species, as almost all strains are able to produce to toxin at extremely high levels. Essential oils (Eos) have been proposed as sustainable and environmentally friendly control methods to reduce fungal proliferation and mycotoxin production in agrofood products. In our group, we have demonstrated that the treatment with *Origanum virens* EO is efficient to control *A. steynii* growth *in vitro* reaching complete inhibition at 500 mg/l. The first aim of this work was to stablish if the treatment with *O. virens* EO had a fungicide or fungistatic effect in *A. steynii* using fluorescence microscopy. Two-day-old mycelium of *A. steynii* was treated during 24 h with the mentioned EO at 500 mg/l. After incubation, mycelium was filtered and stained using the viability fluorophore FUN-1 which is specific to determine fungal viability. The results showed a fungicide effect

of *O. virens* EO, as after treatment, mycelial cells lost viability compared to live control cells without EO. The effect of the treatment on morphology of the hyphal cells was also evaluated using transmission electronic microscopy (TEM). TEM images showed that the presence of *O. virens* EO affects fungal structure at wall level. The cell wall became thicker and the fibrillary membrane, which corresponds to the outer part of the structure, separated from the rest of the wall. The hyphal septa were apparently thinner after treatment and, in some cases, they seemed to be broken. In conclusion, the fungicide effect of *O. virens* EO to *A. steynii* seems to be related to the loss of cell wall integrity. **Acknowledgements**. This work was supported by AGL 2014-53928-C2-2R.

P80

Mycotoxin-sequestering feed additives reduce aflatoxin M1 levels in milk of lactating dairy cows fed aflatoxin

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The objective was to evaluate the efficacy of two dietary mycotoxin sequestrants in reducing aflatoxin M1 (AFM1) concentrations in milk of dairy cows challenged with dietary aflatoxins (Afs). Thirty-two mid-lactation Holstein cows were randomly assigned to the following treatments: (i) 2.8 mg Afs/cow per day (positive control, PC); (ii) 2.8 mg Afs + 100 g of TN (Toxy-Nil)/cow per day (TN); (iii) 2.8 mg Afs + 100 g UP (Unike Plus)/cow per day (UP); and (iv) no Afs and no additives (negative control, NC). For 7 days, treatments were top-dressed twice daily by mixing into the top portion of the TMR at each feeding. After the experimental period, cows were fed the NC diet and clearance of AFM1 via milk was monitored for 7 days. Concentration and mass of AFM1 secreted in milk and in urine were similar between TN and UP but were lower than PC; concentrations in milk averaged 0.2, 0.3, and 0.6±0.1 µg/kg, respectively, and mass secreted in milk averaged 8.1, 9.8, and 20.5±1.7 µg/day. Concentrations in urine averaged 6.9, 7.4, and 14.2±1.5 µg/l, respectively, and mass secreted in urine averaged 225.7, 250.8, and 521.6±53.1 µg/day. Likewise, concentration and mass of free Afs excreted in faeces were similar between TN and UP, but were lower than PC; concentrations averaged 7.7, 8.9, and 12.4±0.6 µg/kg, respectively, and mass excreted averaged 57.8, 69.6, and 95.6±4.8 µg/day. Transfer of Afs from feed to AFM1 in milk was reduced by 63 and 52%, and in urine by 57 and 52% for TN and UP, respectively. Transfer of Afs from feed to free Afs in faeces was reduced by 38 and 26% for TN and UP, respectively. The clearance rate of AFM1 in milk did not differ among PC, TN, and UP. Results indicate that TN or UP significantly reduced Afs absorption and AFM1 in milk of cows consuming a total mixed ration containing approximately 105 µg Afs/kg of diet dry matter.

P81

Efficacy of mycotoxin deactivators on health and growth of swine: a case-challenge study on weaned pigs and meta-analysis

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The efficacy of mycotoxin deactivators on health and growth performance of newly weaned pigs (27 days-old) fed diets naturally contaminated with deoxynivalenol (DON) was investigated. Sixty pigs were housed individually and assigned to 5 treatments for 34 days subdivided into 3 phases: (i) NC (no added deoxynivalenol); (ii) PC (deoxynivalenol at 2 mg/kg); (iii) CYC (PC + clay/yeast culture based product, 0.2%); (iv) CYE (PC + clay/yeast cell wall/plant extracts/antioxidants based product, 0.2%); and (v) CYB (PC + clay/inactivated yeast/botanicals/antioxidants based product, 0.2%). Blood was collected at days 14 and 34. Intestinal mucosa was taken at day 34. Data were analysed using Proc Mixed of SAS with pre-planned contrasts. Deoxynivalenol reduced (P<0.05) ADG in P3. Pigs fed CYC had greater (P<0.05) ADG during overall period, ADFI during P3, and gain/feed during P2 than PC. At day 14, deoxynivalenol reduced (P<0.05) BUN/creatinine and tended to reduce (P=0.088) BUN. Pigs fed CYB tended to have greater (P=0.059) AST than PC. At day 34, pigs fed CYC (P=0.083) and CYB (P=0.068) tended to have lower serum CPK than PC. Pigs fed CYE had lower (P<0.05) BUN/creatinine than PC. Deoxynivalenol tended to increase (P=0.068) malondialdehydes and decrease (P=0.072) glutathione in jejunal mucosa. Pigs fed CYE and CYB had lower (P<0.05) malondialdehydes, whereas pigs fed CYB had greater (P<0.05) glutathione and tended to have lower (P=0.079) jejunal IgA than PC. Pigs fed CYC (P=0.066) and CYE (P=0.099) tended to have lower jejunal IL-8 than PC. In conclusion, deoxynivalenol compromised growth performance and intestinal health. The mycotoxin deactivators could enhance

intestinal health of pigs fed diets with deoxynivalenol without affecting liver function. Finally, a meta-analysis on 14 trials performed on swine at different physiological stages has shown a significant improvement of performance with the supplementation of mycotoxin deactivators.

P82

Fate of *Alternaria* mycotoxins during apple concentrate production

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Apples are one of the major crops in Argentina and are used for internal commerce as well as export trade. Fruits which are not compliant to the envisaged quality standards are transferred to by-products, specifically to fruit concentrates that are applied in different food industries. Apple mouldy core is caused by *Alternaria* species and its incidence is worsened by long-term storage. The mould develops in the centre of the fruit without causing visual external symptoms or lesions, making it difficult to verify fungal presence. In addition, *Alternaria* produces a wide variety of toxic secondary metabolites whose fate during this process remains unknown. The objective of this study was to evaluate the effect of the apple concentrate process on the natural contamination levels of six *Alternaria* mycotoxins, namely alternariol (AOH), alternariol monomethyl ether (AME), tenuazonic acid (TeA), tentoxin (TEN), altenuene (ALT) and altertoxin-I (ATX-I). Six stages (grounding, turbos, decanter muds, pre-concentration, rejection and concentrate) of 5 apple concentrate processes from the Argentinian industry were sampled and analysed, whereof 3 included a clarification stage. The extraction and quantification method was performed according to Walravens *et al.* [J. Agric. Food Chem. 2016, 64: 5102]. Quantifiable levels of AOH, AME, TeA and TEN, except for one that showed TEN at levels below LOQ, were observed in the raw materials from the 5 evaluated processes. ALT and ATX-I were not detected at any stage of the process. The concentration of neutral toxins (AOH, AME, TEN) decreased when peels, seeds and other solid parts of the fruit were eliminated from the flow line (turbo treatment & decanter muds). Nevertheless, the remaining amounts increased again in the pre-concentration stage. On the contrary, the acidic toxin, TeA, with higher affinity for the aqueous phase, showed a minor decrease in the solid removal stages. Both types of processes showed the same effect on mycotoxin quantities until the clarification step, in which all the mycotoxins analysed underwent a significant reduction to non-quantifiable levels during ultrafiltration. Only TeA remained at detectable levels in the final product for one of the three clarified processes. Cloudy processes showed a final contamination with AOH, AME, TEN and TeA in higher levels than their initial concentration. These results indicate that the clarification stage in apple concentrate processes is of crucial importance to significantly reduce *Alternaria* toxins to safe levels in the final products. The major risk is associated with cloudy concentrates, especially if those are intended for infant foods.

P83

Transformation of aflatoxin B1 and zearalenone using an evolved high-redox potential laccase.

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Mycotoxins are the biggest challenge for animal feed producers and therefore, regular monitoring and remediation is necessary. Mycotoxins can be remediated by various methods but enzymes and microbes for deactivating mycotoxins are becoming popular these days. Among enzymes involved in mycotoxin degradation, fungal high-redox potential laccases are promising biocatalysts due to their minor requirements and broad substrate range. We have recently engineered a high-redox potential laccase by consensus design and DNA recombination to enhance its thermostability and substrate scope. In the present study, we have tested the transformation of mycotoxins using the evolved laccase, DooKu variant. The mycotoxins at 2 ppm concentrations were incubated with 500 µl DooKu enzyme at 37°C; with syringaldehyde as redox mediator for 1 h. After incubation, the samples were centrifuged and were analysed by LC-MS/MS based multi-mycotoxin method for quantitation of all mycotoxins (aflatoxin B1, ochratoxin A, zearalenone, deoxynivalenol, fumonisin B1 and T-2 toxin). DooKu mutant demonstrated 73% transformation of aflatoxin B1 at pH 6.5 and 99% transformation of zearalenone at pH 5.0. Therefore, this study shows that DooKu laccase has potential to transform aflatoxin B1 and zearalenone.

P84

Development of a multi-mycotoxins based method for studying adsorption of mycotoxins

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The objective of the study was to develop an LC-MS/MS based method for screening multi-mycotoxins adsorption by binders. We compared methods to adsorb 6 mycotoxins per test tube (aflatoxin B1, fumonisin B1, ochratoxin A, deoxynivalenol, T-2 toxin, zearalenone) with one mycotoxin per tube, at pH 3.0. In this study, organically modified clinoptilolite (OMC) was used as a mycotoxin adsorbent. OMC (triplicates) at a concentration level of 10 mg/ml was incubated with 10 ml pH 3.0 buffer solutions at a fixed concentration (2 ppm) of mycotoxins (6/tube and 1/tube) at 37°C for 60 min in an orbital shaker at 200 rpm. Control samples were prepared using buffer solution and mycotoxins, whereas the test samples were prepared with buffer, mycotoxins and binders. The result quantification/adsorption was done using ^{13}C-labelled mycotoxin analogues and was determined by measuring the mycotoxin in solution using Agilent 6460c LC-MS/MS and calculating the percentage mycotoxins bound.

	DON	AFB1	FB1	T-2	ZEN	OTA
Bias criterium (2.8 σR)	10,02	1,28	6,53	12,54	5,39	3,59
| Y1-Y2|	0,01	0,39	0,04	8,02	3,93	1,67
| Y1-Y2| < 2.8 σR	Yes*	Yes*	Yes*	Yes*	Yes*	Yes*

* The two test results are in agreement.
Y1- test result obtained with single mycotoxin method
Y2- test result obtained with multi-mycotoxin method
σ_R – reproducibility standard deviation

To compare the results from the two methods, bias was calculated |Y1-Y2| < 2.8 σR. If the absolute difference between the two methods does not exceed R (2.8 σR), the two methods agree. With the multi-mycotoxin method the obtained adsorption test results are similar to the single mycotoxin method results. Therefore, this method could be used for screening adsorbents to bind mycotoxins.

P85

ZenA renders ZEN non-oestrogenic in ruminants

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The mycotoxin zearalenone (ZEN) is frequently detected in feed. Due to its structural similarity to oestrogens, it binds to oestrogen receptors, thereby interfering with reproductive functions. Ruminants are assumed to be less sensitive to ZEN than pigs. However, in the rumen, ZEN is metabolized to α-zearalenol (α-ZOL), β-zearalenol (β-ZOL), α-zearalanol (α-ZAL), β-zearalanol (β-ZAL) and zearalanone (ZAN). These metabolites are oestrogenic, with α-ZOL being 60 times as potent as ZEN [EFSA Scientific Opinion, 2016]. The bacterial enzyme zearalenone hydrolase ZenA (ZENzyme®) converts ZEN to the non-oestrogenic compound hydrolysed ZEN (HZEN). HZEN spontaneously de-carboxylates to DHZEN. Here, we investigated the efficacy of ZenA to degrade ZEN in a simulated rumen environment and *in vivo* in dairy cows. For a RUSITEC (rumen simulation technique) experiment, reactor bottles (n=8) were inoculated with 500 ml rumen fluid from slaughtered bulls, 300 ml tap water, 200 ml synthetic saliva (modified McDougall buffer) and 10 g dairy TMR. Culture material of *Fusarium graminearum* was added to achieve a final concentration of 0.3 µM ZEN. ZenA was added to 4 reactors, while the remaining 4 reactors served as control. The reactors were incubated for 24 h at 39°C. To investigate the metabolization of ZEN by the rumen microbiota and ZenA, concentrations of ZEN and its metabolites were measured by HPLC-MS/MS in samples taken after 10, 20 and 30 min, and 1, 2, 3 and 24 h. In the control treatment, ZEN was metabolized to α-ZOL, β-ZOL, β-ZAL and ZAN, with α-ZOL being the main metabolite. In the ZenA treated reactors, HZEN was the main metabolite at each sampling time point, whereas only minute concentrations of α-ZOL were detected. In a 10-day feeding trial, 4 rumen-fistulated Holstein Friesian cows (non-lactating, non-gestating) received (i) ZEN-contaminated feed (5 mg ZEN/cow/day), (ii) ZEN-contaminated feed (5 mg ZEN/cow/day) supplemented with ZenA, or (iii) uncontaminated feed according to an elaborate experimental plan. Rumen fluid and faeces samples were taken daily and analysed for ZEN and its metabolites using HPLC-MS/MS. The inclusion of ZenA in the diet induced a significant (P<0.01) shift from ZEN and α-ZOL to HZEN and DHZEN in rumen fluid and faeces. In conclusion, ZenA degraded ZEN to non-estrogenic HZEN in the rumen, thereby preventing the formation of the highly oestrogenic metabolite α-ZOL. Therefore, application of ZenA as

a feed additive in ruminants is a promising approach to prevent estrogenic effects of ZEN-contaminated feed.

