

ABSTRACTS OF LECTURES & POSTERS

THE
World
Mycotoxin
Forum[®]
15TH
CONFERENCE

WMF
meets
Salzburg

7-9 APRIL 2025
SALZBURG • AUSTRIA

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CONTENTS

Committees		2
Welcome to WMFmeetsSalzburg		3
Social events		4-6
Welcome reception		4
Wine & cheese tasting		5
Conference dinner		6
Programme		7-22
Programme at a glance		7
Conference programme		8-20
Workshop programme		21
Young Scientist Forum		22
Company profiles		23-28
Abstracts of lectures		29-86
Monday 7 April 2025		
Plenary session	The role of digitalization and AI in mycotoxin research and management	30-32
Plenary session	Interactive debate – AI in mycotoxin research and management: Game changer or just hype?	33
Plenary session	Building resilience in regional food systems	34-38
Plenary session	Speed presentations and company pitches	39
Tuesday 8 April 2025		
Session 1	Mycotoxins in One Health perspective – Part 1	40-45
Session 2	Mycotoxin management and risk mitigation: Current status, future opportunities	46-51
Session 3	Mycotoxins in One Health perspective – Part 2	52-56
Session 4	Mycotoxin control at farm level: Towards precision agriculture	57-60
Session 5	Drivers affecting exposure to mycotoxins: What's up, Doc?	61-63
Session 6	Mycotoxin occurrence and control: The focus on fungi	64-67
Session 7	Societal relevance and impact of mycotoxin research	68-70
Session 8	Mycotoxin detection: On-site agri-food applications	71-73
Wednesday 9 April 2025		
Session 9	Late-breaking mycotoxin research	74-77
Session 10	Smart approaches for mycotoxin analysis	78-82
Plenary session	Building a resilient food system in the digital decade - Challenges for mycotoxin research and management	83-86
Abstracts of posters		87-163
Index		88-100
Mycotoxin occurrence and fungal characterization		101-116
Mycotoxins in One Health perspective		117-124
Managing and mitigating mycotoxin risks		124-150
Sampling and analysis		150-163

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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Prof. Chris Elliott

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Queen's University Belfast, UK

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Prof. Chiara Dall'Asta
Dr Sheryl Tittlemier

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University of Parma, Italy
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Mars Global Food Safety Center, China

About The World Mycotoxin Forum®

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

...for a sustainable, safe, and inclusive food future!

WELCOME TO WMF*meets*SALZBURG

The World Mycotoxin Forum® is the leading international meeting series on mycotoxins dedicated to assembling the world's best minds across the spectrum of integrated strategies ensuring the safety and security of the food and feed supply chain. The World Mycotoxin Forum® brings together a holistic conference programme covering the latest issues in mycotoxin management and is targeted at everyone working in the mycotoxin space – researchers, food and feed industry, laboratories, policy makers, and enforcement agencies from around the world.

The common thread running through WMF*meets*Salzburg, the 15th conference of The World Mycotoxin Forum®, is 'building a resilient food system in the digital decade – challenges for mycotoxin research and management'. The conference will offer an excellent way to network, share ideas, and formulate recommendations and conclusions on how to close knowledge gaps. It will include:

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

The aim of this year's conference is to elaborate further on key strategic issues looking forward, amid the current challenges. High-quality speakers, ample time for discussions, and every opportunity to establish rewarding contacts are values the World Mycotoxin Forum® wants to uphold. You are invited to take part in the discussions with participants from different disciplines and meet business relations in your area.

We wish you an active and fruitful meeting!

General conference chairs
Rudolf Krska
Chris Elliott

Steering Committee
Sarah De Saeger
Chiara Dall'Asta
Sheryl Tittlemier

**The list of participants can be found at:
www.WorldMycotoxinForum.org/participants**

About the venue

WMF*meets*Salzburg is held at Salzburg Congress, one of the most remarkable congress venues in Europe. On the verdant edge of the world-famous Mirabell park gardens in the heart of the city, the facility with its tasteful architecture and harmonious design is an ideal venue for the delegates and sponsors of The World Mycotoxin Forum®.

About Salzburg

Wolfgang Amadeus Mozart, the Salzburg Festival and 'The Sound of Music' are just 3 reasons for Salzburg's world fame as a city of music and culture, a UNESCO world heritage site! The baroque city with its uniquely distinct skyline generates special kind of magic that makes attending The World Mycotoxin Forum® an overall experience.

SOCIAL EVENTS

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



Sunday 6 April 2025
18:30 – 20:00

ST. PETER STIFTSKULINARIUM

The Welcome Reception – provided by R-Biopharm – will be held at St. Peter Stiftskulinarium. This free event includes drinks and snacks.

The Stiftskulinarium is situated in the heart of Salzburg old town in the monastery complex of the Benedictine monastery complex of St. Peter's Abbey. Within the abbey's medieval walls, through a narrow archway, and past a landscaped courtyard, lies St. Peter Stiftskulinarium – officially recognized as the oldest existing restaurant in Europe. Originally built as a wine cellar, then expanded into an inn and tavern servicing religious pilgrims and travelers, the restaurant was first mentioned in the year 803, making it over 1,200 years old.

The Welcome Reception provides an excellent opportunity to network, meet old friends and colleagues as well as to make new contacts.

St. Peter Stiftskulinarium
St. Peter Bezirk 1/4
A-5020 Salzburg
www.stpeter.at



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<https://r-bio.ms-hub>

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SAMPLE ID: BR-11071 Aflatoxin: 3.4 ppb
COMMODITY: CORN Deoxynivalenol: 0.8 ppm



The AgraStrip® Pro WATEX® test system enables the rapid and simple on-site quantification of mycotoxins in a variety of agricultural commodities.

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Learn more at www.romerlabs.com



Mycotoxin risk management

Turning science into sustainable solutions

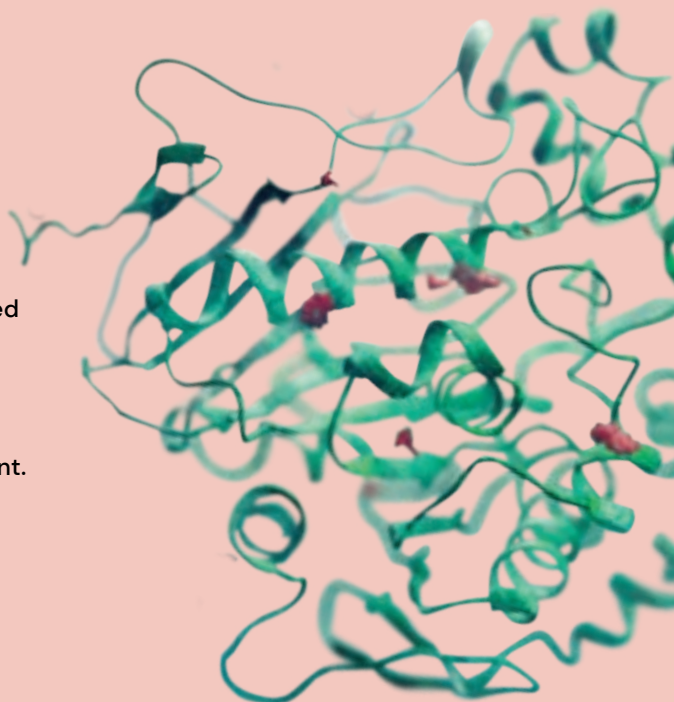


Mycotoxins, found in almost all commodities worldwide, present a significant challenge to animals, impacting both health and performance.

Powered by science, our Mycofix® portfolio is the unique solution to actively defend against multiple mycotoxins using a 3-pronged mitigation strategy including patented enzymatic biotransformation technology.

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WINE & CHEESE TASTING – provided by dsm-firmenich/Romer Labs
(free event)

Monday 7 April 2025
18:00 – 19:00

EXHIBITION FLOOR

dsm-firmenich ●●●



In the good tradition of the World Mycotoxin Forum®, a Wine and Cheese tasting party will be organised. A great way to meet all colleagues from the mycotoxin community.

The Wine & Cheese tasting takes place at the booth of dsm-firmenich/Romer Labs (exhibition space at the 1st floor of Salzburg congress).

CONFERENCE DINNER
(reservations only)

Tuesday 8 April 2025
19:30 – 22:30

STIEGL-KELLER

Welcome to perhaps the most beautiful beer garden and atmospheric restaurant in Mozart's city. A place to enjoy, eat, drink beer, and party! Simply put: If you didn't go to Stiegl-Keller, then you weren't in Salzburg either.

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. To take part, you must wear and show your name badge with the ticket.

Your way to Stiegl-Keller:

The Stiegl-Keller is located within the Salzburg pedestrian zone and can be reached on foot via the Festungsgasse. Address: Festungsgasse 7, 5020 Salzburg



PROGRAMME AT A GLANCE

Sunday 6 April 2025

18:30 – 20:00	Welcome reception – sponsored by R-Biopharm
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Monday 7 April 2025

11:00 – 12:30	Registration & light lunch	EXHIBITION
12:30 – 12:45	OPENING SESSION Welcome to WMFmeetsSalzburg	
12:45 – 14:15	PLENARY SESSION The role of digitalization and AI in mycotoxin research and management	
14:15 – 14:45	INTERACTIVE DEBATE AI in mycotoxin research and management: Game changer or just hype?	
14:45 – 15:15	Networking break & poster viewing	
15:15 – 16:30	PLENARY SESSION Building resilience in regional food systems	
16:30 – 18:00	SPEED PRESENTATIONS AND COMPANY PITCHES (short presentations by selected posters presenters and by sponsors, respectively)	
18:00 – 19:00	Wine & cheese tasting – sponsored by dsm-firmenich and Romer Labs	

Tuesday 8 April 2025

08:30 – 10:30	SESSION 1 Mycotoxins in One Health perspective – Part 1	SESSION 2 Mycotoxin management and risk mitigation: Current status, future opportunities	EXHIBITION
10:30 – 11:00	Networking break & poster viewing		
11:00 – 12:30	SESSION 3 Mycotoxins in One Health perspective – Part 2	SESSION 4 Mycotoxin control at farm level: Towards precision agriculture	
12:30 – 14:00	Lunch break & poster viewing		
12:45 – 13:45	WORKSHOPS		
14:00 – 15:15	SESSION 5 Drivers affecting exposure to mycotoxins: What's up, Doc?	SESSION 6 Mycotoxin occurrence and control: The focus on fungi	
15:15 – 15:45	Networking break & poster viewing		
15:45 – 16:45	SESSION 7 Societal relevance and impact of mycotoxin research	SESSION 8 Mycotoxin detection: On-site agri-food applications	
16:45 – 17:45	WMF YOUNG SCIENTISTS FORUM sponsored by Selko		
19:30 – 22:30	Conference dinner (reservations only)		

Wednesday 9 April 2025

09:00 – 10:30	SESSION 9 Late-breaking mycotoxin research	SESSION 10 Smart approaches for mycotoxin analysis	EXHIBITION
10:30 – 11:00	Networking break & poster viewing		
11:00 – 12:15	PLENARY SESSION Building a resilient food system in the digital decade – Challenges for mycotoxin research and management		
12:15 – 12:30	Best Poster Award and Societal Impact Reward		
12:30 – 13:00	WMFmeetsSalzburg – Top Five Answers Learned		
13:00	CLOSING		
13:30 – 14:30	General Assembly of the International Society for Mycotoxicology		

REGISTRATION & LIGHT LUNCH

11:00 – 12:30

OPENING SESSION

Welcome to *WMFmeetsSalzburg* – 15th conference of The World Mycotoxin Forum

General Conference Chairs:

- Prof. Rudolf Krska, *Department IFA-Tulln, BOKU Vienna, Austria*
- Prof. Chris Elliott, *Institute for Global Food Security, Queen’s University Belfast, UK*

12:30 Introduction to *WMFmeetsSalzburg*

PLENARY SESSION

The role of digitalization and AI in mycotoxin research and management

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

12:45 **The scAInce of chemical risk assessment in food**
 Prof. Thomas Hartung, *Department of Environmental Health and Engineering, Johns Hopkins University, USA*

13:05 **How can AI help mycotoxin research and management?**
 Prof. Ine van der Fels, *Wageningen Food Safety Research and Business Economics Group, Wageningen University & Research, the Netherlands*

13:25 **Harnessing AI for enhanced mycotoxin risk management strategies**
 Dr Ioannis Zachos, *Animal Nutrition and Health R&D Center, dsm-firmenich, Germany*

13:40 **From data to detection: AI-driven mycotoxin detection**
 Dr Alan Inglis, *Hamilton Institute, Maynooth University, Ireland*

13:55 **Harnessing collective intelligence (= AI x human expertise) for food safety in Africa**
 Peter Schelstraete, *Ubuntoo, the Netherlands*

14:15 – 14:45

INTERACTIVE DEBATE

AI in mycotoxin research and management: game changer or just hype?

Across the entire business spectrum, from SMEs to large multinational corporations, people are racking their brains to solve major puzzle pieces:

- *How to get AI work for you?* • *Where do the biggest opportunities lie?*
- *What are the pitfalls and downsides of AI?* • *When to say ‘yes’ (and ‘no’) to AI?*

Moderators:

Prof. Rudolf Krska, *Department IFA-Tulln, BOKU Vienna, Austria*
 Prof. Chris Elliott, *Institute for Global Food Security, Queen’s University Belfast, UK*

The floor will be open for a lively discussion involving Prof. Thomas Hartung, Prof. Ine van der Fels, Dr Ioannis Zachos, Dr Alan Inglis, Peter Schelstraete, and all participants.

14:45 – 15:15

Networking break

PLENARY SESSION**Building resilience in regional food systems**

Chair: Dr Carol Verheecke-Vaessen, *Cranfield University, UK*

15:15 **Mycotoxin awareness: Paving the way to food safety and security in Central America**
Dr Andreia Bianchini, *Department of Food Science and Technology, University of Nebraska-Lincoln, USA*

15:30 **Mycotoxins and climate change: Challenges in South American countries for small farmers' production to increase resilience**
Dr Claudia Foerster, *Institute of Agri-Food, Animal and Environmental Sciences, Universidad de O'Higgins, Chile*

15:45 **FOCUS ON AFRICA:**

- **Food Safety for Africa project, addressing food safety challenges in the African informal sector**
Dr Titilayo Falade, *International Institute of Tropical Agriculture, Nigeria*
- **UP-RISE: Tackling mycotoxin challenges for inclusive and resilient food systems in Africa**
Prof. Sarah De Saeger, *Centre of Excellence in Mycotoxicology and Public Health, Ghent University, Belgium*

16:15 **Climate-friendly, safe, resilience, and sustainable agri-food value chains in the greater Mekong subregion with a focus on mycotoxins**
Dr Awanwee Petchkongkaew, *Thammasat University and International Joint Research Centre on Food Security, Thailand*

16:30 **An integrated approach to reducing mycotoxins in Irish cereal grains**
Prof. Fiona Doohan, *School of Biology and Environmental Science, University College Dublin, Ireland*

PLENARY SESSION**Speed presentations and company pitches**

Short presentations by selected poster presenters to provide an overview of their research and by sponsors to inspire the audience to visit their booths, respectively.

Chair: Dr Lien Thi Kim Phan, *Ho Chi Minh City University of Food Industry, Vietnam*

16:45 **Company pitches**
R-Biopharm – Romer Labs – Adisseo – Bioeasy – Selko

17:15 **Speed presentations**
Baozhu Guo, *ARS-USDA, USA* – Esther Lima de Paiva, *INRAE, France* – Monica Ermolli, *EC-JRC, Italy* – Donato Greco, *ISPA-CNR, Italy*

17:40 **Company pitches**
Biönte – Gold Standard Diagnostics – Patent CO. – EnviroLogix

18:00 – 19:00

WINE & CHEESE TASTING

Sponsored by dsm-firmenich/Romer Labs
Exhibition floor

dsm-firmenich ●●●



SESSION 1

Mycotoxins in One Health perspective – Part 1

To manage the threat posed to health (human, animal, and environmental), a multifaceted 'One Health' approach to mycotoxin research is required.

Chairs: Prof. Marthe De Boevre, *Ghent University, Belgium*
Dr Yueju Zhao, *Mars Global Food Safety Center, China*

08:30 Hazard assessment of mycotoxins for humans and animals: A European perspective in the light of climate change
Dr Isabelle Oswald, *ToxAlim, INRAE, France*

08:45 Differential diagnosis of mycotoxicosis is key for effective risk management
Dr Swamy Haladi, *Trouw Nutrition, India*

09:00 Citrinin in human urine and blood - sources of exposure and impact of food processing
Dr Benedikt Cramer, *Institute for Food Chemistry, Universität Münster, Germany*

09:15 Silent threats: Can mycotoxins fuel neurodegenerative diseases?
Dr Miguel A. Faria, *REQUIMTE-LAQV, University of Porto, Portugal*

09:30 Exploring links between multimycotoxin exposure and colorectal cancer through human biomonitoring and genotoxicity tests
Yasmine Bader, *Centre of Excellence in Mycotoxicology and Public Health, Ghent University, Belgium*

09:45 Unravelling the synergistic impact of exposures to mycotoxins and Epstein Barr virus at early stages of Burkitt lymphomagenesis
Dr Rita Khoueiry, *Epigenomics and Mechanisms Branch, International Agency for Research on Cancer, World Health Organization, France*

10:00 Renal excretion of DON and T-2/HT-2 toxin in humans in an everyday situation
Hannah McKeon, *National Institute for Public Health and the Environment, the Netherlands*

10:15 One Health approaches to mycotoxin research: Impact of beauvericin and enniatin B on aquatic ecosystems and global food safety
Dr Ana Juan-García, *Laboratory of Food Chemistry and Toxicology, University of Valencia, Spain*

10:30 – 11:00

Networking break

Poster viewing

SESSION 2**Mycotoxin management and risk mitigation – Current status, future opportunities****Chairs:** Dr Sheryl Tittlemier, *Canadian Grain Commission, Canada*Dr Gunnar Sundstøl Eriksen, *Norwegian Veterinary Institute, Norway***08:30 Approach to facilitate prioritization of natural toxins for risk management**Prof. Michele Suman, *Barilla SpA and Università Cattolica del Sacro Cuore, Italy***08:45 Left censoring approaches to estimate mycotoxin concentrations in raw materials**Dr Biagio Zaffora, *Nestlé, Switzerland***09:00 A novel mechanism for DON detoxification**Dr Chris Garnham, *London Research & Development Centre, Agriculture and Agri-Food Canada, Canada***09:15 Novel enzymatic approach for decontaminating ochratoxin A in feed and food**Dr Christoph Gonaus, *Animal Nutrition and Health R&D Center, dsm-firmenich, Austria***09:30 A novel enzyme for detoxification of fumonisins**Prof. Manuel Ferrer, *Institute of Catalysis and Petrochemistry, Spanish Council for Scientific Research, Spain* and Dr Jog Raj, *Patent CO., Serbia***09:45 Monitoring complementary *in vitro* and *in vivo* models is key for a proper anti-mycotoxin solution**Dr Damien Prévéraud, *Adisseo, France***10:00 Comparison of multi-strain and single strain biocontrol formulations for mitigation of maize aflatoxin contamination in the USA**Dr Hillary Mehl, *Pest Management & Biological Control Research Unit, ARS-USDA, USA***10:15 Integrating environmental factors and machine learning to predict *Alternaria* mycotoxin contamination in tomatoes**Yimin Zhang, *Business Economics Group, Wageningen University & Research, the Netherlands***10:30 – 11:00****Networking break****Poster viewing**

SESSION 3

Mycotoxins in One Health perspective – Part 2

Chairs: Dr Isabelle Oswald, *INRAE, France*
Thomas Pecqueur, *Cargill, Canada*

11:00 **Global patterns and impact of acute aflatoxicosis: Insights from a systematic review**
Dr Tess Goessens, *Centre of Excellence in Mycotoxicology and Public Health, Ghent University, Belgium*

11:15 **Dietary exposure to DON negatively impacts the production performance of laying hens**
Dr Regiane Santos, *Schothorst Feed Research, the Netherlands*

11:30 **One Health approach to mycotoxin surveillance: Biomarkers of subclinical fumonisins, DON, and zearalenone exposure in poultry**
Dr Revathi Shanmugasundaram, *U.S. National Poultry Research Center, ARS-USDA, USA*

11:45 **Absolute oral bioavailability and quantitative toxicokinetics of *Alternaria* mycotoxins in pigs**
Prof. Siska Croubels, *Department of Pathology, Pharmacology & Zoological Medicine, Ghent University, Belgium*

12:00 **Delving into the field microbiome for mycotoxin mitigation: A case study for the control of *Fusarium* head blight of cereals**
Dr Adeline Picot, *Ecole Supérieure d'Ingénieurs en Agroalimentaire de Bretagne atlantique, Université de Bretagne Occidentale, France*

12:15 **Company pitches**
Impextraco – Olmix – Alltech – Waters | Vicam

12:35 – 14:00
Lunch break

Poster viewing

12:45 – 13:45
WORKSHOPS

- **MS HUB: precision clean-up powered by mycotoxin testing expertise**
Sponsored by R-Biopharm, Germany
Trakl Hall (3rd floor)



- **Tackling mycotoxins: Testing pitfalls, best practices, and a live ZENzyme demo**
Sponsored by dsm-firmenich and Romer Labs
Paracelsus Hall (2nd floor)



For more information on the workshops, see page 21

SESSION 4**Mycotoxin control at farm level – Towards precision agriculture**

Precision agriculture is revolutionizing the agriculture sector all over the world by having many potential benefits in, e.g., crop quality, food safety, and environmental protection. What's up for mycotoxin prevention and control?

Chairs: Prof. Ine van der Fels, *Wageningen Food Safety Research, the Netherlands*
Prof. Carlos A.F. Oliveira, *University of São Paulo, Brazil*

11:00 **Predicting the next outbreak: Models for *Fusarium* head blight and deoxynivalenol**
Prof. Erick DeWolf, *Department of Plant Pathology, Kansas State University, USA*

11:15 **The potential of spectroscopy techniques for *in situ* measurement and mapping of *Fusarium* head blight and DON in cereal crops**
Prof. Abdul Mouazen, *Department of Environment, Ghent University, Belgium*

11:30 **Detection of *Fusarium* head blight in winter wheat fields using imaging spectroscopy and deep learning**
Xinxin Wang, *Wageningen Food Safety Research, the Netherlands*

11:45 **Rapid detection of plant pathogens and fungicide sensitivity to control mycotoxin contamination**
Prof Jonathan West, *Protecting Crops and the Environment Division, Rothamsted Research, UK*

12:00 **The trilogy in mycotoxin management – prediction, detection, and interpretation**
Carrie Maune, *Trilogy Analytical Laboratory, USA*

12:15 **Company pitches**
Charm Sciences – ProGnosis Biotech – Neogen – Pribolab

12:35 – 14:00
Lunch break

Poster viewing

12:45 – 13:45
WORKSHOPS

- **MS HUB: precision clean-up powered by mycotoxin testing expertise**
Sponsored by R-Biopharm, Germany
Trakl Hall (3rd floor)



- **Tackling mycotoxins: Testing pitfalls, best practices, and a live ZENzyme demo**
Sponsored by dsm-firmenich and Romer Labs
Paracelsus Hall (2nd floor)



For more information on the workshops, see page 21

SESSION 5**Drivers affecting exposure to mycotoxins: What's up, Doc?**

With climate change, political crises, and the shift to more plant-based proteins the potential for mycotoxins contaminating food products is only increasing. A selection of hot topics.

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

- 14:00** **Beyond farm to table: When climate and conflict add a risky flavour to your food**
Dr Bert Popping, *FOCOS, Germany*
- 14:15** **Hidden yet far-reaching costs of conflict: Aggravated mycotoxin threats**
Dr Alejandro Ortega-Beltran, *International Institute for Tropical Agriculture, Nigeria*
- 14:30** **Mycotoxin management to face climate change impact on food safety and human health, the match**
Prof. Paola Battilani, *Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Italy*
- 14:45** **Influence of weather and climatic conditions during the growing season on the levels of *Fusarium* mycotoxins in oats in Norway**
Dr Aksel Bernhoft, *Norwegian Veterinary Institute, Norway*
- 15:00** **A survey of mycotoxins and other natural contaminants in plant-based alternatives for meat and dairy products**
Dr Michael Sulyok, *Department IFA-Tulln, BOKU Vienna, Austria*
- 15:15 – 15:45**
Networking break
- Poster viewing**

SESSION 6**Mycotoxin occurrence and control – The focus on fungi**

Chair: Dr Antonio Moretti, *Institute of Sciences of Food Production, CNR, Italy*

14:00 Evolution of regulation of trichothecene toxin production

Dr Robert Proctor, *Mycotoxin Prevention and Applied Microbiology Research, ARS-USDA, USA*

14:15 Scope of taxonomic boundaries: From *Fusarium* genus to populations

Dr Tapani Yli-Mattila, *Department of Life Technologies, University of Turku, Finland*

14:30 Unravelling the puzzle of ochratoxin A production

Dr Silvia Rodríguez-Pires, *Department of Genetics, Physiology and Microbiology, Complutense University of Madrid, Proteomics and Genomics Facility, CIB-CSIC, Spain*

14:45 Host induced gene silencing in reducing pre- and post-harvest aflatoxin contamination in maize

Prof. Zhi-Yan Chen, *Department of Plant Pathology and Crop Physiology, Louisiana State University Agricultural Center, USA*

15:00 Mycobiota and mycotoxins on artisan Italian cheese aged in natural environments: Prevention and control strategies

Dr Antonia Susca, *Institute of Sciences of Food Production, CNR, Italy*

15:15 – 15:45

Networking break

Poster viewing

SESSION 7**Societal relevance and impact of mycotoxin research**

At the same time as the productivity of researchers have become more formalized and institutionalized with increasing emphasis on counting publications in high-ranking journals, citations, h-index, and so on, there is an increased demand on researchers to contribute to what is referred to as societal value, societal relevance, public value, societal impact, and/or similar phenomena.

At the end of the conference, an outstanding presentation considering the societal relevance and impact of the research, will be awarded.

Chair: Dr Lindy J. Rose, Stellenbosch University, South Africa

15:45 Mycotoxin in Africa: A research journey towards achieving food safety and nutrition security

Dr Carla Cervini, Magan Centre of Applied Mycology, Cranfield University, UK

16:00 Mycotoxins as endocrine disruptors in drinking water: Insights from a Belgian cohort study

Dr Tess Goessens and Prof. Marthe De Boevre, Centre of Excellence in Mycotoxicology and Public Health, Ghent University, Belgium

16:15 Uncovering the story of mycotoxin exposure in the UK: analysis of body fluids and hair

Dr Esther Garcia Cela, MycoLab, University of Hertfordshire, UK

16:30 Occurrence of endophyte mycotoxins in cool-season grasses from the Pacific Northwest, United States

Dr Jennifer Durringer, Department of Environmental and Molecular Toxicology, Oregon State University, USA

16:45 – 17:45

WMF Young Scientist Forum

Sponsored by Selko

Paracelsus Hall (2nd floor)



Emerging mycotoxins: Diagnosis, toxicity and management

During this year's Young Scientist Forum, Selko invites young scientists, researchers, and students to share experiences and learn from each other. In today's fast-changing and evolving industry, it can be a challenge to define research needs and connecting them to 'real' scenarios and creating cost-effective solutions for grain and animal producers around the world. How to find the right model for your research? Which biomarkers to apply? *In vitro* or *in vivo*? Single or multi-mycotoxin, or mycotoxins combined with other environmental challenges? What are emerging technologies and how can they improve mycotoxin research in the future?

These and other questions will be discussed during the informal brainstorm session, while enjoying some snacks and drinks. We hope to connect business needs with science and invite those young of age as well as young at heart to join in.

19:30 – 22:30

Conference dinner

For information, see page 6

SESSION 8**Mycotoxin detection – On-site and online agri-food applications**

Chair: Dr Michael Sulyok, *BOKU Vienna, Austria*

15:45 Smarter, faster, on-site: Infrared spectroscopy for mycotoxin detection

Polina Fomina, *Institute of Analytical and Bioanalytical Chemistry, University of Ulm, Germany*

16:00 Portable detection of genetic mycotoxin biosynthesis pathways in crops and food products – System validation

Prof. Monika Szymańska-Czerwińska, *Department of Biotechnology and Nutrigenomics, Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, Poland*

16:15 Highly sensitive rapid lateral flow test for detecting patulin

Dr Boris Polyak, *Gold Standard Diagnostics, USA*

16:30 Application of time-resolved fluorescent immunochromatography in mycotoxin detection

Robin Zhang, *Bioeasy, China*

16:45 – 17:45

WMF Young Scientist Forum

Sponsored by Selko

Paracelsus Hall (2nd floor)



Emerging mycotoxins: Diagnosis, toxicity and management

During this year's Young Scientist Forum, Selko invites young scientists, researchers, and students to share experiences and learn from each other. In today's fast-changing and evolving industry, it can be a challenge to define research needs and connecting them to 'real' scenarios and creating cost-effective solutions for grain and animal producers around the world. How to find the right model for your research? Which biomarkers to apply? *In vitro* or *in vivo*? Single or multi-mycotoxin, or mycotoxins combined with other environmental challenges? What are emerging technologies and how can they improve mycotoxin research in the future?

These and other questions will be discussed during the informal brainstorm session, while enjoying some snacks and drinks. We hope to connect business needs with science and invite those young of age as well as young at heart to join in.

19:30 – 22:30

Conference dinner

For information, see page 6

SESSION 9**Late-breaking mycotoxin research**

Highly significant and timely findings in mycotoxin research that were not available by the regular abstract submission deadline (1 July 2024) of WMFmeetsSalzburg

Chairs: Prof. Sarah De Saeger, *Ghent University, Belgium*
Dr Franz Berthiller, *BOKU Vienna, Austria*

09:00 **Towards resilience of the Robusta coffee production: An Ivorian case study**
Dr Carol Verheecke-Vaessen, *Magan Centre of Applied Mycology, Cranfield University, UK*

09:15 **Unravelling the mysteries of bound ochratoxins**
Dr Franz Berthiller, *Department IFA-Tulln, BOKU Vienna, Austria*

09:30 **Mycotoxins in urine of adults following healthy and sustainable diets: Revealing the dynamics between diet transition and mycotoxin exposure**
Dr Octavian Mihalache, *Department of Food and Drug, University of Parma, Italy*

09:45 **Efficiency of removal of aflatoxin from contaminated peanuts using fluorescence-based sorting technology**
Dr Yueju Zhao, *Mars Global Food Safety Center, Mars Inc., China*

10:00 **Weaning piglet health upon dietary contamination with deoxynivalenol, enniatins, or their combination *in vivo* is connected to altered gut microbiome and gut tissue outcomes *in vitro***
Dr Maria Wiese, *Microbiology and Systems Biology, TNO, the Netherlands*

10:15 **Revolutionizing mycotoxin monitoring: New quality approaches for certified reference materials**
Dr David Steiner, *LVA GmbH, Austria*

10:30 – 11:00
Networking break

Poster viewing

SESSION 10**Smart approaches for mycotoxin analysis**

Chairs: Prof. Michele Suman, *Barilla and Università Cattolica del Sacro Cuore, Italy*
Dr Awanwee Petchkongkaew, *Thammasat University, Thailand*

09:00 Advances in analysis and detection of mycotoxins in dryland crops at the joint FAO/IAEA Centre's Food Safety and Control Laboratory

Dr Christina Vlachou, *Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Austria*

09:15 Automation in mycotoxins analysis: The Wizard of Oz journey from innovation to harmonized data collection

Giulia Rosar, *Gold Standard Diagnostics, Italy*

09:30 Fate of mycotoxins during gluten-free pasta processing: Untargeted ¹³C labelling LC-HRMS based approach

Eleonora Rollo, *Barilla/University of Parma, Italy* and Dr Alexandra Schamann, *FFoQSI, Austria*

09:45 Metabolism of multiple mycotoxins by black soldier fly (*Hermetia illucens*) using an optimized high-throughput LC-MS/MS method

Dr Siegrid De Baere, *Department of Pathology, Pharmacology & Zoological Medicine, Ghent University, Belgium*

10:00 Pulling the thread: A metabolomic approach to unraveling secondary metabolites production in *Fusarium proliferatum*

Irene Picicci, *Department of Food and Drug, University of Parma, Italy*

10:15 A high throughput phenotyping platform for cereal research and breeding programs to identify *Fusarium*-damaged kernels and *Fusarium*-produced mycotoxins

Dr Lipu Wang, *Department of Plant Sciences/Crop Development Centre, University of Saskatchewan, Canada*

10:30 – 11:00

Networking break

Poster viewing

PLENARY SESSION**Building a resilient food system in the digital decade
– Challenges for mycotoxin research and management**

Chairs: Prof. Rudolf Krska, *Department IFA-Tulln, BOKU Vienna, Austria*
 Prof. Chris Elliott, *Institute for Global Food Security, Queen's University Belfast, UK*

11:00 NARESH MAGAN LECTURE AWARD

Proteomic analysis as a tool to unveil effects of control agents on toxigenic *Gnomoniopsis smithogilvyi*, the major chestnut pathogen

Dr María Micaela Álvarez Rubio, *Department of Nutrition and Food Science, Complutense University of Madrid, Spain*

11:15 A byte against blight – AI's impact on mycotoxins

Ronald Niemeijer, *R-Biopharm, Germany*

11:30 Human biomonitoring of mycotoxins: Exciting opportunities and challenges

Dr Marcel Mengelers, *National Institute for Public Health and the Environment, the Netherlands*

11:45 Tech-powered biosolutions: Revolutionizing crop protection using robots, AI and thousands of fungi

Dr Johan Vormsborg Christiansen, *Mycoverse, Denmark*

12:00 Mycotoxin research 4.0: Should we really go omics?