P86

Advanced grain cleaning solutions for mycotoxin reduction

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Mycotoxins in the feed and food chain are a major threat both for human and animal health. Just a few highly contaminated grains can make a whole lot unsafe for use as human or animal nutrition. For efficient mycotoxin reduction, a full value chain approach is needed, starting from measures to prevent contamination in the field, to control measures to avoid mycotoxin production during storage and over the process line, until final consumption. Grain cleaning is the most effective post-harvest mitigation strategy to reduce high levels of mycotoxins due to the efficient removal of mould-infected grains and grain fractions with high mycotoxin content. Several studies have been performed during the last years to investigate the reduction of deoxynivalenol in wheat and barley, ergot in rye, and total aflatoxins in peanuts and maize. In this study, the reduction of *Fusarium* mycotoxins content in maize, i.e., deoxynivalenol (DON), zearalenone (ZEN) and fumonisins (FBs), was tested. Three online cleaning processes were used: (i) mechanical size separation and dust removal by aspiration, (ii) separation based on density differences, and finally (iii) optical sorting. Samples were taken dynamically according to Commission Regulation No. 401/2006 along the entire process line, including cleaned and rejected project streams. Mycotoxin analyses were performed of water-slurry aggregate samples by validated HPLC methods based on immunoaffinity column clean-up of extracts. Selected samples were additionally analysed with LC-MS/MS. The overall reduction rate was up to 55% for DON, up to 100% for ZEN, and up to 65% for FBs. High levels of mycotoxins were found in all rejected fractions. **Acknowledgments**. 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.

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In vitro efficacy assessment of a bentonite-based material acting as a multi-mycotoxin binder

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Adsorbing agents for detoxification proved to be effective in mitigating the effects of mycotoxin contamination in animal feed. Due to the large number of products available on the market, it appears essential to verify the efficacy of these substances. During these tests, it is important not only to demonstrate their performance to reduce the bioavailability of mycotoxins in the digestive tract, but also to ensure there are no unintended detrimental consequences, such as antinutritional effects. The aim of this study was to assess *in vitro* the efficacy of Multiprotect, a bentonite-based product, regarding the adsorption capacity of main mycotoxins at pH 4 and 7, as well as its adsorption speed and its potential interaction with nutrients (vitamins and minerals) adsorption. The binding properties of the bentonite-based product were evaluated by *in vitro* adsorption using single concentration methods towards 1000 ppm of aflatoxin B1, zearalenone, fumonisin B1, ochratoxin, T2 toxin and deoxynivalenol, at pH 4 and 7 in PBS-buffer, during 3 h at 40°C. Adsorption kinetics were performed for aflatoxin and zearalenone after 1, 5 and 10 min. Potential interactions of the product with nutrients were evaluated with phosphorus and vitamin B6, used as minerals and vitamin model, respectively. The products showed binding properties toward all mycotoxins tested, except deoxynivalenol. Values of adsorption varied according to the type of mycotoxins and the pH. Adsorption values ranged from 40 to 99% and from 22 to 99% for mycotoxins adsorbed by bentonite-based materials at pH4 and pH7, respectively. Near 99 and 66% of aflatoxin and zearalenone, respectively, were adsorbed after one min contact with the product. No interaction with mineral and vitamin models has been observed with the product. Overall, this study highlighted important indicators to test the efficacy of an adsorbing agent. The tested product demonstrated a detoxification potential toward a wide spectrum of mycotoxins, as well as a speed binding capacity that may limit the bioavailability of mycotoxins rapidly absorbed in gastrointestinal tract. Finally, it showed a limited antinutritional impact.

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Remediation of deoxynivalenol (DON): developing an *in vitro* model to screen potential detoxifying agents on DON degradation and detoxification in a simulated gastro-intestinal tract of a pig

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Deoxynivalenol (DON) is a trichothecene mycotoxin produced by *Fusarium* species and is occurring worldwide with high incidence in feed. Strategies are needed to eliminate its health risk for livestock and to minimise its economic impact on production. In order to assess the efficacy of potential physical, chemical and biological DON detoxifying agents, a good *in vitro* model is necessary to perform a fast screening of numerous new compounds before *in vivo* trials are set up. In this project, two *in vitro* screening models were developed to screen compounds for DON degradation and detoxification, acting as potential feed additives defined as 'substances for reduction of the contamination of feed by DON' by the European Food Safety Authority (EFSA). The first screening model was performed in a buffer environment (single concentration, isotherm experiment), whereas the second screening model employed a feed matrix in a simulated gastro-intestinal tract (GIT) of a pig, as both matrix and incubation parameters can affect the binding, removal or degradation of DON by the compounds. For each *in vitro* model, residual DON concentration was analysed with ELISA and the residual toxicity with a bioassay using the aquatic water plant *Lemna minor* L. [Vanhoutte, I. *et al.*, 2017. Toxins 9: 63]. Twelve compounds with different modes of action were tested in the *in vitro* buffer model of which three were tested in the *in vitro* feed model. One product seemed very promising in both *in vitro* models and could reduce DON for almost 100% in feed after 6 h. Further *in vitro* experiments have been performed to characterise the most effective use of this additive. Finally, DON metabolites were found with LC-MS/MS at 2, 4 and 6 h. This *in vitro* model seems a promising tool to use as a fast screening method to screen and differentiate compounds detoxifying DON.

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Impacts of climate change on aflatoxins in maize and the dairy production chain

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Aspergillus flavus and related aflatoxin B1 (AFB1) contamination of maize is largely influenced by local weather and agronomical practices, such as the cultivar chosen. Warm and humid conditions as well as droughts facilitate *A. flavus* infection of maize. Climate change will lead to higher temperatures and more variability in rainfall in Europe. Hence, climate change is suggested to increase *A. flavus* infection of maize and resulting AFB1 contamination. This study used a full chain modelling approach to investigate the effects of climate change on AFB1 contamination in maize and dairy cows' feed as well as aflatoxin M1 (AFM1) contamination of dairy cow's milk. To this end, relevant models and input data were linked together in a modelling framework. This framework was applied to a case study for the Netherlands, importing maize grown in Ukraine to be processed into compound feed for dairy cows fed to dairy cows in the Netherlands. Three different climate models and five published AFB1 transfer models were used. Given the selected climate change scenario, model results showed that AFB1 contamination in maize grown in Ukraine and AFM1 contamination in milk produced in the Netherlands was comparable to or higher than the model estimations for the baseline situation. Results varied mainly with the climate model and carryover model considered, and will be presented during the conference. The modelling framework is expected to be helpful for scenario analyses and will provide decision makers with useful results.

P90

Isolation and characterisation of soil-borne bacteria for biocontrol of *Aspergillus flavus*

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Aspergillus flavus is the major fungus responsible for aflatoxin contamination that results in crop losses and aspergillosis in humans. This research study aims to explore the feasibility of using soil-borne bacteria to reduce aflatoxin formation in crops. In this study, non-fungal based biocontrol agents were screened using a modified agar diffusion method and HPLC. Firstly, microorganisms were isolated from the soil of maize and peanut planting areas in China. Secondly, a modified agar diffusion method was

applied to isolate candidate microorganisms with the potential to reduce the growth of *A. flavus* NRRL 3357. The anti-aflatoxin formation activity of these candidates was further confirmed by using an HPLC assay. The candidates were identified by 16s rDNA sequencing, and a subset of non-pathogenic, safe, and effective biocontrol agents were selected. Whole genome sequencing was completed using the Illumina Hiseq platform to further confirm their genetic placement. To date, a total of 163 microorganisms, mainly bacteria and a few yeasts, have been isolated from approximately 2,000 soil microorganisms. Thirty-two of the 163 isolates showed antifungal activity. Based on the 16s rDNA and genomic sequences, 19 of these isolates were *Bacillus* genus, while the remaining 13 isolates were from 11 other genera. Four of the isolates (*Bacillus* sp.) displayed obvious inhibitory effects on growth and aflatoxin production when co-cultured with *A. flavus*.

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In vitro lipooligosaccharide adsorption by bentonite at different biological pH levels

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Lipooligosaccharides (LPS), also known as endotoxins, are key component of outer membrane of gram-negative bacteria, such as *Escherichia coli* (*E. coli*). Symptoms associated with the LPS presence in animal production include reduced feed intake, increased intestinal permeability, hepatic necrosis and increased morbidity. Animals with endotoxemia may reduce the rate of mycotoxin elimination and therefore lengthen the exposure to mycotoxins. In the present study, the adsorption efficacy of smectite-based bentonite was measured *in vitro* using lyophilised amebocyte lysate (LAL) assay. 50 ng/ml *E. coli* LPS with four different bentonite concentrations (0.05, 0.1, 0.2 and 0.4% w/v) were performed at two pH levels (3.0 and 7.0; pH 3.0 to simulate stomach where main digestion occurs, while pH 7.0 to simulate small intestine where main absorption occurs) in three replicates per group. The unbound LPS were measured and expressed as a percentage relative to the negative control (without bentonite). At pH 3.0, the unbound LPS were 58.5, 28.2, 14.0 and 9.2%, respectively, where results (14.0 and 9.2%) of 0.2% (w/v) and 0.4% (w/v) bentonite groups were significantly lower (P<0.05) than others. At pH 7.0, the unbound LPS were 8.6, 21.7, 28.5 and 19.6%, respectively, where results of all groups were not significantly different. Moreover, results (28.2 vs. 21.7%, 14.0 vs. 28.5%, 9.2 vs. 19.6%) of 0.1, 0.2 and 0.4% bentonite groups were not significantly different between two pH levels, indicating that the bound LPS at low pH (3.0) is not expected to desorb at high pH (7.0). In conclusion, smectite-based bentonite showed promising results in the adsorption efficacy on 50 ng/ml *E. coli* LPS at pH 3.0 and 7.0. Further studies should be conducted to confirm the *in vivo* implications of the described results.

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Essential tools to manage grain and flour quality and food safety

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The delivery of quality safe flour products to all customers depends on managing the flour quality through the supply chain for end-users with varied needs. It is important that the quality and value of grain is guaranteed to ensure the full value is realised for the flour. Wheat and wheat flour testing will require tests for mycotoxins, especially deoxynivalenol. As global surface temperatures rise, the prevalence of certain mycotoxins will increase. An internationally recognised and validated laboratory quality program is essential to meet this goal, using international analytical standards and a laboratory proficiency rating programme.

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Utilisation of compost for the degradation of deoxynivalenol

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Annually large amounts of cereals are infested by *Fusarium* and contaminated with deoxynivalenol (DON) globally, the seriously contaminated grains cannot be used as food or feed, resulting in waste of resources and environment pollution. It is important to find new ways to utilise the contaminated cereals. The objective of this study is to determine the possibility of using composts to decontaminate DON in mouldy maize by the methods of submerged or solid state fermentations. Five horse manure composts collected from the University of Guelph were used as microbial source in this study. In the submerged fermentation process, pure DON was added to a sterile tube containing 2 g of compost in 5 ml water to have a DON level at 100 ppm. After incubation at room temperature (23°C) with 100 rpm shaking for 21 days, the supernatant of the mixture was analysed with HPLC. In the solid state fermentation, 1 g of mouldy maize (containing about 200 ppm DON) was mixed with 10 g of compost sample, which was incubated at room temperature under moisture condition. After incubation for 21 days, 5 ml sterile water was added to extract DON from the mixture. The resulting suspension was centrifuged and the supernatant was analysed by HPLC. The pellet was re-extracted with 50% methanol to determine possible binding of DON. DON was completely undetectable after mixing with the compost for 21 days under the submerged processing. Time courses study by addition of extra 10 ppm DON to the submerged processing samples indicated that DON can be degraded within 24 h; epi-DON and keto-DON were found in 1 and 2 samples after 96 h, respectively, indicating that the microbes varied in the samples. After undergoing the solid state fermentation with the compost samples for 21 days, DON in the mouldy maize was degraded by 98.8-100%. DON was not detected from the dried pellet, indicating there was no DON absorption by the compost sample. In summary, compost as a matrix rich in microbes showed high efficiency of degrading DON. The microbes are able to transform DON completely. This is a new method to utilise DON contaminated cereals effectively as recycling resources instead of waste. Also, the method shows a potential to utilise contaminated cereals as organic sources for fertilizer production. Further studies to identify DON degradation microbes are on the way.