Prof. Chiara Dall'Asta, *Department of Food and Drug, University of Parma, Italy*

12:15 Best Poster Award and Societal Impact Reward

To be presented by a Cargill representative

12:30 Top Five Answers Learned

Prof. Rudolf Krska, *Department IFA-Tulln, BOKU Vienna, Austria*

Prof. Chris Elliott, *Institute for Global Food Security, Queen's University Belfast, UK*

Prof. Michele Suman, *Barilla and Università Cattolica del Sacro Cuore, Italy*

13:00 Closing of WMFmeetsSalzburg

Take your packed lunch to eat along the way!

13:30 – 14:30**General Assembly of the International Society for Mycotoxicology**

The International Society for Mycotoxicology (ISM) aims to increase scientific knowledge concerning biology, chemistry and any sciences/ disciplines related to mycotoxins and toxigenic fungi through membership networking, scientific meetings, symposia, discussions, technical courses and publications.

ISM's General Assembly is open to all participants of *WMFmeetsSalzburg*.

WORKSHOPS

TUESDAY 8 APRIL 2025

12:45 – 13:45

TRAKL HALL (3rd floor)

MS HUB: precision clean-up powered by mycotoxin testing expertise

Sponsored by R-Biopharm, Germany

Multi-toxin analysis with LC-MS/MS detection offers many advantages. LC-MS/MS allows for the simultaneous detection of multiple mycotoxins in a single run, making it highly efficient compared to traditional methods that often target individual mycotoxins. Frequently just a minimal sample clean-up is used before injection of the LC-MS/MS, which is very cost-effective, simple, and fast, but has its limitations due to unpredictable matrix effects like signal suppression or signal boosting and lower sensitivity. Besides injecting diluted samples directly into the LC system may increase the need for instrument maintenance. Therefore, we developed the concept of MS-Hub, a combination of precision clean-up and quality assurance tools.

In this workshop, we will present the MS-Hub, which is based on two pillars, focusing on sample clean-up and quality assurance:

- QuantPREP™: Highly effective clean-up tools before LC-MS/MS
- QualiT™: Quality assurance tools, which can be used for matrix-matched calibration

With these offerings, we aim to provide laboratories, researchers, and industry with everything they need for accurate, efficient, and reliable mycotoxin analysis for LC-MS/MS from a single source. The RBR Immunoaffinity Columns and QualiT Pure Solid Phase Columns represent a significant advancement in the field of mycotoxin analysis. Their ability to provide effective clean-up for all kinds of samples, combined with ease of use and versatility, makes them superior to alternative methods. Additionally, the use of matrix-matched calibration with Trilogy mycotoxin quality control materials and standards offers further benefits in terms of accuracy, ease of use, and consistency. By incorporating these tools into your laboratory workflow, you can achieve more accurate, reliable, and efficient mycotoxin analysis.

TUESDAY 8 APRIL 2025

12:45 – 13:45

PARACELSUS HALL (2nd floor)

Tackling mycotoxins: Testing pitfalls, best practices, and a live ZENzyme demo

Sponsored by dsm-firmenich
and Romer Labs

dsm-firmenich ●●●



Step into the role of a 'mycotoxin investigator' as you discover how common testing errors can compromise results and threaten food & feed safety and animal performance. In this interactive session, industry experts from Romer Labs will walk you through the most frequent pitfalls in mycotoxin testing and show you how to avoid them.

Then, move from theory to action with a live, hands-on demonstration of ZENzyme in real time, courtesy of dsm-firmenich ANH. You will see first-hand how this cutting-edge enzyme deactivates zearalenone before your eyes, gaining insider knowledge on verifying its efficacy. By the end of this immersive workshop, you will be equipped not just to spot testing errors, but to employ proven strategies and innovative tools that keep contamination at bay.

WMF YOUNG SCIENTIST FORUM

TUESDAY 8 APRIL 2025

16:45 – 17:45

PARACELSUS HALL (2nd floor)

Emerging mycotoxins: Diagnosis, toxicity and management



During this year's Young Scientist Forum, Selko invites young scientists, researchers, and students to share experiences and learn from each other. In today's fast-changing and evolving industry, it can be a challenge to define research needs and connecting them to 'real' scenarios and creating cost-effective solutions for grain and animal producers around the world. How to find the right model for your research? Which biomarkers to apply? *In vitro* or *in vivo*? Single or multi-mycotoxin, or mycotoxins combined with other environmental challenges? What are emerging technologies and how can they improve mycotoxin research in the future?

These and other questions will be discussed during the informal brainstorm session, while enjoying some snacks and drinks. We hope to connect business needs with science and invite those young of age as well as young at heart to join in.

When it comes to mycotoxin exposure always use the correct tool for the job



Selko's mycotoxin risk management programme gives you the right tools to make decisions based on knowledge and data and allows you to apply mycotoxin control products more precisely.

THE BENEFITS



Bind and eliminate
mycotoxins



Strengthen
intestinal barriers



Modulate immune
response

COMPANY PROFILES

Profiles of the companies presenting pitches

R-Biopharm

<https://r-biopharm.com>



R-Biopharm AG is a family-run business and one of Germany's leading biotechnology companies. The company is dedicated to food safety and offers innovative products for various applications in food and feed analysis. The company operates in two main business areas: food analysis and clinical diagnostics. The R-Biopharm Group provides comprehensive solutions from a single source, including analytical services, contract research, and certification services. The R-Biopharm Group offers a comprehensive range of food analysis services designed to ensure food safety and quality. Here are some key aspects of their services. The R-Biopharm Group's food analysis division is dedicated to food safety and offers comprehensive solutions from a single source. Our product portfolio includes:

- *mycotoxins*, providing immunoaffinity and SPE columns, mycotoxin test kits for detecting mycotoxins in various commodities and QC materials
- *food allergens*, rapid allergen testing using lateral flow tests (LFDs) and ELISA kits
- *genetically modified organisms (GMOs)*, detection and quantification of GMOs in food and feed
- *microbiology*, testing for microbial contamination in food products
- *food adulteration*, ensuring the authenticity and purity of food products
- *residues*, analysing residues of pesticides, veterinary drugs, and other contaminants
- *vitamins*, testing for vitamin content in food products

The R-Biopharm Group also offers specialized services, such as contract research to support laboratory routines. We have a robust service network to provide technical support, training, and after-sales services in various regions.

Romer Labs

<https://www.romerlabs.com>



Romer Labs is a global leader in innovative diagnostic solutions for food and feed safety. With a focus on mycotoxins, food allergens, GMOs, and microbial contaminants, we strive to meet the ever-changing demands of our customers while upholding our reputation for exceptional service. Innovation is at the heart of what we do. We are constantly exploring new technologies and pushing the boundaries of existing ones to simplify workflows, improve accuracy, and enhance reliability. We are committed to developing groundbreaking technology and ensuring that our products and services are always at the forefront of the industry. We have been providing exceptional service to our customers for over 40 years. Our dedicated technical support team is always available to provide assistance, and our technical sales team is committed to helping customers find the right solutions to meet their unique needs. In addition, we operate six analytical services labs across three continents, ensuring that our customers have access to reliable and accurate testing services whenever and wherever they may need them. Our core mission at Romer Labs has always been the same: *Making the World's Food Safer*®. Through our innovative products and exceptional service, we remain dedicated to providing our customers with the tools they need to ensure the safety and quality of their products. Romer Labs is a company of dsm-firmenich.

Adisseo

<https://www.adisseo.com>



Mycotoxin management is not a betting game

Adisseo helps you to identify the risks and adopt the best strategy. From the crop to the feed, mycotoxin production is a cumulative process. It is controlled by several factors (e.g., climatic conditions, agronomic practices). Each mycotoxin has its own model of development, meaning that every year the crops are contaminated differently, both in terms of quantity and mycotoxin type. The risk is therefore ever-present, and ever-changing. A holistic approach is needed to identify the risk and adopt the best strategy. Customers across the globe have been successfully working with our mycotoxin management program for decades. Our MycoMan range of services allows the mycotoxin risk to be identified and

optimal strategies to be developed thanks to the mycotoxin prediction tool, the harvest bulletin, quick or laboratory tests and, finally, our online MycoMan platform. Moreover, Adisseo has also developed a portfolio of solutions in order to propose the best-suited solution to a specific challenge: Unike® Plus, maximum protection against challenges posed by broad-spectrum mycotoxin contamination; Unike®, powerful protection against broad-spectrum mycotoxin contamination; Toxy-Nil®, reliable protection against moderate-level mycotoxin contamination. Adisseo is one of the world's leading experts in feed additives. The group relies on its 6 research centres and its production sites based in Europe, USA, China and Thailand to design, produce and market nutritional solutions for sustainable animal feed. With more than 2,750 employees, it serves around 4,200 customers in over 110 different countries through its global distribution network. In 2023, Adisseo achieved a turnover of over 1.7 billion Euros. Adisseo is one of the main subsidiaries of China National BlueStar, leader in the Chinese chemical industry with nearly 12,500 employees and a turnover of 5.3 billion euros. Adisseo is listed on the Shanghai Stock Exchange.

Bioeasy

<https://en.bioeasy.com>



Shenzhen Bioeasy Biotechnology Co., Ltd., founded in 2007, is a high-tech enterprise engaged in food safety, clinical diagnosis, public safety and other fields. With a focus on rapid detection, we are dedicated to provide our customers with high quality products, services and overall solutions to tackle current and emerging food safety problems, protecting our food from farm to table. Our products include rapid test kits and instruments for detection of antibiotics, aflatoxin, pesticides and other food additive residues, serving clients all over the world in fields like dairy, meat and seafood, feed, grain and oil, food processing, etc. As a member of the AOAC (Association of Official Analytical Chemists) and IAFP (International Association for Food Protection), the products from Bioeasy are very reliable. Ten test kits were selected in China as the national standard test by AQSIQ (China General Administration of Quality Supervision), the Entry-Exit Inspection and Quarantine Bureau because of their excellence in sensitivity, specificity and stability. Our main dairy antibiotics testing products have also been approved by some international prestigious organizations like MPI, ACTALIA and ILVO. etc. We have facilities covering total area of 30000m². 12000m² is used for R & D and manufacturing purposes, including 500 m² Class 10,000 and Class 100,000 cleanrooms and 800 m³ cold storage. Complete platforms have been established for immunology, molecular biology, chemical synthesis, molecular construction and testing equipment development. We gathered a large number of industry-leading biology, chemistry, automation and information technology experts.

Selko

<https://www.selko.com>



Selko is the feed additives brand of Trouw Nutrition, the livestock feed business line of Nutreco. We are committed to developing specialty feed additives that optimise animal performance. Decades of experience, ongoing research, and a commitment to developing functional and more sustainable solutions help our customers' animals achieve their full potential. Backed by sound science and manufactured to meet the highest quality standards, the Selko specialty feed additive portfolio helps support feed safety, antibiotic reduction, trace mineral optimisation, animal health and performance, mycotoxin risk management and much more. Our passionate and driven team is dedicated to solving challenges across the feed-to-food chain. With a presence in 105 countries and manufacturing plants on several continents, we serve feed producers, farmers and home-mixers, integrators, distributors, and food processors. At Selko, we understand that combining the power of nutrition, specialty feed additives and good farm management can transform our industry – and even our planet. Selko's planet-to-plate approach is challenging how today's feed-to-food chain works and helping our customers secure a brighter future.

BIÖNTE

<https://www.bionte.com> | bionte@bionte.com



Mitigating the effects of mycotoxins through research, innovation and technical services

BIÖNTE is a company focused on providing innovative solutions to the challenges posed by mycotoxins to face a more demanding environment, with specialization and technical service as differentiating elements. BIÖNTE is a spin-off of Andrés Pinaluba S.A. (APSA), the parent company of the Pinaluba Group, a multinational conglomerate with 14 companies operating across 7 countries. BIÖNTE commitment is to remain a global benchmark in mitigating the effects of mycotoxins through scientific research, innovation and technical support, delivering effective and sustainable solutions for improved animal nutrition. Each client faces unique challenges – raw materials, target species and regional conditions all present distinct demands. That's why the most effective way to combat mycotoxins is through specialization, tailored solutions and the development of safe, effective and profitable products. At BIÖNTE, we share our expertise and resources with our business partners and clients, enabling them to implement comprehensive, results-driven strategies. Our in-depth market knowledge, understanding of the global challenges in animal nutrition, and the experience and international focus of our technical and commercial team – combined with the technological and operational support of the Pinaluba Group – form the foundation of our work as an independent company.

Gold Standard Diagnostics

<https://www.goldstandarddiagnostics.com>

Automatize mycotoxin analysis: Diagnostic test kits and instruments

Gold Standard Diagnostics is a global provider of fast, reliable, and easy-to-use diagnostic test kits and instruments in the fields of bioanalytical testing for the food, feed, environmental, veterinary and clinical industries. We provide comprehensive solutions for mycotoxin screening, from on-site early detection by rapid tests to a variety of cost-efficient, high-throughput ELISA kits and analysers, and even clean-up devices for laboratories performing confirmational verifications through instrumental analysis. Our on-field trainings and R&D services to customize, verify and validate our customers' applications complete the product offering. Manual mycotoxins analysis, despite the technology that is used, has an intrinsic dependence on human practice. This affects not only the variability and the analysis reliability, but also the data integrity and elaboration. Automation is limiting or removing the human intervention and the possibility to interfere with results, improving the intra- and inter-laboratory precision, and ensuring multi-parameter data collection. These improvements are also necessary to provide the right data to regulatory bodies, AI-based predictive models and internal databases used for local risk assessments. Contact us and we are happy to help you to find the best custom solution adapted to your needs.



Patent CO.

<https://global.patent-co.com>



Patent CO. and its affiliate company agromed Austria GmbH – both subsidiaries of RWA (Raiffeisen Ware Austria) –stand together as a forward-thinking alliance in the global feed additive business to deliver sustainable feed solutions to our clients worldwide. With over 30 years of experience in mycotoxin management, PATENT CO. has established itself as a leader in providing effective solutions to prevent and control mycotoxin contamination in animal feed. The company's extensive expertise ensures high-quality products and services that help protect livestock from the harmful effects of mycotoxins, improving both animal health and productivity. Our mycotoxin management portfolio consists of three products: Minazel®, Minazel® Plus, and Mycoraid. Minazel® is our mycotoxin detoxification solution for combating negative effects of polar mycotoxins. Minazel® Plus is an advanced mycotoxin detoxification solution. Unique organic surface modification enables fast and irreversible adsorption of polar and less polar mycotoxins. Mycoraid is the state-of-the-art solution for improving animal welfare by adsorbing and deactivating mycotoxins that contaminate farm animal's feed. Its safety and efficacy are proven by *in vivo* trials published in peer reviewed journals.

EnviroLogix

<https://www.envirologix.com>



For over 25 years, EnviroLogix has innovated in science and technology for the safety, health, and well-being of society, creating exceptional long-term value for our customers. EnviroLogix has focused on developing risk management systems for mycotoxins, GMOs, and allergens for the grain, feed, and food industry. We take pride in delivering rapid, accurate test results – helping our global customers make informed operational decisions daily. Our comprehensive mycotoxin solutions are streamlined for operator ease-of-use, paired with industry-leading data software solutions to drive insights for customers beyond point-of-use test results. In addition to mycotoxins, EnviroLogix also delivers rapid solutions for allergy, grain processability, and GMO testing on a single easy-to-use platform. Based in the United States, with global sales offices in Brazil, China, and Austria, we are committed to growing with customers by providing and developing quality testing solutions that drive critical decision-making and benefit our customers' operations.

Impextraco

<https://www.impextraco.com>



Impextraco develops & produces feed ingredients that protect animal health and enhance productivity. Our goal is to deliver cost efficient solutions while respecting animal welfare and sustainability. At Impextraco, we ensure you get feed additives that enable you to meet today's demands for healthy and safer food products. Healthy and safe animal products. Minimal environmental pollution. The animal production industry is facing new requirements from the consumer and the regulatory authorities. We at Impextraco, a leading European company, have anticipated this trend several years ago and developed different product solutions that focus on gut health, toxin mitigation and cell protection. Our specialty additive solutions provide functional feed ingredients that are all widely proven *in vivo* with a clear understanding to prove their economical benefit. To make sure you get the best feed ingredients possible, we strongly emphasize on quality control. Our essential ingredients are a comprehensive range of products, including vitamins, micro minerals, amino acids, antioxidants, growth enhancers, anticoccidials, colouring agents, enzymes and organic acid and their salts. All enhancing the nutritional value of the feed in order to bring superior performance to your animal production. Our production, research, warehousing and distribution facilities comply with the highest EU and international quality control standards, such as GMP. They meet the most rigorous quality checks, physical inspections, manufacturing best practices and comprehensive traceability requirements. On top of these practices, we maintained a high flexible organization that facilitates the needs of our customers. What and wherever your needs are, you can safely rely on Impextraco's full commitment to your success.

Olmix

<https://olmix.com>



Olmix is a global company specialized in developing, producing and distributing high value biosourced solutions for livestock and crop farming. Thanks to its 30 years of innovation and its range of finished products and ingredients, Olmix is a privileged partner of farmers, distributors and agri-supply manufacturers on an international scale to enable intelligent, sustainable agriculture. Founded in France in the Brittany region, Olmix employees more than 850 people and operates 10 industrial sites, including a biorefinery specialized in algae processing and 5 R&D centres worldwide. Its animal care division develops innovative technologies to improve animal welfare and hygiene, reduce mycotoxin risks, improve digestive efficiency and boost animals' natural defences while combining economic and environmental performance. In the field of mycotoxin risk, its patented technology, the constant investment in proving the mode of action in well renown research centres and the tools and services offered had led the company to a leading position and a reference in the market .

Alltech

(<https://www.alltech.com>)



The evolution of mycotoxin management strategies and beyond

Founded in 1980 by Irish entrepreneur and scientist Dr. Pearse Lyons, Alltech delivers smarter, more sustainable solutions for agriculture. Our products improve the health and nutrition of plants and animals, resulting in more nutritious food products for people, as well as creating a lesser impact on the environment. With expertise in yeast fermentation, solid-state fermentation, mycotoxins, and the science of nutrigenomics and metabolomics, Alltech is a leading producer of yeast additives, organic trace minerals, feed ingredients, premix and feed. Headquartered in Kentucky, USA, Alltech has a strong presence in all regions of the world, commercially and scientifically, with four bioscience centres and 7 more than 20 research alliances with academic partners, uniting a network of more than 150 scientists. Our 5,000 team members worldwide believe in 'Working Together for a Planet of Plenty™'. By using 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. At Alltech, we believe that effective mycotoxin management is about seeing the whole challenge, from the farm to the feed mill and from risk assessment to feed management. Using a combination of modern management tools, the Alltech® Mycotoxin Management Program provides a complete holistic solution to help producers take control of mycotoxin contamination. The program is built around class-leading risk identification technology, data analysis and insights, and mycotoxin binder solutions designed to reduce the damaging effects of mycotoxins on animal health and production potential. A robust research and development program has helped maintain strong scientific stewardship and leadership through in-depth interaction with key experts in the field. This allowed us to develop a multi-faceted exploration of mycotoxins' impact on animal systems and remediation, using *in silico*, *in vitro*, *ex vivo*, *in situ* and *in vivo* methodologies, pushing the frontiers in search of successful mitigation strategies.

Waters Corporation and VICAM

<https://www.vicam.com>



Leaders in mycotoxin testing solutions

Waters Corporation, a global leader in analytical technologies, and its subsidiary VICAM specialize in advanced solutions for mycotoxin detection. With decades of expertise in chromatography, mass spectrometry, and immunoassay-based methods, Waters and VICAM provide reliable, high-performance tools that help food and agricultural industries ensure compliance with global safety regulations. VICAM, renowned for its rapid and sensitive mycotoxin detection solutions, offers a comprehensive range of immunoaffinity columns, lateral flow strip tests, and fluorometric and HPLC-based methods. These tools enable accurate and efficient screening of mycotoxins such as aflatoxins, ochratoxin A, fumonisins, and DON across diverse food and feed matrices. Waters complements VICAM's portfolio with cutting-edge LC-MS/MS and UPLC solutions, providing laboratories with highly sensitive, quantitative mycotoxin and emerging toxins analyses. Together, Waters and VICAM support global food safety efforts by delivering innovative, easy-to-use, and regulatory-compliant testing solutions that help producers, regulators, and researchers protect public health and maintain product integrity.

Charm Sciences

<https://www.charm.com>



Charm Sciences is the global leader in providing rapid food safety diagnostic tests across many industries. Our reputation was achieved by our commitment to our customers' needs and by developing reliable, simple, and innovative technologies that meet different regional guidance and regulations around the world. Vertical integration is a hallmark of our business, with reagent and equipment manufacturing, software design, and firmware design located in one of our three manufacturing facilities in Massachusetts, USA. As food safety risks evolve, Charm offers customized solutions to develop faster, user-friendly test solutions to proactively prevent contamination. Charm's portfolio includes test kits and systems for pesticides, alkaline phosphatase, pathogens, end product microbial assessment, allergen control, water quality, antibiotics, ATP sanitation verification, and mycotoxins. Our Rosa® portfolio includes aflatoxin, DON, fumonisin, ochratoxin, T-2/HT-2, and zearalenone tests with options using water-based single extraction for

multiple toxins. Cutting-edge data management allows customers to link test results with real-time corrective action. Charm products serve the food, beverage, water, pharmaceutical, medical, personal care, environmental, and industrial markets in more than 100 countries. Our goal – and source of pride – is to provide peace of mind using Charm’s technology, meet our customers’ food safety needs and protect their brand.

ProGnosis Biotech

<https://www.prognosis-biotech.com>



Advancing mycotoxin determination with innovation and sustainability

ProGnosis Biotech is dedicated to high-performance mycotoxin detection, providing industries with fast, accurate, and reliable solutions to ensure food and feed safety. These ELISA and lateral flow tests help businesses comply with international regulations, reducing contamination risks and safeguarding consumer health. The Quantum and Symmetric Mycotoxin test series, with their ultra-fast quantification and common extraction protocols for all mycotoxins, offer efficient, reproducible results across diverse matrices. The ELISA Mycotoxin tests (Bio-shield and 1-Standard) provide an additional layer of high sensitivity and cost-effective screening, making it ideal for detecting mycotoxins in complex food and feed samples. The 5-minute Bio-Shield ELISA tests simplify workflows, supporting rapid, large-scale testing with uncompromised precision, while being USDA approved. Additionally, Bio-Shield M1 ES, designed for aflatoxin M1 detection in milk, has earned the ILVO validation, ensuring compliance with dairy safety standards. Committed to sustainability, ProGnosis Biotech integrates green chemistry into its Symmetric and Quantum Green series, replacing organic solvents with aqueous extraction methods, minimizing environmental impact and enhancing lab safety. With strong in-house R&D, the company internally develops its own antibodies, hardware, and software, ensuring full control over quality and innovation. Real-time and easily shareable results are achieved with the use of an Android mobile application and cloud-based connectivity, improving traceability and decision-making across the supply chain. Operating in over 80 countries, ProGnosis Biotech continues to shape the future of mycotoxin analysis, combining scientific expertise and technical advancements with sustainable, practical solutions.

Neogen

<https://www.neogen.com>



At Neogen, we partner with our customers to protect and enhance the world’s level of food and animal safety. By offering a diverse suite of solutions for the food, beverage, animal protein and agriculture industries, Neogen empowers our customers to safeguard their brands and create better products. Providing support from grains to finished goods, Neogen now offer more mycotoxin testing support than ever before. Our solutions combine accuracy and flexibility to help ensure reactive testing at all stages of production. From harvest and grain storage to processing and final products, we’re committed to supporting you with mycotoxin testing.

Pribolab

<https://www.pribolab.com>



Pribolab Pte. Ltd. focuses on analytical laboratory consumables and instruments for food and feed safety. Products cover mycotoxins, cyanotoxins and marine toxins, food allergens, GMO, food compositional analysis, vitamins, veterinary drug residues, etc. We adhere to technological innovation for high quality and professional products and services.

ELEVATE YOUR MYCOTOXIN MANAGEMENT

MYCOMAN, the all-in-one digital platform to:

Stay one step ahead and act proactively

Take faster and better-informed decisions

Secure animal performance and save costs

Benefit from Adisseo expertise



Anticipate mycotoxin contamination



Screen your feed



Protect your animals



Improve your process



Expand your knowledge



TRY IT OUT!

YOUR FOOD SAFETY CONCERN ***OUR DEDICATION***

Bioeasy as the global leading supplier of rapid detection in food safety since 2007, we have the widest ranges of test kits available, we can customize different detection limits and develop new test kit according to your local market demand. With good quality, high accuracy robust and affordable product, we help you to smooth your business trading all over the world!



Milk safety



Mycotoxin Screening



Antibiotic Honey Screening



Meat/Aquatic Product Free of Antibiotics



Pesticide Rapid Test



LECTURE ABSTRACTS

PLENARY SESSION THE ROLE OF DIGITALIZATION AND AI IN MYCOTOXIN RESEARCH AND MANAGEMENT

The scAInce of chemical risk assessment

Thomas Hartung^{1,2}

¹ Johns Hopkins Bloomberg School of Public Health and Whiting School of Engineering, USA

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Mycotoxins, toxic secondary metabolites produced by fungi, pose significant risks to food safety, public health, and agricultural economies. Traditional mycotoxin detection, risk assessment, and mitigation rely on labour-intensive, costly approaches. Artificial Intelligence (AI) presents transformative potential across multiple domains of mycotoxin research. AI-driven spectroscopy, imaging techniques, and machine learning models significantly enhance detection accuracy and sensitivity in analytical methods, while predictive algorithms analyse environmental and agricultural data to forecast contamination risks. In toxicology, AI facilitates *in silico* studies and refines risk assessments by integrating multi-omics data. For mitigation, AI accelerates discovery of detoxification methods and optimizes biocontrol agents. Supply chain applications include blockchain-based traceability and fraud detection. AI also supports regulatory harmonization through policy analysis and improved risk communication. Despite challenges in data availability and model interpretability, interdisciplinary collaboration between academia, industry, and regulatory bodies will drive AI innovation in mycotoxin research. By embracing these technologies, researchers can enhance food safety, reduce economic losses, and advance public health protection.

How can AI help mycotoxin research and management?

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Mycotoxins are a widespread global problem; they have been reported to be present in 60 to 80% of food crops and it is estimated that 25% of food crops are contaminated with at least one mycotoxin in concentrations above EU or CODEX limits. The effects of climate change, such as increased temperature and relative humidity seem to increase both the frequency and severity of mycotoxin contamination. Traditional management of mycotoxins includes sampling and analyses for mycotoxins, good agricultural and manufacturing practices, and applying HACCP procedures. But, given the changes in the food systems and its environment, mycotoxin management needs to be continuously improved. The use of predictive modelling as the basis for early warning systems is increasingly getting attention. In addition, lately, the use of artificial intelligence (AI) in various aspects of mycotoxin management is promising.

In this presentation, the state-of-the art of the use of AI in different fields of mycotoxin management will be provided, as well as an outlook to the future. The presentation will dive into the three different categories of using AI. First, the use of AI for predictive modelling of mycotoxins will be presented, both for pre-harvest and post-harvest applications, diving into how AI could be built upon classic prediction models, that are mainly based on weather variables such as temperature, and relative humidity. Secondly, novelties in predictive modelling will be presented, particularly the addition of satellite images to better predict mycotoxins pre-harvest, as well as the use of imaging analysis to detect mycotoxins both pre-harvest and post-harvest. Thirdly, the integration of AI with analytical detection of mycotoxins will be presented.

Harnessing AI for enhanced mycotoxin risk management strategies

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The integration of artificial intelligence (AI) in biosciences presents numerous opportunities for developing innovative tools to effectively manage mycotoxin risks. Our research leverages machine learning (ML) for mycotoxin forecasting, enzyme development, and on-farm efficacy testing.

Accurate mycotoxin forecasting enables optimal intervention strategies before harvest. Utilizing extensive mycotoxin occurrence data (>200,000 samples) and weather data, we have developed ML-driven empirical models that predict mycotoxin contamination several weeks in advance. For instance, our deoxynivalenol (DON) forecast model categorizes risk levels into four distinct categories: moderate, medium, medium-high, and high.

We also use machine learning based software tools for developing mycotoxin-degrading enzymes. These enzymes offer a promising solution for mitigating the adverse effects of mycotoxins, toxic compounds produced by certain fungi that contaminate crops, posing serious health risks to humans and animals. Industrially, the application of mycotoxin-degrading enzymes can enhance food and feed safety, reduce economic losses due to crop contamination, and improve overall supply chain integrity. The advent of new tools such as AlphaFold offers tremendous potential to accelerate the development of effective degrading enzymes for these challenging mycotoxins. AlphaFold's groundbreaking structure prediction capabilities save months to years of time and resources, providing an unprecedented leap forward in enzyme discovery. The optimism surrounding these advancements is well-founded, as machine-learning tools revolutionize the field, opening new horizons for creating highly efficient mycotoxin-degrading enzymes. Though this field is still in an early stage of development, we present insights from proof-of-concept studies on various mycotoxinases, demonstrating the potential and paving the way for future innovations.

We also employ precision livestock farming technologies in feeding trials to assess the effects of mycotoxins and the efficacy of our mycotoxin-degrading enzymes. Automated scales and feeders facilitate individual-level monitoring of growth and feed consumption over time. Furthermore, AI and computer vision are utilized for real-time monitoring of animal behaviour and key indicators of pig health and welfare.

The application of AI and ML supports a quicker development of more precise tools to predict mycotoxins, to study their effects and to develop strategies for counteraction.

From data to detection: AI-driven mycotoxin detection

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Mycotoxins are toxic compounds produced by fungi that pose serious risks to food safety, global trade, and public health. Traditional detection methods, while reliable, are often slow and resource-intensive, limiting their feasibility for large-scale screening. In recent years, artificial intelligence (AI) and machine learning (ML) have emerged as powerful tools for improving mycotoxin detection, offering rapid, cost-effective, and scalable solutions. This talk explores the latest AI-driven approaches to detecting and predicting mycotoxin contamination in crops, highlighting key advancements, challenges, and future directions. We discuss the role of spectral imaging, spatiotemporal modelling, and deep learning in mycotoxin analysis, along with the need for open data and reproducibility in ML research. By bridging

the gap between computational intelligence and agricultural safety, AI-driven mycotoxin detection has the potential to transform food safety practices and regulatory frameworks worldwide.

Harnessing Collective Intelligence (=AI x human expertise) for food safety in Africa

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This presentation explores how human expertise and AI can work together to enhance access to practical food safety knowledge in Africa.

The Fourth Industrial Revolution has brought unprecedented access to information, a trend accelerated by the rise of Large Language Models (LLMs). These models mimic natural language to answer questions, yet they often lack credible sources, rely on biased datasets, and provide uncurated information. As part of the Horizon Europe project 'Food Safety for Africa', we are addressing these shortcomings by developing a hybrid system that integrates a rigorously curated knowledge database with LLM technology. This database, built using AI-powered deep search and expert curation, focuses on key topics such as aflatoxins, pesticide residues, and food safety governance in Africa.

By combining verified knowledge with LLMs' natural language processing, we aim to deliver accurate, accessible, and credible insights to food safety professionals. However, challenges remain:

- Tacit knowledge – practical, experience-based food safety expertise – is difficult to capture and requires dedicated resources.
- Connecting existing knowledge repositories is essential to creating a broader, AI-readable ecosystem for food safety solutions.

As AI evolves – especially with the emergence of AI agents – integrating relevant databases and numeric data will be crucial for tackling complex food safety challenges, including mycotoxins and contamination risks.

INTERACTIVE DEBATE

AI IN MYCOTOXIN RESEARCH AND MANAGEMENT: GAME CHANGER OR JUST HYPE?

Across the entire business spectrum, from SMEs to large multinational corporations, people are racking their brains to solve major puzzle pieces:

- How to get AI work for you?
- Where do the biggest opportunities lie?
- What are the pitfalls and downsides of AI?
- When to say 'yes' (and 'no') to AI?

Moderators:

Prof. Rudolf Krska, Department IFA-Tulln, BKU Vienna, Austria

Prof. Chris Elliott, Institute for Global Food Security, Queen's University Belfast, UK

The floor will be open for a lively discussion involving:

Prof. Thomas Hartung, Johns Hopkins University, USA

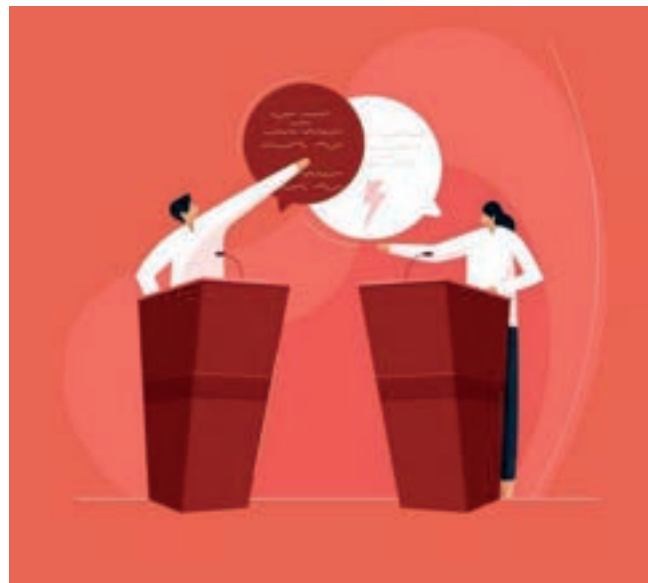
Prof. Ine van der Fels, Wageningen Food Safety Research, the Netherlands

Dr Ioannis Zachos, dsm-firmenich, Germany

Dr Alan Inglis, Maynooth University, Ireland

Peter Schelstraete, Ubuntu, the Netherlands

.....AND ALL PARTICIPANTS.