SAMPLING AND ANALYSIS
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P94
Microscale thermophoresis for non-competitive measurement of HT-2 toxin in beer
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Due to their thermal stability mycotoxins can withstand different processing conditions. For that reason, they can be transmitted, e.g., from contaminated malt into beer. Mycotoxin occurrence in cereals for beer production has been reported frequently as well as mycotoxins in bottled beer. Rapid methods for the detection of the mycotoxin contamination are needed for the efficient screening of high number of samples with fewer resources than required by the sophisticated analytical methods. Immunodiagnostic methods can provide tools for on-site screening preceding the further confirmatory analysis. Recombinant antibodies offer advantages over the traditionally used poly- or monoclonal antibodies in immunoassays. Recombinant antibody fragments can be isolated *in vitro* from the antibody gene libraries displayed on bacteriophages and produced cost-efficiently in large scale in *E. coli* bacteria. Recombinant antibodies can also be further engineered to meet the requirements of a certain application, e.g., by improving their affinity, specificity or stability. Recombinant antibody technology also allows the development of anti-immune complex antibodies that enable otherwise unachievable sandwich type of assay for small molecules, such as mycotoxins. Previously, we have reported a non-competitive sandwich assay for HT-2 toxin in FRET format (wheat) [Arola, H. et al., 2016. Anal. Chem. 88: 2446] and simple ELISA (wheat, oats and barley) [Arola, H. et al., 2017. Toxins 9: 145] based on anti-immune complex antibodies. Here, we demonstrate microscale thermophoresis (MST) method for non-competitive HT-2 toxin measurement in beer. The technology is compared with ELISA and SPR in terms of sensitivity and tolerability for matrix effects. While the assay affinity (Kd value) in SPR method was 25 nM (10.5 ng/ml) in buffer, the similar value in MST method was 6 nM (2.5 ng/ml). The assay was applied to three different types of beer; lager, wheat beer and stout and Kd values of 8.9 nM (3.5 ng/ml), 4.5 nM (1.9 ng/ml) and 8.8 nM (3.7 ng/ml) were obtained, respectively. The primary results suggest that MST technology could be applied to mycotoxin determination from complex sample matrices.

P95
Extraction of mycotoxins from vegetal oils using natural deep eutectic solvents: a green alternative to conventional methods
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Food analysis techniques have improved significantly in recent years however, the use of organic solvents remains widespread for both extraction and purification processes. Many of them present risks to human and animal health and for the environment, in particular due to their volatility (e.g., acetonitrile, methanol, isopropanol, hexane). Therefore, their replacement by non-hazardous and biodegradable solvents has focused a great deal of attention in green chemistry. In particular, the extraction of mycotoxins from foodstuffs, avoiding the use of traditional solvents, requires a significant effort as these contaminants are found in very complex matrices and at very low concentration levels. Moreover, the molecular structure and physicochemical properties of these toxins are very heterogeneous, and the optimisation of an environmentally friendly methodology that allows their simultaneous and efficient extraction from different foodstuffs is a challenge. In this work we report the development of a simple, rapid, green and sustainable liquid-liquid microextraction method, based on the use of natural deep eutectic solvents (DES) [Lie, Y. *et al.*, 2018. J. Nat. Prod. 81: 679] and liquid chromatography with diode array and fluorescence detection (LC-DAD-FLD) for the analysis of four mycotoxins, namely deoxynivalenol, alternariol, ochratoxin A and zearalenone, in vegetable oils. The optimisation of the composition of the DES (choline chloride (ChCl) in various molar proportions, glucose, ethylene glycol, malonic acid or urea) as well as the extraction conditions has been carried out applying experimental design methodologies. Initially, a fractional factorial design (FFD) was used to explore the effects of several controllable factors on the mycotoxins' extraction efficiency including, water content (%) in the DES mixture, volume of extractant (µL), sample size (mg), extraction temperature (ºC) and time (min). The most significant factors were identified by ANOVA analysis and further optimised with the aid of a circumscribed central composition design (CCCD) and the desirability function. Under optimum conditions, recoveries of the target mycotoxins ranged between 100 and 108% (except for zearalenone,

36%) and the limits of quantification of the DES-LC-DAD-FLD method, without further clean-up or preconcentration, were always lower than the maximum residue limits established by Commission Regulation (EC) No 1881/2006 in food matrices, with precisions lower than 8%. The method has been validated in rice oil and other vegetable cooking oils according to the Commission Regulation (EC) No 401/2006 [Pradanas-González, F. *et al.*, manuscript in preparation]. **Acknowledgements**. This study was supported by the Ministry of Economy and Competitiveness (Ministerio de Ciencia, Innovación y Universidades, CTQ2015-69278-C2 and RTI2018-096410-B-C21).

P96

Lateral flow test to detect ergot alkaloids

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Ergot alkaloids are a range of compounds that are produced by the fungi of *Claviceps* genus on a wide variety of grains and are classed as mycotoxins. Their presence within food commodities has damaging and detrimental effects once consumed and therefore should be routinely analysed. As recommended by Commission Recommendation 2012/154/EU, the Reveal® Q+ MAX for ergot alkaloids kit has been developed to detect the six main ergot alkaloids and their epimer form, i.e., ergometrine/ergometrinine, ergosine/ergosinine, ergotamine/ergotaminine, ergocristine/ergocristinine, ergocornine/ergocorninine, and ergocryptine/ergocryptinine. Neogen has designed this fast lateral flow device to detect the presence of ergot alkaloids in grain from 50-5,000 ppb. The use of traditional organic solvents has been eliminated with Neogen's aqueous extraction procedure, resulting in a safer protocol which is environmentally friendly. The ergot alkaloids lateral flow device is a competitive lateral flow assay which uses a mix of bespoke antibodies coating gold colloid nanoparticles, along with the presence of additional antibodies and ergot proteins in the test and control lines to obtain a result. The use of control and test lines to determine the result insures the operator of each device's functionality. Results are returning in rapid time through the novel aqueous extraction of the ergots from ground grains of rye or wheat in under five minutes followed by a sample run time of eight minutes. Coupled with the use of Neogen's new Raptor® Integrated Analysis Platform, the test provides quantifiable results whilst removing operator subjectivity of scoring visual test lines.

P97

The effect of grind and extraction size on deoxynivalenol result variability

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Deoxynivalenol (DON) is a common problem in areas growing barley in North America. Each year levels fluctuate from low to high. One thing remains constant however, sample preparation for this commodity is critical for obtaining accurate DON results. This study evaluates sample preparation of barley containing DON. Sample preparation is a critical part of the total analytical process. The difference in sample grind size, as well as the amount of sample extracted contributes to the overall result variability. An evaluation was conducted to compare the extraction of barley naturally contaminated with DON utilising different sample grind and different sample extraction weights. The naturally contaminated barley was ground to various mesh sizes, homogenised and various sample sizes where extracted. The extractions were performed using acetonitrile/water (84/16) with a 1 h on Eberbach shaker. The extracts were then analysed by LC-MS/MS. Data presented shows the effect grind size and sample extraction size has on DON results variability.

P98

The effect of grind and extraction size on zearalenone result variability

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In the 2018 maize crop, zearalenone was found in several areas of maize growing regions in the USA. In many years, zearalenone is detected at low levels in areas of the USA, however, in 2018 the contamination levels were found to be higher than in typical years. These types of crop years highlight the importance of proper sample preparation for zearalenone in maize. Sample preparation of products being tested for Zearalenone is a critical part of the total analytical process. The difference in sample grind size, as well as the amount of sample extracted can also contribute to the overall result variability. An evaluation was conducted to compare the extraction of maize naturally contaminated with

zearalenone utilising different sample grind and different sample extraction weights. The naturally contaminated maize was ground to various mesh sizes, homogenised and various sample sizes where extracted. The extractions were performed using acetonitrile/water (84/16) with a 1 hour on Eberbach shaker. The extracts were then analysed by LC-MS/MS. Data presented shows the effect grind size and sample extraction size has on zearalenone result variability.

P99
Looking beyond the horizon
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In South America, scientific discussions on mycotoxins started in the late 90s. Little was known about mycotoxins. Technical training, dissemination activities, and awareness at the level of industry, government and academia were the focus from the beginning. The first mycotoxins selected for study were aflatoxin B1, B2, G1 and G2. As the years went by, aflatoxin M1, ochratoxin A, zearalenone, fumonisins B1 and B2, deoxynivalenol and patulin were incorporated. The critical mass of professionals devoted to the development of techniques, publications, participation in discussion activities, dissemination and projects grew every year. This path led to having national regulations. Uruguay was a pioneer in regulations including the mentioned mycotoxins in foods and feed, plus ergot alkaloids. Up to date, each country in South America has its own regulation or standard to follow. To comply with mandated limits, laboratories have needed to advance in technology from methods with TLC detection to HPLC with fluorescence detectors, UV detectors, and then DAD, arriving finally at UPLC-MS/MS. The evolution in analytical techniques was also accompanied by demands to demonstrate the quality assurance of analytical results. The increase in the requirements for the validation of methods to comply with ISO/IEC 17025:2017, drastically increased analytical costs. Samples of assigned value for the control of the batch of analysis, maintenance and updating of the system veracity and precision, calibrated equipment and evidence of the training of analysts is essential to report a result. In addition to ensure comparability amongst laboratories, standards of known purity, certified calibration standards, ISO/IEC 17043:2010 certified reference materials, are required and there seems to be an ever-growing demand for these. The metrological Institutes are participating in a project meet this demand. The CBKT project is designed to allow the International Bureau of Weights and Measures (BIPM) and National Metrology Institutes (NMIs) to work together to strengthen mycotoxin metrology infrastructure; provide knowledge transfer to scientists developing capabilities in this area; and enable NMIs to characterise selected pure mycotoxin materials, provide mycotoxin calibrants and matrix reference material and proficiency test materials to support mycotoxin testing laboratories within their countries. From South America, Argentina, Brazil, Colombia and Uruguay are partners. In the near horizon, it is intended that these reference materials will be produced and available in the region, with the purpose to ensure that analytical results are comparable and fit for purpose in supporting trade and safety of food, issues which are of concern for all countries.

P100
Simplifying multi-toxin testing using a single extract for five common mycotoxins
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Lateral flow has become an important first step in the defence against mycotoxin contamination in food and feed products. With increasing regulations and awareness of mycotoxins around the world one of the largest problems with lateral flow has become its ability to test multiple toxins on the same sample efficiently. Vicam’s unified Aqua Premix extraction procedures have solved this problem by allowing a single extract to test for aflatoxin, deoxynivalenol, fumonisin, ochratoxin, and zearalenone. The platform consists of devices that specifically target each of these five toxins. By ensuring that each device can work using the same simple extraction procedure it is possible to test for any one of these toxins or all of them by only performing one extraction. After extraction the operator must only transfer 100 microliters of their extract to each of the test devices of their choice. These streamlined procedures enable the operator to test for one, all or any combination of the five toxins listed above while performing only one extract on their sample. By allowing the operator to pick and choose which tests they wish to perform on a single extraction it is possible to save time, reduce cost and eliminate unnecessary waste that is associated with performing multiple extractions on a sample. With each test being developed to target a specific type of mycotoxin it should not be surprising that the performance of these tests is not impacted at all if there are multiple toxins in a sample. The tests themselves are designed to have no cross reactivity with the other toxins allowing for confidence in the results being reported. The goal of

this extraction procedure is to enable the end user to test for whichever toxin they are required to test for without complicating the process by which they test.