**PLENARY SESSION
BUILDING RESILIENCE IN REGIONAL FOOD SYSTEMS**

Mycotoxin awareness: Paving the way to food safety and Security in Central America

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Maize (*Zea mays*) is a staple in many Central American countries and it is known to be prone to mycotoxin contamination when improper management occurs during planting, harvesting, drying and storage. Some of these practices may be rooted in traditional agriculture that has been passed down from generation to generation. Therefore, an assessment on maize handling practices as well as an evaluation of mycotoxin occurrence and exposure in Guatemala and Honduras were conducted.

The agricultural practices survey revealed that farmers in this region prefer to plant criolla (native) maize (81-96%), although non-native varieties were also grown and consumed. Following harvest, sun drying is the most used method for moisture control. In Guatemala, 49% of farmers indicated mishandling of grain moisture as the main cause of losses, leading to insect infestation and fungal growth. Similarly in Honduras, farmers attributed their losses to inadequate drying and pest damage. Although aware of this, up to 28% of the producers in Honduras reported not performing preventive grain inspections in the course of storage. Moreover, 65% of farmers reported performing pest control during storage but only as a corrective measure.

Given that producers continue to rely on traditional practices, and corrective rather than preventive measures, the likelihood of low-quality product and risk for mycotoxin contamination is exacerbated. Indeed, in Guatemala aflatoxin was present in maize from all evaluated farms at 1.0 to 85.3 ppb, while fumonisin was detected on samples from 52% of the farms at 0.4 to 31.0 ppm. Average mycotoxin exposure, based on daily maize consumption, was above the recommended maximum intake for aflatoxin. Estimated daily intake was 0.01 to 0.85 µg/kg of body weight per day for aflatoxin and 2.9 to 310.0 µg/kg of body weight per day for fumonisin. In Honduras, fumonisins were detected in 97% of maize samples destined for human consumption at levels ranging from 0.25 to 41.0 ppm. Aflatoxins were detected in 17% of maize samples for human consumption with concentrations ranging between 1.0 and 490.0 ppb. The average probable daily intake of aflatoxins in Honduras ranged between zero and 0.26 µg/kg body weight/day, while for fumonisins the average probable daily intake fluctuated between 16.6 and 53.2 µg/kg body weight/day.

Efforts to raise awareness regarding mycotoxin contamination and education around the best agricultural practices to prevent mould growth and toxin production were made in both countries. To accomplish this, educational materials were developed in the local language using illustrations in which farmers and their families could identify themselves to help with engagement and adoption. Additionally, testing protocols were developed and equipment provided to establish testing centres in both countries. More than 250 local extension educators, university students and staff were trained with the goal of building capacity in the region regarding mould and mycotoxin prevention and testing. Information collected in both countries was also shared with local industry and government to help raise awareness around this issue. Only by engaging and educating all sectors of the food production and processing chains the quality and safety of maize consumed in Central America can be safeguarded, which is essential to ensuring food security in this region.

Mycotoxins and climate change: Challenges in South American countries for small farmers' production to increase resilience

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South America is a continent that extends from a broad equatorial zone in the north to a narrow sub-Arctic zone in the south, including tropical to temperate, arid, and cold climates. This variable climate is ideal for different types of fungal growth and mycotoxin production. Furthermore, the continent's economy is centred on exporting natural resources, including diverse food products prone to mycotoxins, like nuts, coffee, and cacao, and industrial crops, such as maize, wheat, soybeans, rice, quinoa, and cotton. South America is experiencing the effects of climate change, including extreme weather events and changes in temperature and precipitation patterns. Climate change is expected to increase and modify mycotoxin contamination, and only a slight elevation of CO₂ levels will stimulate the growth of mycotoxin-producing fungi.

In the region, there has been an increased risk for mycotoxin contamination of maize, wheat, and other grain species. Most studies have been conducted to find mycotoxins in grains and cereals, with an increasing analysis of aflatoxin M1 in milk. Most of them were the 'traditional and regulated mycotoxins' like aflatoxins, ochratoxin A, fumonisins, deoxynivalenol, and zearalenone, found with variable occurrences depending on the region, climatic conditions, and methodology used. Emerging and modified toxins like alternariol, beauvericin, enniatins, moniliformin, fusaric acid, and others have been studied only in Argentina and Brazil, where some studies have shown high occurrences.

Challenges observed in South American countries regarding mycotoxin research included increasing food surveillance, internal mycotoxin regulation, biomonitoring analysis, and enhancing transdisciplinary research. Effective connections and collaboration between disciplines and sectors in South American countries are urgently needed. Also, it was demonstrated that strategies to reduce mycotoxin contamination of foodstuffs require a multifaceted approach combining pre- and postharvest interventions. In this regard, farmers in most countries lack awareness and knowledge of mycotoxins' occurrence, control, and prevention and climate change's increasing effects on those risks.

Chile meets seven of the nine criteria for vulnerability to climate change. For this reason, the national agricultural sector is highly vulnerable to climate change. Empirical results reveal that the most effective way to reduce barriers to adaptation is to improve access to information, while a highly relevant mode to increase the intensity of adaptation is to encourage social networking. A current study in Chile concluded that farmers respond differently by engaging in proactive or reactive adaptation behaviour, concluding that developing and enhancing social connections and access to information will enable efficient and effective adaptation within agriculture. Efforts have been proposed to evaluate the attitude, norms, behaviour, and intentions of farmers in the O'Higgins region regarding food safety practices and increase resilience to emerging risks due to climate change through educational programs for small and medium farmers. We also intend to provide evidence-based information to design training programs, recommendations, and policies that encourage adopting food safety practices and improve farmers' knowledge and adaptive capacity to emerging food risks like mycotoxins.

Food Safety for Africa project, addressing food safety challenges in the African informal sector

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The World Health Organization and the African Union report that there are 600 million cases of foodborne disease globally, resulting in 420,000 deaths annually, and 91 million cases within Africa, leading to 137,000 deaths each year. The high burden of foodborne disease in Africa is largely attributed to the informal sector, where food safety mechanisms are not well established.

There are significant gaps in policies, awareness, monitoring, and enforcement within the informal sector, necessitating strategic and innovative interventions. To address these challenges, a recently established four-year EU HORIZON co-funded initiative, the Food Safety for Africa Project, is designing, testing, and implementing targeted interventions.

This project leverages various innovations, structures, and mechanisms, including the food convergence innovation approach, the implementation of emerging and well-established food safety practices through use cases in the informal sector, the development of food safety databases and a knowledge platform, and the mezzanine approach, among others. With the collaboration of 16 consortium partners from Africa, Europe and North America, the project aims to improve food safety across eight countries in Africa.

UP-RISE: Tackling mycotoxin challenges for inclusive and resilient food systems in Africa

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The EU-African Union (AU) Partnership for Resilient, Inclusive, and Safe Food Systems for Everyone (UP-RISE) is a Horizon Europe project aimed at improving and strengthening the African Food Safety System (AFSS). Mycotoxins pose significant health risks in Africa, including liver cancer, stunting in children, and immune suppression, while also causing economic losses due to reduced crop value and trade restrictions. By focusing on fermented food value chains of high economic and nutritional relevance that are susceptible to mycotoxin contamination - specifically aflatoxins, fumonisins, deoxynivalenol, zearalenone, and ochratoxin A - UP-RISE seeks to enhance food safety from farm to fork. The project led by Ghent University in collaboration with 13 EU and AU partners, aligns with the AU Food Safety Strategy for Africa, concentrating its efforts in three AU regions with fieldwork in five target member states: Benin, Côte d'Ivoire, Nigeria, Kenya, and South Africa.

With an estimated 75% of intra-regional food trade in sub-Saharan Africa occurring through informal markets, there is an urgent need to address food safety risks associated with limited infrastructure and capacity. UP-RISE is dedicated to collaborating with informal food business operators, providing science-based solutions to reduce mycotoxin occurrence, prolong shelf life, and minimize food waste.

UP-RISE is structured around five key building blocks: (i) roadmaps for a shared quality culture and possible integration of the informal sector in the AFSS; (ii) strengthening the food safety regulatory framework with focus on mycotoxins in both formal and informal sectors; (iii) early warning to prevent mycotoxin contamination and adapt to climate change; (iv) prevention of food losses and improving food safety by providing innovative microbiome-based solutions for mycotoxin reduction and nutritious food;

and (v) co-creation, training and mentoring. The project's co-created solutions will be demonstrated in five representative fermented food product value chains based on maize, millet, sorghum, and milk, and implemented in 10 SME business cases within the target AU member states.

Integrating social sciences with technological advancements, UP-RISE is further strengthened by an Accelerator Platform, comprising key stakeholders such as food safety authorities, farmers, consumers, and trade organizations. Long-term sustainability is ensured through the establishment of Training Hubs, the UP-RISE PhD Community, an AU-EU Microbial Biobank Network, early warning systems, a risk assessment toolkit, and a regulatory food safety model.

This presentation will provide an overview of the UP-RISE activities, with a particular focus on achievements from the first year, including value chain analyses of selected crops, crop sampling, and progress in business case selection.

Acknowledgements

The UP-RISE project received funding from the European Union under the grant agreement 101136649.

Climate-friendly, safe, resilience, and sustainable agri-food value chains in the Greater Mekong Subregion with a focus on mycotoxins

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The Greater Mekong subregion (GMS) was established in 1992 with the assistance from Asian Development Bank (ADB). It comprises of 6 countries, Cambodia, the People's Republic of China (PRC, specifically Yunnan Province and Guangxi Zhuang Autonomous Region), Lao People's Democratic Republic (Lao PDR), Myanmar, Thailand, and Viet Nam. The vision of GMS in 2030 is to have a "more integrated, prosperous, sustainable and inclusive subregion".

The GMS holds irreplaceable natural and cultural riches and is considered as one of the world's most significant biodiversity hotspots. The region is an important food provider in global terms and the site of many large-scale construction projects with social and economic implications. Currently, the GMS faces significant challenges in transforming its agrifood systems. While significant progress in addressing the needs and challenges of farmers and agrifood system stakeholders remains to be done, ongoing efforts in innovation and policy reform are paving the way for a more sustainable and resilient future.

The 3rd GMS Agriculture Minister's Meeting which was held in Kunming during 19-22 November, 2024, was a pivotal platform for discussing innovative and resilience approaches to agriculture in the face of multiple climate and environmental challenges. All member countries agreed on the proposed plan to promote higher food safety and quality standards for expanding exports; encourage climate- and environment-friendly production practices along the value chain, as well as sustain natural assets with a focus on small-scale farmers and micro, small, and medium-sized agro-enterprises; and support food security response and recovery efforts in the medium and long terms in lieu of the adverse impact of COVID-19 on agricultural supply chains.

Lastly, this presentation will explore details about emerging contaminants related to climate change in GMS region.

An integrated approach to reducing mycotoxins in Irish cereal grains

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Mycotox-I is all-Island project focused on developing tools to reduce mycotoxin risk in Irish cereals. This project is predominantly focused on oats due to its importance to Irish agriculture and the difficulties with predicting a problem in this crop (unlike wheat and barley, mycotoxin contamination often occurs in the absence of any visual symptoms of fungal contamination). A mapping study confirmed the hazard points in the production chain and from this we selected the most critical control points for monitoring and controlling mycotoxin contamination of grain and milled products. Standardised state of the art analytical tools were developed and used in all-Island surveys, in glasshouse and in field experiments to detect and quantify toxigenic fungi and mycotoxins in cereal produce. Field-based surveys determined the prevalence of mycotoxic fungi and mycotoxin levels in Irish cereal grains. Post-harvest surveys determined the prevalence of mycotoxins in milled grain products. Results from glasshouse and field-based studies are currently being used to the potential of integrated disease management systems to reduce mycotoxin levels under current and future climatic scenarios. Based on the survey results, consumer studies are assessing the risk of exposure. Using state of the art machine learning technology, and building on available tools and incorporating weather, varietal and input data, a new decision support system is currently being tailored for Irish agricultural systems in order to support growers and processors reduce the mycotoxin loads in food produce. The results to date within Mycotox-I as well as advances in enhancing host resistance to mycotoxin accumulation in Irish cereal grain will be presented.

PLENARY SESSION
SPEED PRESENTATIONS

Short presentations (5-minutes) by selected poster presenters to provide an overview of their research.

The abstracts can be found in the section 'Poster abstracts' (pages 87-163).

P36

Searching the mitigation silver-bullet by understanding aflatoxin contamination and drought stress in crops

Baozhu Guo

Crop Genetics and Breeding Research Unit, USDA-ARS, USA

P42

Combined effects of arsenic and aflatoxin B1 present in infant foods: analysis in different target organs

Esther Lima de Paiva^{1,2}

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P53

Knowledge centre for global food and nutrition security (KC-FNS)

Monica G.L. Ermolli

European Commission – Joint Research Centre, Directorate D, Sustainable Resources, Italy

P58

The use of mycotoxin binders: A sustainable strategy to support the feed and dairy industry

Donato Greco

Institute of Sciences of Food Production, CNR, Italy

SESSION 1 MYCOTOXINS IN ONE HEALTH PERSPECTIVE – PART 1

Hazard assessment of mycotoxins for humans and animals: a European perspective in the light of climate change

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Because of their harmful effects and their common occurrence in food and feed, it is necessary to establish toxicological reference values for humans but also for the different farm and companion animal species exposed to these contaminants. At the European level, the establishment of these toxicological reference values is performed by the European Food Safety Agency (EFSA) and published in various scientific opinions. To characterize the hazard, published data on their toxicokinetics and toxicity are scrutinized to determine the human health-based guideline values (HBGVs) and to identify reference points (RPs) for animal category and species. We will describe how these values are constructed and summarizes the human tolerable daily intake (TDI) for the *Fusarium* toxins (deoxynivalenol, nivalenol, T-2 and HT-2 toxins, zearalenone and fumonisins) but also for ochratoxin A, and ergot alkaloids. For carcinogenic mycotoxins such as aflatoxins, it is not possible to establish TDI and the risk for human health is determined by the margin of exposure between the benchmark dose's lower confidence limit (BMDL) and the exposure. We will also be detailed, when they could be determined, the RPs for these mycotoxins for ruminants (dairy cow, heifer, beef cattle, steer, sheep, goat), poultry (chicken, duck, turkey), porcine, solipeds, fish, rabbits, cats and dogs.

To conclude the difficulties and challenges involved in establishing TDIs and/or RPs will be addressed in light of climate change. We will especially discuss (i) the lack of data (for example on emerging mycotoxins), and their quality, (ii) the availability of species- or sex-specific data, (iii) the consideration of clinical cases or a new, more sensitive endpoints, and (iv) the establishment of grouped TDIs/RPs and the consideration of possible interactions between mycotoxins.

Differential diagnosis of mycotoxicosis is key for effective risk management

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Mycotoxicosis is a toxic condition in animals caused by consuming feed contaminated with mycotoxins, which are toxic metabolites produced by certain fungi. Depending on the amount and the duration of the toxin intake, mycotoxicosis can be expressed as acute, subacute and chronic toxicity. The concentrations of mycotoxins required to cause these conditions can vary based on the animal species and the mycotoxin in question. For example, deoxynivalenol (DON) can induce noticeable intake depression symptoms of DON toxicity in pigs are obvious, poultry continue to consume DON-contaminated feed for longer duration leading to much severe systemic toxicity.

One of the most difficult aspects of mycotoxicosis is the lack of classical animal symptoms. This makes it harder to diagnose the challenge timely leading to economic losses. To avoid such losses, animal producers must take the help of veterinarians, nutritionist and farm managers to conduct differential diagnosis (DD) of mycotoxicosis. A differential diagnosis is a list of possible conditions that share the same symptoms in the animals. This list is not the final diagnosis, but a theory as to what is potentially can cause these symptoms. The table indicates DD for gizzard erosions observed in poultry.

Gizzard erosion causes	
Biogenic amines	Histamine 'black vomit' (fish and fish products)
Gizzerosine	Overheated fish meal
Mycotoxins	DON, T-2, fumonisins, CPA, others
Vitamin deficiencies	B1, B6, E
Rancid fats	Extended storage of fats under unsuitable conditions
<i>Clastridium perfringens</i>	In broiler chickens
Chemical/physical damage	Self-explanatory
Adenovirus-associated causes	Various strains

Many more mycotoxicosis conditions that deserve DD and the potential diagnostic tools that can help in the final confirmatory diagnosis will be discussed.

Citrinin in human urine and blood - sources of exposure and impact of food processing

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A discrepancy seems to exist between the high occurrence rate of citrinin and its metabolite dihydrocitrinone in human urine and its occurrence in food in Europe: out of 1000 samples analysed for citrinin, only 8% were tested positive for citrinin [1]. Among these, most were cereal- based, with processed samples showing significantly lower citrinin levels than unprocessed ones. In contrast, recent studies have found occurrence rates of citrinin and its metabolite in over 50% of urine samples from adults in Belgium and Germany [2,3]. Given the relative short biological half-life of approximately 8 hours [4], these findings suggest a more frequent exposure to citrinin than would be expected from food analysis.

Possible explanations for this disparity include the formation of modified or matrix-bound citrinin through reactions with food components or by dimerization. Notably, covalent binding of citrinin to carbohydrates or reactive side chains of amino acids may play a significant role, potentially leading to hidden sources of citrinin [5,6]. Such bound forms of citrinin could access the gastrointestinal tract, where intestinal enzymes or the gut microbiome may cleave these bonds, liberating the parent compound or its derivatives.

The different reactions of CIT with food constituents, starting from model systems to classical food production are presented. These data are combined with results from food sample analysis and compared with food data from high exposure regions, such as Bangladesh.

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Silent threats: Can mycotoxins fuel neurodegenerative diseases?

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Neurodegenerative diseases (NDs), including Alzheimer's disease (AD) and Parkinson's disease (PD), pose significant global health challenges, with increasing prevalence and socioeconomic burden. While genetic factors contribute to disease risk, environmental and dietary exposures to toxicants are modifiable risk factors for disease progression. Among these, mycotoxins are prevalent and unavoidable dietary contaminants, posing a significant risk due to chronic lifetime exposure through contaminated food sources. While their hepatotoxic, nephrotoxic, and carcinogenic effects are well-documented, emerging evidence suggests they also contribute to neurotoxicity. Mycotoxins can cross the blood-brain barrier (BBB), accumulate in brain tissue, and disrupt central nervous system (CNS) function.

This presentation examines the neurotoxic effects of major mycotoxins, including ochratoxin A (OTA), aflatoxin B1 (AFB1), and T-2 toxin, and their potential role in neurodegenerative diseases. Growing epidemiological evidence links dietary and environmental mycotoxin exposure to increased AD and PD risk. Molecular mechanisms underlying mycotoxin-induced neurotoxicity are discussed, focusing on key pathways such as BBB disruption, oxidative stress, mitochondrial dysfunction, reactive oxygen species (ROS) overproduction, neuronal apoptosis, chronic neuroinflammation via microglial and astrocyte activation, and neurotransmitter dysregulation. Additionally, toxicological interactions between mycotoxins and other food contaminants will be explored.

Advancements in toxicological evaluation, including new approach methodologies (NAMs) and adverse outcome pathways (AOPs), provide promising tools for assessing food contaminant-related neurotoxicity under realistic exposure scenarios. Addressing the cumulative and synergistic effects of food contaminant mixtures remains a critical research and regulatory priority. This presentation aims to provide a comprehensive overview of current findings, emphasize the need for further research to confirm causality, and identify potential interventions against mycotoxin-induced neurotoxicity.

Exploring links between multi-mycotoxin exposure and colorectal cancer through human biomonitoring and genotoxicity tests

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Research has shown that dietary exposure to mycotoxins, which are mainly found in cereal-based products, vegetables, non-alcoholic beverages, fruits, nuts and seeds, has the potential to disturb gut health, particularly affecting the intestinal epithelium, and disturbing the balance and metabolism of gut microbes. Additionally, *in vitro* and *in vivo* experiments have demonstrated that certain mycotoxins such as deoxynivalenol, are able to exacerbate DNA damage induced by colibactin-producing *E. coli* strains [1]. To expand on these pioneering findings, this research sought to explore the link between the exposure to multiple mycotoxins and colorectal carcinoma (CRC). First, 1,900 plasma samples of CRC cases and matched controls, originating from the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, were analysed on 39 different mycotoxins using protein precipitation with acetonitrile followed by ultrahigh performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS, Xevo™ TQ-XS). A subsequent step involved the additional screening for aflatoxin-lysine, a validated biomarker for chronic exposure, able to provide insights into the long-term exposure, which is of particular interest in the development of CRC. Next, retrieved mycotoxins were screened for

genotoxicity by the formation of micronuclei on human colorectal adenocarcinoma (caco-2) cells using flow cytometry. Finally, correlations between both single and multiple mycotoxin exposure, and the development of CRC, was assessed using principal component analysis (PCA) in RStudio. Resulting biomonitoring showed the presence of ochratoxin A, enniatins, citrinin, tenuazonic acid, deoxynivalenol, cyclopiazonic acid and aflatoxins, quantified at ppt-ppb level. Out of the latter, genotoxic potential was found for deoxynivalenol, tenuazonic acid and aflatoxin B1. Finally, genotoxicity results and results of the PCA analysis, will be presented at the conference.

Acknowledgements

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Unravelling the synergistic impact of exposures to mycotoxins and Epstein Barr virus at early stages of Burkitt lymphomagenesis.

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Burkitt lymphoma (BL) is an aggressive malignancy, with its endemic form (eBL) strongly associated with Epstein-Barr virus (EBV). However, EBV infection alone is insufficient for carcinogenesis, suggesting the involvement of additional cofactors. In regions where BL is endemic, children experience chronic exposure to mycotoxins, starting in utero and persisting throughout life. While both mycotoxins and oncogenic viruses are known to modulate immune responses, impact the epigenome and increase cancer risk, their combined impact on eBL pathogenesis and its underpinning mechanisms remains poorly understood.

We employed a dual approach to investigate the interplay between EBV and mycotoxin exposure:

- Molecular epidemiology approach – A longitudinal mother-child cohort in rural Burkina Faso was established to assess early-life exposure to mycotoxins and EBV infection. Ochratoxin A (OTA) was the most prevalent mycotoxin detected and significantly associated with EBV infection. Genome-wide methylation analyses of infant blood revealed 1,615 differentially methylated regions (DMRs) linked to OTA and 8,663 DMRs associated with EBV, with combined exposure amplifying epigenetic changes. Pathway enrichment analyses revealed the association of differentially methylated genes with pathways related to immune regulation and cancer, with notable overlap with BL-associated methylation patterns.
- Functional studies in B cells and murine models – Transcriptomic profiling of B cells exposed to aflatoxin B1 (AFB1) revealed upregulation of the chemokine ligand 22 (CCL22), an

immunomodulatory molecule. Further mechanistic studies using *in vitro* B cell cultures and humanized mice model demonstrated that combined exposure to AFB1 and EBV synergistically stimulated CCL22 via NF- κ B activation. EBV latent proteins, including LMP1, were key contributors to this effect. Importantly, CCL22 upregulation enhanced EBV infection and viral gene expression, while neutralizing CCL22 function *in vitro* and *in vivo* significantly restricted EBV infection and dissemination.

Our findings highlight novel mechanisms by which mycotoxin exposure and EBV infection interact to promote early immunological and epigenetic alterations relevant to eBL development. This might corroborate what happens in African sub-Saharan regions where children are first exposed to mycotoxins including AFB1 and OTA through food contamination that could deregulate their immune response and increase their susceptibility to EBV infection and EBV-associated diseases. This would explain in part the high prevalence of eBL in those regions.

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Renal excretion of deoxynivalenol and T-2/HT-2 toxin in humans in an everyday situation

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Deoxynivalenol (DON), T-2 toxin (T-2) and HT-2 toxin (HT-2) are mycotoxins that can contaminate food, especially cereals. Exposure to the mycotoxins has mainly been estimated using dietary exposure assessment, however, human biomonitoring (HBM) presents another valuable approach. Currently, both exposure assessment approaches present uncertainty. Combining data from both approaches is a comprehensive strategy to obtain a better understanding of real-life exposure and it can provide insights into the strengths and limitations of the exposure methods. The aim of this research was to explore the relationship between daily intake and urinary excretion of DON, T-2 and HT-2 over time in a group of adults.

The mycotoxins, DON, T-2 and HT-2, and their predominant metabolites were analysed in 24-h urine samples of Norwegian adults using LC-MS/MS. Dietary exposure of DON, T-2 and HT-2 was calculated using 24-h weighed dietary records and concentration data in food. Statistical models were developed and fit to estimate the excreted fraction ($f_{\text{abs_excr}}$) and residence time parameters for DON and T-2/HT-2.

For DON, the estimated time in which 97.5% of the ingested DON was excreted as DON-15-GlcA was 12 h, and the elimination half-life was 4 h. Based on the estimated mean $f_{\text{abs_excr}}$ of 0.44 (equivalent to 44%), the mean reversed dosimetry factor of DON-15-GlcA was 2.28 which can be used to calculate the amount of total DON intake in an everyday situation, based on the excreted amount of DON-15-GlcA. For T-2/HT-2, the estimated time in which 97.5% of the ingested T-2/HT-2 was excreted as total HT-2 (HT-2 and its glucuronides) was 14 h, and the elimination half-life was 4 h. A mean $f_{\text{abs_excr}}$ of 0.18, equivalent to 18%, was estimated indicating that approximately 20% of the external exposure can be traced back in the urine within 24 h. A mean reversed dosimetry factor was not calculated for total HT-2 due to several uncertainties identified which require further investigation.

One Health approaches to mycotoxin research: Impact of beauvericin and enniatin B on aquatic ecosystems and global food safety

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Mycotoxins in agriculture and animal feed pose an increasing risk to human health, animal farm welfare, aquatic ecosystems, and environmental integrity – all within the One Health framework. Their impact in aquatic ecosystems achieves invertebrate populations set as excellent natural biomarkers. Studies on aquatic invertebrates, such as *Daphnia magna* and zebrafish (*Danio rerio*), indicate that mycotoxins like beauvericin (BEA), enniatin A (ENN A), enniatin B (ENN B), gliotoxin (GTX), and ochratoxin A (OTA) induce both acute and chronic toxicity. These effects include impaired survival, altered behaviour, reproductive challenges, and changes in gene expression. This study presents findings on the effects of BEA and ENN B, particularly their impact on heart rate and reproduction in *D. magna*, as well as their toxicity, influence on gene expression, and effects on growth. These disruptions can cascade through the food chain, ultimately affecting animal populations and human food safety. Moreover, co-exposure to multiple mycotoxins can lead to complex toxicological interactions, making it essential to assess both individual and combined effects. An integrated risk assessment is crucial, considering both food safety and environmental impact. Effective mitigation requires cross-sector collaboration and comprehensive management strategies. Multidisciplinary research is key to balancing resources for the health of humans, animals, and ecosystems.

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SESSION 2 MYCOTOXIN MANAGEMENT AND RISK MITIGATION – CURRENT STATUS, FUTURE OPPORTUNITIES

Approach to facilitate prioritization of natural toxins for risk management

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In its simplest form, risk is the product of hazard (i.e., toxic potency of a chemical) and exposure (or dose). 'Hazard-based' decision-making is based solely on hazard without any consideration of exposure. The development of mitigation strategies should prioritize mycotoxins that regularly occur at undesirable levels in commonly consumed commodities, wherein both the toxicological profiles and effectiveness of mitigation are understood with a reasonable degree of certainty. The International Life Sciences Institute Europe (ILSI Europe) Food Contaminants Task Force is firmly committed to contributing to the understanding of the issues of mycotoxins affecting the different points of the food chain.

This presentation will illustrate the final outcomes of a 2-year project, launched exactly in occasion of the previous World Mycotoxin Forum 2023, devoted to establishing a framework for the prioritization of mycotoxins found in food following a risk-based approach (Decision Tree). A specific case study was based on the following selected mycotoxins: ochratoxin A, deoxynivalenol, T-2/HT-2 toxins, zearalenone, ergot alkaloids, and aflatoxins. This was done in parallel to the correspondent identification of main grains (wheat) and grain-based product categories driving exposure of adults within the different EU countries. In particular, the risk prioritization has been conducted with the following main steps/objectives:

- calculate the contribution of each mycotoxin to the exposure from the main wheat-based food categories to overall exposure (all age groups except infants);
- calculate the contribution of each mycotoxin to the exposure from the main wheat-based food categories to toxicological HBGV (health-based guidance values) and POD (point of departure) (all age groups except infants);
- assign a severity score for the pivotal effect of each mycotoxin;
- scaling and prioritizing mycotoxins in terms of risk-ranking with respect to this calculated health impact for each wheat-based food category; and
- delineate the right path for and consequent mitigation opportunities.

Therefore, based on the scale of risk to consumers, and the potential for risk mitigation, this framework enables the differentiation between mycotoxins where risk management action is both warranted and likely to be effective based on available evidence.

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Left-censoring approaches to estimate mycotoxin concentrations in raw materials

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The objective of this study is to evaluate different left-censoring approaches for estimating key statistics related to mycotoxin concentrations in raw materials used for food production. The focus is on exploring reliable alternatives to commonly used substitution methods, such as the Medium (MB) and Upper Bound (UB) approaches, which are known to introduce bias in concentration estimations. Through extensive simulations involving different theoretical distributions, levels of censoring, and sample sizes, we assessed multiple left-censoring approaches and compared the results with those obtained from substitution methods. The findings from this study are the following:

- Substitution methods exhibit bias in estimating the mean, median, and percentiles, particularly when the censoring proportion exceeds 80% or when the sample size is small.
- Non-parametric approaches, such as Regression on Order Statistics (ROS), outperform other methods regardless of the censoring proportion or sample size.
- Most methods tend to underestimate the variance, but non-parametric approaches are less affected by censoring. Most methods demonstrate good performances in estimating the 75th and 95th percentiles.
- Parametric approaches tend to perform poorly, especially when the theoretical distribution deviates from the actual distribution. High levels of censoring can cause parametric methods to diverge, leading to less reliable results.

In addition to simulations, we identified real-world use cases involving mycotoxins (e.g., aflatoxins and T-2/HT-2) with varying levels of censoring. Although lacking a ground truth, we observed that the Lower Bound approach underestimated mean/median concentrations compared to non-parametric approaches. Conversely, the Medium and Upper Bound approaches overestimated concentrations relative to Regression on Order Statistics.

While estimating statistics with censoring levels above 80% is discouraged, certain statistical tools such as exceedances, copulas, bootstrap, and Bayesian statistics can provide valuable insights and are further investigated. We also discuss extensions to the ROS approach to account for the age of the data and improve estimations in cases of high censoring proportions. A novel approach to estimate sums of contaminants is proposed for cases when regulatory limits are on the sum of multiple contaminants.

The proposed methodologies can be applied to estimate concentrations of contaminants other than mycotoxins when high levels of censoring are present. They are particularly useful when reliable estimation of contaminant percentiles is required, such as in risk and exposure assessment scenarios.

A novel mechanism for DON detoxification

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The mycotoxin deoxynivalenol (DON) is a chronic problem in cereals in temperate areas worldwide. Above regulatory levels, DON contamination can result in significant economic loss both to the primary producer and the feed industry in terms of increased costs. Here we report the enzymatic biotransformation of DON to a novel stable metabolite by a soil-borne strain of *Bacillus subtilis*. Proteomic analysis of activity-enriched protein fractions from this *B. subtilis* strain identified the glycosyltransferase YjiC as the putative enzyme responsible for the observed DON biotransformation. Liquid chromatography high resolution tandem mass spectrometry and NMR spectroscopic analysis

demonstrated that YjiC glycosylates DON at the 7-hydroxyl position, producing the novel metabolite DON-8,15-hemiketal-7-glucoside (HKDON7G). In toxicity experiments, duckweed exposed to 20 µM HKDON7G showed no phytotoxicity when compared to DON. Stability testing of HKDON7G demonstrated that it is significantly more resistant to enzymatic and microbial hydrolysis compared to DON-3-glucoside. This study is the first to report a chemical modification to the 7-hydroxyl position of DON and presents a novel mechanism for the detoxification of DON-contaminated feed.

Novel enzymatic approach for decontaminating ochratoxin A in feed and food

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Ochratoxin A (OTA), a mycotoxin, has been shown to cause various adverse effects on animal health, including nephrotoxicity, hepatotoxicity, immunosuppression, neurotoxicity, and carcinogenicity. To enhance animal welfare and performance, we are developing enzymatic mitigation strategies against OTA in animal feed. Enzymes offer a highly specific and tailored solution for OTA degradation, ensuring the toxin is irreversibly broken down and cannot be reactivated.

This study presents two distinct approaches to identify novel enzymes capable of degrading OTA. In the *in-silico* approach, ligands structurally similar to OTA were used to computationally screen for potential proteins. Five candidate enzymes were empirically tested, and one was confirmed to degrade OTA (1). The second approach involved isolating enzymes from the lysate of the microorganism *Stenotrophomonas* sp. 043-1a, known for its OTA degradation ability. Enzymes in the purified lysate were identified via peptide fragment fingerprinting. Two enzymes, a metallo-dependent amidohydrolase and an S9-peptidase, were shown to catalyse the degradation of OTA into ochratoxin alpha, with the latter being the first reported member of its family with this activity (2).

Our most effective enzyme from *in vitro* assessments was subsequently tested *in vivo* in piglets by adding it to contaminated feed. After 14 days on the experimental diet, OTA accumulation in blood and kidneys was reduced by more than 50% compared to the control group without the enzyme. This represents, to our knowledge, the first reported *in-vivo* proof of concept for enzymatic OTA mitigation (3). Furthermore, the enzyme was used for detoxifying OTA in various food matrices.

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A novel enzyme for detoxification of fumonisins

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Fungi in grain and animal feed produce mycotoxins, posing a significant challenge for producers due to their harmful effects on both humans and animals. Fumonisins, among these mycotoxins, contaminate various foods and feed matrices, leading to substantial economic losses in the agri-food sector. While mechanical, thermal, or chemical methods exist for mycotoxin removal, they can generate dangerous by-products. Enzymatic detoxification presents a promising alternative. However, given the limited enzymatic fumonisin detoxification methods available, alternative approaches are necessary to effectively address this challenge in the agri-food industry. However, both the methods for searching such enzymes and the available benchmarks are limited. Here we introduce an enzyme for degrading

fumonisin, along with the metagenomic-based pipeline to develop it. The enzyme efficiently degrades fumonisin B1 (FB1) as well as fumonisin B2 (FB2) and fumonisin B3 (FB3), at different concentrations and under various conditions (1). Preferred conditions include a pH range of 5.0 to 8.0, temperatures from 30°C to 90°C, and fumonisins ranging from 1 ppm to 90 ppm in pure form and in naturally contaminated maize matrix. The enzyme's applications extend to raw materials, food and feed products. In terms of sequence, this enzyme is only less than 15% like the sole known enzyme capable of degrading fumonisin B1 toxin, FumD (WO2010031101A1). Coupled with its high performance in stability against pH and thermal conditions, and its effective degradation capability of fumonisins under real and industrial application conditions, it becomes a candidate for the development of FB1-free animal feed.

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Monitoring complementary *in vitro* and *in vivo* models is key for a proper anti-mycotoxin solution

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Since 2010, the European Food Safety Authority (EFSA) has established guidelines for assessing additives in the functional group substances for the reduction of feed contamination by mycotoxins. These guidelines conclude that efficacy can only be fully demonstrated through *in vivo* studies, as *in vitro* studies do not sufficiently replicate the conditions of the digestive tract, the differences in metabolism among target animals, or practical application scenarios. Instead, *in vitro* studies are primarily considered screening tools that may also provide insights into an additive's mode of action.

To compare *in vitro* and *in vivo* approaches, we conducted a binding assay using the same mycotoxin concentrations and additive inclusion levels as in a parallel broiler study that assessed performance and health biomarkers. Briefly, the mycotoxin binding protocol involved incubating a mixture of binder and buffer (pH 3) containing mycotoxins at 37°C for 3 h under constant agitation. After incubation, centrifugation separated the binder with bound toxins from the unbound fraction. The concentration of unbound mycotoxins in the supernatant, along with a control mycotoxin solution containing no binder, was analysed by HPLC. Binding efficiency was calculated using the following formulas: Amount of toxin in centrifuged solution (unbound)/ amount of toxin in the standard x 100= % unbound toxin 100 - % unbound toxin = % bound toxin. The same experiment was repeated at pH 6.5 with the binder containing the bound toxin (desorption procedure) and the % desorption is calculated. Efficiency is then calculated using the following formula: % bound toxin – % desorption.

In parallel, a 42-day *in vivo* study was conducted using 1,200 one-day-old Ross 308 male broilers, randomly assigned to six treatments with eight pens per treatment (25 birds/pen). The treatments included: a negative control (NC) with low residual mycotoxins, a positive control (PC) containing 814 ppb DON, 165 ppb ZEN, 96 ppb T2, 2,140 ppb FUM, and 32 ppb AFB1 (matching the *in vitro* assay conditions), and four groups receiving mycotoxin deactivators at different inclusion levels: UP and MD1 (1 kg/t) and UN and MD2 (1.5 kg/t). Zootechnical parameters and health biomarkers in blood, liver, and intestine were evaluated.

Results from the *in vitro* study showed strong binding efficacy for AFB1 (>99.8%), moderate binding for FUM (17.2-27.6%) and ZEN (7.7-25.4%), and minimal binding for trichothecene mycotoxins, such as DON (0.1-9.0%) and T-2 (1.6-5.7%). In the *in vivo* study, mycotoxin exposure significantly impaired feed conversion ratio (FCR) by 2.85% (NC vs. PC). Among the tested additives, UP improved FCR by 2.47% compared to PC, whereas MD1 showed no effect. Similarly, at a higher inclusion level (1.5 kg/t), UN improved FCR by 1.45%, while MD2 exhibited no improvement compared to PC. Overall, the comparison between *in vitro* and *in vivo* models in a mycotoxin co-contamination scenario showed no correlation between the two approaches. Thus, *in vitro* assays should not be considered predictive models for broiler performance in a co-contamination model.

Comparison of multi-strain and single strain biocontrol formulations for mitigation of maize aflatoxin contamination in the USA

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Aflatoxin contamination of maize grain causes significant economic losses in the southern United States, especially in the state of Texas. Though two aflatoxin biocontrol products have been commercially available in the USA for over two decades, neither one was developed specifically for maize, and both are based on a single non-aflatoxigenic strain. Four non-aflatoxigenic *Aspergillus flavus* genotypes that commonly occur in association with Texas maize were characterized and developed into a formulated multi-strain biocontrol product that was registered by the U.S. Environmental Protection Agency in 2023. The ability of mixtures versus individual biocontrol strains to displace aflatoxigenic *A. flavus* and reduce aflatoxin contamination of maize was compared in both the laboratory and field studies under different biotic and abiotic conditions. In the laboratory, antagonistic interactions among the multiple biocontrol strains reduced their overall growth and sporulation compared to individual strains, but this did not compromise their ability to competitively displace aflatoxin producers and reduce aflatoxin concentrations in inoculated maize grain. When co-inoculated on different crop hosts and at different temperatures, the dominance of individual biocontrol strains within mixtures varied, but overall displacement of aflatoxigenic *A. flavus* was similar across treatments. This suggests mixtures of biocontrol strains may be effective across a broad range of biotic and abiotic conditions due to differential adaptation of individual genotypes within the mixtures. Similarly, when the multi-strain biocontrol formulation was applied to maize fields, proportions of the soil and crop-associated *A. flavus* comprised of each genotype at harvest varied across years and regions with variable environmental conditions. Though the multi-strain formulation displaced aflatoxin producers and reduced contamination by over 80%, displacement was greater for single strain formulations. This was likely due to the dominance of the single strain formulation genotypes in the soil prior to application that had resulted from multi-year, area-wide applications in the region. Though additional years of sampling in treated fields are needed to evaluate long-term efficacy, the new multi-strain formulation developed for Texas maize provides producers with an additional tool to mitigate crop aflatoxin contamination.

Integrating environmental factors and machine learning to predict *Alternaria* mycotoxin contamination in tomatoes

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Tomatoes, a widely cultivated vegetable, are highly susceptible to *Alternaria* species infection during their cultivation period. Climate change further exacerbates this susceptibility by altering environmental conditions favourable to fungal development and mycotoxin production. *Alternaria* mycotoxins, including alternariol (AOH), alternariol monomethyl ether (AME), and tenuazonic acid (TeA), have emerged as significant threats to public health. This study aimed to develop a machine learning algorithm for predicting the contamination probability of *Alternaria* mycotoxins in tomatoes by integrating fungal growth and mycotoxin production simulation models. A dataset on *Alternaria* mycotoxin concentrations was collected from six individual tomato cultivations in China, based on three inoculation levels under various environmental conditions. Combined with data on agronomic factors and meteorological information, the biological contamination risk index was computed from fungal growth and mycotoxin

production simulation models. These were then linked with input data and multiple threshold limits to predict mycotoxin contamination probability. The dataset, collected in April and September, was split into a model training set (70%) and an internal validation set (30%). Using the training data, the random forest algorithm was applied to train the model. Data from May and June were used for external validation. The model gives a high prediction accuracy on the probability of the presence of each of the three *Alternaria* mycotoxins in tomatoes: 0.97-0.99 accuracy for internal validation and 0.67-0.82 accuracy for external validation, depending on the *Alternaria* mycotoxin type. The predicted specificity was sensitive to shifts in climate conditions and the threshold limits applied to classify contamination. To our knowledge, this is the first predictive model for *Alternaria* mycotoxins in tomatoes. With predictions on mycotoxin presence, this model could assist growers and advisors in maintaining the production of tomatoes, carrying out risk-based monitoring, and ensuring food safety.

SESSION 3 MYCOTOXINS IN ONE HEALTH PERSPECTIVE – PART 2

Global patterns and impact of acute aflatoxicosis: Insights from a systematic review

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Aflatoxins are mycotoxins produced by *Aspergillus* fungi in crops intended for food and feed. Acute exposure to high levels of aflatoxin B1, one of the most toxic mycotoxins, can result in severe poisoning, defined as acute aflatoxicosis, which manifests as acute hepatic failure followed by death in severe cases. Unlike chronic exposure for liver cancer, global burden estimates for acute aflatoxicosis remain non-existent, hindering effective risk management and prevention strategies. As such, this systematic review examined global evidence on the incidence, mortality, and symptoms of acute aflatoxicosis among humans from 1990 to 2023. A comprehensive search across multiple databases (PubMed, Web of Science, Embase, Scopus and INASP) and grey literature identified 11,539 references, of which nine studies met the inclusion criteria. Included studies varied in design, geographic region, population age, and aflatoxin analysis methods. Reported cases ranged from 1 to 317, with aflatoxin concentrations varying widely, from 10 to 51,100 µg/kg in food, from 36 to 209,000 pg/mg albumin in serum, and from 19 to 18,521 pg/g in tissue. Only one outbreak provided sufficient data to estimate an attack rate of 8 cases per 100,000. Mortality rates ranged between 16.2 and 76.5%, with the highest impact observed among children under 15 and adults over 40. Common symptoms included vomiting (77-100%), jaundice (88-100%), and abdominal pain (8-87%). Despite a generally low risk of bias, the variability in study methods and the lack of standardized reporting limited accurate burden estimation. Findings underscore the ongoing public health threat posed by acute aflatoxicosis, particularly in African countries, and highlight the urgent need for improved surveillance, standardized reporting, and strengthened early warning systems to mitigate its impact.

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Dietary exposure to deoxynivalenol negatively impacts the production performance of laying hens

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The negative impact of deoxynivalenol (DON) on broiler chicken is well documented. However, there is a scarcity of studies on laying hens exposed to diets naturally contaminated with this mycotoxin. Given the impact of DON on intestinal health, it's reasonable to anticipate that exposure to DON will also affect laying hens' production performance and egg quality. This study aimed to evaluate the effect of dietary exposure to a naturally DON-contaminated diet on laying hens' performance. 600 56-week-old Dekalb White laying hens were fed a marginally contaminated diet (control; CON) or a diet containing 2,450 ppb DON (DON) for a period of 16 weeks. The experiment had a randomised complete block design with two treatments and six replicates (i.e., pens). We prepared the DON-contaminated diet using a naturally contaminated corn batch, resulting in a multi-contaminated diet. However, DON was the predominant mycotoxin. The laying hens had *ad libitum* access to feed and fresh drinking water. Each replicate consisted of 50 hens in a pen. During the 16-week trial, some technical measurements were performed, i.e., feed intake per pen was recorded weekly, laying rate per pen was expressed per week, egg weight and mass were recorded per pen weekly, and feed conversion ratio (FCR) was calculated weekly. At weeks 64 and 72 of age, eggs (10 per pen) were collected for quality analysis, including egg yolk colour. Furthermore, samples of the intestines, eggshell gland, and ovaries of laying hens were collected for integrity and function analyses. There was a significant decrease in the egg yolk colour when the laying hens were fed the DON diet for 8- or 16-weeks, compared with control, and this was probably a result of low carotenoid levels in the DON-contaminated corn. There was no difference in relative organ weight or the number of large (8–35 mm) and small (5-8 mm) yellow follicles. However, after 16 weeks of exposure (72 weeks of age), a significant decrease in the number of large (1-4 mm) white follicles was observed. This may indicate a degeneration of the ovarian reserve. Finally, FCR was already impaired after 8 weeks of exposure to DON, and the same negative impact was observed after 12 weeks of DON dietary exposure. Although no differences in FCR were observed after 16 weeks of exposure, this recovery was not able to compensate for the losses observed in the overall experimental period.

One Health approach to mycotoxin surveillance: Biomarkers of subclinical fumonisins, DON, and zearalenone exposure in poultry

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The One Health concept provides a holistic approach for addressing mycotoxin problems among animals, humans, and the environment, thereby providing a comprehensive solution for tackling mycotoxin exposure. Mycotoxins are secondary metabolites present in both pre- and postharvest crops, feed, and food commodities. According to the Biomin 2024 mycotoxin survey, 90% of maize and maize by-products collected within the USA are contaminated with more than one mycotoxin. With maize being an integral part of poultry feed in the USA, the economic loss due to fumonisins (FUM), deoxynivalenol (DON), zearalenone (ZEN), and aflatoxin B1 (AFB) is estimated to be \$ 900 million per year. FUM, DON, ZEN, and AFB account for at least 95% of the confirmed mycotoxicosis in the midwestern USA. Mycotoxins are metabolized to various degradation derivatives in several organs. In poultry species, DON is metabolized to DOM-1, DON-3a-sulfate, DON-3-GlcAc, and DON-15-GlcAc, while FUM is metabolized to hydroxy-FUM B1. However, there is a challenge in quantifying DON metabolites using LC-MS/MS in broilers because their limits of quantification (LOQ) are well below in chicken blood, excreta, and chyme samples. Therefore, it is critical to find suitable biomarkers for DON toxicity in poultry. Further, LC-MS/MS is unable to determine phase I and II mycotoxin metabolites and interaction products for which commercial standards are lacking. In poultry, similar to DON, the serum sphinganine to sphingosine ratio has been identified as a biomarker of FUM toxicity. However, FUM contamination

was identified in the chicken feed, liver, and muscle; the birds did not show any clinical signs of mycotoxicity. Thereby questioning the validity of utilizing FUM content in the feed or biological samples as a biomarker for FUM contamination in poultry. This leads to underestimation of mycotoxin levels in foodstuffs and potentially increased health risks to consumers. MicroRNAs (miRNAs) have emerged in recent years as a promising new class of biomarker for monitoring toxicity. miRNAs have been identified as desirable molecular biomarkers of various toxins because miRNAs are responsive to acute environmental cues, often altering their expression prior to pathophysiological changes. We identified nine potential liver miRNAs: gga-let-7a-5p (14.17-fold), gga-miR-9-5p (7.05-fold), gga-miR-217-5p (16.87-fold), gga-miR-133a-3p (7.41-fold), and gga-miR-215-5p (6.93-fold) were upregulated in response to combined doses of 21.0 FB1 + 3.0 DON + 1.0 ZEA mg/kg diet. Further, significant increases of serum aspartate aminotransferase (AST) and 58% and 73% creatine kinase (CK) levels, and gut permeability ($p < 0.05$) in response to different concentrations of mycotoxins exposure well below the FDA guidelines. In conclusion, serum FITC-d, AST, CK, and liver miRNAs could serve as potential biomarkers for detecting combined mycotoxin concentration doses above 1 mg/kg diet in broiler chickens as early as d14. Thereby, miRNAs act as multipurpose biomarkers in toxicodynamics, and studying miRNAs will help to biomonitoring mycotoxin toxicity in poultry.

Absolute oral bioavailability and quantitative toxicokinetics of *Alternaria* mycotoxins in pigs

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The mycotoxins alternariol (AOH), alternariol monomethyl ether (AME) and tenuazonic acid (TEA), produced by *Alternaria* spp., are common contaminants of food and feed, and are a potential threat for animal and human health. An array of toxic effects have been reported, including cytotoxic, genotoxic, mutagenic, immunosuppressive and endocrine disruptive properties (1). Despite these data, the European Food Safety Authority (EFSA) could not yet set risk-based guidance values in food and feed, because of important data gaps. Instead, the European Commission issued indicative levels for AOH, AME and TeA above which investigations should be performed either on the factors leading to the presence of *Alternaria* toxins or on the effects of food processing (2). In particular, the presence in cereal-based foods for infants and young children should be monitored. To date, the most prominent data gaps for this comprehensive risk assessment concern information on the *in vivo* absorption, distribution, metabolism and excretion (ADME) and toxicokinetic behaviour of AOH, AME and TEA.

Therefore, we aimed to determine the absolute oral bioavailability, quantitative toxicokinetic characteristics and biotransformation of AOH, AME and TEA *in vivo* in pigs. Pigs are currently considered a suitable translational animal model for humans, including piglets for children. Cross-over toxicokinetic trials were performed with both intravenous (IV) and oral administration of AOH and AME at 2 mg/kg body weight (bw), and for TEA at 0.05 mg/kg bw. Plasma concentration-time profiles of these mycotoxins as well as their phase I and II metabolites in *vena jugularis* samples were studied using UPLC-MS/MS and LC-HRMS. Furthermore, plasma from the *vena portae* was analysed to evaluate presystemic biotransformation, a key factor in explaining low oral bioavailability. Urine was collected to determine both the urinary excretion and metabolite profiles.

Our results reveal a low absolute oral bioavailability of AOH (15%) and AME (9%), caused by a low absorption and/or extensive first-pass metabolism to mainly phase II, and to a lesser extent phase I metabolites. Quantitative toxicokinetic modelling of the IV data showed a high total body clearance (CL) for both AOH and AME (12.9 and 16.8 l/(h*kg bw), respectively), a high volume of distribution (V_d) (4.97 and 5.15 l/kg bw, respectively) and a short elimination half-life (t_{1/2el}) of 0.16 and 0.21 h, respectively (3). These AOH and AME toxicokinetic data are in sharp contrast to those for TEA. The latter showed a complete oral bioavailability, a low CL of 448.4 ml/(h*kg bw) and a low V_d of 325.8 ml/kg bw, resulting in a t_{1/2el} of 0.51 h (4). Using *in vitro* cytotoxicity as endpoint, it can be concluded that AOH and AME which have the highest cytotoxicity, show a low systemic exposure due to limited oral absorption and/or first-pass metabolism. In marked contrast, TEA – with a lower cytotoxicity – shows a high systemic exposure due to complete oral absorption and a limited metabolism.

These novel findings will contribute to the risk assessment of AOH, AME and TEA. Moreover, they can aid in the development of candidate biomarkers of *Alternaria* toxin exposure in biomonitoring studies.

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Delving into the field microbiome for mycotoxin mitigation: a case study for the control of *Fusarium* head blight of cereals

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Fusarium head blight (FHB) is a devastating fungal disease of small-grain cereals, including wheat, mainly caused by members of *Fusarium* spp. complexes. Beyond significantly reduced yields, the main consequence is grain contamination with mycotoxins, including type A and B trichothecenes and zearalenone, produced by toxigenic *Fusarium* spp. The predominant role of the interactions within the *Fusarium* communities as well as with members of the phytomicrobiome in disease onset and development has gained increasing attention. Understanding the diversity and dynamics of bacterial and fungal communities across different substrates colonized by *Fusarium* spp. in wheat fields can provide valuable insights into disease ecology and lead to the discovery of native microorganisms with biocontrol potential. In this study, the bacterial and fungal communities associated with soil, maize residues, and wheat grains, were studied based on metabarcoding sequencing of 16S rRNA and ITS2 regions in six wheat fields over two years and characterized by different levels of FHB disease pressure and mycotoxin contamination. Overall, the diversity and composition of microbial communities were primarily influenced by substrate type followed by geographic origins of fields and sampling time, notably for grains and residues while the soil microbiome was less impacted by environmental fluctuations. In addition, we found several taxa either strongly negatively correlated to *Fusarium* spp. and/or to levels of *Fusarium* DNA or mycotoxins in grains or residues, including *Cladosporium*, *Epicoccum*, *Paenibacillus*, *Curtobacterium*, *Pseudomonas*, *Pantoea*, and *Sphingomonas*, which could be potential antagonistic agents against *Fusarium* spp. Furthermore, we built a collection of over 1,600 bacterial and fungal isolates from the field microbiome. This entire collection was screened for antagonistic activity against several *Fusarium graminearum* strains, as well as *F. avenaceum* and *F. poae* (predominant species found in our samples) on wheat-based agar media and surface-disinfected wheat grains. We identified about ten bacterial and fungal isolates with antifungal activity, including species of *Pantoea* and *Epicoccum*, which were also among the potential biocontrol agents identified through metabarcoding data. The isolate *Trichoderma gamsii* proved to be the most promising candidate, with complete

inhibition of *Fusarium* growth on grains and residues, as well as suppression of mycotoxin production. Altogether, these findings provide novel insights into the field microbiome functioning and their complex interactions with the *Fusarium* communities, ultimately contributing to *Fusarium* suppression and mycotoxin mitigation.

SESSION 4 MYCOTOXIN CONTROL AT FARM LEVEL – TOWARDS PRECISION AGRICULTURE

Predicting the next outbreak: Models for *Fusarium* head blight and deoxynivalenol

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The collaborative effort to forecast epidemics of *Fusarium* head blight (FHB) and deoxynivalenol (DON) in wheat and barley has made considerable progress in recent years. Collaborative work supported by the USDA – US Wheat and Barley Scab Initiative includes researchers and extension specialists from many of the key wheat producing regions of the United States. The goal of this effort is to predict when and where FHB and DON are likely to emerge as significant production problems so that farmers can take action to manage the disease and minimize the risk of unacceptable levels of DON in grain. This team began developing predictive models more than 20 years ago. These initial modelling efforts were based on 50 observations of disease from 4 states. In recent years, the collaboration has expanded the dataset available for modelling to more than 1,200 cases. These observations incorporate information from additional production environments addressing conditions in more than 20 states. The current models also allow wheat growers to customize the estimates of disease risk to account for differences in winter wheat vs. spring wheat production systems, and the level genetic resistance present in the varieties of wheat they are currently growing. The continued effort to gather new observations also helps ensure the long-term stability of the forecasting models within changing climates.

Recent advances in model development focused on building ensembles of predictive models based on Random Forests (RF) machine learning algorithms. This modelling approach improved overall prediction accuracy of the forecasts relative to previous generations of modelling. The RF modelling approach yielded multiple models with sensitivity and specificity greater than 80%. The RF models also provided useful insights into weather patterns that favor the development of FHB epidemics. For example, variables describing the stability of temperature prior to crop anthesis were the most selected by the RF models indicating considerable predictive value of this information. Although the exact influence of temperature stability is unknown, it is likely that these periods of stable temperature are linked to cloudy days with frequent, light rainfall that favor disease development. FHB development is known to be sensitive to atmospheric moisture with severe outbreaks favoured by extended periods of wet conditions. This information was represented in the models in variables such as relative humidity, dew point and vapor pressure deficit, and these variables were critical predictors of FHB epidemics. The predictive models for FHB are currently deployed covering approximately 2/3 of the United States via the Fusarium Head Blight Prediction Center. These web-based tools provide daily estimates of disease risk in 35 states. There were also important advances with the web-based tools used to deploy the forecasting models. Recent user surveys of the forecasting system helped document the value of the information to small grain producers in the United States. These surveys indicated that 89% of the users thought that the information improved the profitability of their farm. The users also reported that the forecasting system helped them avoid unnecessary fungicide applications and estimated that the value of the information exceeded \$ 70 million (USD) annually.

The potential of spectroscopy techniques for *in situ* measurement and mapping of *Fusarium* head blight and DON in cereal crops

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Advancements in smart agricultural technology are providing powerful tools to address critical challenges in crop production. Among these, *Fusarium* head blight (FHB) presents a significant threat to cereal crops, reducing yields and contaminating grains with deoxynivalenol (DON), a harmful mycotoxin. Conventional approaches for detecting FHB and DON include manual visual inspections and laboratory techniques such as high-performance liquid chromatography (HPLC) and gas chromatography (GC). While accurate, these methods are resource-intensive, time-consuming, and impractical for large-scale applications. The need for innovative, scalable, and non-invasive solutions is evident.

Spectroscopy techniques, particularly hyperspectral imaging (HSI), have emerged as a promising alternative. HSI combines imaging and spectroscopy to capture detailed spatial and spectral information, enabling the identification of subtle differences in crop health. This work explores the potential of a mobile (on-line) HIS (350-900 nm) coupled with machine learning modelling techniques for prediction and mapping of FHB and DON at the field scale, addressing the limitations of traditional methods. A hyperspectral camera mounted on a tractor was used in wheat fields in Belgium and Lithuania to collect on-line spectral data across multiple wavelengths. Advanced machine learning models were applied to analyse the spectral data. For FHB detection, extra trees regression (ETR), random forest regression (RFR), support vector regression (SVR), and a one-dimensional convolutional neural network (1DCNN) were utilized, with ETR achieving 80% accuracy in predicting infected wheat ears. For DON classification, Light Gradient Boosting Machine (LGBM) and Decision Tree Classifier (DTC) were employed, with LGBM achieving an impressive 90.5% accuracy. The classification of DON contamination was based on thresholds aligned with European Union (EU) regulations, dividing contamination levels into three categories, namely, Class 1 for human consumption, Class 2 for animal feed, and Class 3 for biofuel production. This approach enabled precise spatial mapping of DON contamination hotspots, highlighting the immense potential of HSI for providing real-time, actionable insights. By integrating hyperspectral imaging with machine learning, this study demonstrates a scalable and non-invasive solution for FHB management, offering farmers tools to optimize harvesting strategies while ensuring food safety.

Detection of *Fusarium* head blight in winter wheat fields using imaging spectroscopy and deep learning

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Fusarium head blight (FHB) is a plant disease caused by various species of the *Fusarium* fungus. *Fusarium* spp. can produce mycotoxins in small grain cereals potentially posing a risk to human and animal health and leading to major economic losses. A reliable site-specific precise FHB early warning model is therefore needed to ensure food and feed safety by early detection of contamination hotspots, enabling effective and efficient fungicide applications, and providing FHB prevention management advice. Such precision farming techniques contribute to environmentally friendly production and a sustainable agriculture.

This study developed a predictive model for on-site FHB infection in wheat, using imaging spectroscopy and deep learning. Data were collected from an experiment farm in the Netherlands in 2021 composed of (i) experimental plot inoculated with *Fusarium* spp. (52.5m*3m) and (ii) control plot (52.5m*3m) not

inoculated with *Fusarium* spp. and sprayed with standard fungicides. Hyperspectral images were collected from both the experimental and control plots. Ground truth locations of *Fusarium* infected wheat ears and healthy ears were collected by visual observations by an expert. Deep learning approaches (pretrained YOLOv5 and DeepMAC on Global Wheat Head Detection dataset) were applied to segment wheat ears, and XGBoost was used to analyse the hyperspectral information related to wheat ears and make prediction of fusarium infected wheat ear and healthy wheat ear. Accuracy and F1 score were used to evaluate the performance of the predictive model. Deep learning approaches can detect and segment the ears of wheat by applying the pretrained models. The predictive model can automatically identify if a spot in the wheat field is infected or not with accuracy and F1 scores higher than 90%. Significant differences in spectral reflectance, for wavelengths between 600-800 nm were observed between infected and healthy wheat ears.

This model facilitates the early detection of *Fusarium* spp. at crop level, enabling the farmers to apply targeted control measures such as fungicide spraying, limiting subsequent mycotoxin contamination, and contributing to safe food and feed. Advice on efficient fungicide application will reduce chemical pollution in the water and soil for a sustainable farming system and reduce crop losses and increase food safety.

Rapid detection of plant pathogens and fungicide sensitivity to control mycotoxin contamination

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A number of plant pathogens, such as *Claviceps purpurea* (ergot) and *Fusarium graminearum* or *F. culmorum* (head blight or scab), produce mycotoxins in crops before harvest. Although some success has been made in selectively segregating ergots or *Fusarium*-infected grains from healthy grain post-harvest, it is still possible for mycotoxins to enter the food chain. An additional approach to improve crop protection can be made by detecting airborne spores that initiate grain infections in order to target fungicide use precisely or to identify high-risk locations. Airborne spores of these pathogens can be sporadic and difficult to predict using weather-based models. Infection by *C. purpurea* is prevented if pollination has occurred, while *Fusarium* infection can be prevented by earlier infection by non-mycotoxin-producing fungi, such as *Microdochium nivale*. Therefore, the precise timing of airborne inoculum in relation to predicted flower time, weather conditions and presence of other pathogens can be used to estimate mycotoxin risk.

Previously, air samples had to be sent to a lab for analysis by DNA extraction and qPCR but user-friendly and rapid testing is now possible using portable devices compatible with immunological or DNA-based assays such as LAMP. Appropriate DNA-based assays can also inform on fungicide resistance issues to allow selection of effective modes of action. Although LAMP assays have been published for *C. purpurea* and *F. graminearum*, those were for assays using wet reagents. For field-use in a user-friendly kit, it is necessary to stabilise some of the reagents into a solid, which can be kept in a sealed tube or microfluidic chip at room temperature for many months ahead of use. This can then be added to a liquid mastermix along with disrupted spores collected by an air sampler, such as a rotorod or high-volume cyclone. An additional filtering step can be used if a lot of soil or heavily pigmented dust is present to avoid inhibition of the assay.

Work is currently underway to test the dry LAMP assays for *Claviceps* and *Fusarium* species. Tests already completed on other pathogens (*Botrytis cinerea* and *Phytophthora infestans*) show how rapid on-site testing can improve crop protection. Automated systems are under evaluation using a range of methods such as lateral flow strips, LAMP assays and also microscope image recognition systems to identify airborne spores.

The trilogy in mycotoxin management – Prediction, detection and interpretation.

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Mycotoxin management is a complex issue that has producers questioning if ‘mycotoxins’ and ‘management’ should even appear in the same sentence. There are so many variables and a multitude of challenging factors today that producers must evaluate each time they plant a crop. Mycotoxin production in their fields is one challenge they hope they can avoid and not manage at all. Unfortunately, research in the past decade has indicated that future years will experience both increased toxin production and toxin production in more areas of the world, causing concern for all stakeholders.

Many predictive models have been developed and published in the past several years. There are a variety of approaches to predictive models. Each model has its own unique method of deriving predictions. Some are more regionally based; some have taken historical data from specific boundaries to formulate mathematical models or machine learning. All models have the same desired outcome: predict, preharvest, potential mycotoxin issues. Information on toxin potential allows producers/stakeholders to alter harvest methods, prepare for co-mingling possibilities, secure mycotoxin binders for animal production options, and make financial decisions on buying/selling/storing products. Prediction offers options currently not widely available.

This model uses a field-by-field approach to toxin risk prediction utilizing parameters specific to each geographic field. Toxin production in each field is a unique path each crop year depending on the specific parameters per field. While there may be factors that are key indicators of what is possible, such as weather patterns, crop/toxin history patterns, in this model, the specifics of each field are incorporated to predict the toxin probability by location. The predictive model presented focuses on the specific parameters of each field to serve as inputs for evaluating and then predicting the risk potential for mycotoxin contamination prior to harvest. With pre-harvest predictions, producers have the possibilities to make decisions on harvest practices, storage considerations and selling options before harvest.

SESSION 5

DRIVERS AFFECTING EXPOSURE TO MYCOTOXINS: WHAT'S UP, DOC?

Beyond farm to table: When climate and conflict add a risky flavour to your food

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Food should be many things – delicious, nutritious, and safe. But what happens when the journey from farm to table takes an unexpected detour through climate chaos and geopolitical turbulence?

In this presentation, we will embark on a whistle-stop tour of how climate change is (literally) cranking up the heat on staple crops, creating the perfect storm for mycotoxin contamination. From drought-stressed maize to heatwave-ripened wheat, rising temperatures and erratic weather patterns are turning food safety into a high-stakes game. Meanwhile, supply chain disruptions – courtesy of 'special operations', trade conflicts, and economic instability – are forcing producers and suppliers to take shortcuts, sometimes at the cost of safety. After all, when supply chains get tangled, risk is not just on the menu; it is the main course.

We will explore how these twin threats – climate change and global conflict – are shaping mycotoxin risk, why your morning coffee or that tempting chocolate bar might come with an extra undesirable flavour, and what we can (and should) do about it. So, if you're ready to go beyond the usual food safety narrative and dive into a world where risk assessment meets global upheaval, pull up a chair – just be sure to check for mould first.

Hidden yet far-reaching costs of conflict: Aggravated mycotoxin threats

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Conflicts worldwide not only cause profound human suffering but also exacerbate food security and food safety challenges. Many conflict-affected regions produce staple crops such as wheat, maize, and rice. As a result of conflict, global grain supplies have been disrupted. These disruptions force importing countries to seek alternative suppliers, or boost local production, often in tropical and subtropical areas where climatic conditions favour mycotoxin contamination of crops, particularly aflatoxins and fumonisins. Aflatoxins and fumonisins contribute to severe health issues, including liver cancer, stunted growth, and immunosuppression, while restricting market access for contaminated crops. Further, supply chain disruptions – such as blocked trade routes, transportation delays, and prolonged storage – further increase mycotoxin accumulation. If production to meet gaps is done in tropical regions, appropriate safeguards must be put in place. Integrated mycotoxin management strategies combining pre- and post-harvest interventions, improved storage, and enhanced monitoring can help ensure food safety. Scaling these strategies requires coordination among governments, agricultural organizations, humanitarian agencies, donors, and industry. Public-private partnerships, financial incentives for farmers, and expanded food safety policies in low- and middle-income countries (LMICs) can drive adoption of mycotoxin control measures. Emerging approaches like Food Convergence Innovation (FCI) integrate data, technology, and cross-sector collaboration to address food safety challenges. This systems-based strategy aligns technical, institutional, and policy solutions to ensure the production and distribution of safe, high-quality food. As global conflicts persist, prioritizing food safety alongside food security is critical to safeguarding public health, stabilizing markets, and building resilience against future disruptions.