P101

Development of an UPLC-MS/MS method for the quantitative determination of six aflatoxins in different food matrices of animal origin

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Aflatoxins (Afs) are mycotoxins produced mainly by *Aspergillus flavus*, *A. parasiticus* and *A. nomius*, which frequently contaminate food and animal feeds, especially in (sub)tropical countries. Afs have been shown to be carcinogenic and immunosuppressive in mammals. The four major Afs are aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1), and G2 (AFG2). AFB1 and AFB2 can be transformed in the body by hydroxylation to aflatoxin M1 (AFM1) and M2 (AFM2), respectively. If animals consume contaminated feeds, Afs can be transferred as the parent components and/or metabolites to edible tissues and products, such as eggs and milk, which ultimately can reach the human food chain. Therefore, Afs pose a substantial health risk to humans consuming animal-derived products. Currently, the European Union has set a maximum level for AFM1 in milk (0.05 µg/kg; Commission Regulation (EU) No 165/2010). Dietary adsorbents, such as bentonite clay, have been used for reducing Afs exposure in animals and carry- over to edible tissues. To investigate the efficacy of adding a bentonite clay to animal diets in reducing the concentration of AFB1, AFB2, AFG1, AFG2 and their major metabolites AFM1 and AFM2 in animal-derived foods (chicken muscle and liver, chicken eggs and cattle milk), a selective and sensitive analytical method based on ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) has been developed. Chromatography was performed on an Acquity HSS-T3 column (100 x 2.1 mm i.d., dp: 1.8 µm) using water and methanol as mobile phase. A gradient elution was performed. $^{13}C_{17}$-AFB1 and $^{13}C_{17}$-AFM1 were used as internal standards. Sample preparation consisted of a liquid extraction using 1% formic acid in acetonitrile, followed by a further purification using QuEChERS (muscle tissue), Oasis® Ostro (egg), QuEChERS in combination with Oasis® Ostro (liver tissue) and Oasis® PriME HLB (milk). The UPLC-MS/MS method was validated in accordance with Commission Decision 2002/657/EC and the following parameters were evaluated: linearity, accuracy, within-day and between-day precision, limit of quantification, limit of detection, specificity and carry-over. Results will be presented. **Acknowledgements**. This research was conducted within the ERA-NET LEAP-Agri MycoSafe-South project and was funded by the Kenian Ministry of Education Science and Technology (MOEST), the Belgian F.R.S.-Fonds de la Recherche Scientifique and the Belgian Science Policy Office through the contract BL/02/LeapAgri 01.

P102

Beyond mycotoxins: pyrrolizidine alkaloids, where are we and where are we going?

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The European Commission has recently reiterated the interest and concern for pyrrolizidine alkaloids (PAs) in relation to the opinion of the European Food Safety Authority that has declared the possible risk in terms of public health and to the recent new reference point (237 µg/kg body weight per day).
At legislative level, not only PAs as contaminants are under discussion but also the specific matrices – above all food supplements– the reference MRLs and their expression. There is also interest and concern about the decisions that will soon be taken to limit the content of PAs where necessary. For this reason, the whole food chain is progressively intensifying the controls on PAs and needs to rely on sound methods, which are selective, sensitive and above all reliable. Mérieux NutriSciences has a multi-residual LC-MS/MS method for the determination of 31 PAs, widely validated on numerous matrices of which some results are given as examples. Despite the excellent performances (with respect to set goals), the challenge remains open for complex matrices such as dehydrated / dried plant extracts (typical of many food supplements), which are potential sources of matrix effect, interference and co-elution. To overcome this problem with a view to continuous improvement, Mérieux NutriSciences Research is working on a possible solution that would imply the reduction of PAs oxidised forms [Kowalczyk, Z. *et al.*, 2018. Food Anal. Methods 11: 1345; Kowalczyk, E. and Kwiatek, J., 2018. J. Vet. Res. 62: 183].. The results obtained so far have proved to be correct from the analytical point of view

and promising for costs reduction as well as for the overall complexity of the analysis and the global control over the contamination of PAs in food.

P103

European Union Reference Laboratory for mycotoxins & plant toxins in food and feed: uniform testing for safe food

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Wageningen Food Safety Research hosts the European Union Reference Laboratory (EURL) mycotoxins & plant toxins in food and feed since March 1, 2018. Tasks and activities of the EURL, as laid down in Regulation (EU) 2017/625, are to provide technical and scientific assistance on analysis to the National Reference Laboratories in the EU member states and to the European Commission. Legal limits on mycotoxins and plant toxins in food and feed in the EU are laid down in Commission Regulation (EC) No 1881/2006, Directive 2002/32/EC and Commission Recommendation 2006/576/EC, and their amendments. EU regulation on mycotoxins focusses on aflatoxins, deoxynivalenol, zearalenone, ochratoxin A, fumonisins and ergots in food and feed and patulin in food. Current legal limits refer to the inherent plant toxins erucic acid and tropane alkaloids in food, hydrocyanic acid in food and feed and free gossypol, theobromine, vinyl thiooxazolidone and volatile mustard oil in feed. Furthermore, the presence of seeds of several harmful botanicals are limited in feed. New or extended EU legislation is foreseen for the mycotoxins ergot alkaloids, and for the plant toxins pyrrolizidine alkaloids and tropane alkaloids. The work program of the EURL for mycotoxins & plant toxins will be discussed during the meeting. Background will be given on tasks and responsibilities of the EURL, how the work programme is designed, focus on compounds and which methods will be developed and extended. The results of two proficiency tests, on deoxynivalenol and related compounds and pyrrolizidine alkaloids, will be discussed in more detail.

P104

EURL proficiency test for DON, acetyl-DONs, and DON-3G in cereals

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Wageningen Food Safety Research is EU Reference Laboratory (EURL) for mycotoxins & plant toxins in food and feed. One task is to ensure comparability and reliability of national reference laboratories (NRLs) assigned by the EU member states. NRLs perform analyses to enforce legal limits and for risk assessment by national authorities and the European Food Safety Authority (EFSA). One of the tools for this is to organise proficiency tests (PT). A PT was organised in 2018 for the quantification of deoxynivalenol (DON), 3-acetyl-DON, 15-acetyl-DON, and DON-3-glucoside (DON-3G). Legal limits for DON are established in Commission Regulation (EC) No 1881/2006 and Directive 2002/32/EC. In addition, monitoring of the acetyl-DONs and DON-3G is recommended by EFSA since they contribute to the overall exposure of DON. A group-TDI of 1 µg/kg bw per day for the sum of the four DON forms has been established, and a group-ArfD of 8 µg/kg bw per eating occasion. Two food/feed materials, wheat and maize, were prepared, spiked with DON, 3-acetyl-DON and 15-acetyl-DON, and wheat also with DON-3G. Both materials were sufficiently homogeneous and stable during the course of the PT. Fifty laboratories from 29 countries participated (NRLs from all EU member states and Official Laboratories). Laboratories analysed the samples using their routine methods (using LC-MS/MS, with or without clean-up (two-third) or LC-UV with IAC clean-up). The assigned values, derived from the consensus of the submitted results, ranged from 35 to 750 µg/kg for the various mycotoxins. The proficiency of the participants was assessed through z-scores, calculated using the assigned value and a relative target standard deviation of 25%. All participants submitted results for DON and obtained satisfactory z-scores in almost all cases. Acetyl-DONs and DON-3G were covered by less than half and less than one third of the laboratories, respectively. The laboratories that reported results for these mycotoxins had adequate performance in ≥79%. Four false positives and two false negatives were reported, all related to 15-acetyl-DON. The quantitative performance of the participants was generally good, but extension of the scope is needed (and lower LOQs in some cases) to align with EFSA monitoring recommendations. In a relatively limited number of cases, a follow up is needed regarding questionable or unsatisfactory z-scores and false positive/false negative results. **Acknowledgements**. This project was financially supported by the Netherlands Ministry of Agriculture, Nature and Food Quality and the European Commission (DG SANTE).

P105
Influence of capsule shells on the analysis of citrinin in red yeast rice food supplements using LC-MS/MS
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Citrinin (CIT) is a nephrotoxic, hepatotoxic and cytotoxic mycotoxin produced by the fungi *Aspergillus*, *Penicillium* and *Monascus*. CIT is generally produced during storage and can therefore contaminate products, such as grains, beans, fruits, herbs, spices, etc. CIT can also be produced as a contaminant when rice is fermented with *Monascus* for the production of monacolin K. For this food supplement, described as red yeast rice (RYR), the EU has a legal limit of 2,000 µg/kg for CIT in RYR (Commission Regulation (EU) No 212/2014). RYR is present on the market as powder in a cellulose-based capsule or as oil suspension in a gelatinous capsule. Aim of this study was to estimate the influence of the capsule on the analytical method using LC-MS/MS for quantification of CIT in RYR. The matrix types (shell, content and whole capsules) were evaluated separately. Depending on the RYR food supplement, the sample shell contribution to the total weight of the whole capsules varied in the range of 15-22%. The shell typically contained less than 1% of the total content of CIT. Shells, content and whole capsules were analysed and the results showed that the shell did not significantly affect analytical performance of the method. Therefore, RYR food supplements can be analysed with shells. The method was validated and applied to 11 RYR food supplement samples, obtained from internet shops in the Netherlands. Two (~18%) samples contained CIT but far below the legal limit. **Acknowledgements**. This project was financially supported by the Netherlands Ministry of Agriculture, Nature and Food Quality and the European Commission (DG SANTE).

P106
Analysis of spices using IMMUNOPREP® online ochratoxin cartridges
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Ochratoxin A is produced by moulds of the genera *Aspergillus* and *Penicillium*. Such fungi thrive in a warm damp environment. Food and feed are susceptible to fungal contamination during growth, harvest and storage. Ochratoxin can be found in a wide range of food commodities including spices and coffee and European legislative levels are currently in place. In terms of analysis, immunoaffinity clean-up is already well established in official methods (AOAC International and CEN Standards) for use in the analysis of a diverse range of complex matrices for all the regulated mycotoxins, including aflatoxins and ochratoxin A. These immunoaffinity column methods have been rigorously validated and have been applied to a variety of spice samples as immunoaffinity clean-up is particularly effective in removing all pigments from the sample to allow accurate quantification by HPLC or LC-MS/MS. IMMUNOPREP® online automated affinity cartridges have been developed which offer the same benefits as immunoaffinity column clean-up. Automating analysis offers the added advantage of allowing large scale laboratories to meet increasing pressures with the fastest turn-around times. Sample preparation methods have been validated and tested for the analysis of difficult commodities such as spices and coffee for the automated determination of ochratoxin.

P107
Verification of multi-toxin immunoaffinity columns in determination of mycotoxins in animal feed and swine urine by UHPLC-MS/MS
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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 this study, a bile and urine samples were analysed in order to determine recoveries for legislated recoveries as well as a wide range of non-legislated mycotoxins. 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.

P108

Analysis of mycotoxins in cannabis and related products using multi-toxin immunoaffinity clean-up in conjunction with HPLC-FLD

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More than 40 years ago, a paper published in the scientific journal Mycopathologia showed that under favourable conditions, *Aspergillus flavus* and *A. parasiticus* could flourish and produce aflatoxins on marijuana. Uncured marijuana plant material or inadequately processed marijuana could offer the right conditions for fungal growth. There appears to have been little or no follow-up to these observations, probably as at that time they largely concerned the safety of an illegal substance. However, legalisation of consumption of cannabis products in Canada and also in several States of the USA, for medicinal purposes and in some cases recreationally now brings safety to the fore. Cannabis now needs to be scrutinised for residues and contaminants to the same extent as food or pharmaceutical products. This means applying the same safety standards for levels of mycotoxins as apply to foodstuffs and conducting routine monitoring to ensure standards are maintained for products placed on the market. Fortunately, in terms of analysis, immunoaffinity clean-up is already well established in official methods (AOAC International and CEN Standards) for use in the analysis of a diverse range of complex matrices for all the regulated mycotoxins including aflatoxins and ochratoxin A. These immunoaffinity column methods have been rigorously validated and have been applied to a variety of botanical products, such as herbal medicines, which have matrix similarities to marijuana. Now, recent work by R-Biopharm has demonstrated that multi-toxin immunoaffinity columns such as AO ZON PREP® provide excellent clean-up of marijuana samples when spiked and can be used with LC-fluorescence. The use of immunoaffinity columns results in excellent clean-up and better chromatography as well as having the added benefits of improving productivity and lowering overall analysis costs when compared to using single toxin immunoaffinity columns.

P109

Efficient automated analysis of mycotoxins in cannabis and related products using online immunoaffinity clean-up conjunction with HPLC-FLD

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More than 40 years ago a paper published in the scientific journal Mycopathologia showed that under favourable conditions, *Aspergillus flavus* and *A. parasiticus* could flourish and produce aflatoxins on marijuana. Uncured marijuana plant material or inadequately processed marijuana could offer the right conditions for fungal growth. There appears to have been little or no follow-up to these observations, probably as at that time they largely concerned the safety of an illegal substance. However, legalisation of consumption of cannabis products in Canada and also in several States of the USA, for medicinal purposes and in some cases recreationally now brings safety to the fore. Cannabis now needs to be scrutinised for residues and contaminants to the same extent as food or pharmaceutical products. This means applying the same safety standards for levels of mycotoxins as apply to foodstuffs and conducting routine monitoring to ensure standards are maintained for products placed on the market. Fortunately, in terms of analysis, immunoaffinity clean-up is already well established in official methods (AOAC International and CEN Standards) for use in the analysis of a diverse range of complex matrices for all the regulated mycotoxins including aflatoxins and ochratoxin A. These immunoaffinity column methods have been rigorously validated and have been applied to a variety of botanical products, such as herbal medicines, which have matrix similarities to marijuana. Now, recent work by R-Biopharm has demonstrated that AFLAPREP® and OCHRAPREP®, AFLAOCHRA PREP® columns provide excellent clean-up of marijuana samples when spiked and can be used with LC-fluorescence. In addition, IMMUNOPREP® ONLINE automated cartridges have been developed to allow large scale laboratories to meet increasing pressures to produce high quality results with the fastest turn-around times.