Mycotoxin management to face climate change impact on food safety and human health, the match

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Climate change (CC) has become an increasingly paramount topic in relation to mycotoxin contamination of foodstuff; it interferes with the ongoing aim of a world without hunger and emphasises food insecurity in areas already exposed. In 2024, global temperatures exceeded the critical 1.5°C threshold, making concerns regarding food safety risks more consistent.

To face challenges related to CC, research efforts should focus on exploring topics that require further knowledge acquisition. The interaction between abiotic stresses, plant-fungi interaction and mycotoxin production, fungi co-occurrence and prevalence/emergence of certain fungal species, as along implementing biocontrol agents, are among the main research fields to be considered and combined with existing knowledge. Further, existing predictive models are gaining more interest among the stakeholders, and the transfer to other crops when the fungus is the same or the development of new models for new fungi, with potential expansion of validation to more countries, has become urgent.

A strong and multi-actor partnership is needed to contribute to (i) the prediction and mitigation of risk related to fungi and mycotoxin occurrence, (ii) the assessment of mycotoxins exposure in humans (concerning different diets) and animals, and (iii) the implementation of proper risk management measures. Artificial intelligence (AI) can be helpful and all data and algorithms could be optimally managed through an AI mycotoxin management Platform as reference support for all food system actors with tailored predictions, recommendations, and mitigation approaches. By using this platform, the agri-food researchers, farmers, industry stakeholders, and policymakers, involved through a multi-actor framework, will be assisted in taking threat-mitigation initiatives and in decision-making, both in the short- and strategic long-term planning.

This match, intended with the double meaning of (i) compete against, in this case adapt of be resilient to CC, and (ii) provide something suitable for a particular situation/purpose, intended as finding the best mitigation actions shared with all stakeholders, is the focus of the MYMATCH project, recently granted by the European Commission.

Influence of weather and climatic conditions during the growing season on the levels of *Fusarium* mycotoxins in oats in Norway

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Fusarium mycotoxins in cereals constitute major problems for animal and human health worldwide. The causative *Fusarium* mould species infect cereal plants in the field. Deoxynivalenol (DON) and T-2 toxin/HT-2 toxin are the *Fusarium* mycotoxins considered of most concern in Norwegian cereal grains. Whereas DON is primarily produced by *F. graminearum* and *F. culmorum*, T-2 and HT-2 are mainly caused by *F. langsethiae*, *F. poae* and *F. sporotrichioides*. As the various *Fusarium* species have different micro climatic optimum for growth and mycotoxin production, the local weather during the growing season, such as precipitation, relative humidity and temperature influence considerably on the *Fusarium* pattern and the levels of mycotoxins produced. Of particular importance are shown to be weather conditions during the period of cereal anthesis as well as the last couple of weeks before harvest. Of the cereal species cultivated in Norway, oats are clearly the most contaminated with DON and T-2/HT-2. The Norwegian Veterinary Institute in collaboration with the Norwegian Food Safety

Authority have conducted annual surveillance programme on mycotoxins in cereal, with main emphasis on oats, since 2002. Samples are collected from the main cereal cultivation areas in the southeast and mid Norway. The mycotoxin levels vary year by year without any temporal trends. The annual mean of DON in oats have varied from minimum 80 µg/kg (2022) to a maximum of 2,400 µg/kg (2012). The corresponding mean of T-2+HT-2 in oats varied between 60 µg/kg (2008) to 390 µg/kg (2005). The relation between mycotoxin concentrations in oats and the local weather conditions during the growing season through these 22 years will be presented and discussed.

A Survey of mycotoxins and other natural contaminants in plant-based alternatives for meat- and dairy products

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Plant-based alternatives for meat and dairy products are becoming more and more popular, even among non-vegetarians and non-vegans, due to health benefits and a decreased carbon footprint. However, these beneficial aspects might be outweighed by the fact that mycotoxins and other natural contaminants such as tropane alkaloids are frequently present in raw materials such as soybeans, peas and other legumes (1,2). Indeed, an increased exposure to ochratoxin A of vegans compared to omnivores has been found recently (3).

However, regulatory limits are neither set for the abovementioned raw materials nor for the related products and there are only a few publications focusing on the occurrence of mycotoxins in plant-based alternatives sold on the market. All these studies are slightly limited by a generally low sample number and a focus on soy-based burgers, whereas occurrence data for products based on seitan, pea or fungal protein are even more scarce. In addition, the range of analytes only covers mycotoxins addressed by regulatory limits, *Alternaria* toxins and a few emerging mycotoxins like beauvericin and enniatins.

This study aims at filling the current gap of knowledge by applying an extended version of our multi-analyte method based on liquid chromatography coupled to tandem mass spectrometry (4) to an extensive set of finished vegan foodstuffs deriving from different raw materials. We will present the occurrence data of selected toxins and other secondary metabolites with highest prevalence, (as e.g. we have frequently found cytochalasins in raw soybeans) and which shall serve as a basis for a priority list for upcoming risk assessment.

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SESSION 6 MYCOTOXIN OCCURRENCE AND CONTROL – THE FOCUS ON FUNGI

Evolution of regulation of trichothecene toxin production

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The mycotoxins of most concern to food and feed safety include multiple *Fusarium* trichothecenes. Although trichothecenes produced by other fungi are typically of less concern, knowledge of the biochemistry and genetics of their biosynthesis can provide valuable insights into those produced by *Fusarium*. Comparisons of *Fusarium* and *Trichoderma* species indicate that the *TRI6* and *TRI10* genes act together to regulate transcription of other trichothecene biosynthetic genes (*TRI*) and thereby control where and when the toxins are produced. Genome sequence analyses of diverse fungi indicate *TRI6/TRI10*-mediated regulation of trichothecene biosynthesis is widespread and likely the ancestral condition. However, the analyses also indicate exceptions to or variation in *TRI6/TRI10*-mediated regulation. For example, trichothecene-producing fungi in the family *Clavicipitaceae* lack *TRI6* and *TRI10*. In addition, some *Fusarium* species have a third transcriptional regulatory gene, *TRI21*, that activates expression of *TRI* genes that are unique to *Fusarium* and that confer the ability to produce unique trichothecene analogues. In the most economically important trichothecene-producing species of *Fusarium*, however, *TRI21* has been lost and its regulatory function replaced by *TRI6* and *TRI10*. Although paralogs of multiple *TRI* genes occur in some trichothecene-producing fungi, paralogs of *TRI6* and *TRI10* occur most frequently. Phylogenetic analyses suggest that some of the paralogs have arisen by gene duplication while others have been acquired by horizontal gene transfer. Whether and how *TRI6* or *TRI10* paralogs within the same organism differ in function remains to be determined. Despite the marked variation in genetic regulation of trichothecene biosynthesis among fungal genera, available evidence indicates that single copies of *TRI6* and *TRI10* regulate biosynthesis in *Fusarium* species of major concern to food and feed safety.

Scope of taxonomic boundaries: From *Fusarium* genus to populations

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DNA-based phylogenetic methods have revolutionized the taxonomy of toxigenic and pathogenic species of *Fusarium*. Recently, DNA-based methods have also been used to investigate population biology of *Fusarium* species.

Fusarium graminearum is the most common cause of *Fusarium* head blight (FHB) and trichothecene (mainly DON) contamination in wheat, barley, and oats. It is also an important cause of mycotoxin contamination, seedling blight, and root, stalk and ear rot of maize. Previous analyses have identified significant differences in genetic variation as well as trichothecene chemotype frequencies among phylogenetically distinct populations of the fungus. Here, we describe results from recent investigations of *F. graminearum* populations. Based on genetic variation at variable number tandem repeat (VNTR) markers, we have identified two highly differentiated and geographically structured populations in Europe, hereafter referred to Eurasian population 1 (E1) and 2 (E2) (1). Isolates from northern Europe were almost exclusively from the E1 population and had the 3ADON (3-acetyl-deoxynivalenol) trichothecene genotype. They were also most common and produced highest levels of DON in oats (2).

In contrast, almost all isolates from southern and eastern Europe were from population E2, had the 15ADON (15-acetyldeoxynivalenol) genotype, and were most common and produced highest levels of DON in wheat. The E2 population also predominated in the Russian Far East, where 3ADON and 15ADON genotypes occurred at nearly equal frequencies. Population E1 harboured substantially less genetic diversity than population E2, indicative of a selective sweep or recent introduction and subsequent range expansion in northern Europe. When these two Eurasian populations were analysed together with previously described genetic populations from North America (NA1 and NA2), NA2 and E2 were identified as a single population, consistent with the hypotheses that NA2 was recently introduced into North America from Eurasia. Additionally, more than 10% of E2 isolates from the Russian Far East and southern Europe were closely related to North American population NA1, indicating recent introductions of NA1 into parts of Eurasia.

Based on the whole genome single nucleotide polymorphism (SNP) analysis of strains representing all major global wheat growing regions (3) there is a third Eurasian population of *F. graminearum* (population E3) that has 15ADON genotype and is sympatric with population E2 in western Europe. According to the latest global SNP analysis from all wheat-growing regions, there are at least five major populations of *F. graminearum*, which have been influenced by recent transcontinental introductions (4). Two of these populations (NA1 and NA3) likely originated in North America, two others (E3 and E2) likely originated by adaptation to wheat in Europe and Asia, and a fifth population (E1) likely originated by adaptation to oats in northern Europe. The newly designated E3 population, which is related to population E1, is abundant in Western Europe and Korea and is present in South Africa, Australia and South America. Long-range dispersal has likely distributed populations NA1, E2/NA2 and E3 on multiple continents.

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Unravelling the puzzle of ochratoxin A production

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Ochratoxin A (OTA) is a widespread mycotoxin that poses severe risks to human and animal health. Its presence in agricultural products results in substantial economic losses. OTA contamination impacts various commodities, including cocoa, coffee, grapes, wine, cereals, and cereal-based foods. It is produced by various fungal species, predominantly within the genera *Aspergillus* and *Penicillium*. Among these, *Aspergillus steynii* has attracted particular attention due to its ability to produce notably high levels of OTA. Controlling OTA contamination relies mainly on preventing mycotoxin production and detoxification. However, the current limitations of decontamination methods emphasize the need for more effective preventive strategies, which require a deeper understanding of fungal biology and the regulation of biosynthetic genes.

In this study, we investigated the functionality of OTA hal and OTA bZIP of *A. steynii* by determining its subcellular localization using bioinformatics, genetic tools, and fluorescence microscopy. Potential binding sites of OTA bZIP were predicted using AlphaFold3 to gain insights into its molecular interactions. To analyse its regulatory role, we employed a heterologous system in *Aspergillus nidulans*, where gene replacement at the *InuA* (inulinase) locus with *OTAh_{hal}* or *OTA_{bZIP}* from *A. steynii* was

achieved via PEG/CaCl₂-mediated transformation. Correct insertion was verified through PCR genotyping, and protein expression was confirmed by immunodetection. Fluorescence microscopy of GFP-tagged fusion proteins revealed distinct subcellular localization patterns; OTAhal::GFP was detected in the cytoplasm, while the transcription factor OTAbZIP::GFP was localized in the nucleus. Moreover, OTA bZIP was found to regulate the expression of the halogenase gene, confirming its role in OTA biosynthesis. The use of *A. nidulans* as a heterologous host demonstrated its potential for functional studies on secondary metabolism. These findings confirm the nuclear localization of OTA bZIP and its regulatory effect on OTA biosynthesis. Overall, these results contribute to a deeper understanding of OTA biosynthesis regulation, potentially aiding in the development of strategies to control mycotoxin contamination.

Acknowledgements

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Host induced gene silencing in reducing pre- and post-harvest aflatoxin contamination in maize

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Maize is susceptible to *Aspergillus flavus* infection and subsequent aflatoxin contamination both before and after harvest. Aflatoxins are the most potent naturally occurring carcinogens and consumption of aflatoxin contaminated food has been associated with increased liver cancer and other serious health concerns in human. Breeders in the past several decades made a lot of progress in identifying resistance. However, incorporating these resistance traits into commercial background has been proved to be difficult. While biocontrol using atoxigenic *A. flavus* to manage aflatoxin contamination under field conditions has gained popularity, its success depends a lot on the time of application and the environmental conditions. Here, we demonstrate that RNA interference (RNAi)-based Host Induced Gene Silencing (HIGS) can offer another approach to reduce aflatoxin contamination in maize. RNAi regulates plant growth, development and defence through sequence-specific degradation of transcripts of the target gene that are homologous to its small interfering RNAs (siRNA). RNAi not only can silence the expression of target genes within an organism but also can silence the expression of homologous genes in the pathogens that invade the organism. In our laboratory, *A. flavus* alkaline protease (Alk), polygalacturonase (P2c), O-methyltransferase (OmtA), and versicolorin dehydrogenase (Ver-1) genes were selected, cloned into a HIGS vector, and transformed into maize B104 line to determine whether the HIGS approach can be used to suppress the growth and toxin production by *A. flavus* during infection of maize. Our data show that homozygous transgenic lines in B104 background consistently reduce aflatoxin production up to 93.7% compared to the controls in our multi-year field inoculation studies. F1 crosses of these lines with elite inbred lines have also showed significantly enhanced aflatoxin resistance and supported less fungal growth and toxin production during post-harvest under aflatoxin production-inductive conditions, demonstrating HIGS is a viable approach to reduce aflatoxin contamination in maize and other susceptible crops. Currently, homozygous transgenic maize lines in elite background have been produced through at least five rounds of backcrossing and two rounds of self-pollination for further evaluation of their aflatoxin resistance and other agronomical traits under field conditions.

Mycobiota and mycotoxins on artisan Italian cheese aged in natural environments: Prevention and control strategies

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Ripened cheese is a very popular fermented food around the world, for which fungi are used as secondary starters to improve and typify sensorial traits. Traditional cheese, often made with artisanal and natural protocols, are perceived as more authentic and healthier than industrial products, and are characterized by spontaneous, and therefore uncontrolled, fungal colonization of cheese surface.

The mycobiota of natural environments is often a source of desirable fungi, but it can also be a source for toxigenic species contamination, including those able to produce ochratoxin A (OTA). Indeed, the contamination of Italian cheese by OTA is widely reported, and its occurrence is mostly related to the growth on the cheese surface of *Aspergillus westerdijkiae*, the most frequent species associated to OTA production. Because of fungal growth and OTA production in *A. westerdijkiae* is variable and can be affected by genetic and ecophysiological factors related to the cheese composition and environmental conditions, the prevention of contamination remains the most effective strategy to mitigate OTA occurrence.

Therefore, we developed early and efficient methods for the detection of *A. westerdijkiae*, based on LAMP and MS-Nose techniques. These tools allow a rapid risk monitoring of potential OTA contamination in aged cheese, with the aim to ensure safer and healthier products for consumers. In addition, we tested the effect of *Debariomyces hansenii* on cheese-like medium, confirming its capability to reduce both *A. westerdijkiae* growth and OTA production.

SESSION 7

SOCIETAL RELEVANCE AND IMPACT OF MYCOTOXIN RESEARCH

Mycotoxin in Africa: A research journey towards achieving food safety and nutrition security

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Mycotoxin contamination remains a major threat to food safety and nutrition security in Africa, particularly in staple crops such as maize, peanuts, and sorghum. Aflatoxins and other mycotoxins compromise human and animal health, reduce agricultural productivity, and cause substantial economic losses. This presentation explores ongoing efforts at Cranfield University conducted by the Applied Mycology Group, to mitigate mycotoxin risks in African food systems through interdisciplinary approaches, combining sustainable control strategies, technological innovations, and knowledge transfer activities.

The NutriNuts project (UKRI, 2019-2023) targeted aflatoxin reduction in Ethiopian peanuts used for therapeutic food products. Key interventions included a vertical passive solar dryer to replace traditional ground drying, a hand-operated sheller to eliminate the unsafe 'soaking' method, and the formulation of aflatoxin-safe peanut-based products. Meanwhile, the EWA-Belt project (EU Horizon, 2020-2025) addresses agricultural sustainability challenges in East and West Africa, including post-harvest mycotoxin control. Main findings include the use of slow-release sulphur dioxide bags and an early detection system for mycotoxin monitoring during storage.

Future research must prioritise scalable, cost-effective solutions that translate scientific advancements into practical applications. Collaboration between researchers, policymakers, and government bodies is essential to building resilient food systems across Africa.

Mycotoxins as endocrine disruptors in drinking water: insights from a Belgian cohort study

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Endocrine disrupting compounds are associated with chronic diseases such as hormonal cancers, metabolic disorders, and reproductive issues. Their persistence in drinking water is largely due to wastewater treatment systems being unable to remove them effectively at low concentrations, making continuous monitoring crucial. Certain mycotoxins, including zearalenone (ZEN) and its metabolites, have been linked to endocrine-related conditions such as breast, ovarian, and endometrial cancer, as well as premature puberty or thelarche. Within the AquaGlance study, the presence of these compounds was investigated in bottled and tap water from 150 households across different geographical areas as part of the Ghent Longitudinal Observational Research Investigating Aging (GLORIA®) cohort – the first large-scale prospective Belgian cohort study aiming to assess the interaction between genetic and environmental factors in age-related health outcomes over 20 years with 20,000 participants.

A multiresidue solid-phase extraction (SPE) and ultra-high performance liquid chromatography-high resolution mass spectrometry (UHPLC-HRMS, Orbitrap Exploris 120) method was developed, achieving detection limits (LODs) between 0.5 and 4.4 ng/l, and quantification limits (LOQs) between 1 and 5 ng/l, for ZEN and its metabolites. Results showed that α -zearalanol was present in 45% of bottled water samples at concentrations ranging between LOQ and 53 ng/l. Statistical analysis using an Ordinary Least Squares regression model indicated that spring water contained significantly higher levels of α -zearalanol than mineral water ($\beta=1.7449$, $p=0.003$). In tap water, ZEN and α -zearalanol were detected in 31 and 81% of samples, respectively, with concentrations ranging between LOQ and 120 ng/l (mean = 8 ng/l) for ZEN, and up to 296 ng/l (mean = 41 ng/l) for α -zearalanol. Suspect screening revealed additional presence of α -zearalanol in 30% of samples. While detected concentrations remain generally low when compared to regulatory limits in food, concerns persist regarding the chronic exposure through drinking water and the potential 'cocktail effects' with other contaminants. Within the GLORIA® cohort, health risks associated with mycotoxins a.o. will be further investigated by assessing both external and internal exposure through dietary and drinking water questionnaires, as well as biomonitoring. This comprehensive exposome-based approach will offer a detailed understanding of long-term human exposure and its potential health impacts.

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Uncovering the story of mycotoxin exposure in the UK: Analysis of body fluids and hair

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The research community, industry, and policymakers have made significant investments to mitigate mycotoxin contamination in the UK. Strong regulations prevent contaminated food from reaching consumers, but recent studies have shown that UK children's exposure to ochratoxin A (OTA) and zearalenone (ZEA) still exceeds the maximum tolerable daily intakes recommended by the European Food Safety Authority before 2020. Understanding the real exposure to mycotoxins is challenging due to consumers' lack of awareness of these natural chemical contaminants, the diversity of diets, and the varying amounts of consumed products. Thus, the aim of this work was to unfold mycotoxin awareness among consumers and develop a reliable method for detecting biomarkers for mycotoxins in human samples such as urine, human breast milk, and hair to accurately assess population exposure. In order to better comprehend the dietary habits, the participants who donated samples completed a questionnaire recall, indicating food preferences and awareness of contaminants.

Different analytical methods were tested to evaluate the performance of three extraction techniques for subsequent high-performance liquid chromatography analysis: (i) solid phase extraction (SPE) ready-to-use columns; (ii) QuEChERS method; and (iii) dilute-and-shoot method. The targeted mycotoxins included ochratoxin A, α -ochratoxin, zearalenone, α -zearalenol, β -zearalenol, α -zearalanol, β -zearalanol, deoxynivalenol, deepoxy-deoxynivalenol, 3-acetyl-deoxynivalenol and deoxynivalenol-3-glucoside.

Moreover, this research provides valuable insights into the extent of mycotoxin exposure among adults and infants considering the intricate correlation between dietary patterns and presence of mycotoxin biomarkers in, urine, hair and breastmilk. The transfer of toxins into milk and urine were investigated in previous studies, but few attempts to correlate the long-term exposure by analysing infants' hair was carried out. By raising awareness and improving detection methods, we can work towards ensuring the safety of food products and protecting public health.

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Occurrence of endophyte mycotoxins in cool-season grasses from the Pacific Northwest, United States

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Endophyte-infected cool-season forage grasses are a significant nutritional resource when grazed in pastures for livestock managers in the United States. In addition, hay/straw material from endophyte-infected grass seed fields from the Pacific Northwest is baled and exported to global locations, making knowledge of mycotoxin concentrations and relative toxicological impacts on livestock essential to ensuring safe feed throughout the supply chain. The Endophyte Service Laboratory (ESL) tests approximately 3,000 samples per year for ergot alkaloids (ergovaline) and lolitrem B, providing a service to both feed exporters and livestock owners regarding mycotoxin contamination while also connecting and communicating information to all relevant stakeholders.

Tall fescue (*Festuca arundinaceum*) is the most commonly submitted grass species; ergovaline concentrations averaged 127 ng/g in submitted samples but have ranged from <100-1,534 ng/g over the last two years (1,486 samples). The endophyte in perennial ryegrass (*Lolium perenne*) can express both lolitrem B and ergovaline; lolitrem B and ergovaline concentrations averaged 569 and 146 ng/g but ranged from <100-4,763 and <100-2,443 ng/g over the last two years, respectively (1,514 samples). Samples exceeding established thresholds of toxicity (for beef cattle) were monitored and were 5% and 7% for ergovaline (responsible for summer slump/fescue foot) in tall fescue and perennial ryegrass, respectively; and 7% for lolitrem B (responsible for ryegrass staggers). Caveats around this being a voluntary testing program (and therefore not a reflection of randomized sampling throughout the region) and that both turf and forage varieties are mixed within the samples needs to be considered when interpreting this data.

Upon receipt of results, personnel in the laboratory are available to growers/livestock managers for consultation that may guide decision making in selling, procuring and/or feeding of contaminated material. Additionally, the ESL provides educational/outreach efforts that impact agronomic and feed practices, collaborating with global partners to support the transmission of reliable contemporary knowledge of mycotoxins originating in endophyte-infected grass seed materials.

SESSION 8 MYCOTOXIN DETECTION – ON-SITE AND ONLINE AGRI-FOOD APPLICATIONS

Smarter, faster, on-site: Infrared spectroscopy for mycotoxin detection

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In the time of climate crisis accompanied by global warming and unpredictable rainfalls, more challenges than ever arise in food production. In particular, these can be attributed to the growth of plant pathogens at cereals exemplified by species such as *Fusarium graminearum* and *Fusarium culmorum*. These pathogens produce toxic secondary metabolites known as mycotoxins, which are considered a major global food safety issue. Resulting, there is an increasing demand for analytical methods that facilitate rapid, sustainable and on-site contamination detection and monitoring.

This presentation will discuss innovative spectroscopic solutions for real-time mycotoxin detection along the food supply chain. A handheld IR-ATR analyser has been developed for rapid on-site screening at points-of-need such as farms or during transportation and in storage facilities. Complementarily, a high-fidelity laser-based analyser system provides confirmatory analysis at goods reception points and laboratories. The performance of these devices for detecting mycotoxins is demonstrated showcasing their efficiency and reliability. Additionally, the potential for expanding the application scenarios to pesticide monitoring will be highlighted offering a promising approach for enhanced food safety and quality control.

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Portable detection of genetic mycotoxin biosynthesis pathways in crops and food products – System validation

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Monitoring pathogenic fungi capable of mycotoxin biosynthesis presents a major challenge in agriculture and food safety. Mycotoxin contamination affects 60-80% of agricultural and food products, posing significant health and economic risks. Aflatoxins, in particular, can cause acute toxicity (fever, vomiting, diarrhoea, hepatitis) and chronic conditions such as liver cancer and immune suppression. Given these risks, developing and implementing rapid detection systems for early risk assessment is essential.

The Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences is actively advancing technologies to mitigate the risks associated with food contamination. In response to market demands, Bioaccure Ltd. (Poland) has developed a portable, non-laboratory system for the rapid detection of fungi responsible for mycotoxin biosynthesis. The system comprises a four-channel real-

time portable thermal cycler (PCR:smart®), a dedicated application for device operation, lyophilized qPCR assays, and a simplified DNA extraction protocol. The research is focused on integrating mobile diagnostic approaches based on molecular biology techniques into routine mycotoxin monitoring. To achieve this, Bioaccure has developed qPCR assays that allow for broad-spectrum detection of mycotoxin-producing fungi by focusing on conserved biosynthetic genes rather than species-specific markers. By targeting *norA*, *norB*, and *affK* for aflatoxins, and *tri101* for trichothecenes type A and B Bioaccure's qPCR assays enable the identification of multiple pathogenic fungal species that share these toxin-producing pathways. This enhances detection sensitivity and applicability across diverse agricultural and food safety settings. Furthermore, the PCR: smart® system, in combination with direct PCR reagents, enables an optimization of a rapid DNA isolation that eliminates the need for laboratory-based sample preparation.

The Polish Academy of Sciences conducts extensive research on implementing this technology under non-laboratory conditions and optimizes an analytical protocol for field applications. Initial validation studies have demonstrated 100% specificity of the qPCR assays in detecting genetic pathways involved in mycotoxin biosynthesis in biological samples, with a >98% reproducibility rate compared to laboratory-based qPCR platforms (CFX96 System, Bio-Rad Laboratories). These findings underscore the potential of the portable detection system for on-site monitoring of mycotoxin contamination, providing a rapid and reliable tool for food safety management.

Highly sensitive rapid lateral flow test for detecting patulin

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Patulin is a harmful secondary metabolite produced by certain moulds. It poses a significant food safety risk due to its contamination of fruits and processed products. Exposure to patulin can lead to a range of health issues, from gastrointestinal discomfort to severe long-term effects, such as immunotoxicity and genotoxicity. Therefore, monitoring patulin levels is essential for compliance with regulatory standards and safeguarding consumer health.

Regulatory authorities, including the U.S. Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA), have established maximum allowable concentrations of patulin: 50 µg/kg for apple juice and juice-based beverages, 25 µg/kg for solid apple products, and 10 µg/kg for foods intended for infants and young children. Detecting patulin is challenging due to its low concentrations and the complexity of food matrices. In addition, its thermal resistance complicates its management, as thermal treatments used in food production do not effectively eliminate patulin. Furthermore, moulds can continue to produce patulin even at low temperatures, preventing levels from being kept lower, which underscores the need for reliable detection methods.

This study presents the development of a novel dipstick assay designed for the rapid, semi-quantitative, and highly sensitive detection of patulin in apple and pear-based products, including juices, sauces, and cider. Using a competitive immunoassay format with a robust monoclonal antibody, the test achieves a sensitivity range of 488 µg/kg, depending on the food matrix. The method's reliability has been validated through spike recovery experiments, precision assessments, and correlation studies across multiple batches of test strips. To our knowledge, this represents the first lateral flow immunoassay specifically targeting patulin, providing a promising new tool for enhancing food safety and preventing the consumption of contaminated products.

Application of time-resolved fluorescent immunochromatography in mycotoxin detection

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Time-resolved fluorescence immunochromatography (TRFIC) is an advanced detection technique that combines immunochromatographic assays with time-resolved fluorescence to enhance sensitivity and specificity. This method minimizes background interference and provides highly accurate quantification of mycotoxins in food and feed samples. Compared to conventional immunoassays, TRFIC offers improved detection limits, and broader applicability.

This presentation will explore the principles, advantages, and practical applications of TRFIC in mycotoxin detection, highlighting its potential for ensuring food safety and regulatory compliance.

SESSION 9 LATE-BREAKING MYCOTOXIN RESEARCH

Towards resilience of the Robusta coffee production: an Ivorian case study

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Ivory Coast produces 66 million kilograms of Robusta coffee every year. This production is impacted by the contamination of Ochratoxin A (OTA) mainly produced by *Aspergillus westerdijkiae* and *Aspergillus carbonarius*. With an EU maximum limit of 3 µg/kg for roasted coffee and above 5 µg/kg for soluble coffee there is a need to develop innovative and resilient solutions to prevent OTA accumulation within the Robusta coffee supply chain.

Following a review on the current mitigation strategies along the coffee supply chain. This study first identified both high OTA producers in Ivory Coast coffee cherries and candidate biocontrol agents. The potential of indigenous yeasts as biocontrol agents was further explored and confirmed in *in vitro* conditions. For the first time, the most promising candidates were also investigated for their capacity to prevent OTA production considering predicting climate change conditions. This novel approach led to the selection of one biocontrol candidate (*Meyerozyma caribbica* Y4) with the best resilience with reduction of both growth (50%) and OTA production (70%) under all the scenarios tested.

This research is pivotal in the pursuit of climate-resilient strategies for mycotoxin management, contributing to both food safety and agricultural sustainability.

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Unravelling the mysteries of bound ochratoxins

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Ochratoxin A (OTA) is a nephrotoxic, immunosuppressive and neurotoxic mycotoxin, produced by various fungi of the genus *Aspergillus* and *Penicillium*. Its frequent occurrence in food products such as cereals, spices, dried fruits and coffee poses public health risks in particular to infants and children. The European Food Safety Agency panel on Contaminants in the Food Chain stated in their latest opinion that “more data on occurrence and toxicity of modified OTA are needed” (1).

Modified forms of OTA of potential concern can arise during food processing (e.g., 2'R-OTA during coffee roasting) (2) and due to plant metabolism (e.g. 4-OH-OTA or 4-OH-OTA-glucoside) (3). Studies with plants are currently limited to cell cultures and it is not yet fully understood which metabolites are formed in culture plants, such as maize, and to which extent. In addition, OTA might be bound, e.g., to proteins and little is known about the occurrence and bioavailability of bound forms.

We screened 53 fungal strains to assess OTA production in several semi-synthetic liquid media at different light conditions. Highest yields were obtained in Czapek yeast autolysate medium with strains of *Aspergillus westerdijkiae* and *Aspergillus steynii* during continuous darkness. We then used $^{13}\text{C}_6$ -glucose to produce fully ^{13}C labelled yeast and subsequently fully ^{13}C labelled yeast extract. The labelled sugar and yeast extract was used to synthesize ^{13}C -OTA in milligram amounts. The compound was purified using liquid-liquid extraction, solid-phase extraction and preparative HPLC. The resulting ^{13}C -OTA will be mixed with natural OTA to treat mini-maize plants during flowering. High resolution mass spectrometry and MetExtract II data processing (4) will then be employed to determine all soluble OTA metabolites of maize.

Acknowledgements

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Mycotoxins in urine of adults following healthy and sustainable diets: Revealing the dynamics between diet transition and mycotoxin exposure

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The aim of this study was to investigate the relationship between mycotoxin exposure and dietary patterns in a cohort of 300 Italian adults. Urine samples, part the OPCT study from the ERC-StG project PREDICT-CARE (Grant Agreement No. 950050) together with food frequency questionnaires (FFQ), were analysed to stratify the consumers based on their dietary patterns. We quantified 24 mycotoxins and their metabolites in human urine using an ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method. The targeted mycotoxins included aflatoxins (AFB1, AFB2, AFG1, AFG2), alternariol (AOH), alternariol monomethyl ether (AME), tentoxin (TEN), fumonisins (FB1, FB2), HT-2/T-2 toxins, ochratoxin A (OTA), ochratoxin alpha (OT α), zearalenone (ZEN), alpha-zearalenol (α -ZEL), beta-zearalenol (β -ZEL), zearalanone (ZAN), deoxynivalenol (DON), deepoxy-deoxynivalenol (DOM-1), enniatins (ENNB, ENNB1, ENNA, ENNA1), and beauvericin (BEA). Mycotoxin levels were reported both unadjusted and adjusted for creatinine, ensuring accurate exposure assessment across individuals. Consumers' exposure to mycotoxins was stratified by gender, age, and adherence to a Mediterranean (MedDiet), provegetarian (PVG), and a plant-based diet (PBD). Using established dietary indices from literature, consumers' adherence to these diets was classified as low, medium, or high. Additionally, PVG and PBD diets were further divided into general, healthful, and unhealthful patterns, providing a nuanced analysis of how dietary choices impact mycotoxin exposure. Understanding these dynamics is essential for public health to ensure that sustainability and food safety go hand in hand. The findings could inform dietary guidelines and public health strategies aimed at mitigating mycotoxin exposure.

Efficiency of removal of aflatoxin from contaminated peanuts using fluorescence-based sorting technology

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Widespread aflatoxin contamination in peanuts poses a significant global threat to food safety and human health, leading to severe consequences that range from acute toxicity to chronic diseases. To address this issue, various technologies have been explored, with fluorescence-based sorting technology emerging as a promising candidate. This technology utilizes the fluorescent signatures under UV light of aflatoxin and kojic acid derivative (formed through the oxidation of kojic acid, another metabolite produced by aflatoxin-producing fungi) to distinguish between contaminated and uncontaminated peanuts.

The present study evaluated the efficiency of fluorescence-based sorting technology on an industrial scale using two emerging sorters, both equipped with fluorescence technology, in two peanut processing enterprises. The results indicate that the technology can efficiently remove contaminated peanuts, with aflatoxins highly concentrated in the rejections after sorting by both sorters. At appropriate sorting settings, the technology achieved over 80% sensitivity, specificity, and accuracy. Both sorters demonstrated stability during the sorting process, with a coefficient of variation (CV) for the weight of the repeated test reject being less than 15%. The loss rate was less than 5% and primarily depended on the rejection settings of the machine, which were determined based on the contamination level of the peanuts.

Weaning piglet health upon dietary contamination with deoxynivalenol, enniatins, or their combination *in vivo* is connected to altered gut microbiome and gut tissue outcomes *in vitro*

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Apart from damaging pig intestinal integrity, deoxynivalenol (DON) may affect gut-microbiome axis. Moreover, advanced analytical techniques identified additional mycotoxins like enniatins (ENNs), although their toxicity remains unclear. The aim of this study was to apply an *ex vivo* gut tissue InTESTine™ platform and *in vitro* i-screen® gut microbiome characterization method in combination with an *in vivo* mycotoxin exposure to understand mycotoxin-microbiome, mycotoxin-gut tissue, and microbiome-gut tissue interactions.

Four experimental diets were provided during 14 days (D) to weaned piglets: a control (CON) diet marginally contaminated with mycotoxins or diets naturally contaminated with 0.7 mg/kg DON (DON), 0.8 mg/kg ENNs (ENNs), or their combination (DON+ENNs). Faecal samples were collected at D0, 6, and 14, and colon tissue was collected at D6 and 14. Gut microbial communities derived from faecal samples were studied and cultured in the *in vitro* i-screen® microbiome screening platform (48 h). Gut barrier and viability were studied in colon tissue samples in the *ex vivo* gut tissue InTESTine™ platform (6 hours). Additionally, i-screen® fermented samples were added to InTESTine™. When fed the DON+ENNs diet, piglets had poor growth and increased susceptibility to infections. Mycotoxins affected the microbiome *in vivo* and *in vitro*. *In vivo*, the alpha diversity, Shannon and Chao indices, was significantly increased after 14 days of ENNs exposure. The *in vivo* results were maintained *in vitro*, showing that the microbial diversity of ENN and DON+ENNs *in vivo*-treated samples differed more strongly from CON than DON treated samples. Significantly increased lactate dehydrogenase release indicated worsened gut tissue viability by DON after 6 days and by all mycotoxin conditions after 14 days of exposure. Gut barrier leakage of paracellular and transcellular small (mannitol and caffeine) and large (FITC-dextran 4 kDa (FD4)) molecules was more pronounced after 6 than 14 days of exposure. DON negatively affected tissue transport functionality by reducing the transport rate of both small molecules resulting in a decreased caffeine over mannitol transport ratio. ENNs mainly increased paracellular transport of mannitol and FD4 after 6 days of exposure. DON+ENNs exposure did not reflect of augment individual exposure to DON or ENNs effects. Combination of D14 gut tissue with D6 microbial metabolites (i-screen®) more closely resembled D6 observations on tissue transport and permeability whereas tissue viability more closely resembled D14 observations.

Based on the results, the i-screen® and InTESTine™ platforms can help to unravel mycotoxin-microbiome, mycotoxin-gut tissue, and microbiome-gut tissue interactions and understand *in vivo* trial outcomes.

Revolutionizing mycotoxin monitoring: New quality approaches for certified reference materials

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Ensuring food and feed safety relies on the precise quantification of mycotoxins, toxic fungal metabolites that pose severe risks to human and animal health. Central to this effort is the availability of high-quality mycotoxin reference materials, which underpin the accuracy of analytical methods and the reliability of data used in regulatory frameworks, risk assessments, and supply chain monitoring. Despite the ISO 17034 standard's efforts to streamline technical and managerial requirements for certified reference material production, significant challenges persist, particularly in purity determination, contamination management, and stability monitoring.

This study critically examines the existing practices and highlights gaps in the quality assurance of liquid mycotoxin reference standards. Leveraging advanced techniques such as two-dimensional nuclear magnetic resonance (2D-NMR), liquid chromatography-tandem mass spectrometry (LC-MS/MS), and high-resolution mass spectrometry (HRMS), we assessed 30 reference standards encompassing aflatoxin B1, deoxynivalenol, and zearalenone from 10 global manufacturers. Our findings revealed a significant prevalence of structural analogue contaminants and cross-contamination among suppliers, underscoring the necessity of rigorous pre- and post-production quality control systems. We propose a novel quality management framework that redefines the standards for reference material production. This approach integrates enhanced purity determination using quantitative nuclear magnetic resonance (q-NMR), a tiered stability monitoring protocol, and systematic contamination assessments. Additionally, the study discusses the implications of ISO Guide 34's transition to ISO 17034, highlighting its streamlined management requirements but identifying unresolved technical issues that impact reference material reliability.

The findings emphasize the role of robust quality assurance as the cornerstone of laboratory data integrity, with implications extending to regulatory compliance, international trade, and consumer safety. By addressing the current limitations and implementing innovative quality protocols, this work aims to set a new benchmark for the production of certified reference materials, ensuring their suitability for diverse applications in mycotoxin analysis.

SESSION 10 SMART APPROACHES FOR MYCOTOXIN ANALYSIS

Advances in analysis and detection of mycotoxins in dryland crops at the Joint FAO/IAEA Centre's Food Safety and Control Laboratory

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Dryland farming encompasses about 70% of the global cultivated land and contributes significantly to food security, providing much of the world's grain supply and supporting livelihoods of producers and consumers worldwide. Some dryland crops, such as millet and pulses, are receiving growing attention, particularly because of their resilience to climate change. However, shifting climate conditions can create environments conducive to mycotoxin-producing fungi, compromising food and feed safety and nutritional security, endangering international trade, and increasing the risk of food rejection and loss.

The Food Safety and Control Laboratory (FSCL) of the Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture in Seibersdorf, Austria, assists the Member States of FAO and IAEA in improving laboratory practices and methodologies in the area of food safety through R&D and technology transfer on analytical methods based on nuclear and complementary techniques. Within the project 'Ensuring food security and safety by future-proofing dryland crops under climate change', FSCL is developing, validating and transferring to the Member States laboratories rapid screening and confirmatory methodologies, to support robust monitoring of climate-related hazards in dryland crops.

Cost-effective electrochemical immunosensors using screen-printed electrodes are being developed and are appropriate to be used in the field, integrated into early detection and alert systems, but also within the framework of regular food controls. Sensors for screening the sum of aflatoxins B1, B2, G1 and G2 in pistachio (1) and the sum of fumonisins B1, B2 and B3 in maize have been already validated and optimized. Regarding confirmatory approaches, FSCL focuses on multi-analyte methods, making efforts to simplify the sample preparation procedure. Recently developed method protocols included the determination of aflatoxins and fumonisins in maize, millet and cassava by LC-MS/MS, while a newly adopted technique, the supercritical fluid chromatography coupled to tandem mass spectrometry, using carbon dioxide (CO₂) as mobile phase together with methanol as modifier, provides strengthened analytical capabilities, allowing for higher performance and sensitivity for greener analysis.

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Automation in mycotoxins analysis: The Wizard of Oz journey from innovation to harmonized data collection.

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Automation in mycotoxin analysis represents a transformative step forward, addressing the limitations of traditional manual processes with improvements in precision, data integrity, and standardization. Routine testing, from farm samples to laboratory confirmations, currently depends on technologies such

as immunoassays, near-infrared (NIR) spectroscopy, liquid chromatography, and mass spectrometry, each subject to variability based on human handling. The adoption of automation can be seen as a 'Yellow Brick Road' – a structured, incremental journey toward harmonized, high-quality data collection that minimizes operator variability, enhances consistency across labs and regions, and aligns with regulatory requirements and internal quality systems.

Choosing to go down this path introduces a new level of data integrity and control. Automated traceability and validation processes not only enhance immediate data reliability but also establish long-term analytical robustness by standardizing every stage of sample handling and analysis, reducing human error through built-in data traceability. Much like the 'Tin Man in The Wizard of Oz', automation incorporates 'heart' into the process, capturing and protecting data with an objectivity and precision that manual handling cannot consistently match. In addition, automated, standardized data collection feeds AI-driven predictive models, which can support advanced contamination risk assessments in food and feed safety, reinforcing the value of real-time, integrated data. These AI-driven models empower food and feed safety stakeholders to make timely, informed decisions, enhancing real-time risk assessment and response.

Despite these strengths, automation – like the 'Wizard behind the curtain' – has limitations. While data from early case studies highlight benefits such as +35% increase in analytical capacity (+ overnight run capability), -50% reduction in consumables used (i.e., reagents, tips, purification columns and filters), and a general increase in throughput and significant reduction in cross-operator variability, ELISA automation does not eliminate analytical biases. Adapting these systems to diverse lab environments and maintaining the technology over time requires substantial investment, as well as ongoing training and system validation. However, these challenges are balanced by the tangible improvements in reproducibility and accuracy that the full solution, merged with services and reagents, provides, offering a more reliable framework for complex analyses.

The journey toward fully harmonized automation in mycotoxin testing, the 'Emerald City' of this path, is within reach, a vision achievable only through collaborative efforts among laboratories, industry stakeholders, and regulatory bodies. Building on current capabilities will allow automation to fulfil its promises, inspiring continued innovation for future applications and establishing a robust foundation for industry-wide data integrity.

Fate of mycotoxins during gluten-free pasta processing: Untargeted ¹³C-labelling LC-HRMS based approach.

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Our multidisciplinary academic-industrial research compages started several years ago to conduct in-depth research in order to investigate and understand how the fungal toxins behave during various food production chains. Based on the previous experience and current knowledge in this area, it is assumed that the toxins could degrade or be modified due to exposure to energetic and mechanical conditions during the process and thus form novel modified mycotoxins. However, reduction of mycotoxin level does not have to necessarily result in a mitigation of toxicological effects. Nowadays, most of the information on the novel degradation products of mycotoxins is still missing; identification and investigation of their structure and toxicity potential is of a high interest for risk assessment strategies.

To study the fate of mycotoxins during food processing is a very challenging task for several reasons: (i) characterization and structural identification of degradation products in complex matrix is difficult; (ii) matrix composition varies over the production steps; (iii) availability of analytical standards is limited; and (iv) preparation of 'in-house' analytical standards in larger amounts needed for structural confirmation and for toxicological assessment, is costly and time-consuming. Therefore, most of the published studies were mainly focused on the detection of the parent mycotoxins and calculation of the rate of decline over the production.

In this study, the untargeted stable isotope labelling (SIL)-LC-HRMS approach was applied: material used for food production is treated with a mixture of non-labelled and ¹³C-labelled standard of mycotoxin, intermediate products and final pasta are then analysed by liquid chromatography-high resolution mass spectrometry (LC-HRMS). Detected pair of signals originated from non-labelled and isotopically labelled compounds are extracted from the full-scan chromatogram. Results on aflatoxins and fumonisins fates along this industrial process will be presented.

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Metabolism of multiple mycotoxins by black soldier fly (*Hermetia illucens*) using an optimized high-throughput UHPLC-MS/MS method

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Among the most important safety risks for the feed industry and stability of the feed supply chain are mycotoxins. Apart from the current mitigation strategies for mycotoxins, which include the use of mycotoxin binders or biotransforming agents, there is an urgent need for efficient mitigation strategies to upgrade the valorisation of batches of highly multi-mycotoxin contaminated organic waste. Preliminary data suggest that edible insects might have the capacity to bioconvert mycotoxins in non- or less toxic metabolites. In addition, insects are efficient bioconvertors that can turn low value organic waste into high value edible feed products for poultry and pigs. This can close the nutrient loop, ensuring a circular agro-industrial model. The most frequently used insect for the waste bioconversion process is currently the black soldier fly (BSF, *Hermetia illucens*).

This research project aimed: (i) to develop and validate a high-throughput ultra-high performance liquid chromatography (UHPLC) tandem mass spectrometry (MS/MS) method for the analysis of two well known *Fusarium* mycotoxins, i.e., deoxynivalenol (DON) and fumonisins (FBs), and potential phase-I and -II metabolites in BSF larvae and frass; and (ii) to evaluate the metabolization pathways and safety of the detoxification approach of DON and FBs by BSF larvae. Following a feeding experiment with artificially DON or FBs (FB1+FB2+FB3) contaminated Gainesville diet to BSF larvae at levels corresponding with 0.25, 0.5, 1, 2 and 4 times the recommended EU level in feed (1), samples of remaining frass and 12-day old larvae were harvested and stored at -20°C. Upon lyophilisation and

crushing, 0.5 g aliquots of frass and larvae were wetted and subjected to a liquid extraction using 1% formic acid in acetonitrile. After appropriate dilution, the final extract was analysed using an in-house validated UHPLC-MS/MS method (2). Limit of quantification values were 1 µg/kg for FBs, DON and metabolites in the diluted extracts of larvae and frass, respectively. Measured concentrations of DON ranged between 0.02-0.40 mg/kg ww and 0.10-5.4 mg/kg ww in larvae and frass, respectively. Measured concentrations of total FBs (sum of FB1, FB2 and FB3) ranged between 0.05-1.83 mg/kg wet weight (ww) and 0.36-2.83 mg/kg ww in larvae and frass of the different treatment groups. The bioaccumulation factor was 0.018±0.005 and 0.007±0.003 for DON and FBs, respectively. The molar mass balance at the end of the experiment was 5.4±2.8 % for DON and 6.2±2.3 % for FBs (sum of parent FB1, FB2 and FB3). No phase-I or -II metabolites of DON were detected in frass or larvae samples. Partially hydrolysed and fully hydrolysed metabolites of FBs were detected in both larvae and frass, suggesting substantial metabolization of parent FBs. Metabolisation pathways could be further explored in the future using UHPLC high-resolution mass spectrometry experiments.

This study provided insights into the capacity of BSF larvae to detoxify and convert *Fusarium* mycotoxins in metabolites, highlighting their potential as sustainable solutions for transforming contaminated organic waste into safe, high-quality feed products.

Acknowledgements

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Pulling the thread: a metabolomic approach to unraveling secondary metabolites production in *Fusarium proliferatum*

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Deciphering the complexity of mycotoxigenic fungi secondary metabolism demands sophisticated and advanced strategies. In recent years, significant progress has been made in characterizing the genes within biosynthetic gene clusters responsible for producing mycotoxins and other secondary metabolites. However, many of the enzymes they encode remain unlinked to specific end products, and their associated biosynthetic pathways are yet to be fully elucidated. In the same way, while several studies have explored fungal species-specific chemical potential through a genomic approach, few have conducted comprehensive metabolome profiling for the same purpose. Besides, while some genera and species have been more thoroughly characterized, others lack any information. *Fusarium proliferatum*, a globally distributed filamentous fungus infecting a wide range of hosts, is among the most significant producers of fumonisins, which are toxic disruptors of the sphingolipid metabolism in humans and animals. Despite being a key representative species of *Fusarium* genus chemo-diversity, its metabolome still remains underexplored.

Under this framework, this study investigated the *in vitro* secondary metabolism of nine *Fusarium proliferatum* strains isolated from date palm (*Phoenix dactylifera*) in Tunisia. The strains were cultivated in triplicate on autoclaved rice and PDA substrates. Then, the polar fraction obtained after a biphasic solid-liquid extraction (SLE) was analysed through liquid chromatography-tandem mass spectrometry (LC-MS/MS) and liquid chromatography-traveling wave ion mobility spectrometry-high-resolution mass spectrometry (LC-TWIMS-HRMS). LC-MS/MS allowed the sensitive targeted detection of B-series fumonisins (FBs) in order to classify the analysed *Fusarium proliferatum* strains as FBs non-producers, low-producers, and producers. Then, targeted and suspect approaches – using an in-house database built from literature and online sources in the latter case – were proposed for metabolite identification through LC-TWIMS-HRMS. The workflow allowed the tentative identification of more than 50

compounds, mostly related to fumonisins' metabolism, as well as other polyketides, non-ribosomal peptides (i.e., beauvericins), and terpene derivatives. Results showed different metabolic profiles for FBs-producing and non-producing strains, especially in fumonisin intermediates accumulation. Interestingly, also the FB4 deaminated form (fumonisin Py4), whose reduced toxicity has been previously reported, accumulated in FBs non-producing strains. Additionally, substrate-dependent differences were also observed, further disclosing the metabolic plasticity of these strains. Understanding what underlies this diverse production and the shifts in fungal metabolism could provide insights for mycotoxin reduction future strategies.

This study represents a step toward unravelling the chemical potential of *F. proliferatum*. Integrating these results with other omics approaches could be key to fully comprehend how metabolite production is modulated in this species.

A high throughput phenotyping platform for cereal research and breeding programs to identify *Fusarium*-damaged kernels and *Fusarium*-produced mycotoxins

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Fusarium head blight (FHB), caused by *Fusarium* spp., is a destructive disease of wheat. FHB affects kernel development, resulting in lightweight, chalky white, shrunken kernels covered with white or pink mycelia; these are known as *Fusarium*-damaged kernels (FDKs). Infected kernels are frequently contaminated with *Fusarium*-produced mycotoxins, especially deoxynivalenol (DON). FHB significantly reduces grain yield and quality, resulting in hundreds of millions of dollars in losses annually in Canada. Breeding cultivars with high disease resistance and low mycotoxin contamination is a priority for wheat breeders. However, traditional FDK and DON measurement methods are time-consuming, labour-intensive, and of variable accuracy; improvements are needed for large-scale screening in breeding programs.

In this study, two high throughput phenotyping tools were developed for FDK and DON measurement. A fast chromatography (FC) – tandem mass spectrometry (MS/MS) method was developed for DON quantification. It employs a one-step acetonitrile extraction protocol with a short guard column to reduce complexity, cost and analysis time. In addition, a high-throughput single-kernel screening tool was developed to assess FDK through automated image acquisition and analysis. It non-destructively images and analyses samples composed of several hundred seeds, taking close-up top and side images of individual kernels. Meanwhile a customized convolutional neural network (CNN) model was developed and trained to process, count, and analyse vast amounts of scanned sample images. Preliminary results with the CNN model are promising; it can automatically determine the percentage of FDK in much less time than visual assessment.

PLENARY SESSION

BUILDING A RESILIENT FOOD SYSTEM IN THE DIGITAL DECADE – CHALLENGES FOR MYCOTOXIN RESEARCH AND MANAGEMENT

Naresh Magan Lecture Award

Proteomic analysis as a tool to unveil the effects of control agents on toxigenic *Gnomoniosis smithogilvyi*, the major chestnut pathogen

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Chestnut fruit is a seasonal product, commercialised as fresh or processed ready-to-use products. The storage of chestnuts is a challenge due to their nutritional richness and high water content which create conditions that are conducive to fungal infections and insect infestations. *Gnomoniopsis smithogilvyi* (syn. *Gnomoniopsis castaneae* Tamietti) has led to significant losses in various chestnut species, in particular, *Castanea sativa* in Europe. In previous research, this fungus has been described as a 3-nitropropionic acid and diploidiatoxin mycotoxin producer. Four antifungal agents, such as *Bacillus amyloliquefaciens* QST 713 (Serenade®ASO), *B. amyloliquefaciens* CIMO-BCA1, and the fungicide Horizon® (tebuconazole), have shown to reduce the growth of *G. smithogilvyi*. However, under sub-inhibitory concentrations, all the antifungal agents enhanced mycotoxin production. Proteomics can clarify the mould's physiology and the impact of antifungal agents on the mould's metabolism. Thus, this study aimed to assess the impact of Horizon®, Serenade®, and *B. amyloliquefaciens* CIMO-BCA1 on the proteome of *G. smithogilvyi* to unveil their modes of action and decipher why the mould counteracts by increasing the mycotoxin production.

For this, the mycelium close to the inhibition zone provoked by antifungals was macroscopically and microscopically observed. Proteins were extracted and analysed using a Q-Exactive plus Orbitrap. The results did not elucidate specific proteins involved in the mycotoxin biosynthesis, but these agents provoked different stress on the mould, mainly affecting the cell wall and antioxidant response, which points to the mycotoxins overproduction as a defence mechanism. The biocontrol agent CIMO-BCA1 acts similarly to Horizon® (tebuconazole). Therefore, CIMO-BCA1 could replace tebuconazole, thus reducing the use of this environmentally toxic antifungal in agriculture to which numerous fungi present resistances in both agricultural and clinical settings. The results also revealed different responses on the mould's metabolism when co-cultured with the two *B. amyloliquefaciens* strains, showing different modes of action of each bacterium, which opens the possibility of combining both. In conclusion, these results unveil different modes of action of the treatments that could help reduce the use of toxic chemicals to combat plant pathogens worldwide.

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A byte against blight – AI's Impact on mycotoxins

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Artificial intelligence (AI) – technology mimicking human cognition through learning and decision-making – is revolutionizing mycotoxin management in agriculture and food safety. This presentation explores AI's evolution from its origins to its modern applications, driven by machine learning and big data. Currently, AI enhances mycotoxin analysis via rapid spectral data processing, predicts contamination risks using environmental models, ensures food safety through real-time sorting, and supports precision agriculture with drone-based monitoring. External factors amplify its importance: rising plant-based food trends increase reliance on mycotoxin-prone crops, climate change and unpredictable weather exacerbate mould growth, and weather-driven trade flow shifts complicate supply chain safety.

Looking ahead, AI could enable global risk mapping, portable on-site testing, comprehensive supply chain oversight, and smart farming innovations, such as autonomous interventions and resistant crop breeding. While challenges such as data access and collaboration remain, AI's integration promises safer food systems and resilient agriculture. This presentation highlights AI's transformative potential against the persistent threat of mycotoxins.

Human biomonitoring of mycotoxins: Exciting opportunities and challenges

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Knowledge and information on human exposure to mycotoxins is important for risk assessment and health impact assessment. Human biomonitoring (HBM) is an instrument for the direct measurement of internal exposure to chemicals from different sources and by different pathways. Internal exposure is quantified by biomarkers which can be measured in bodily tissues or fluids (e.g. blood, urine or hair). These biomarkers can be indicators of exposure, effect or susceptibility. Examples of mycotoxin biomarkers will be given during the presentation.

The risk assessment of (internal) mycotoxin exposure can be based on comparing internal concentrations of parent compound(s) and/or metabolites(s) with available or derivable human biomonitoring-guidance values (HBM-GVs). Otherwise, after toxicokinetic dosimetry, based on comparing estimated dietary intakes with external guidance values (e.g., tolerable daily intake). Both approaches comprise their own uncertainties. Preferably, HBM-GVs are based on dose-effect relationships observed in humans. Alternatively, HBM-GV derivation is based on a defined external toxicity reference value which in many cases is based on a point of departure derived from animal studies. Opportunities for mycotoxin risk assessment lie in epidemiological studies (for dose-effect relationships), HBM studies (for exposure data) and human intervention studies (for toxicokinetics). Challenges are related to the quality of these studies and to a lesser extent to the number of well performed studies.

In the past decade(s) attention has been (mainly) paid to the exposure of mycotoxins in humans but with progress on the applicability of effect biomarkers and increasing interest on human health effects, attention nowadays focuses on effect biomarkers and assessing the health impact for certain (sub)populations. Only in cases where exposure is substantially higher than an internal or external guidance value, health effects related to adverse outcomes may be observed. Due to an increasing

interest in developing so-called adverse outcome pathways (AOPs), opportunities lie in finding relevant 'early' effect biomarkers related to earlier key events in the AOP.

Most consumers are exposed, on a regular basis, to a mixture of mycotoxins and the mixture risk assessment of combined (internal) exposure to mycotoxins with a common effect is consequently more relevant than the risk assessment of single mycotoxin exposure. Depending on the level of availability of HBM data (including uncertainty), this mixture risk assessment can be carried out using a tiered approach. The desired levels (from low to high tier) encompass different opportunities and challenges.

Tech-powered biosolutions: Revolutionizing crop protection using robots, AI and thousands of fungi

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Crop diseases caused by fungi or pseudofungi are one of the major threats against global food security. However, important diseases are kept in check, mostly by per- and polyfluoroalkyl (PFAS) related forever-chemicals that cause both environmental and health concerns. Specifically, potato production in Northern Europe and North America is heavily reliant on the usage of forever-chemicals, typically receiving 10-12 treatments per season, with a combination of pesticides, to keep disease development in check. In addition, regulatory authorities in Europe are working towards a ban of the use of PFAS-related pesticides, leaving potato farmers helpless for the production of food for tomorrow.

In Mycoverse, a spinout from The Technical University of Denmark, we leverage a fungal strain collection of almost 15,000 isolates to discover novel bioactives that can replace forever-chemicals. We have developed high throughput discovery pipelines against common crop pathogens using robotics, where we can screen thousands of fungal isolates weekly. We have also implemented high throughput metabolomics workflows to deselect isolates that produce known mycotoxins early in the pipeline and efficiently query our metabolomics database for correlations between bioactivity and metabolite leads.

As a recent academic project, we have built and verified our discovery platform towards wheat pathogens with great success but have now shifted focus towards the widespread potato pathogen *Phytophthora infestans*.

Mycotoxin research 4.0: Should we really go omics?

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The advent of omics technologies has revolutionized various fields of biological research, and mycotoxin research is no exception. Recent advancements in this field frequently incorporate one or more omics methodologies, often combined with advance chemometrics or AI-driven approaches.

While analytical chemists are nowadays well-equipped for the detection of regulated and emerging mycotoxins, and a range of rapid methods are quickly becoming available for in-site/on-site application, traditional approaches fall short in capturing the intricate processes of mycotoxin biosynthesis and contamination. In this context, omics technologies offer comprehensive insights into these processes by enabling the detailed analysis of fungal genomes, gene expression patterns, protein interactions, and metabolic pathways.

Recent studies have demonstrated the potential of multi-omics approaches in identifying key biomarkers for mycotoxin detection and understanding the factors affecting the plant-pathogen interaction. These integrated approaches not only enhance our ability to detect and quantify mycotoxins but also pave the

way for the development of innovative strategies to mitigate mycotoxins occurrence in crops. However, omics technologies do not come without challenges and severe limitations. The complexity and high cost of multi-omics data integration can be prohibitive, requiring advanced computational tools and expertise. Data interpretation can be challenging due to the sheer volume of information generated, which may include irrelevant or redundant data. Not lastly, the variability in sample preparation and data acquisition methods can lead to inconsistencies and reproducibility issues.

This work explores the applications of omics in current mycotoxin research, highlighting their potential to transform our understanding of the problem, but also discussing limitations and challenges to be embraced by researchers in order to effectively taking up and exploit the full potential of omics technique in the field.

POSTER ABSTRACTS

POSTER INDEX

P1 – P30

MYCOTOXIN OCCURRENCE AND FUNGAL CHARACTERIZATION

- P1 Fumonisin contamination in maize from Nebraska: A multiyear snapshot
Ram Kumar Shrestha¹, Tamra A. Jackson-Ziems², Jayne Stratton¹, Heather Hallen-Adams¹ and **Andreia Bianchini**¹
¹Department of Food Science and Technology, University of Nebraska-Lincoln, USA; ²Department of Plant Pathology, University of Nebraska-Lincoln, USA
- P2 Monitoring the occurrence of aflatoxin B1 in natural products
Maria Antonia Calori, Leticia Medeiros Naval, Raphaela Romeu Rosa, Ivani Valarini Zambello and Aline Silva Mello Cesar
Department of Food Science and Technology, University of São Paulo, Brazil
- P3 Extending *Fusarium proliferatum* metabolite identification by combining LC-TWIMS-HRMS and FBMN
Guillem Campmajó¹, Irene Picicci¹, Antonio Moretti² and Chiara Dall'Asta¹
¹Department of Food and Drug, University of Parma, Italy; ²Institute of Sciences of Food Production, CNR, Italy
- P4 Evaluation of climate change on the presence of mycotoxins on food matrices for the last 10 years
Roger Collantes Farrés and Josep Calderón
Laboratori de l'Agència de Salut Pública de Barcelona, Spain
- P5 Diversity of mycotoxins in stored paddy rice: Contamination patterns in the Mekong Delta, Vietnam
Lien Thi Kim Phan^{1,3}, Thuy Thi Ngoc Nguyen¹, Thien Thi Thanh Tran¹, **Yen Thi Dang**¹ and Sarah De Saeger^{2,3}
¹Faculty of Food Science and Technology, Ho Chi Minh city University of Industry and Trade, Vietnam; ²Department of Bioanalysis, Ghent University, Belgium; ³Mytox-South@, International Thematic Network, Ghent University, Belgium
- P6 Mycotoxin contamination of cereals and fermented foods across African regions in the last 10 years (2014-2024)
Annalisa De Girolamo¹, Sheila Okott², Kokeb T. Hadush³, Mohammed Merhdie^{4,5}, Edson Coffi⁶, Julianah Odukoya⁷, Lethicia Manizan⁸, Vincenzo Lippolis¹, Sarah De Saeger² and Antonio Moretti¹ + UP-RISE consortium
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- P7 Mycotoxin prevalence in UK horse feed
Robert Furmage and Gemma White
Volac International Ltd., UK
- P8 Mycotoxin contamination in animal feed – UK and Ireland survey 2024
Robert Furmage, Gemma White and Angelika Borkowska
Volac International Ltd., UK
- P9 Survey: Do bale storage conditions have an effect on exposure to field mycotoxins?
Hsueh Iui Ho, **Robert Furmage**¹ and Gemma White
Volac International Ltd., UK

- P10 Occurrence of 16 mycotoxins in spices and herbs commercialized in Italy
Lucia Gambacorta¹, K. Gialluisi¹, M. Nicoletti², A. Moretti¹ and M. Solfrizzo¹
¹Institute of Sciences of Food Production, CNR, Italy; ²Ladisa Srl – Ristorazione, Italy
- P11 Studies of the growth and aflatoxin production of *Aspergillus parasiticus* on in-shell, shelled and split almonds
B. Szonyi¹, G. Huang², T. Birmingham² and **Dawit Gizachew**¹
¹Department of Chemistry and Physics, Purdue University Northwest, USA; ²Almond Board of California, USA
- P12 Analysis of mycotoxin data from 2020-2024: There exists a pattern in global mycotoxin contamination in raw materials and complete feeds
Avinash Bhat¹, Pim Kleinhoven² and **Swamy Haladi**²
¹Masterlab, the Netherlands; ²Selko, the Netherlands
- P13 Analysis of prevalence of mycotoxins in Asian region during 2024
Avinash Bhat¹, Pim Kleinhoven² and **Swamy Haladi**²
¹Masterlab, the Netherlands; ²Selko, the Netherlands
- P14 Analysis of prevalence of mycotoxins in Latin American region during 2024
Avinash Bhat¹, Pim Kleinhoven² and **Swamy Haladi**²
¹Masterlab, the Netherlands; ²Selko, the Netherlands
- P15 Analysis of prevalence of mycotoxins in European region during 2024
Avinash Bhat¹, Pim Kleinhoven² and **Swamy Haladi**²
¹Masterlab, the Netherlands; ²Selko, the Netherlands
- P16 Regional distribution of *Fusarium* mycotoxins and their metabolites in maize cultivated in Croatia
Tina Lešić¹, Manuela Zadravec², Sanja Furmeg³, Nina Kudumija¹, Irena Perković⁴, Hrvoje Krajina⁴, Mirta Vukičević⁴ and Ana Vulić¹
¹Laboratory for Analytical Chemistry and ²Laboratory for Feed Microbiology, Croatia; ³Veterinary Institute Križevci and ⁴Veterinary Institute Vinkovci, Croatian Veterinary Institute, Croatia
- P17 Presence of *Aspergillus* species in Serbia
Milica Lučev, Ana Obradović¹, Slavica Stanković¹, Vesna Krnjaja², and Goran Stanković¹
¹Maize Research Institute Zemun Polje, Serbia; ²Institute for Animal Husbandry, Serbia
- P18 Characterization of *Fusarium graminearum* species complex originated from maize kernels in Serbia
Ana Obradović¹, Vesna Krnjaja², Milica Lučev¹, Goran Stanković¹ and Slavica Stanković¹
¹Maize Research Institute Zemun Polje, Serbia; ²Institute for Animal Husbandry, Serbia
- P19 Study of mycotoxin occurrence in Spanish cereal fields
Carmen Erena Ortega^{1,2}, Jéssica Gil Serna¹, **Belén Patiño Álvarez**¹, Ángel Medina Vaya² and Andrea Patriarca²
¹Departamento Genética fisiología y Microbiología, Universidad Complutense de Madrid, Spain; ²Applied Mycology Group, Cranfield University, UK
- P20 Leveraging extensive mycotoxin analysis data for accurate contamination trends and risk management
Clement Soulet, Juniti Hanamoto and **Thomas Pecqueur**
Cargill Animal Nutrition & Health, Canada
- P21 Occurrence of multiple mycotoxins in various fibre sources
Jog Raj, Hunor Farkaš, Svetlana Čujić, Tobias Steiner, Goran Grubješić and Marko Vasiljević
Patent CO. DOO., Serbia