P110
A rapid and simple LC-MS/MS method for the quantification of the EU regulated mycotoxins in cereal-based products
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Mycotoxins are toxic secondary metabolites produced by various mould species found to infect a variety of agricultural commodities and processed food. Herein, we developed and validated a quantitative method for the analysis of aflatoxins B1, B2, G1 and G2, fumonisins B1 and B2, deoxynivalenol, toxins T-2 and HT-2, zearalenone, ochratoxin A and nivalenol in wheat grain flour, based on a high sensitivity LC-MS/MS instrument. The ultimate sensitivity and robustness of the Xevo TQ-XS allowed the extreme simplification of the sample treatment process, which consists in a rapid solvent extraction and dilution of the matrix without the need for time consuming pre-concentration or clean-up steps. Both external and internal standard calibration methods were evaluated as part of this study. All regression equations showed coefficients of determination (R^2) between 0.9941 and 1.0000, and percentage residuals lower than 20% across the full calibration range. Method LOQs were adopted as the lowest point of the linear ranges, which bracket the EU maximum permitted limits (MPLs). When using the internal standard method, percentage recoveries lie within the range 94-105%, whilst RSD% (n=7) were below 10% for all analytes. Thus, trueness and precision of the method were well within the criteria set by Commission Regulation (EC) No 1881/2006 and subsequent amendments. Matrix effects ranging from >30% signal suppression for nivalenol, to >1000% signal enhancement for ochratoxin A were encountered. The incorporation of ^{13}C-labelled internal standards within the analytical workflow leads to enhanced method performance and is therefore recommended as an efficient approach to correct for both matrix effects, and the inevitable analyte losses during the sample preparation. The method performance fulfils the EU regulations also when applied to oatmeal, and to a mixture of different flours (rice, potato, tapioca, maize and buckwheat), and it appears to be potentially transferable to different types of commodities commonly contaminated with mycotoxins, including dried spices.

P111
ST: SniffTox – system for the detection of mycotoxins with electronic nose technology
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Agri-food companies need to guarantee the safety of their matrices during the entire production chain. For this reason, it is important to rapidly check for the presence of mycotoxins in seeds, which are harmful to human and animal health. The start-up ATPr&d has developed the SniffTox (ST) service due to which it is possible to define a database useful for the detection of mycotoxins through the e(lectronic)-nose technology, in addition to the creation of an ICT high-tech system for sharing and integrating the results in the main business management systems (ERP, enterprise resources planning) and in the cloud. The ST service allows to drastically reduce the analysis time, obtaining results, with minimum sample pre-treatment. ST has been developed to detect multiple mycotoxins in maize. The sample taken from the probes is ground and placed inside a vial, closed by a rubberised stopper. After a few minutes, the cap is pierced by a needle connected to the tube of the instrument and ST starts analysing the sample. In 60 s, the olfactory profile detected by 10 MOS (metal oxide semiconductor) sensors is recorded. The ST algorithm (based on neural networks) processes the result in real time, comparing it with the database experimentally created and provided a result that identifies the type and quantity of the mycotoxins present in the sample. The ST instrument is portable and does not require laboratory solvents; therefore, it can be carried out directly by non-expert operators also outside the laboratories. The total execution time is only 15 min and the results of the analysis can be transmitted simultaneously to all the company functions involved: quality control, acceptance, storage and production. Finally, the costs are lower than all other rapid analysis methods present on the market nowadays. The development of the ST 1.0 service is under completion even if exhaustive evaluation will require a larger dataset to perform a validation procedure. Therefore, e-nose associated with a specific database and algorithm seem to be a promising rapid/screening method to detect contamination by mycotoxins in maize kernel stocks. Once the method has been validated, the final phase involves the installations and operational tests at client companies. The ST 2.0 service will focus on making the instrument even more portable, for its direct use in the field, connected to a dedicated app.

P112

Validation of the Reveal® Q+ for DON method for quantitative determination of deoxynivalenol in grains and grain products

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A Performance Tested Method^{SM} validation was conducted on Neogen's Reveal® Q+ for DON quantitative immunochromatographic test for determining deoxynivalenol contamination in grains. The results showed the test to be accurate and consistent, and the test has been accepted for PTM status and assigned PTM number 071901. The test's performance was validated on naturally contaminated maize and wheat samples. Across a reference level range of 0.5 to 34.5 ppm, mean recovery ranged from 90 to 104%. The limit of detection was calculated as 0.014 ppm in maize and 0.037 ppm in wheat, and the limit of quantitation was calculated as 0.042 ppm in maize and 0.11 ppm in wheat. Both commodities had a linearity R^2 value of 0.999. Spiked samples of eight additional grains were analysed from 0.5 to 30 ppm, with mean recovery ranging from 90 to 109%. Cross-reactivity tests showed no detection of or interference by other mycotoxins. All primary validation results were supported by independent laboratory testing, and consistency and stability studies showed consistent lot-to-lot performance across the test's 18-month expiry period.

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Measuring the sum – a novel screening method for ergot alkaloids in food

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Ergot alkaloids play a significant role in human history for more than 1000 years and are part of the most common contaminants of food and feed worldwide. Their high toxicity against humans and livestock even at low concentrations causes a high demand for quick and reliable analytics even though no European limits for ergot alkaloids have been determined yet. Currently the most common methods for the quantitation of the six major ergot alkaloids and their corresponding stereoisomers are HPLC based, using either fluorescence or mass spectrometric detection. Whereas these conventional detection methods measure each compound individually, a novel approach is to transfer all ergot alkaloids to one basic structure, which could be measured as a sum parameter. Since all ergots contain a lysergic acid amide moiety and a differing peptide component, cleaving the molecule into a simple lysergic acid derivative, which could be quantified via either HPLC-FLD or MS/MS, is intended. To clean up the cleavage reaction mixture molecularly imprinted polymers (MIPs) are a simple and effective way to separate the desired structure. Due to the selectivity of MIPs, an improved matrix separation is expected, which results in fewer interferences in the FLD and the possibility to measure samples with more complex matrices. When fully developed, the novel method could overcome some major drawbacks of the conventional detection methods. Higher throughput and the need for less well-trained personnel are just two advantages, that should lead to a quick and cheap quantitation of ergot alkaloids. First results of this project will be presented. **Acknowledgements**. Funded by the German ZIM program (Zentrales Innovationsprogramm Mittelstand) of the Federal Ministry for Economic Affairs and Energy.

P114

Investigation into the performance of a NIR equipment on the prediction of mycotoxins in silo-stored lots of maize

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The present study was aimed at evaluating the performance of a near infrared spectroscopy (NIR) equipment on the prediction of mycotoxins in silo-stored lots of maize. We analysed 240 samples from 4 silos, which were collected with the aid of a pneumatic probe using 2 sampling processes, A and B. In process A, three collective samples were taken (upper, middle and lower third of silo depth); in process B, only one sample composed of grains from the whole depth of the silo was obtained. Five points were collected from each silo: surface centre and centre of each surface quadrant (north, south, east and west). Analysis of aflatoxin B1 (AFB1), zearalenone (ZEN) and deoxynivalenol (DON) were performed by high performance liquid chromatography coupled to mass spectrometry (LC-MS/MS) using an Infinity 1200 series HPLC (Agilent, USA), coupled to a 5500 QTRAP mass spectrometer (Applied Biosystems, USA). Spectra were obtained via a NIR model XDS equipment (Foss, Denmark).

The spectrum of each sample was sent to the Pegasus Science Olimpo® platform to obtain the results of mycotoxicological prediction. Contamination of the positive samples analysed by LC-MS/MS was 5-26 µg/kg for AFB1 and 30-418 µg/kg for ZEN. For the NIR predictions, the contamination range of the positive samples was 5-17 µg/kg for AFB1 and 30-107 µg/kg for ZEN. The value of the samples analysed for DON was lower than the NIR quantification limit (QL) (QL=350 µg/kg). The Z-score of the results via NIR was calculated for the evaluation, taking the LC-MS/MS results as standard. Data were classified as satisfactory, questionable and unsatisfactory, being satisfactory in 81, 90 and 100% of the samples for AFB1, ZEA and DON, respectively. The average concentration of each silo for the analysis through LC-MS/MS and prediction via NIR were: silo 1 (AFB1, 1.8 and 3.4 µg/kg; ZEA, 17 and 34 µg/kg); silo 2 (AFB1, 1.3 and 4 µg/kg; ZEA, 23 and 25 µg/kg); silo 3 (AFB1, 6 and 7 µg/kg; ZEA, 46 and 57 µg/kg); and silo 4 (AFB1, 4 and 5 µg/kg; ZEA, 54 and 44 µg/kg). It may be concluded that the NIR methodology can be used as a practical, accurate, fast and non-destructive mycotoxicological monitoring tool for lots of maize stored in silos.

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Development of antibodies for the mycotoxin citreoviridin

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Citreoviridin (CTV) was discovered many years ago as one of the yellow rice toxins. It is produced by a variety of species, including *Penicillium citreonigrum*, *Aspergillus terreus*, and *Eupenicillium ochrosalmoneum*. It is known to cause acute toxicity in mice, rats, guinea pigs, and ducklings. One of the sites of action of CTV is mitochondrial ATPase. Intoxication with CTV is marked by ascending paralysis, disturbances of the central nervous system, and respiratory arrest. Consumption of CTV has been associated with acute cardiac beriberi, manifested as neurological symptoms and heart failure. Chronic exposure has been suggested to be a potential trigger of Keshan disease. While it is known primarily for its association with rice, CTV has also been found in maize and pecan nuts. There are very few reports of antibodies that have been developed against this toxin. We have developed and characterised two sensitive monoclonal antibodies for CTV, which were given the shortened designations '2-2' and '2-4'. In competitive antigen-immobilised enzyme-linked immunosorbent assays (CI-ELISAs) in buffer, the observed IC_{50}s were 11 and 18 ng/ml for 2-2 and 2-4, respectively. Both antibodies were relatively tolerant to the presence of methanol. When CTV standards were prepared in 20% methanol, the IC_{50}s increased only slightly, to 15 and 21 ng/ml, respectively. The antibodies give indications that they may also perform well at higher methanol concentrations, however methanol levels greater than 30% tended to precipitate the serum albumins added to promote antibody stability. Cross-reactivity with three compounds having structural similarity to portions of the CTV molecule was minimal. Results suggest that these antibodies will be sensitive enough to find application in immunoassays and biosensors for CTV in commodities, such as rice and maize. **Acknowledgements**. This work has been supported by the USDA-ARS project number 5010-42000-0049-00D and by the Health and Labour Sciences Research Grants (Research on Food Safety, H28-shokuhin-ippan-004) from the Ministry of Health, Labour and Welfare of Japan.

P116

Near infrared hyperspectral imaging for deoxynivalenol detection in wheat grain

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Selection of deoxynivalenol (DON) non-contaminated batches of wheat or, at least, batches contaminated below the legal EU limit for unprocessed cereals, still remains a challenge for the food and feed industries. Spectroscopic detection techniques are already widely used in food and feed industries for the determination of organic compounds, such as proteins, moisture, starch and pigments. The low concentration range for DON makes it very challenging for NIR quantitative analysis. In particular, Fourier Transform near- and mid-infrared (FT-NIR and FT-MIR) spectroscopy has been tentatively used for the detection of DON in wheat. Hyperspectral imaging (HSI) combined with spectroscopy represents a new non-destructive methodology. Its advantage is that it provides spectral information at each spatial pixel on a sample, thus it may have higher analytical potential. HSI-NIR has been already proposed by some authors for assessment of *Fusarium* damaged kernels percentage, and DON presence over given levels. Our aim was to standardise the methodology used for HSI-NIR image acquisition in whole wheat kernels in order to have a precise method to screen samples for DON

concentration above the legal EU limit. The impact of the pixel selection technique, intra-day repeatability, between-day repeatability, kernel orientation, and kernel location in the screening area was assessed. It was concluded that kernel orientation has an effect on reflectance spectra, but it is lower than the difference between DON contaminated and uncontaminated samples. Also, inter-day repeatability may affect the collected spectra, thus this would imply the need to scan reference samples along with test samples in every run set. Then, the pre-treated spectra from 24 samples (72 images) in the range <LOD to 2,682.8 µg/kg were analysed using PLS in order to test the possibility to quantify the level of DON. However, although the calibration model led to a R of 0.91, a SEP of 325.8 and a RMSEP of 323.3 µg/kg for a full-cross validation, an independent test set showed an R of 0.54, a SEP of 1143.9 and a RMSEP of 1665.3 µg/kg, which is above the legal EU limit, thus quantification seems initially unfeasible. Additionally, 150 samples were scanned, from which 75 were used as calibration set and the other 75 as the test set for discriminant analysis in order to discriminate samples below or above the legal limit (1,250 µg/kg). Results showed a 73.3% of correctly classified samples in the calibration set but the percentage decreased to 62.6 for the test samples. **Acknowledgements**. The authors are grateful to the University of Lleida (predoctoral grant), and to the Spanish Ministry of Science, Innovation and Universities (Project AGL2017-87755-R) for funding this work.

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A rapid SERS detection of ochratoxin A in wine

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Ochratoxin A (OTA) is a toxigenic molecule produced by fungi belonging to the genera *Penicillium* and *Aspergillus*. In humans, nephrotoxic, hepatotoxic, immunotoxic, and teratogenic effects can occur with exposure to OTA. Additionally, the International Agency for Research on Cancer classifies OTA in group 2B: possibly carcinogenic to humans [Heussner, A.H. and Bingle, L.E., 2015. Toxins 7: 4253]. A principal source of OTA exposure in humans comes from the consumption of wine, a derivative of grapes. Ochratoxigenic species have been found in soil and organic matter in temperate and tropical regions and have been shown to contaminate grapes during the growing period of berries [Ailsa, D. *et al.*, 2007. Int. J. Food Microbiol. 119: 84]. Different techniques are employed for the detection of OTA in food and beverages. Liquid chromatography-mass spectroscopy, high-performance liquid chromatography, and gas chromatography are most often used today. These techniques are time-consuming, expensive, and require trained personnel [Cigić, I.K. and Prosen, H., 2009. Int. J. Molec. Sci. 10: 62]. Surface-enhanced Raman spectroscopy (SERS), a variant of the Raman spectroscopy, has emerged as a high specificity and rapid method for determining structural information of solid samples and aqueous solutions using metallic nanosubstrates. SERS requires minimum sample preparation and is highly sensitive, therefore it is being considered as a powerful technique for food inspection [Yang, T. *et al.*, 2017. In: Reference Module in Food Science]. Currently, SERS has already been demonstrated as a good tool to detect other mycotoxins (aflatoxin, citrinin) [Lee, K. *et al.*, 2014. J. Agric. Food Chem. 62:4466; Singh, D. *et al.*, 2013. J. Hazard. Mater. 265C: 89]. The potential of SERS was investigated to develop an accelerated spectroscopic method as an alternative technique for ochratoxin A detection in wine. A simple two-step extraction protocol that involves a rapid organic extraction and simultaneous formation of a highly effective and innovative SERS substrate (Ag mirror) was applied. Strong Raman bands associated with ochratoxin A and changes in wine induced by ochratoxin A contamination were observed in different spectroscopic regions. The partial least square (PLS) models showed a higher predictive accuracy with stronger correlation coefficients (r=0.9906) and a higher sensitivity with lower limits of detection of 10 µg/kg. The proposed SERS method would be a more effective and efficient analytical tool, with a higher accuracy and lower constraints, for ochratoxin A analysis in wine compared to other existing spectroscopic and chromatographic methods.

P118

Novel strategies to ochratoxin A antibody generation and immunochemical analysis in wine and grape juice samples

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Ochratoxin A (OTA) is classified by the International Agency for Research on Cancer as potentially carcinogenic to humans (group 2B). This mycotoxin can be present in different foodstuffs such as coffee, rice, beer, and wine, and due to its high chemical stability, it remains unaltered during food processing. Consequently, the European Food Safety Authority established a tolerable weekly intake of 0.12 µg/kg

of body weight. Structurally, OTA is characterised by a phenylalanine and a dihydroisocoumarin moiety linked by an amide bond, and by a free carboxyl group. The analysis of OTA in foodstuffs is usually carried out by high-performance liquid chromatography and fluorescence detection, with a previous clean-up and concentration step using immunoaffinity columns. Alternatively, immunochemical assays are commercialised nowadays for OTA determination, such as microplate-based kits using the enzyme-linked immunosorbent assay (ELISA) or dipsticks using the lateral flow immunochromatography method. Due to the low molecular weight of OTA, protein bioconjugates are required for antibody generation and competitive assay development. During the past years, many papers have been published concerning antibody generation and immunoassay development for OTA analysis. However, in all those studies, bioconjugates of the mycotoxin were prepared by activation of the native carboxylate group for covalent linking to the amine groups of the protein. In the present study, a novel strategy was followed to prepare the immunising conjugates of OTA. Three functionalised OTA derivatives (haptens) in which the linker arm was located at alternative sites of the molecular framework were obtained by total synthesis; thus, different spatial orientations of the molecule could be studied. The antigenic capacity of the three haptens and OTA itself was compared by raising rabbit polyclonal antibodies. Then, a collection of high-affinity monoclonal antibodies to OTA were generated from mice immunised with the two best performing immunogens. Particularly, the so-called monoclonal OTAb#311 is very likely the best antibody produced so far in terms of specificity and affinity to OTA, showing an IC50 value of 0.07 nM. Competitive ELISA tests were optimised for the analysis of this mycotoxin in wine and grape juice samples. Good precision and accuracy values were obtained using OTA fortified matrices. Finally, analysis of a certified wine sample containing 0.5 µg/l OTA was used for validation of the developed immunoassay, showing a 90% recovery.

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Interlaboratory variability in quantitative determination of mycotoxins

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Quantitative determination of mycotoxins in food or feed samples by different laboratories inevitably results in variation of results, even when analysing portions of the same homogenised sample, and even when using the same or equivalent methods of analysis. In the field of mycotoxins, it is still common to use the (modified) Horwitz equation [Horwitz W. *et al.*, 1980. J. Assoc. Off. Anal. Chem. 63: 1344; Thompson, M., 2000. Analyst 125: 385] to predict the achievable inter-laboratory variability. In its modified form [2] the predicted inter-laboratory variability expressed as relative standard deviation (RSD_R) is 22% for levels ≤120 µg/kg, and then decreases with increasing levels (e.g., 18% at 500 µg/kg and 11% at 10,000 µg/kg). In part, method performance criteria for mycotoxins in Commission Regulation (EC) No 401/2006 have been based on the Horwitz approach. In this work, the actual inter-laboratory variability of quantitative determination of mycotoxins was investigated. This was done using the robust standard deviations as observed in proficiency tests (PTs) organised between 2013 and 2018. For this, results from PTs organised by the EURL for mycotoxins and by Fapas were used. Since PT data were used, analytical methods varied and included both LC-MS/MS and classical LC-UV-based methods, reflecting current routine practices. The data set contained more than 750 PT RSD_R-values from a wide variety of mycotoxin/matrix/level combinations. Mycotoxins included were mostly the regulated ones, matrices varied from wheat to black pepper and milk, and levels ranged from 0.03 to 10,000 µg/kg. An assessment was made to reveal any dependencies of the RSD_R on the concentration, the mycotoxin, or the matrix. In contrast to the Horwitz prediction, no relationship of the RSD_R with concentration was observed. In addition, there was also hardly any effect of the mycotoxin or the matrix. The median RSD_R was 22%, the 75th percentile 26%. Apparently, when suitable validated methods are used, and laboratories are experienced, similar method performances can be obtained irrespective the mycotoxin, matrix or concentration. Based on this outcome, it would make sense to set a fixed generic method performance criterion for the RSD_R of 25%, rather than the mycotoxins/concentration dependent RSD_Rs from current legislation. In line with this, for evaluation of PT results, the use of a fixed fit-for-purpose target standard deviation for proficiency assessment of 25% is proposed instead of the Horwitz-based values. **Acknowledgements**. This project was financially supported by the Netherlands Ministry of Agriculture, Nature and Food Quality and the European Commission (DG SANTE).

P120

A five-minute ELISA, using mycotoxin-free one standard and precalibrated curve for the quantification of mycotoxins

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The rapid quantification of mycotoxins (aflatoxins (Afs), deoxynivalenol (DON), zearalenone (ZEN), ochratoxin A (OTA), fumonisins, T-2/HT-2) in grains, without handling harmful mycotoxins, constitutes a challenge. It is also important to develop techniques, with high accuracy and repeatability, having also low LOD, LOQ and coefficient of variation (CV). Consequently, the use of a mycotoxin-free 5-minute ELISA system, using one mycotoxin-free standard and precalibrated curve could be an essential tool for the quantification of mycotoxins. The aim of this study was to evaluate the recovery levels of aflatoxin B1 (AFB1), Afs, DON, ZEN, fumonisins, OTA and T-2/ H-T2 in grains using a 5-minute ELISA with one mycotoxin-free standard after a single extraction. The levels of AFB1, Afs, DON, ZEN, OTA, fumonisins, T-2/HT-2 were determined using the One Standard 5-minute ELISAs (Prognosis Biotech SA) Bio-Shield One Standard B1 (B4948, B4948005), Total (B4348, B4348009), DON (B4548, B4548006), ZON (B4448, B4448005), Ochratoxin (B4248, B4248004), Fumonisin (B4748, B4748003), and T-2/HT-2 (B4848, B4848005), respectively. All methods were used with or without standards. A single extraction with methanol 70% was used. Mycotoxin-free samples were chosen and spiked at three levels (including the LOQ) with a mixed mycotoxin solution. Reference materials from Fapas were also analysed. The recovery and CV in all spiked samples and reference materials lied within acceptable range and were consistent either with or without the use of standards. Furthermore, the LOQ did not show significant difference between the paired techniques. The innovative mycotoxin-free 5-minute ELISA system, using one standard and precalibrated curve, gives acceptable recovery and CV. Using a single extraction, the rapid toxin-free methods are effortless, accurate and cost-effective, providing unique advantages in the quantification of mycotoxins.

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Validation of an innovative 5-minute lateral flow test for the quantification of ochratoxin A in wine samples

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In the analysis of wine, there are several ELISA tests that either detect, semi-quantify or quantify ochratoxin A (OTA) (EU limit, 2 μg/kg) with a total procedure time of 60-90 min. Regarding the lateral flow technology, the quantification of OTA in wine is quite limited with a procedure time of 10 min and most of the products available are only qualitative. Intensely coloured matrices, such as wine, require special pre-treatment, including organic solvents for extraction, laboratory equipment and scientific technicians. The aim of this work was to determine the OTA levels in spiked wine samples with a new, simple and innovative 5-minute lateral flow assay, comparing the recovery results with ELISA. A quantitative 5-minute lateral flow test (Symmetric Ochratoxin Wine, B6148, Lot B6148002) and an ELISA test (Bio-shield Ochratoxin Wine, B6196, Lot B6196004) from ProGnosis Biotech SA were used to determine the OTA levels in wine samples. Considering the physical and chemical properties of wine, both methods use a dilution normalization requiring no previous treatment. Twelve OTA-free wine samples originating from different varieties (Sauvignon Blanc, Riesling, Grüner Veltliner, Grenache Rouge, Zinfandel, Cabernet Sauvignon, Pinotage, Merlot, Crianza and the Greek Xynomavro, Agiorgitiko and Lagorthi) were chosen from the global market and were spiked with OTA. Quality control materials were also used, and the recovery of samples was calculated. Using the 5-minute symmetric lateral flow assay for spiked wine samples of different varieties or for quality control materials, the recovery and CV were identical with ELISAs. The level of 1.5 ppb of OTA was easy to be detected visually, making the assay suitable for a qualitative test, too. This innovative lateral flow device constitutes the most simple, rapid and accurate tool in the quantification of OTA in wine samples, providing results comparable to those of a quantitative ELISA.

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Quantification of all mycotoxins using symmetric lateral flow technology and one step multi-toxin aqueous extraction

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The use of state-of-the-art features for the quantification of all the major mycotoxins (aflatoxin B1, B2, G1, G2, deoxynivalenol (DON), zearalenone (ZEN), ochratoxin A (OTA), fumonisins, T-2/HT-2) in grains constitutes the biggest challenge in rapid mycotoxin analysis. In addition, an aqueous extraction solution could eliminate the adverse effects of organic solvents on users involved in this analysis. A lateral flow method that uses a multi-toxin aqueous extraction, with very high accuracy and repeatability, having also low LOD, LOQ and coefficient of variation (CV), is considered to be an essential tool in mycotoxin analysis. The aim of this study was to evaluate the recovery levels of total Aflatoxins, DON, ZEN, fumonisins, OTA and T-2/ H-T2 in maize and animal feed samples using one single aqueous extraction and lateral flow symmetric technology. The levels of all mycotoxins were determined by the 5-minute symmetric green lateral flow assays (Prognosis Biotech SA), using one single aqueous extraction. In detail, the levels of total aflatoxins, DON, ZEN, OTA, fumonisins, T-2/HT-2 were determined using the Symmetric Total Green 0-30/S3448/S3448004, Ochratoxin Green/S6048/S6048004, DON Green/ S4048/S4048008, ZON Green/S5048/S5048004, Fumonisin Green/S7048/S7048005, and T-2/HT-2 Green/ S8048/ S8048004, respectively. Different reference materials from Fapas were used, including all mycotoxins. The recovery levels and CV were acceptable. The recovery results were also in agreement with those of different Fapas multi-mycotoxin ring tests. For the first time, a single aqueous extraction can be used for all the mycotoxins providing unique advantages to the users in terms of cost and time-saving, while symmetric technology signifies the transition of lateral flow sticks from being a low-esteemed screening tool into a reliable confirmatory method.