- P22 Fumonisin production in onion (*Allium cepa*) inoculated with *Fusarium proliferatum*
Sari Rämö¹, Sadikshya Ghimire², Minna Haapalainen² and Satu Latvala¹
¹Natural Resources Institute Finland (Luke), Finland; ²Department of Agricultural Sciences, University of Helsinki, Finland
- P23 Mycotoxin occurrence in European grains: an update on prevalence and economic impacts
Dinda Raraswati, Xinxin Wang, Marlous Focker and H.J. van der Fels-Klerx
 Wageningen Food Safety Research, Wageningen University & Research, the Netherlands
- P24 Occurrence of regulated and non-regulated mycotoxins in plant-based foods from the UK by liquid chromatography coupled to mass spectrometry in tandem (UHPLC-MS/MS)
Raquel Torrijos^{1,2}, Octavian Augustin Mihalache¹, Chiara Dall'Asta¹ and Ángel Medina³
¹Department of Food and Drug, University of Parma, Italy; ²Laboratory of Food Chemistry and Toxicology, University of Valencia, Spain; ³Magan Centre of Applied Mycology, Cranfield University, UK
- P25 Fumonisin B1 in cereals grain in Serbia
Slavica Stankovic¹, Ana Obradovic¹, Vesna Krnjaja², Milica Lucev¹ and Goran Stankovic¹
¹Maize Research Institute Zemun Polje, Serbia; ²Institute for Animal Husbandry, Serbia
- P26 Insights on world emerging and masked mycotoxins 2024
Ines Taschl¹, Doris Hartinger² and Anneliese Mueller¹
¹Animal Nutrition & Health and ²Animal Nutrition & Health R&D Center, dsm-firmenich, Austria
- P27 Uptake of beauvericin, deoxynivalenol, zearalenone and other mycotoxins by black soldier fly larvae growing on contaminated maize and market waste
Marcus Trentzsch¹, Carolyne Kipkoeh¹, Jakob Kühn¹, Christoph Gottschalk¹, Julia Jaster-Keller¹, John M. Wesonga², Ronald Maul³, Christoph Hutzler¹ and Stefan Weigel¹
¹Department for Safety in the Food Chain, German Federal Institute for Risk Assessment (BfR), Germany; ²Department of Horticulture and Food, Jomo Kenyatta University of Agriculture and Technology, Kenya; ³Department of Safety and Quality of Milk and Fish Products, Max Rubner-Institut, Federal Research Institute of Nutrition and Food, Germany
- P28 Aflatoxin contamination in maize from Bangladesh: A multi-mycotoxin analysis of harvested and stored grains
 Meher Nigad Nipa, **Carol Verheecke-Vaessen**, Angel Medina Vaya and Andrea Patriarca
 Magan Centre of Applied Mycology, Cranfield University, UK
- P29 Occurrence of deoxynivalenol and its conjugates in cereals harvested in Croatia
Ana Vulić¹, S. Furmeg², T. Lešić¹, N. Kudumija¹, M. Sokolović³, M. Berendika³, V. Jaki Tkalec² and M. Zadavec⁴
¹Laboratory for Analytical Chemistry, ²Veterinary Institute Križevci, ³Poultry Center and ⁴Laboratory for Feed Microbiology, Croatian Veterinary Institute, Croatia
- P30 European grain monitoring – An important risk management tool for the cereals industry using the example of enniatins and beauvericin
Lena Woelk¹, Bärbel Kniel², Peter Köhler², Maximilian Moser² and Peter Haarbeck¹
¹German Cereal Processing, Milling and Starch Industries` Association, Germany and ²Biotask AG, Germany

MYCOTOXINS IN ONE HEALTH PERSPECTIVE

- P31 Exploring the effects of fumonisin B mycotoxins on rabbits: Cell membrane fatty acid composition and organ histopathology
Omeralfaroug Ali¹, Zsolt Gerencsér², Krisztian Balogh³, Miklós Mézes³, Róbert Glávits⁵, Edward Agyarko¹, Melinda Kovács^{1,4} and András Szabó^{1,4}
¹Institute of Physiology and Animal Nutrition, Department of Animal Physiology and Health, ²Institute of Agricultural and Food Economics, ³Department of Feed Safety, Institute of Physiology and Nutrition, and ⁴Mycotoxins in the Food Chain Research Group, Hungarian University of Agriculture and Life Sciences; ⁵Autopsy Ltd., Hungary
- P32 Contamination of feed silos as a source of mycotoxicological contamination – Impact on productivity and biochemical parameters of blood of domestic pigs
Michał Dąbrowski¹, Małgorzata Gugolek¹, Adam Okorski², Krzysztof Jankowski², Krzysztof Karpiesiuk³, Wojciech Kozera³ and Łukasz Zielonka¹
¹Department of Veterinary Prevention and Feed Hygiene, ²Department of Entomology, Phytopathology and Molecular Diagnostics, and ³Department of Pig Breeding, University of Warmia and Mazury in Olsztyn, Poland
- P33 Blood levels of zearalenone, thyroid-stimulating hormone, and thyroid hormones in patients with colorectal cancer
Magdalena Gajęcka, Łukasz Zielonka, Michał Dąbrowski and Maciej T. Gajęcki
 Department of Veterinary Prevention and Feed Hygiene, University of Warmia and Mazury in Olsztyn, Poland
- P34 Differential modulation of the lipid signature in *Zea mays* L. resistant and susceptible inbred lines following *F. verticillioides* infection
Noemi Gesteiro^{1,3}, Laura Carbonell-Rozas^{1#}, Laura Righetti², Rogelio Santiago³, Ana Butrón³ and Chiara Dall'Asta¹
¹Department of Food and Drug, University of Parma, Italy; ²Laboratory of Organic Chemistry, Wageningen University & Research, the Netherlands; ³Misión Biológica de Galicia, Sede de Pontevedra, Spain
- P35 Impact of two probiotics (*Lactobacillus plantarum* and *Bifidobacterium animalis*) on productivity and selected parameters of blood morphology in swine fed with feed naturally contaminated with deoxynivalenol
Małgorzata Gugolek¹, Michał Dąbrowski¹, Krzysztof Karpiesiuk², Wojciech Kozera² and Łukasz Zielonka¹
¹Department of Veterinary Prevention and Feed Hygiene and ²Department of Pig Breeding, University of Warmia and Mazury in Olsztyn, Poland
- P36 Searching the mitigation silver-bullet by understanding aflatoxin contamination and drought stress in crops
Baozhu Guo¹, Jake C. Fountain², Liming Yang³ and Corley Holbrook¹
¹Crop Genetics and Breeding Research Unit, USDA-ARS, USA; ²Department of Plant Pathology, University of Georgia, USA; ³College of Biology and Environment, Nanjing Forestry University, Nanjing, P.R. China
- P37 *In ovo* effect of single and combined aflatoxin B1 and B2 on growth performance, antioxidant systems, and energy metabolism genes of broiler chickens
Martha Cebile Jobe and Mulunda Mwanza
 Department of Animal Health, North-West University, South Africa
- P38 Unlocking diversity: Towards a comprehensive framework for physiologically based pharmacokinetic population qualification in Sub-Saharan Africa
Orphélie Lootens¹⁻⁴, Marthe De Boevre^{1,3,4}, Jan Van Bocxlaer², Sarah De Saeger^{1,3-5} and An Vermeulen²
¹Centre of Excellence in Mycotoxicology and Public Health, Ghent University, Belgium; ²Laboratory of Medical Biochemistry and Clinical Analysis, Ghent University, Belgium; ³MYTOX-

SOUTH[®], International Thematic Network, Belgium; ⁴Cancer Research Institute Ghent, Belgium;
⁵Department of Biotechnology and Food Technology, University of Johannesburg, South Africa

- P39 Mycotoxin-induced dysbiosis: Effects of sub-chronical doses of ochratoxin a and aflatoxin B1 on beneficial and pathogenic gut microorganisms
Alessandra Marcon Gasperini¹, Damaris Christine Landgraf^{1,2}, Carmen Vidaechea^{1,3}, Daniele Sartori² and Esther Garcia-Cela¹
¹MycoLab, Clinical, Pharmaceutical and Biological Sciences, University of Hertfordshire, UK;
²Departamento de Bioquímica e Biotecnologia, Universidade Estadual de Londrina, Brazil;
³Facultad de Ciencias Experimentales, Universidad Francisco de Vitoria, Spain
- P40 The toxicokinetics of the mycotoxin T-2 toxin in human volunteers following a single oral exposure: Study design
Hannah McKeon, Corinne Sprong and Marcel Mengelers
National Institute for Public Health and the Environment (RIVM), the Netherlands
- P41 Unraveling the impact of multiple mycotoxin exposures on post-kidney transplant outcomes through uniting epidemiological and multi-omics designs
Truong Nguyen^{1,2}, Alfonso Narvaez³, Roger P. Gascón¹, Tim J. Knobbe⁴, Johannes R. Björk⁵, Alexander Stockdale⁶, Tess Goessens¹, Stephan J.L. Bakker^{4*}, Sarah De Saeger^{1,7}, Marthe De Boevre¹
¹Centre of Excellence in Mycotoxicology and Public Health, Ghent University, Belgium; ²Cancer Research Institute Ghent, Belgium; ³Laboratory of Food Chemistry and Toxicology, University of Valencia, Spain; ⁴Department of Internal Medicine and ⁵Department of Gastroenterology and Hepatology, University Medical Centre Groningen, the Netherlands; ⁶National Institute for Health and Care Research, University of Liverpool, UK; ⁷Faculty of Science, University of Johannesburg, South Africa
- P42 Combined effects of arsenic and aflatoxin B1 present in infant foods: analysis in different target organs
Esther Lima de Paiva^{1,2}, Olivia Labat¹, Philippe Pinton¹, Carlos Augusto Fernandes de Oliveira², Olivier Puel¹ and Isabelle P. Oswald¹
¹Toxalim – Research Centre in Food Toxicology, University of Toulouse, INRAE, ENVT, INP-Purpan, France; ²Faculty of Animal Science and Food Engineering, University of São Paulo, Brazil
- P43 Evaluating the impact of intermittent multi-mycotoxin exposure on layer breeder performance, egg quality, and hatchability: insights from an intervention study
M. Caballero¹, K. Palanisamy¹ and **Si Trung Tran**²
¹EW Nutrition, Germany; ²EW Nutrition SEAP, Vietnam

MANAGING AND MITIGATING MYCOTOXIN RISKS

- P44 Synergistic antifungal effects of ammonium propionate and medium chain fatty acids
Eugenio Alcalde¹, Jan Dijksterhuis², Pim Kleinhoven¹, Sandra van Kuijk¹, Anouk H.G. Wolters³ and Paul G. Bruinenberg⁴
¹Selko, the Netherlands; ²Westerdijk Fungal Biodiversity Institute, the Netherlands; ³Department of Biomedical Sciences, University Medical Center Groningen, the Netherlands; ⁴Trouw Nutrition R&D, the Netherlands
- P45 Combination of physical and biological methods to mitigate aflatoxin M1 in milk
 Jaqueline Garda Buffon¹, Eliana Badiale Furlong¹ and **Andreia Bianchini**²
¹Laboratory of Mycotoxin and Food Science, Federal University of Rio Grande, Brazil; ²Department of Food Science and Technology, University of Nebraska-Lincoln, USA
- P46 Review of yeast cell wall mode of action and benefits on aflatoxin contamination
Melina Bonato, Fernando Augusto de Souza, William Lima Santiago dos Reis and Céline Coutolleau
 ICC Brazil, Brazil
- P47 Prediction of aflatoxin contamination outbreaks in Texas maize by using mechanistic and machine learning models
Lina Castano-Duque¹, Angela Avila², Joshua Blackstock³, Edwin Winzeler³, Alex Nanez², Matthew Lebar¹, Phillip Ray Owens³, James Lindsay⁴, Kanniah Rajasekaran¹ and Jianzhong Su²
¹Southern Regional Research Center, ARS-USDA, USA; ²University of Texas, USA; ³Dale Bumpers Small Farms Research Center, ARS-USDA, USA; ⁴Office of National Programs, ARS-USDA, USA
- P48 Exploring the potential of Ery 4 laccase-mediator system for aflatoxin B1 and zearalenone degradation: *In vitro* efficacy with mechanistic insights and application in contaminated maize
Biancamaria Ciasca, Martina Loi, Veronica Maria Teresa Lattanzio, Giuseppina Mulè, Antonio Moretti and Miriam Haidukowski
 Institute of Sciences of Food Production, CNR, Italy
- P49 A comparative study of methods for determining minimum inhibitory concentrations in *Aspergillus* species
 Bettina Kitti Salamon¹, Enikő Makkai¹⁻³, Zoltán Gazdag³ and **Árpád Czéh**^{1,2}
¹Institute of Chemistry, External Department of Applied Molecular Sciences – Mycotoxin Research Group, University of Pécs, Hungary; ²Soft Flow Ltd., Hungary; ³Institute of Biology, Department of Molecular Biology and Microbiology, University of Pécs, Hungary
- P50 Biocontrol potential of yeast-produced volatile organic compounds against *Aspergillus carbonarius*
 Pitchapa Eamlaor, Chananwat Kortheerakul, Panan Rerngsamran and **Cheewanun Dachoupakan Sirisomboon**
 Department of Microbiology, University, Bangkok, Thailand
- P51 MYTOX-SOUTH®, a sustainable scientific network for global food safety.
Esther De Rycke^{1,2}, Marthe De Boevre^{1,2} and Sarah De Saeger^{1,2,3}
¹Centre of Excellence in Mycotoxicology and Public Health, Department of Bio-analysis, and ²International Thematic Network MYTOX-SOUTH®, Ghent University, Belgium; ³Department of Biotechnology and Food Technology, University of Johannesburg, South Africa
- P52 Biocatalysts against the zearalenone mycotoxin
Nikolett Emődi¹, Kinga Nyíri¹ and Zsófia Bata²
¹Department of Applied Biotechnology and Food Science, Budapest University of Technology and Economics, Hungary; ²Dr. Bata Ltd. Research and Development Laboratory, Hungary

- P53 Knowledge centre for global food and nutrition security (KC-FNS)
Monica G.L. Ermolli, Carlo Rega and Felix Rembold
European Commission – Joint Research Centre, Directorate D, Sustainable Resources, Italy
- P54 Food safety risks (mycotoxins and heavy metal contamination) associated with ginger and aflatoxin decontamination via probiotics
Lydia Ibrahim^{1,2}, Kolawole Banwo¹ and **Titilayo D.O. Falade**²
¹Department of Microbiology, University of Ibadan, Nigeria; ²International Institute of Tropical Agriculture, Nigeria
- P55 Effectiveness of monitoring aflatoxins along the feed and dairy supply chain
H.J. van der Fels-Klerx and X. Wang
Wageningen University & Research, the Netherlands
- P56 Mycotoxin predictions for cereal and maize worldwide extension
Alain Froment, Alexandre Nussbaumer and Vincent Godet
Syngenta, France
- P57 Potential of the yeast *Hyphopichia burtonii* for the decontamination of mycotoxins
Luis Javier Romero García, Belén Patiño and **Jéssica Gil-Serna**
Department of Genetics, Physiology and Microbiology, Complutense University of Madrid, Spain
- P58 The use of mycotoxin binders: A sustainable strategy to support the feed and dairy industry
Donato Greco¹, Vito D'Ascanio¹, Mariagrazia Abbasciano¹, Milena Brasca¹, Erminio Trevisi², Vincenzo Lopreiato³ and Giuseppina Avantaggiato¹
¹Institute of Sciences of Food Production, CNR, Italy; ²Department of Animal Science, Food and Nutrition, Università Cattolica del Sacro Cuore, Italy; ³Department of Veterinary Sciences, University of Messina, Italy
- P59 Prediction of emerging mycotoxins: Comparison of six machine learning models
Nina M.C. Hommels¹, J.H. Bonestroo¹ and H.J. van der Fels-Klerx^{1,2}
¹Business Economics Group, Wageningen University and Research, the Netherlands; ²Wageningen Food Safety Research, Wageningen University and Research, the Netherlands
- P60 Differential *in vitro* capabilities of mycotoxin binders to mitigate DON damage in IPEC-J2 cell9
Karina Horgan, Niall Browne and Ciara McDermott
Alltech Bioscience Centre, Ireland
- P61 Understanding the molecular mechanism of a fumonisin esterase by kinetic and structural studies
Dániel J. Incze¹, Zsófia Molnár^{1,3}, Gergely N. Nagy^{3,4}, Ibolya Leveles^{3,4}, Beáta G. Vértessy^{3,4}, László Poppe^{1,5} and Zsófia Bata²
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- P62 Mitigation of negative effects of enniatin in creep feed and post-weaning diets in piglets
Sandra van Kuijk¹, Guanlin Wang¹, Anouschka Middelkoop², Regiane R. Santos² and Swamy Haladi¹
¹Trouw Nutrition, the Netherlands; ²Schothorst Feed Research, the Netherlands
- P63 Comparison of the effects of commercially available fungicides and a novel compound on mycotoxin-producing *Fusarium species*
Enikő Makkai¹⁻³, András Vida², Tibor Bartók⁴, Attila K. Horváth⁵, György Csekő⁵, Árpád Czéh^{2,3} and Zoltán Gazdag¹
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Molecular Sciences, University of Pécs, Hungary; ⁴Fumizol Ltd., Hungary; ⁵Institute of Chemistry, Department of General and Inorganic Chemistry, University of Pécs, Hungary

- P64 Adsorption of zearalenone by smectite-based materials of different surface chemistry
Jakub Matusik and Klaudia Dziewiątka
Faculty of Geology, Geophysics and Environmental Protection, AGH University of Krakow, Poland
- P65 Smectite-based modified materials for adsorption of emerging mycotoxins: Alternariol, enniatin B1, and beauvericin
Klaudia Dziewiątka¹, **Jakub Matusik**¹, Małgorzata Gbylik-Sikorska², Aneta Matras² and Piotr Jedziniak²
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- P66 Mycotoxins and predictive models in Africa.
Mohammed Mahdi, Julia Höhler and Ine van der Fels-Klerx
Wageningen University and Research, the Netherlands
- P67 Bioprospecting and valorisation of citrus crops to obtain preharvest and post-harvest biocontrol agents against mycotoxigenic fungi
Jorge Calpe, Ana Moreno, Rosa Vazquez, Elisa Soriano, Sergio Hernandez, Victor Dopazo, Juan Manuel Quiles, Carlos Luz, Laura Escriva and **Giuseppe Meca**
Department of Preventive Medicine and Public Health, Food Sciences, Toxicology and Forensic Medicine, University of Valencia, Spain
- P68 Inhibitive effect of *Urginea epigea* methanolic extract and silver/zinc oxide nanoparticles on *Aspergillus* and aflatoxin production
Mulunda Mwanza and Martha C. Jobe
Department of Animal Health, North-West University, South Africa
- P69 The investigation of effectiveness of different concentrations of the mycotoxin detoxification agent added to broiler feed, in the presence of T-2 toxin, on performance, organ mass and the residues T-2 toxin and its metabolites in the broiler tissues
Jelena Nedeljković Trailović¹, Marko Vasiljević², Hunor Farkaš², Jog Roj², Branko Petrujkić¹, Stamen Radulović¹, Gorana Popović¹ and Dragoljub Jovanović¹
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- P70 Gut microbiota maturation and diversity in broilers: the impact of deoxynivalenol contamination
Clément Soulet, Jean De Oliveira and **Thomas Pecqueur**
Cargill Animal Nutrition and Health, Canada
- P71 Efficacy of bentonite-based toxin binder in mitigating the effects of aflatoxin B1 on the growth performance of tilapia
Phuc Hoang¹, May Moh Moh Khin¹, Loc Tran¹, **Lane Pineda**², Swamy Haladi², Guan-lin Wang² and Saravanan Subramanian²
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- P72 A mycotoxin binder containing bentonite and yeast cell wall fractions improves the performance of laying hens exposed to multiple mycotoxins
V. Malathi¹, **Lane Pineda**², R. Bhargava¹, V. Kavitha Rani¹, Guan-Lin Wang², Swamy Haladi²
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- P73 Remediation of emerging mycotoxins using a premium mycotoxin remediation product
Jog Raj, Hunor Farkaš, Svetlana Čujić, Zdenka Jakovčević, and Marko Vasiljević
Patent CO, DOO., Serbia
- P74 Degradation of ochratoxin A by the strain *Stenotrophomonas acidaminiphila* PAFO/6
Zdenka Jakovčević, **Jog Raj**, Hunor Farkaš, Svetlana Čujić and Marko Vasiljević
Patent CO, DOO., Serbia

- P75 *Stenocarpella maydis* ear rot and diplodiatoxin in maize grain production areas of South Africa
Belinda Janse van Rensburg¹, P. Dikhoba¹, B.C. Flett^{1,†} and Hannalien Meyer²
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- P76 The effect of conservation cropping systems used by small-scale farmers in KwaZulu-Natal, South-Africa, on mycotoxigenic fungi and mycotoxin incidence in maize grain
Belinda Janse van Rensburg¹, B.C. Flett¹, H. Njom¹ and E. Kruger²
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- P77 Evaluation of an anti-mycotoxin agent on mycotoxin binding and ruminal chemical profiles in ruminants: an *in vitro* study
Insaf Riahi¹, Eva León¹, Oscar Castro¹, Erica Fiorbelli², Marco Lapris², Michela Errico², Gabriele Rocchetti² and Antonio Gallo².
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- P78 *Ex vivo* efficacy trial of an anti-mycotoxin agent in counteracting the detrimental effects of *Fusarium* mycotoxins in porcine ileal organoid monolayers
Insaf Riahi¹, Raquel Codina¹, Óscar Castro¹, Francesc Molist² and Regiane R Santos²
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- P79 The effect of curcumin and silymarin in mitigating the oxidative stress induced by deoxynivalenol in hepatic cells
Insaf Riahi¹, Meritxell Sadurní¹, Raquel Codina¹, Ignacio Montagud¹, Laura Escrivá² and Giuseppe Meca²
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- P80 Evaluation of the immunomodulatory capacity of an anti-mycotoxin agent for aquatic species containing phytochemicals and emulsifiers in gastrointestinal cells
Insaf Riahi¹, R. Codina¹, A. Moreno², J. Calpe², V. D'Opazo² and G. Meca²
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- P81 The impact of deoxynivalenol and enniatins on brain and gut of weaned piglets, and the effect of an algoclay-based decontaminant in counteracting the negative effect of these mycotoxins
Xiaonan Guan¹, Aneliya Milanova², Ekaterina Vachkova², Valerija Petrova², Maria A Rodriguez³, **Leandro Royo**³, Cendrine Nicoletti⁴, Marc Maresca⁴, Francesc Molist¹ and Regiane R Santos¹
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- P82 Association of preharvest practices with multimycotoxin contamination in sorghum (*Sorghum bicolor*) in Northwest Ethiopia
Jemal Awol Sadik^{1,2}, B. Vermeulen³, L. Righetti^{3,4}, N. Fentahun⁵, I.D. Brouwer^{6,7}, M. Tessema⁸, M. Abera⁹ and H.J. van der Fels-Klerx^{1,3}
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- P83 Innovative enzyme solutions: advancing ochratoxin a detoxification in broilers
Dian Schatzmayr, Barbara Doupovec, Christoph Gonaus, Barbara Streit and Shreenath Prasad
dsm-firmenich, Animal Nutrition and Health R&D Center Tulln, Austria
- P84 Effects of bacteria-based anti-biotoxins supplementation on post-weaning gilt performances, blood parameters, gut health and microbiome, in the presence of feed contaminated with deoxynivalenol and zearalenone
Eduardo Micotti da Gloria¹, **Clarisse Techer**², Andre Mayer³ and Alexandre Brame²
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- P85 Mitigation of mycotoxin effects on broiler performance and intestinal health using a *Bacillus*-based anti-biotoxins solution
Djepa Obekouo Marie¹, Kouakou N'goran David Vincent¹, **Clarisse Techer**², Alexandre Brame² and Abou Koné²
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- P86 A global strategy using ingredients with complementary mode of actions to limit mycotoxins adverse effects on animals
Clarisse Techer
miXscience, France
- P87 Biocontrol potential of VOCs-producing *Hanseniaspora uvarum* L793 and *Metschnikowia pulcherrima* L672 against *Aspergillus flavus* M114 isolated from fig (*Ficus Carica* L.)
Raquel Torrijos^{1,2}, Paula Tejero³, Ana Martínez³, Guillem Campmajó¹, Chiara Dall'Asta¹, Alejandro Hernández³, Alberto Martín³ and Alicia Rodríguez³
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- P88 Ambrosia (2024-2027): Bridging knowledge, communication, and action for food safety in a changing climate
Fady Mohareb¹, **Carol Verheecke-Vaessen**², Kostas Koutsomanis³, Amanda Maycock⁴, Spyros Fountas⁵ and Christopher Brewster⁶
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- P89 Two mycotoxin-mitigating products improve the reproductive performance of sows exposed to *Fusarium* mycotoxins
Karolina Von Zuben Augusto¹, Rhuan Chaves², Igor Donzeles², Guan-Lin Wang¹, Swamy Haladi¹ and Vinícius Cantarelli³,
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- P90 Development of a feed additive (zearalenone detoxifier) using yeast enzymes
Krzysztof Waśkiewicz¹, Michał Dąbrowski², Łukasz Zielonka² and Michał Łuczyński¹
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- P91 A novel yeast cell wall based preparation with improved mitigation efficacy toward deoxynivalenol and fusaric acid
Alexandros Yiannikouris, Ursula Fox, Aaron Kirkland and Josh Perry
Alltech Inc., USA

SAMPLING AND ANALYSIS

- P92 Linking *Fusarium* damaged kernels and deoxynivalenol contamination in wheat: A synchrotron-based X-ray imaging approach
Sheila M.P. Andrade¹, Xiao Fan Ding³, Lipu Wang¹, Ruijiao Kang³, Maria Alejandra Oviedo-Ludena¹, Chithra Karunakaran⁴, Ning Zhu^{2,4} and H. Randy Kutcher¹
¹Department of Plant Sciences and ²Division of Biomedical Engineering, University of Saskatchewan, Canada; ³Xuchang Vocational and Technical College, China; ⁴Canadian Light Source Inc., Canada
- P93 Analysis of multi-mycotoxins including emerging mycotoxins in cereals, finished feeds and food matrices by liquid chromatography-tandem quadrupole mass spectrometry with lower limits of detection
Julie Brunkhorst¹, Emilee Easter¹, Jordan Steinberg¹ and Holly Lee²
¹Trilogy Analytical Laboratory, USA; ²Sciex, Canada
- P94 Use of flexible scope accreditation under ISO/IEC 17025 for the analysis of mycotoxins in new food matrixes
Josep Calderón and Roger Collantes
 Agència de Salut Pública de Barcelona, Spain
- P95 Improving internal laboratory processes and sustainability using rapid online automation for aflatoxin analysis
 Sebastian Wissmueller, Alexander Burkon, Markus Kopp, Elizabeth Manning, Naomi McLachlan, **Carol Donnelly** and Siobhan Kelly
 R-Biopharm Rhône, UK
- P96 Leveraging the full potential of LC-MS/MS to study the impact of climate change on mycotoxin occurrence
Stephan Freitag¹, Michael Sulyok¹, Elisabeth Reiter², Maximilian Lipp², Klemens Mechtler² and Rudolf Krska^{1,3}
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- P97 LC-MS matrix effect and correction using ¹³C stable isotope internal standards
Robert Furnage
 Volac International Ltd., UK
- P98 Advanced tools for rapid and on-site detection of ochratoxin in the food chain from farm to fork (the OTASens project)
 Carmen Campanale, **Donato Greco**, Elisa Santovito, Vito D'Ascanio, Mariagrazia Abbasciano and Giuseppina Avantaggiato
 Institute of Sciences of Food Production, CNR, Italy
- P99 Development of an ultra-fast, green and sustainable LC-MS/MS method for the analysis of multiple mycotoxins in feed and food
Brett Greer^{1,4}, Qiqi He¹, Anne Nugent¹, Sufyan Pandor³, Olivier Chevallier³ and Christopher Elliott^{1,2}
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- P100 Using aflatoxin spiked maize kernels for testing sample preparation
Claus Holm¹, Tamás Szörényi², Adrienn Lajkó², Miklós Harasztia², Darwin Palima¹ and Andrés Vida²
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- P101 Multi-mycotoxin analyses by UPLC-MS/MS in wheat: Situation in Wallonia in 2023 and 2024
Camille Jonard, Philippe Vermeulen., Bruno Godin and Sébastien Gofflot
Department Knowledge and Valorization of Agricultural Products, Walloon Agricultural Research Centre, Belgium
- P102 Development of analytical method for multi-mycotoxins using rapid sample preparation technique for food safety
Youngwoon Kang¹, Hee Joong Kim¹, Hee Won Lee¹, Seung Jung Shin¹, Jong Hoon Ahn¹ and Junghyuck Suh¹
Korea Food Contaminants Division, National Institute of Food and Drug Safety Evaluation, Ministry of Food and Drug Safety, Korea
- P103 Targeted analysis of 1000 fungal metabolites using UPLC-Orbitrap-HRMS: Method transfer and optimization challenges
Lidija Kenjeric^{1,2}, Bernhard Seidl^{2,3}, Maria Doppler³, Michael Sulyok², Rainer Schuhmacher² and Rudolf Krska^{1,2,4}
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- P104 High-throughput, fully automated sample preparation for contaminant analysis
Mareike Reichel, Sarah Kulas, Mareike Rathke, Clara John, Jan Sebastian Mänz
Eurofins WEJ Contaminants GmbH, Germany
- P105 Developing of a MS-eNose tool for the early detection of ochratoxigenic *Aspergillus westerdijkiae* on traditional Italian caciocavallo during ripening process
Francesco Longobardi¹, Salvatore Cervellieri², Antonia Susca², Pamela Anelli², Massimo Ferrara², Thomas Netti², Miriam Haidukowski², Antonio Moretti² and Vincenzo Lippolis²
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- P106 Increase performance whilst reducing operating costs: Combining immunoaffinity clean-up with automation
Elizabeth Manning, **Claire Milligan** and Helen Cameron
R-Biopharm Rhône, UK
- P107 Validation of single extraction method for the simultaneous analysis of mycotoxins using immunoaffinity columns
Dave Leeman¹, Andy Allan¹, Helen Cameron¹, Carol Donnelly¹, **Claire Milligan**¹, Adam Tramaseur², Joanna Stratton² and Susan MacDonald²
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- P108 New lower EU legislative levels for *Fusarium* toxins: Improving limits of detection with baby food using a multi-toxin immunoaffinity column for sample clean-up
Dave Leeman, **Claire Milligan** and Siobhan Kelly
R-Biopharm Rhône, UK
- P109 Quantum Ochratoxin Green is a rapid and novel lateral flow device for quantification of ochratoxin in grains, cereals and nuts: Identification of toxin-free samples in just 2 min
Georgios Papageorgiou, A. Ntantasios, V. Meras, C. Mpramis, S. Laporda, A. Alevra, M. Begolli and S. Iliopoulou
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- P110 development of a high-sensitivity ELISA method for aflatoxin b1 detection in human blood serum
Georgios Papageorgiou, Konstantina Badra, Ioannis Gkantounas, Konstantinos Lakis, Maria-Eleni Mavrommati and Antonios Ntantasios
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- P111 A quantitative analysis of aflatoxin B1, B2, G1, G2 and ochratoxin A in food samples via 2D-LC-MS/MS
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- P112 Quantitation of mycotoxins and tropane alkaloids in food using triple quadrupole and QTRAP™ technology
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- P113 Hydrolysis of T-2 toxin in aqueous maize extracts
Ben Strong and Ron Sarver
 Neogen Corporation, USA
- P114 Multi-year inter-laboratory HPLC and LCMS results for analysis of naturally incurred aflatoxin containing reference materials in ground maize
 Ronald Sarver, Alex Kostin and **Benjamin Strong**
 Neogen Corporation, USA
- P115 Approval of the AgraStrip® Pro Total Aflatoxin WATEX® test kit for FGIS Certification
Martin Witek, Barbara Cvak, Sonja Kraus, Donna Houchins and Luis M. Fidalgo
 Romer Labs, Austria
- P116 Validation of a lateral flow device for ochratoxin A detection in roasted coffee
Martin Witek, Jasmin Kraus, Cecilia Korpela and Anna-Mariia Nikolaieva
 Romer Labs, Austria
- P117 Accelerated and parallel mycotoxin clean-up by thermal elution – higher sensitivity and faster processing
Frederik N. Wuppermann and Uwe R. Aulwurm
 LCTech GmbH, Germany
- P118 Rapid analysis of ochratoxin A in cocoa powder (cocoa cake) using Ochra-V™ Max method
Justine Yu, Jianmin Liu and Lingyun Chen
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MYCOTOXIN OCCURRENCE AND FUNGAL CHARACTERIZATION

P1

Fumonisin contamination in maize from Nebraska: A multiyear snapshot

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Fumonisin are among the most toxic mycotoxins, primarily produced by *Fusarium proliferatum* and *F. verticillioides*. They are involved in disrupting sphingolipid metabolism and inhibiting ceramide synthase. Exposure to these mycotoxins can cause equine leukoencephalomalacia and porcine pulmonary edema. Fumonisin have also been associated with esophageal cancer and neural tube defects in humans. Notably, maize is known as the most susceptible cereal to fumonisin contamination; fumonisin contamination in maize can pose a significant food safety concern. Nebraska, the third largest maize producer in the United States, produced 1.73 billion bushels of maize in the year 2023. In this multi-year study, fumonisin levels in maize were monitored from 2022 to 2024 across the major maize-producing counties in Nebraska. A total of 134 maize samples were analysed using a fluorometer-based fumonisin test. Results indicated that 70.1% of the maize was positive for fumonisin, with an average of 1.97 ppm. The total fumonisin concentration ranged from 0.25 to 19 ppm, and the average among the positive samples was 2.80 ppm. The percentage of samples exceeding 4.0 ppm fumonisins was 12.7%. Further, crop damage by insects and weather events, including hail and wind, significantly affected fumonisin contamination in maize grain. This dataset suggests a reasonable risk of fumonisins associated with maize produced in Nebraska and indicates the necessity to effectively mitigate affected crops intended for human consumption and animal feed. An integrated approach, including optimal agronomic practices, better pest management, improved storage conditions, and intensive surveillance and remediation in high-risk zones, can effectively minimize contamination risks and improve the overall quality of maize in Nebraska.