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Validation of an innovative ultra-fast 5-minute ELISA for the quantification of mycotoxins

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The rapid quantification of all the major mycotoxins (aflatoxin B1, B2, G1, G2, deoxynivalenol (DON), zearalenone (ZEN), ochratoxin A (OTA), fumonisins, T-2/HT-2) in grains, using a single extract, constitutes a challenge. A time-saving assay with high accuracy and repeatability, having also low LOD, LOQ and coefficient of variation (CV), can be a valuable tool in mycotoxin analysis. Consequently, the use of a single extract in a 5-minute next generation ELISA is of utmost importance for the quantification of mycotoxins in grains. The aim of this study was to evaluate the recovery levels of aflatoxin B1 (AFB1), total aflatoxins (AFs), DOn, ZEN, fumonisins, OTA and T-2/ H-T2 in spiked samples and reference materials using a 5-minute ELISA with one single extract. The levels of AFB1, AFs, DON, ZEN, OTA, fumonisins, T-2/HT-2 were determined using the 5-minute ELISA (Prognosis Biotech SA) Bio-Shield B1 5/B5048/B5048004, Total 5/B5148/B5148005, DON 5/B5248 /B5248005, ZEN 5/B5348/B5348003, Ochratoxin 5/B5448/B5448003, Fumonisin 5/B5548/B5548004, and T2/H-T2 5/B5648/ B5648004, respectively. A single extraction was used with methanol 70% and the same extracts were used in all 5-minute methods. Mycotoxin-free samples were chosen and spiked with a mixed mycotoxin solution. Reference materials were also analysed. The determination of the mycotoxin levels in the spiked samples showed that the recovery levels were acceptable. The results were also confirmed by analysing the reference materials. The CV of all samples was also within acceptable range. The innovative 5-minute Bio-Shield ELISA technology gives acceptable recovery and CV levels using a single extraction. The ultra-fast ELISA methods are effortless and accurate providing unique advantages in terms of time-saving.

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Mimotope-based immunoassays for mycotoxin detection

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The exceptional ability of epitope-mimicking peptides, or mimotopes, to imitate the epitope of an antigen and thus bind to same antibody paratope, presents an intriguing alternative to overcome some of the limitations of competitive immunoassays. As they bind to the same antibody paratope as the antigen and elicit a similar antibody response, epitope mimics can be used as the competitor instead of the labelled antigen in applications where the conjugation of the target to a carrier molecule is challenging, or it can cause toxicity to the user. In this work, we present the development of mimotopes and various mimotope-based immunoassays for the detection of fumonisins and zearalenone, mycotoxins commonly found as natural contaminants in maize and other foodstuffs. Mimotopes were selected from phage-displayed peptide library, and after identifying the target specific clones for both mycotoxins, their epitope-mimicking nature was demonstrated in phage-based competitive immunoassays. As an alternative for the phage-borne peptide, recombinant fusion proteins, as well as the synthetic counterpart of the peptides, have been later developed and applied to mycotoxin detection. The development of microarray-based immunoassay [Peltomaa, R. *et al.*, 2017. Anal. Chem. 89: 6216] and magnetic bead-based assay for fumonisins detection using the synthetic peptide with a biotin linker provided good sensitivity with detection limits of 11.1 and 0.029 ng/ml, respectively. On the other hand, we have constructed recombinant fusion proteins consisting of the mimotopes with fluorescent or bioluminescent proteins which could be directly used as the tracer in the immunoassay without the need of secondary antibodies or further labelling. Heterogeneous immunoassays using the mimotopes tagged with a yellow fluorescent protein (YFP) or *Gaussia* luciferase resulted in good analytical sensitivities in a rapid and simple assay concept. Furthermore, the YFP-tagged fumonisins mimotope was used to develop a homogeneous fluorescence quenching immunoassay based on gold nanoparticles (AuNPs). The approach demonstrated high sensitivity for fumonisin detection in only 20 min with a detection limit of 1.1 ng/ml, and negligible matrix effect in 5% wheat extract [Peltomaa, R. *et al.*, 2018. ACS Nano 12: 11333]. **Acknowledgements**. This study was supported by the Ministry of Economy and Competitiveness (Ministerio de Ciencia, Innovación y Universidades, CTQ2015-69278-C2 and RTI2018-096410-B-C21). R.P. acknowledges UCM for a predoctoral grant.

P125

Nanoparticle-based mycotoxin detection in food and agricultural products

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Food and crop contamination with mycotoxins are a severe health risk for consumers and cause high economic losses worldwide. Currently, different methods can be used to detect mycotoxins, such as aflatoxins, ochratoxins, deoxynivalenol and others within different sample matrices. Recently, the interest in multiplex detection of several mycotoxins in one sample has strongly increased since synergistic effects of different mycotoxins were discovered. Developments in chromatographic methods as liquid chromatography in combination with tandem mass spectrometry (LC-MS/MS) opens a new field in simultaneous mycotoxin detection in one single run. However, such laboratory-based analytical approaches are time-consuming and expensive. Rapid immuno-based methods as, e.g., enzyme-linked immunosorbent assays (ELISA) or especially lateral flow devices (LFD) seem to be an alternative for routinely sample testing. Although ELISA-based quantification shows a high sensitivity as well as a high sample throughput, a laboratory- based procedure with the required equipment is not suitable for fast on-field screening. LFA-based detection of toxins has the great advantage regarding easy handling and fast on-site testing. However, those assays typically have a lower sensitivity making it difficult to detect contaminations in the lower range close to regulatory limit. Furthermore, quantitative measurements are not possible in most cases. Here, we describe, a novel, portable and high-sensitive method based on a competitive magnetic immunodetection (cMID) approach. For this, monoclonal antibodies against various mycotoxins were coupled to superparamagnetic nanoparticles. These nanoparticles were then added to samples containing mycotoxins at different concentrations where they specifically bound to the mycotoxin molecules. Subsequently, a magnetic separation step was employed to separate and

concentrate the nanoparticles together with the captured mycotoxin molecules as well as removing sample debris. Afterwards, these nanoparticles-mycotoxin aggregates were applied onto special immunofiltration columns containing a matrix coated with a mycotoxin conjugate. By flushing the nanoparticles through the immunofiltration column by gravity flow, particles enrich inside the matrix due to a competitive binding reaction depending on the amount of pre-captured mycotoxins. Enriched magnetic particles were then detected by means of magnetic frequency mixing technology, using a low- and a high-frequency magnetic excitation field. Based on calibration measurements, the detected signal can be attributed to the amount of captured mycotoxin enabling a fast, high-sensitive and quantitative detection comparable to laboratory-based methods. Based on our findings it can be concluded that the competitive magnetic immunodetection is a powerful analytic tool to reduce the risk of processing contaminated food and agricultural products.

P126

Application of an enzyme-linked immunosorbent assay to the detection of all prevalent twelve ergot alkaloids in cereals and cereal based animal feed proposed for European Commission regulation

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The determination of ergot alkaloids in cereals and feed is important for food and feed safety as they are undesirable contaminants and can cause adverse health effects in humans and animals. The European Commission (EC) is considering implementation of maximum levels for a total of twelve main ergot alkaloids in cereal grains for human consumption. Recently, the draft of the proposed legislation with the proposed matrices was released. In this context, the availability of screening methods allowing the detection of ergot alkaloids from proposed cereal grains and their corresponding milling products is relevant. Aiming to the detection of the twelve ergot alkaloids (ergotamine, ergocristine, ergocryptine, ergocornine, ergometrine, ergosine and their inine forms) in cereal grains, cereal milling product and cereal-based feed, as proposed by the EC, this study reports the analytical performance of an enzyme-linked immunosorbent assay (ELISA) for the detection of these ergot alkaloids in the proposed matrices. The competitive ELISA was validated according to Commission Regulation (EU) No 519/2014 for proposed in draft legislation matrices. Cereal grains, cereal milling products and feed samples were extracted by a single generic solid/liquid extraction. Screening results were semi-quantitative. The ELISA presented limits of detection (LODs) ≤50ppb for the proposed ergot alkaloids in rye, oat, wheat, spelt, cereal-based feed. The overall intra-assay precision, expressed as CV (%), was <10%. The assessment of cereal and cereal based feed samples (n=20) with this ELISA and LC-MS or HPLC-MS/MS showed an overall correlation of 96% for positive naturally contaminated samples (n=11). The correct assessment was also obtained for remaining samples (n=9), which screened ≤29 ppb on ELISA and read below confirmatory method LOD (either 24 or 36 ppb). The analysis of naturally contaminated rye flour samples through Fapas proficiency testing scheme showed correlation of 95% between total ergot alkaloids assigned concentration and the ELISA. In conclusion, the data indicate applicability of the reported ELISA to the detection of twelve predominant ergot alkaloids in cereal grains, milling products and cereal-based feed. This application facilitates the testing process and allows the detection of the proposed ergot alkaloids in the proposed matrices considered by the EC.

P127

Innovative multiplex technology, challenges and the future of mycotoxin determination for predominant, masked and emerging mycotoxins

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The continuous development of knowledge and availability of the most up-to-date risk assessments report on co-exposure, effect of low-level cocktails, metabolites and conjugated forms of predominant mycotoxins is of pivotal importance for both the human and animal food chains. The need for testing the most predominant toxins is well described extensively in scientific reports and implemented by regulatory authorities globally. Maximum residue limits and/or guidance levels are well established for aflatoxins, fumonisin, deoxynivalenol, zearalenone, ochratoxin and T-2/HT-2 toxins across various global jurisdictions. Information about unregulated mycotoxins, called emerging toxins, which includes ergot alkaloids have a potential to cause serious health concerns to humans and animals. Various well-established strategies ensuring the safety and security of the food and feed supply chain are currently being used globally. Depending on the matrix and testing throughput required, simple, fast qualitative screening tests or highly sensitive, specific and precise quantitative analyses are utilised. Generally, commercial technologies available to the industry lack the capacity for multiplex detection, which

increases the screening capacity and allows the detection of multi-mycotoxin contamination per sample. The trend of increasing number of scientific reports and growing health risks needs to be swiftly followed by the new, integrated strategies for prevention and control of mycotoxin contamination of raw materials and finished food and feed products. An innovative multi-mycotoxin multiplex screening approach, using biochip array technology, applicable to the food and feed industry, research and regulatory authorities will be presented. With this application, after a single step extraction, the feed sample is applied to the biochip which contains multiple discrete test sites allowing for a multi-mycotoxin comprehensive screening from a single sample. The use of the dedicated biochip analyser Evidence Investigator enables the analysis of up to 54 biochips at a time. This easy to use technology has been evaluated by multiple multi-mycotoxin contaminated certified reference materials and proficiency tests and showed optimal analytical performance proving functionality of the technology. Further validated performance data of the multi-mycotoxin array has been peer reviewed and published [Plotan, M. *et al.*, 2016. J. AOAC Int. 99: 878]. An overview of the challenges for both well-established and emerging mycotoxins determination will be discussed highlighting the issues experienced by science authorities, screening test manufacturers, confirmatory test providers, raw material suppliers, food/feed manufacturers and regulatory authorities. The future of mycotoxin control lies in continued productive collaboration between academia, all establishments involved in the safe production of food and feed and the regulatory authorities as well.

P128

The future lab – towards fully automated mycotoxin analysis

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One of the challenges in mycotoxin analysis is the comprehensive manual sample preparation. Both rapid on-site and reference laboratory methods need trained personal to carry out time-consuming sample extraction, clean-up and measurement. Within the last years, more and more automation solutions have been developed. First, partially automated systems focused on the integration of laborious clean-up steps into measurement systems. Examples are combined devices for aflatoxin or ochratoxin A detection by on-line immunoaffinity clean-up – HPLC-FD or comprehensive chromatographic solutions by online-SPE or 2D-LC. However, the trend is towards fully-automated systems. For laboratory reference methods, a food and feed sample preparation robot was set up at Eurofins in Hamburg. Three robotic arms handle up to 400 samples per day. From spiking with standards, over extraction, salt dispensing, centrifugation, phase separation, and filtration to filling the measurement solution in a vial and labelling it, every step is fully automatically carried out. Only remaining manual steps are placing the samples on the entrance conveyer belt and taking out the ready prepared measurement vials. Comprehensive software enables to run multiple different methods in parallel. Even matrix-dependent individual sample treatment and thus, new approaches to design methods at the same time as robust and as cost-efficient as possible become feasible. Whereas the robotic lab measures several square meters, automation of on-site rapid test bases upon miniaturisation. Microfluidic cartridges were developed for automated mycotoxin control in the grain industry. The disposable lab-on-a-chip system includes an extraction vessel, dilution and detection chambers. Up to four mycotoxins can simultaneously be detected by means of fluorescent immunoassays. All required solvents and buffers are stored on the chip. A small spectroscopic device can run the chip and measure the result within ~10 min. For future labs, the described automation solutions and development of new sensor systems will pave the way for mycotoxin results at the push of a button. **Acknowledgements**. This work was supported by the Federal Ministry of Education and Research of Germany (BmBF) within KOMBISPEC (funding code 13N13943).

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Quantitative determination of emerging mycotoxins in biological matrices

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Beauvericin (BEA) and enniatins (enniatin A, A1, B and B1, ENNs) are mycotoxins produced by *Fusarium* spp. (mainly BEA, *F. proliferatum, F. subglutinans*, *F. verticillioides* or *F. oxysporum*; ENNs, *F. avenaceum*, *F. oxysporum*, *F. poae* or *F. tricinctum*). Those compounds are cyclic hexadepsipeptides that occur frequently in cereal grains and cereal-based products. BEA and ENNs are often considered as emerging mycotoxins, since no regulatory limits are applied. They can lead to cytotoxic effects, an increase of oxidative stress and cause cell apoptosis [Gruber-Dorninger *et al.*, 2016. J. Agric. Food Chem. 65: 7052]. In order to study the exposure of animals to BEA and ENNs an analytical procedure

for the simultaneous determination of ENNs and BEA in porcine liver tissue has been developed. The method is based on a solid-liquid extraction of tissue samples. The extracts have been analysed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) equipped with an electrospray ionisation (ESI) interface on a QTrap 6500+ (AB Sciex). The positive ionisation mode was used. Chromatographic separation of all 5 analytes was achieved on a Zorbax Eclipse Plus C18 column (RRHD 1.8 µm, Agilent Technologies) within 1 min. The method was established and validated in-house by spiking different concentration levels of the compounds of interest in blank pig liver tissue samples. Satisfactory results were obtained in regard to the method performance parameters specificity, linearity, accuracy and precision.

P130

Mycotoxins determination by LC-MS/MS in beans, maize and soybeans stored under controlled atmospheric conditions

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This study aimed to develop and validate analytical methods for the determination of mycotoxins in regular beans, maize and soybeans and later apply them to assess mycotoxins occurrence in samples stored in controlled atmospheric conditions. Extraction of the mycotoxins was performed with the QuEChERS approach, which was optimised specifically for each commodity in order to provide recoveries in the range of 70 to 120% and RSD ≤ 20%. After optimisation, the methods were validated for regular beans and soybeans (validation for maize was already done before). Amounts of 5 g ground samples were extracted with 10 ml of acetonitrile containing 1% formic acid for regular beans and maize, and 5% formic acid for soybeans. To remove water and to induce phase partitioning, 5 g $MgSO_4$ were added. Before injection into the LC-MS/MS system, the extracts were diluted 2-fold with methanol directly into the vials. The commodities were subject to 15 different treatments in controlled atmospheric conditions for four cultivars of regular beans (Fepagro 26, Garapiá, Cowpea white and Red Cowpea), four cultivars of soybeans (Brasmax Ultra IPRO, Brasmax Desafio RR, Brasmax Bonus IPRO and Tec IRGA 6070), and 6 different treatments for maize. The treatments differed in storage temperature (20, 25 and 30ºC) and in atmospheric conditions (O_2 and CO_2 concentrations). Mycotoxins were not detected in white and red cultivars of Cowpea beans and in soybean cultivars Brasmax Desafio RR and Brasmax Ultra IPRO. Otherwise, aflatoxin B1 (AFB1) was detected in regular beans and soybean cultivars. Mycotoxins were also not detected in the cultivars of the beans Fepagro 26 and Garapiá, when stored at temperatures ≤ 20 ºC. For the soybean cultivars Brasmax Bonus IPRO and Tec IRGA, AFB1 was detected in all studied treatments, indicating that the storage conditions were not effective to prevent the contamination of the samples. Mycotoxins AFB1, fumonisins B1 and B2 were detected in maize samples, but fumonisin B1 was not detected in stored maize with a moisture content of 14% at 30ºC in ambient atmosphere.

P131

Validation of LC-MS/MS based multi-toxin methods – a suggestion for reducing the workload and focusing on the data that are essential

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Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) has become the most intensively used instrumental technique for the quantitative determination of mycotoxins in food and feed samples. However, there is a lack of official guidance for determination of method performance parameters that focus on matrix effects and that at same time scrutinise the applicability of more general guidance documents to multi-target analysis. For instance, the criterion given in SANTE demands matrix matching if the response in matrix is decreased or increased by more than 20% compared to the solvent standard, but it is not clear whether extreme suppression by, e.g., a factor of 5 or even 10 is still accepted provided it is properly compensated. In addition, it might not be absolute but rather relative matrix effects that are the main limitation for the performance of an LC-MS based multi-method, particularly as they cannot be compensated by matrix-matched calibration. It is emphasised that the workload associated with the validation of such a method requires to find approaches to reduce the analytical burden by, e.g.,

pooling matrices for validation. A particular challenge in connection with a method covering several hundreds of analytes is the consumption of time for evaluation of raw data, which is particularly true for concentrations near the LOQ, which requires manual inspection of the chromatograms. Based on method performance data we have obtained for 550 secondary metabolites in seven different matrices, we have identified validation experiments which can be skipped in order to significantly reduce the workload required for in-house validation and revalidation upon transfer to a new matrix.

P132
Verification of the performance of two ELISA test kits for aflatoxin M1 in milk and dairy products
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I'screen AFLA M1 and I'screen AFLA M1 milk ELISA kits have been object of several published studies in the last 15 years, demonstrating accuracy and robustness, both in skimmed and unskimmed milk testing, and therefore they can be considered a reliable screening method for control bodies and dairy industries. In this poster, an overall overview of the kits performances during the years is presented, adding the very latest results obtained for batches produced in different sites. Specificity and sensitivity on raw milk samples were compared among batches produced from 2005 till today, showing high reproducibility of results. Trueness in the analysis of control materials in different batches is compliant and comparable, pointing out high lot-to-lot consistency with mean recoveries ranging from 90% to 100%. External quality assessment during a period of 10 years by Tecna participation to proficiency tests organised by Test Veritas, Fapas and AIA, shows 100% of <1.21 z-scores. In 2017, 19 participants to the proficiency test Progetto Trieste (Test Veritas), all using Tecna kits, obtained z-score values between -1.11 and +1.14, while the other 21 participants, using other screening methods, had z-score values between -3.22 and +2.23.

P133
Reliable quantitative determination of aflatoxin B1 in several matrices by rapid classic and master curve calibrated ELISA kits
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Screening of mycotoxins by ELISA kits is a widespread practice thanks to their reliability, sensitivity, precision and little time required for testing. These features are not always accompanied by consistent cost effectiveness, because a calibration curve has to be run in each experiment even when analysing a limited number of samples. The comparison between the performances of Celer Afla B1 and the corresponding B ZERO Afla B1 ELISA kit for the determination of aflatoxin B1, denies the belief that accuracy can be achieved only running calibration solutions in the same analytical session. A large number of matrices were tested for aflatoxin B1 using these two kits, that differ from each other for running a complete calibration curve in the assay (Celer Afla B1) or running only the zero standard (B ZERO Afla B1). In this last case, B/B0 value of the samples are interpolated on a virtual master curve, whose B/B0 values are provided in the kit certificate. Results obtained in several analytical sessions run with extracts from maize, maize germ, high moisture maize, silage, sorghum, soy, soybean meal, feed, DDGS, hazelnuts, almonds, peanuts, pistachio, dried fruits, indicate that accuracy and precision are equivalent in the two kind of assays, thanks to stability of the reagents and assay robustness. The Master Curve approach gives therefore a reliable and cost-effective tool for screening analysis when the number of samples is medium to low.

P134
Determination of fumonisins B1 and B2 in maize via Fourier Transform near infrared spectroscopy
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Given the global importance of maize, the study of novel techniques to efficiently monitor the mycotoxicological parameters to determine the quality of the grain in a fast and reliable manner is mandatory. This work aimed at developing a speedy and trustworthy alternative methodology to analyse fumonisins B1 and B2 (FB1 and FB2) in maize via Fourier Transform near infrared spectroscopy (FT-NIR). Fumonisins are secondary metabolites produced by fungi of the genus *Fusarium* which are highly prevalent in maize. Such ingredient is one of the major constituents of feeds, and the presence of these

toxic substances causes a diversity of negative effects on the animals. Firstly, the samples used to create the calibration model were chosen; all levels of contamination routinely found in the field were included. The technique of diffuse reflectance was applied, and the spectral data were correlated with the values of FB1 and FB2 through the partial least squares regression method. Data obtained through chemical analyses based on liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) were used as reference to build the calibration model, as well as the infrared spectra with different mathematical pre-treatments. Four hundred and thirty samples of ground maize of different origins and varieties were utilised. Then, 50 samples which did not belong to the database were used for external validation. The models were evaluated separately, one for FB1 and another for FB2. The best models were found with pre-treatment using Karl Norris gap derivative, with 2nd derivation and gap size 4. The calibration results for FB1 and FB2 were, respectively: correlation coefficient (r_{cal}), 0.98 and 0.97; coefficient of determination (r^2_{cal}), 0.97 and 0.94; root mean square error of prediction ($RMSEP_{cal}$), 431 and 239; and residual prediction deviation (RPD_{cal}), 5.9 and 3.3. The prediction samples were compared with the reference method and the Student's t-test was applied; no statistical difference was found between the groups (P=0.835), thus indicating a satisfactory predictive ability and confirming the potential of FT-NIR to predict fumonisins in maize. Thus, this work provides an alternative methodology for the analyses of fumonisins, which can deliver reliable results that may be feasibly applied in the field.

P135

Lateral flow-based method – fit for screening feed ingredients for aflatoxin?

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Lateral flow mycotoxin testing is increasingly used in the quality control of raw materials and feed. Like every test method, lateral flow tests can generate false-negative or false-positive results. The rate of the false-negative and false-positive results determines whether the use of the test is cost-effective. To determine the rate of false-negative and false-positive results the European Union has set down specific performance requirements for quantitative screening tests, such as the lateral flow method. In our study, we have validated Mycomaster, a lateral flow-based method, for testing aflatoxins in animal and feed ingredients following the protocol described in Commission Regulation (EU) No 519/2014. The performance of the test was evaluated using natural contaminated maize at screening target concentrations (STC) close to EU maximum permitted levels in feed materials. Twenty maize-samples were tested on five different days. The cut-off value was determined and additionally the percentage false-negative and false-positive results. Additionally, we tested the robustness of the test by setting up a ring test based on ISO/IEC 17043:2010. For this ring test, natural aflatoxin contaminated maize samples were sent out to 28 participants in Europe, Asia and America. Eight participants were using Mycomaster. The performance of the participating laboratories was evaluated comparing the obtained analysis results with the assigned value and the performance criteria for confirmatory methods described in Commission Regulation (EU) No 519/2014. All participants using Mycomaster were generating results within acceptable range, as they met the performance criteria for aflatoxins. The average aflatoxin concentration was 19 µg/kg, with a standard deviation of 4 µg/kg.

P136

Matrix effect in multi-mycotoxin analysis of samples from button mushroom (*Agaricus bisporus*) cultivation

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Mycotoxins may appear in complex matrices of button mushroom (*Agaricus bisporus*) cultivation [Branà, M.T. *et al.*, 2017. PloS ONE 12: e0182574]. Nutrition content of compost is excellent for different microorganisms that could transform mycotoxins via metabolism defending the growing mushroom from mycotoxin absorption. For the purpose of multi-mycotoxin determination, liquid chromatography (HPLC or UHPLC) coupled to mass spectrometry (MS/MS) is the method of choice. The sample extraction must be well chosen in order to extract wide range of mycotoxins and their metabolites also decrease the co-eluting interfering compounds (Malachová, A. *et al.*, 2014. J. Chromatogr. A 1362: 145). In our study, we evaluated the applicability of 'dilute and shoot' and modified citrate-QuEChERS extraction techniques for the multi-mycotoxin determination of samples from mushroom cultivation origin, paying special attention to matrix effects and their correction possibilities. The recovery efficiency (RE) of LB medium, button mushroom and compost was ≥60% for 13 investigated mycotoxins except for DON-3G. From the wheat straw, T-2 toxin, 3-acetyl-DON, 15-acetyl-DON and DAS mycotoxins could be extracted

with low efficiency (≤60%) by both extraction methods. The result of cluster analysis shows that the neat solvent (MeCN), LB medium and wheat straw matrices form well-separated clusters with respect of RE, referring to significant impact of these matrices on extractability (Fisher's exact test, $P<0.05$). In our study, the four matrices had a suppressive effect on ionisation in almost all target mycotoxins, fumonisin B1 meant to be an exception. We also investigated isotopic labelled native mycotoxin standards spiked after extraction as an internal standard, as a possible compensation of matrix effect regardless of native as well as metabolite mycotoxins. We compared the effect of different matrices for the signal of ^{13}C15 DON to DON metabolites, such as DOM-1, DON-3G, 3-acetyl-DON, 15-acetyl-DON and ^{13}C24 T-2 to T2 metabolites, such as HT-2 and T2-triol. The Student's pared T-test showed no significant differences in matrix effects between ^{13}C-labelled parent toxins and their metabolites ($P<0.05$).

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