P2

Monitoring the occurrence of aflatoxin B1 in natural products

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The global consumption of natural products is on the rise, driven by their aromatic profiles, flavour-enhancing qualities, as well as their functional and medicinal properties. Consequently, there is a growing consumer demand for foods recognized for their health-promoting nutrients. Among these products, bee pollen, green tea (*Camellia sinensis*), and cinnamon (*Cinnamomum verum*) stand out. During the production stages of these products, contamination by toxigenic fungi may occur. Given the health risks associated with aflatoxin B1 (AFB1), this study aims to monitor and quantify its occurrence in these products marketed in a region of São Paulo state, Brazil. To date, six bee pollen, five green tea, and five cinnamon samples have been analysed using acetone:water (85:15) as the extraction solvent and an immunoaffinity column for cleanup. Detection and quantification were performed using HPLC with post-column photochemical derivatization and fluorescence detector. The limit of detection for AFB1 in the three products is approximately 0.15 µg/kg, and the recovery at concentrations of 1.6 and 4.5 µg/kg ranged from 80% to 115%. Among the analysed samples, three bee pollen (50%), two green tea (40%), and two cinnamon (40%) samples were detected with AFB1, with concentrations below the theoretical limit of quantification approximately 0.5 µg/kg. Although only a few samples have been analysed so far, the percentage of positive samples, even at low concentrations, suggests a potential for AFB1 production in the studied products, as well as a contribution to dietary exposure to this toxin, highlighting the need for continuous monitoring and risk assessment.

P3

Extending *Fusarium proliferatum* metabolite identification by combining LC-TWIMS-HRMS and FBMN
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Fusarium proliferatum, a member of the *Fusarium fujikuroi* species complex, is a toxigenic filamentous fungus characterised by an extensive and diverse host range. This species is best known for producing fumonisins, one of the most prevalent classes of mycotoxins, especially in staple foods such as rice or maize, posing significant concern to human and animal health. Additionally, it synthesises other toxic metabolites (e.g., beauvericins, moniliformin, fusarins) along with various structurally distinct families of secondary metabolites. However, metabolomic insights on its secondary metabolism are still missing, and several molecules remain undetected and uncharacterised. At the same time, the intraspecific differential production of mycotoxins and related compounds, the accumulation of their intermediates, and the production of metabolic derivatives have not been systematically explored. In this context, deciphering the fungal metabolome – uncovering specific underlying mechanisms and behaviours of the fungus – requires combining accurate and advanced analytical techniques with proper processing tools. This study evaluated the potential of combining suspect and non-targeted liquid chromatography-traveling wave ion mobility spectrometry-high-resolution mass spectrometry (LC-TWIMS-HRMS) with feature-based molecular networking (FBMN) to map *Fusarium proliferatum* polar. Hence, 13 *Fusarium proliferatum* strains inoculated in triplicate on autoclaved rice were analysed. The strains comprised Fumonisin Bs (FBs) non-producers, low-producers, and producers. After sample treatment, the polar fraction was analysed through LC-TWIMS-HRMS under instrumental conditions favouring the LC separation and electrospray ionisation (ESI) of fumonisin-related metabolites. Moreover, the HRMS detection was carried out in the positive ESI mode and combined the full-scan and data-independent analysis (DIA) MS modes (m/z range from 100 to 1,000). Targeted and suspect LC-TWIMS-HRMS data processing allowed the tentative identification of the main *Fusarium proliferatum* toxic secondary metabolites, using reference analytical standards and an in-house database containing HRMS and TWIMS data of species-specific metabolites. Furthermore, FBMN analysis was also carried out to expand metabolite identification through a non-targeted approach. As a result, five molecular networks were related to fumonisin metabolism in the FBs-producing strains, one to beauvericin, and two to FBs-non-producing strains characteristic patterns. For instance, changes in rice glycerophosphocholine composition were observed between FBs-producing and non-producing strains, suggesting that medium composition may play a role in the shifts in fumonisin metabolism. Therefore, the present study highlights the potential of applying LC-TWIMS-HRMS and FBMN as a valid tool for comparing molecular features across fungal strains with different chemical phenotypes, revealing biosynthetic pathway intermediates or products, and inferring the identities of related unknowns.

P4

Evaluation of climate change on the presence of mycotoxins on food matrices for the last 10 years

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Mycotoxins are regulated since many years, currently in Commission Regulation (EU) 2023/915. Some of the mycotoxins included in this document are deoxynivalenol, zearalenone, fumonisins B1 and B2, T-2/HT-2 toxins, aflatoxins, and ochratoxin. Maximum residue limits are defined for different food matrices, such as cereals and nuts. The Laboratori de l'Agència de Salut Pública de Barcelona (LASPB) has analysed mycotoxins in food samples such as cereals, nuts, dried fruits, spices, and many more matrices, for more than 25 years. The methods are currently included in the scope of the accreditation of the laboratory following ISO/IEC 17025 requirements. The extraction is carried out with three different methods, depending on the type of mycotoxins. For aflatoxins B and G and ochratoxin, an extraction with polar solvents and a clean-up using immunoaffinity columns and determination by LC-FLD is carried out. For the other toxins, a QuEChERS technology has been optimised, while the instrumental method is based on LC-MS/MS. The presence of mycotoxins in food is related to the presence of fungi during cultivation, storage, and to many other processes. The increment of the temperature due to climate change may affect the increment of fungi in food commodities, resulting in the production of more mycotoxins. In the present work, the results of mycotoxin analysis by the LASPB during the last 10 years of official campaigns, will be studied in correlation with increasing temperatures.

P5

Diversity of mycotoxins in stored paddy rice: Contamination patterns in the Mekong Delta, Vietnam
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Rice (*Oryza sativa* L.) is the most important food worldwide and a vital staple food in Vietnam. However, 15% of rice is lost annually during post-harvest due to fungal growth and mycotoxins contamination. This study aimed to evaluate mycotoxin contamination in stored paddy rice collected in 2018, 2019 and 2022 in six provinces in the Mekong Delta, Vietnam, using LC-MS/MS. The results revealed that 47% of the samples were contaminated with 12 types of mycotoxins. The prevalence of these mycotoxins was 30% (ZEN), 10% (FUS or MON), 6% (BEA or AFB2), 4% (AFG1), 3% (AFB1), 2% (AFG2 or FB1) and 1% (OTA or AME or ENB). Among the provinces, stored paddy rice from Kien Giang had the highest contamination (80%), and then Ben Tre (67%), Long An (63%), An Giang (58%), Dong Thap (44%) and Can Tho (19%). Remarkably, paddy rice collected in 2022 was usually contaminated with emerging mycotoxins with a higher incidence of co-occurrence. These mycotoxins typically co-occurred in combinations as two mycotoxins (2% of the samples were contaminated with FUS-BEA or ZEN), three mycotoxins (2-4% of the samples were contaminated with ZEN or MON-FUS-BEA), four mycotoxins (2-6% of the samples contaminated with ZEN-FUS-BEA-ENB or MON), and five mycotoxins (2% of the samples contaminated with ZEN-FUS-BEA-MON-FB1). Additionally, five stored paddy rice samples were contaminated with AFB1, OTA, and ZEN exceeding Vietnamese regulatory limits for unprocessed rice. Our findings provide valuable insights into mycotoxin contamination across different years and growing regions in the Mekong Delta, Vietnam. This study could give essential information to stakeholders as policy makers or food safety authorities, etc., to inform strategies to mitigate these toxins in the near future and underscores the importance of monitoring rice production.

P6

Mycotoxin contamination of cereals and fermented foods across African regions in the last 10 years (2014-2024)

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Mycotoxins are toxic secondary fungal metabolites contaminating various foods and agricultural products worldwide and posing serious threat to global food security. The most harmful mycotoxins include aflatoxins, fumonisins, trichothecenes, ochratoxin A and zearalenone produced from the genera *Aspergillus*, *Fusarium* and *Penicillium*. Favourable weather conditions such as high temperatures and humidity, as well as other factors like grain damage, poor postharvest handling and unappropriated storage conditions make cereal crops susceptible to fungal growth and mycotoxin contamination. In Africa, aflatoxins and fumonisins are considered the major mycotoxins occurring widespread in maize, sorghum and millet, that are among the most important staple food crops across the country. Furthermore, the presence of the other main mycotoxins, deoxynivalenol, ochratoxin A and zearalenone, have also been often reported in African cereals even though in limited surveys. The levels of mycotoxins in crops, in Africa, frequently exceed the maximum limit set by the European Union and many countries in African lack effective strategies to control their level in food and feeds. Therefore, the sourcing of high-quality raw materials and producing high-quality products are challenges due to mycotoxin contamination. Then, it is important for all African countries to develop effective strategies to monitor the harmful mycotoxins and integrate their management into food value chain policies and development frameworks to enable effective mitigation strategies. The aim of this work is to provide a map on the status of the major mycotoxin contaminating cereals and fermented foods in Africa in the last 10 years (2014-2024). The main process of literature searching employed the online database and was based on: (i) defining research questions; (ii) screening and selection of papers; (iii) classification of selected papers based on geographical origin (African countries) of samples, type of food, mycotoxins detected;

and (iv) data synthesis and analysis to identify challenges and gaps in their management and provide useful recommendations enhancing food safety governance in Africa. Acknowledgements. This research was financially supported by the European HORIZON project UP-RISE: EU-AU Partnership for Resilient, Inclusive and Safe food systems for Everyone (Grant Agreement No. 101136649).

P7

Mycotoxin prevalence in UK horse feed

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Mycotoxin contamination in animal feed is well documented in production species such as cattle, pigs, and poultry, yet its prevalence in horse feed, particularly in the UK, is not as well researched. This study aimed to assess the exposure of UK horses to mycotoxins by analysing 268 samples of raw materials, forages, and finished feeds collected from commercial sources between February and September 2024. Samples were analysed for 18 different mycotoxins with results indicating widespread contamination. 78% of samples tested positive for at least one mycotoxin. Forage samples had the lowest contamination rates, with only 33% testing positive, and most containing low levels of toxins. In contrast, cereals and compound feeds showed significantly higher contamination rates, with 89% and 96% testing positive, respectively. Overall, 28% of all samples presented at least a medium risk to horses, with 10% posing a very high risk. The primary mycotoxins detected were produced by *Fusarium* and *Penicillium* species, consistent with the temperate UK climate. T-2 and HT-2 toxins were particularly prevalent, found in 100% of oat samples and 68% of all positive samples, posing a significant risk due to their toxicity. These findings show that UK horses are frequently exposed to potentially harmful mycotoxins, particularly through oats and oat-based products, which form a key component of their diets.

P8

Mycotoxin contamination in animal feed – UK and Ireland survey 2024

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Mycotoxin contamination in animal feed is a persistent concern for livestock producers in the UK and Ireland. This survey aimed to assess mycotoxin prevalence across different feed types and animal species from 622 commercial samples sent to Volac International for analysis. Samples included complete feeds (43%), homegrown forage (28%), cereals (15%), and other feed materials (14%). Samples were analysed for 18 mycotoxins using Volac International's ISO 17025-accredited LCMS method, with results categorized into risk levels according to the Volac Mycocheck Risk Assessment. Overall, 77% of all samples tested positive for at least one mycotoxin, though most were scored as low risk. However, over a quarter of the samples (27%) were scored as medium risk or higher, with 13% presenting a high or very high risk. Contamination rates and sample risk varied significantly by species. Poultry feed had the highest rate of positive samples (100%) with 54% being scored medium risk or higher. Conversely, swine feed had the lowest prevalence (40%) and the lowest risk samples with only 10% at medium risk or higher. Dairy and beef feeds showed contamination rates of 71% and 78%, respectively, with 13% and 17% of samples presenting a medium or higher risk. Despite 2024 not being a high-risk year for mycotoxin production, contamination patterns differed significantly across species, likely due to feed composition. These findings highlight the need for continued species-specific monitoring and risk assessment to safeguard livestock health and productivity.

P9

Survey: Do bale storage conditions have an effect on exposure to field mycotoxins?

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By 2050, it is estimated by the World Resource Institute that the global population will have risen to 10 billion people; the demand for agricultural crops and animal feed is thus projected to rise by 84%. How to meet the growing food demand for an expanding population has raised concerns about the quality and safety of animal feed as hazardous substances entering the food/feed chain can directly impact livestock productivity. Fungal growth on crops and subsequent contamination of crops with 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. It is known that storage conditions can influence the mycotoxin contamination profile, particularly mycotoxins produced by *Penicillium* or *Aspergillus* species. Studies investigating mycotoxin profile during storage have principally focused on the storage of cereal grains as they are consumed directly by livestock and humans. Straw bales (produced from cereal crops) are used in farming principally as bedding, however, animals are known to consume the straw (Terre *et al.*, 2007. *Animal Feed Science and Technology* 137: 115). From our previous survey in 2018, we identified that 80.3% of straw samples contained 1 or more mycotoxin, while 14.1% of samples had 3 or more mycotoxins indicating that straw can contribute to the exposure of livestock to mycotoxins (Furmage *et al.*, 2019. *The World Mycotoxin Forum*, 14-16 October 2019, Belfast, UK, p.119). The aim of this survey was to determine whether bale storage conditions had an effect on the mycotoxin contamination profile of straw bales. Straw bales were sampled from 3 farms in the UK within close proximity of each geographical location and analysed for the presence of mycotoxins using a Waters LC/MS running a multi-mycotoxin detection method over a period of a year. The most frequently detected mycotoxin was deoxynivalenol (DON). Interestingly, straw bales on one farm that had no visible detection of mycotoxins over the first 4 months of the year, showed the presence of mycotoxins after 5 months of storage suggesting that storage conditions of straw bales may have an effect on the mycotoxin profile of field mycotoxins. While more research is required to further validate these results, this survey indicates that field mycotoxins profile in straw bales can also be affected by storage conditions.

P10

Occurrence of 16 mycotoxins in spices and herbs commercialized in Italy

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Aflatoxins (AFB1, AFB2, AFG1, AFG2), ochratoxin A (OTA), fumonisins (FB1, FB2), T-2 and HT-2 toxins (HT-2, T-2), zearalenone (ZEA), deoxynivalenol (DON), tenuazonic acid (TeA), alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT) and tentoxin (TTX) were analysed in 100 samples of spices and herbs commercialized in Italy. Only aflatoxins and OTA are regulated in Europe for some spices (*Capsicum* spp., *Piper* spp., *Myristica fragrans*, *Zingiber officinale*, *Curcuma longa*) and their mixtures with herbs. A double solvent extraction was used for principal mycotoxins, whereas a single solvent extraction was used for *Alternaria* mycotoxins. The extracts of the two groups of mycotoxins were separately analysed by UPLC-MS/MS and matrix assisted calibration after one night refrigeration, centrifugation and microfiltration. The limits of detection (LOD) of the methods ranged from 0.072 µg/kg (ZEA) to 70.6 µg/kg (DON). High percentage of samples (79%) were found contaminated by 1-7 mycotoxins. Aflatoxins were detected in 9% and 5% of spices and herbs, respectively and OTA in 14% of spices and 0% of herbs. Only one sample of spices (cloves) showed an AFB1 value (9 µg/kg) higher than the European limit. No sample of spices and herbs were contaminated by OTA beyond the limit. Within the non-regulated mycotoxins, ZEA was the most occurring (67%). All samples were negative to ALT. Mean levels of non-regulated mycotoxins in positive samples of spices ranged from 1,813.9 µg/kg (FB1) to 4.6 µg/kg (ZEA), in herbs they ranged from 131.4 µg/kg (FB2) to 2.5 µg/kg (ZEA) and in mix of spices they ranged from 1071.73 µg/kg (FB1) to 2.6 µg/kg (ZEA). The spices with higher mycotoxin levels were garlic, red pepper, flax seeds and paprika, whereas within the herbs they were basil and sage. This study provides large information on the natural occurrence of multi-mycotoxins (regulated and not regulated) in spices and herbs consumed in Italy, in view of possible future regulation of some mycotoxins such as FBs and TEA which occurred at the highest levels, i.e., 6,692 µg/kg and 12,612 µg/kg, respectively. Furthermore, the high percentage of positive samples and the high levels of some mycotoxins observed in this study demonstrate the high susceptibility of spices and herbs to multi-mycotoxins contamination. These results represent a good starting point to assess human exposure to multi-mycotoxins from spices and herbs in Italy and require further studies to establish safe limits for not yet regulated mycotoxins that will protect human health.

P11

Studies of the growth and aflatoxin production of *Aspergillus parasiticus* on in-shell, shelled and split almonds

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Aspergillus parasiticus is a fungus that can infect almonds and produce carcinogenic aflatoxins (AF). The objective of this study was to determine the growth and total AF production of *A. parasiticus* on three types of almond kernels (in-shell, shelled and split) under different combinations of temperature (20, 27 and 35 °C), water activity (0.65, 0.75, 0.80, 0.85, 0.90, 0.95, 0.99 a_w) and incubation period (10, 20 and 30 days). The in-shell kernels supported the least amount of growth and AF production. The fungus grew moderately at 0.90 and 0.95 a_w and produced moderate (<50 µg/kg) amount of total AF at 0.95 a_w on in-shell almonds. On shelled kernels, growth was also limited to 0.90 and 0.95 a_w . Aflatoxin production reached high levels at 0.95 a_w at all three temperatures on shelled almonds (324.3, 325.4, and 139.8 µg/kg at 20, 27 and 35 °C, respectively). The fungus grew rapidly and produced high levels of AF (>300 µg/kg) on split kernels over a wide range of conditions (0.80 to 0.95 a_w and 20 to 35 °C). Contour plots revealed that the optimum conditions for AF production on split kernels were at 0.90-0.95 a_w and 20-27 °C. Aflatoxin production also depended on incubation time. By day 30, AF production on split kernels exceeded 300 µg/kg at 0.80 a_w at all temperatures. There was no fungal growth and AF production at 0.65 and 0.75 a_w . Transit studies using data loggers placed inside boxes of almonds indicated that the relative humidity levels inside the boxes remained in the range of 44.5 to 61.9%, and the temperature ranged from 12.3 to 30.7°C, during shipping over the ocean. These relative humidity levels during ocean transit ensure a low water activity (<0.65 a_w) for the almonds. Our study showed that *A. parasiticus* was not able to grow and produce aflatoxins at 0.65 a_w on almonds for a six-month observation period at any of the study temperatures. Consequently, environmental conditions during ocean transit are not favourable for the growth and AF production of *A. parasiticus* on almonds. The results of this study show the kernel types and conditions where the threat of spoilage due to *A. parasiticus* on almonds is high. The risk of infestation by *A. parasiticus* during storage and transportation can be reduced by sorting damaged kernels and maintaining low water activity (<0.75 a_w).

P12

Analysis of mycotoxin data from 2020-2024: There exists a pattern in global mycotoxin contamination in raw materials and complete feeds

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The contamination of big six mycotoxins: aflatoxin (AF), deoxynivalenol (DON), fumonisins (FB), ochratoxin (OTA), T-2 and HT-2 toxin (T2HT2) and zearalenone (ZEA) during 2020 to 2024 were analysed. A total of 267910 samples comprising of 275010 total analyses from 59 countries globally were analysed. The samples were acquired from various farms, feed mills, integration operations, and various Nutreco laboratories. The mycotoxin analysis was carried out by using ELISA, lateral flow devices and LC-MSMS techniques. The analysis indicated the presence of unique cyclic pattern in AF, OTA, DON and T2HT2 contamination. AF, OTA and T2HT2 exhibited annual cyclic pattern, while DON exhibited a cyclic pattern comprising of roughly 1.75 years. ZEA contamination exhibited a non distinct pattern, while FB exhibited an increasing trend approaching a plateau. The analysis also revealed highest level of global AF contamination occurred in the last quarter of 2022 reaching a global average of around 11.5 µg/kg. The data also revealed an increasing trend of AF contamination starting from 2020. A very different and distinct contamination patterns were observed when data were segregated according to the geographical distribution. In Asia, Africa and Latin American region AF contamination is very prominent and distinct. North America was dominant in DON contamination. FB contamination in Asia and African region showing downward pattern while in Europe it was showing upswing trend. Climate change, movement of materials from region to region play an important role in regional mycotoxin trend.

P13

Analysis of prevalence of mycotoxins in Asian region during 2024

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The contamination levels of big six mycotoxins: aflatoxin (AF), deoxynivalenol (DON), fumonisins (FB), ochratoxin (OTA), T2 and HT2 toxin (T2HT2) and zearalenone (ZEA), in 5,810 samples comprising of raw materials and finished feeds from Asian region collected during 2024 were analysed for animal feed raw materials and finished feeds. Aflatoxin was the most important mycotoxin analysed in the region. The median AF concentration varied between 3-45 µg/kg, while average value varied between 4-49 µg/kg. The maximum average contamination was recorded in ruminant feed. The median DON concentration varied up to 350 µg/kg while the average varied from <100-1,340 µg/kg. The highest level of average DON contamination was recorded in grain byproducts. The median FB concentration varied between <100-1,200 µg/kg, while average varied between <100-1,689 µg/kg. Grains were contaminated with highest average concentration of which had also highest median concentration, followed by poultry feed with average concentration of 1,507 µg/kg. The median OTA concentration varied between 1-12 µg/kg, while average was 2-16 µg/kg. OTA was found to be major contaminant of protein meals. Protein meals had highest median and average OTA concentration. The median concentration level of T2HT2 toxin remained low between 1-5 µg/kg, while the average T2HT2 concentration varying between 1-11 µg/kg. Maximum average concentration of T2HT2 was recorded in grain byproducts. The median concentration for ZEA varied between <2.5-48.5 µg/kg, while the average concentration of ZEA varied between 6-71 µg/kg. Maximum level of ZEA contamination was observed in grain byproducts. Highest aflatoxin contamination was observed in January while lowest monthly variation in aflatoxin levels was observed during April. The OTA also showed highest contamination during January. Highest DON contamination was observed around May to July. Highest level of FB contamination was observed in January while lowest contamination was observed in August. ZEA contamination was peaked in May.

P14

Analysis of prevalence of mycotoxins in Latin American region during 2024

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The contamination levels of Big six mycotoxins: aflatoxin (AF), deoxynivalenol (DON), fumonisins (FB), ochratoxin (OTA), T2 and HT2 toxin (T2HT2) and zearalenone (ZEA), in 18614 samples comprising of raw materials and finished feeds from Latin American region collected during 2024 were analysed. The median AF concentration varied between <1-8.5 µg/kg, while average value varied between 1-12 µg/kg. The maximum average contamination was recorded in silages followed by grain products (9 µg/kg). The median DON concentration varied up to 300 µg/kg while the average varied from <100-648 µg/kg. The median FB concentration varied between <100-1,200 µg/kg, while average varied between 207-1,604 µg/kg. Poultry feed was contaminated with highest average concentration of which had also highest median concentration. The median OTA concentration varied between 1-7 µg/kg, while average was 2-50 µg/kg. OTA was found to be major contaminant of grains and grain byproducts. A maximum of 2,467 µg/kg level of OTA was recorded in grain samples. The median concentration level of T2HT2 toxin remained between <1-8 µg/kg, while the average T2HT2 concentration varying between <1-86 µg/kg. Maximum average concentration of T2HT2 was recorded in grain byproducts. The median concentration for ZEA varied between <2.5-48.5 µg/kg, while the average concentration of ZEA varied between 6-71 µg/kg. Maximum level of ZEA contamination was observed in grain byproducts. Highest aflatoxin contamination was observed in September while lowest monthly variation in aflatoxin levels was observed in November. The OTA also showed similar pattern as AF. Highest DON contamination was observed during March and lowest in November. FB contamination pattern had three distinct peaks in February, June and November, where highest level contamination was observed during February peak. Lowest FB contamination was observed during April and August. ZEA showed continuous decreasing trend from January to December. T2HT2 contamination was peaked in August while lowest level was observed in June.

P15

Analysis of prevalence of mycotoxins in European region during 2024

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The contamination levels of Big six mycotoxins: aflatoxin (AF), deoxynivalenol (DON), fumonisins (FB), ochratoxin (OTA), T2 and HT2 toxin (T2HT2) and zearalenone (ZEA), in 40,217 samples comprising of raw materials, silages, total mixed ration (TMR) and finished feeds from European region collected during 2024 were analysed. The median concentration for AF was 0.9-1 µg/kg for all types of commodities. The average value varied between 1-4 µg/kg. The grain byproducts and protein meals had highest average contamination. Among the feed categories, poultry feed had highest level of AF contamination (3 µg/kg). DON is a major concern for European region. The median DON contamination varied from <100-562 µg/kg while the average varied from <100-1,039 µg/kg. Highest level of DON contamination recorded was 15,509 µg/kg in grain byproducts. Recent days FBs have emerged as one of the major contaminants in the southern Europe. The median contamination level was <100-438 µg/kg while the average was between <100-2,486 µg/kg. The highest level of FB contamination was recorded in grain byproducts and silages (24,435 µg/kg). Among the finished feeds, pig feeds recorded highest average contamination level of 468 µg/kg. Ochratoxin contamination remained insignificant in all commodities with median 1-2.5 µg/kg and average contamination of 1-2 µg/kg. The median and average T2HT2 contamination varied between 5-38 and 5-83 µg/kg respectively. TMR had highest median level of T2HT2 contamination. Highest T2HT2 concentration was recorded in silages (1,536 µg/kg). TMR recorded highest level of ZEA contamination with median and average of 63.5 and 111 µg/kg respectively. The mycotoxin data reveals a pattern or trend in contamination over the entire year. The mycotoxin contamination trend in feed samples were mainly influenced by contamination in grains and grain byproducts. AF contamination in grains had more influence on AF contamination in feeds. Contamination of DON in feeds was more influenced by contamination in grain byproducts. FB and T2HT2 contamination in feed were influenced by contamination of both grains and grain byproducts.

P16

Regional distribution of *Fusarium* mycotoxins and their metabolites in maize cultivated in Croatia

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During maize growth, the crop is prone to contamination by moulds, particularly from the *Fusarium* genus, which can produce mycotoxins that can adversely affect human health. The prevalence of mycotoxins in maize is influenced by seasonal and local weather conditions during the growth phase. This study analysed 62 maize samples collected from October till November 2024 from family-owned farms in three regions of Croatia – eastern (n=32), northern (n=15), and central (n=15) – prior to storage. The samples were analysed for the concentrations of 15 *Fusarium* mycotoxins, including zearalenone (ZEA) and its metabolites ZEA-14-O-β-glucoside and ZEA-14-sulfate, nivalenol (NIV), deoxynivalenol (DON) and its metabolites (3-acetyl-DON, 15-acetyl-DON, and DON-3-glucoside), fumonisin B1 (FUMB1), fumonisin B2 (FUMB2), T-2 and HT-2 toxins, diacetoxyscirpenol (DAS), neosolaniol (NEO), and fusarenon-X (FUS-X) using the LC-MS/MS (liquid chromatography-tandem mass spectrometry) method. Five mycotoxins – ZEA, ZEA-14-glucoside, DAS, NEO, and FUS-X – were not detected in any of the samples. FUMB1 was the most prevalent mycotoxin across all three regions, with average concentrations ranging from 1000 to 1,500 µg/kg, and only 6% of the samples were free from contamination. The occurrence of mycotoxins varied by region: in the eastern region, FUMB2 (47%) and DON (16%) were the second most prevalent, while in the northern region, HT-2 (40%), T-2 (27%), and DON (27%) were the most abundant. In the central region, DON (60%) was the second most prevalent mycotoxin, with T-2, HT-2, FUMB2, and DON metabolites found at similar prevalence rates of 30-40%. The high contamination with FUM and DON in general can be attributed to moderate temperatures and significant rainfall in Croatia during September while crops were still on the field. Regional differences in mycotoxin prevalence, such as the lower occurrence of T-2 and HT-2 toxins in the eastern region, may be explained by local weather conditions, such as the drier conditions in August in that region. Four samples contaminated with summed T-2 and HT-2 toxins and five with summed fumonisins (FUMs) exceeded the European Union regulatory limits for unprocessed maize grains. While mycotoxin

metabolites are not yet regulated by legislation, there is growing interest from the European Food Safety Authority (EFSA) in their monitoring. Continued surveillance of *Fusarium* mycotoxins, particularly those that are not yet regulated, will provide valuable insights into trends in mycotoxin occurrence in relation to climatic conditions.

P17

Presence of *Aspergillus* species in Serbia

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Aspergillus species are important fungi that cause various plant diseases and also produce mycotoxins that can be harmful to human and animal health. In Serbia, maize and small grains are key crops, so studying the occurrence of *Aspergillus* species on these crops is of particular importance for agricultural production and food safety. The aim of this study was to identify *Aspergillus* species present on maize and small grains in different agro-ecological conditions in Serbia, as well as to assess their impact on crop quality and potential mycotoxin production. Samples of maize and stubble grains from various locations in Serbia were tested during the growing season in the period 2022-2024, especially during the ripening and harvest phases. From the isolated samples, standard molecular methods (PCR, sequencing) were used to identify the species of the genus *Aspergillus* present. The results showed that the most abundant species were *Aspergillus flavus* and *Aspergillus parasiticus*, which are notable for their high ability to synthesize aflatoxins. *Aspergillus flavus* was most abundant on maize, while species of the genus *Aspergillus* section *Nigri* were more common on small grains, such as wheat and barley. These results suggest a high incidence of crop contamination by *Aspergillus* species, which poses a potential risk to food safety, as the mycotoxins produced by these species can cause serious health problems. Given climate change, which may increase the frequency of droughts and high temperatures, the risk of *Aspergillus* contamination is expected to increase, indicating the need for the implementation of effective preventive and control strategies in maize and small grain production in Serbia.

P18

Characterization of *Fusarium graminearum* species complex originated from maize kernels in Serbia

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The species *Fusarium graminearum* Schwabe is one of the most important and widespread species of the genus *Fusarium* both in the world and in Serbia. This species can directly affect yield reduction as well as grain quality because it has the ability to synthesize a large number of mycotoxins, of which the most important are trichothecenes type B deoxynivalenol (DON), its acetyl-ester derivatives (3ADON and 15ADON), nivalenol (NIV) and zearalenone (ZEA). Using phylogenetic analysis and the GCPSR method (genealogical matching of phylogenetic species), 15 different phylogenetic species were discovered within the *Fusarium graminearum* species complex (FGSC) (Sarver *et al.*, 2011. Fungal Genetics and Biology 48:1096). The aim of this research was to determine the species within the FGSC in Serbia. In this research, 37 isolates of this complex, isolated from maize kernels from 10 different locations in Serbia were studied. Phylogenetic analysis of four selected sequences of two genomic regions of *TEF-1α* (MF974400, MF974405, MF974408, MF974409) and *histone H3* gene (MF999140, MF999145, MF999148, MF999149), revealed that three isolates were identified as *Fusarium graminearum sensu stricto* and one isolate as *F. boothii*. (MF974409, MF999149). It was found that these two genomic regions were sufficiently informative to distinguish the *F. boothii* species within FGSC. Based on chemical and molecular analyses, it was confirmed that all tested isolates belonged to chemotype 15ADON. Since previous research has shown that climate change is the main cause of the appearance of new and potentially more toxic species, future research must pay special attention to changes within the FGSC population.

P19

Study of mycotoxin occurrence in Spanish cereal fields

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Mycotoxins are harmful secondary metabolites produced by certain fungal species, capable of contaminating agricultural products and raising significant concerns for human and animal health. Additionally, they are increasingly influenced by climate change, which alters fungal growth patterns and toxin production. Higher temperatures, more humidity, and extreme weather make crops like oat, wheat, and barley more likely to be contaminated by mycotoxigenic fungi. In Spain, this might affect the risk of mycotoxin outbreaks, threatening agricultural productivity and food safety in critical regions related to cereal production. In this work, we analysed zearalenone, nivalenol and deoxynivalenol levels in thirty soil and cereal samples collected in 2023 from a variety of Spanish regions. Mycotoxins were extracted following Malachová *et al.*, 2014 (Journal of Chromatography A 2014, 1362: 145) for cereals and Muñoz *et al.*, 2015 (Mycotoxin Research 31: 191) with modifications for soil. LC-MS/MS was used for identification and quantification, ensuring high sensitivity and specificity. Calibration curves were prepared with certified standards, and quality control included blanks and positive samples to validate protocols. Limit of detection (LOD) and quantification were also calculated for both methods. Zearalenone was the most frequently detected mycotoxin in soil, found in 70% of samples, with a peak concentration of 6.29 ng/g. In cereals was found in 27% of samples, (maximum: 7.47 ng/g). Nivalenol was detected in six soil samples (maximum: 1.95 ng/g). Moreover, it was found in only one cereal sample (6.27 ng/ml). Deoxynivalenol was detected in both soil (16%) and cereals (30%) samples. Upper levels were 4.79 ng/g and 7.24 ng/g, respectively. For zearalenone, 20% of the studied samples showed contamination in the corresponding soil and cereal samples. One possible explanation is that toxigenic fungi produce mycotoxins in the soil, which are subsequently absorbed by plants through their roots. These mycotoxins might then be transported to different parts of the plant, eventually accumulating in various tissues. To our knowledge, this is the first study to investigate the occurrence of mycotoxins in Spanish soil samples. Our results reveal detectable contamination in the studied crops, which poses a significant risk to food safety. In this context, understanding the mechanism by which mycotoxins are taken up from soil to plants is essential for developing effective strategies to mitigate contamination in agricultural products and protect public health.

P20

Leveraging extensive mycotoxin analysis data for accurate contamination trends and risk management
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Mycotoxin contaminations are inherently dynamic, exhibiting significant year-to-year variations and notable differences even between neighbouring countries. While interpreting contamination trends over large regions is insightful, the ability to conduct geographically precise analyses is far more beneficial. This precision, however, necessitates a database with a vast quantity of results to ensure real significance and true interpretability. Cargill's analysis database, the largest of its kind globally, encompasses over 375,000 analyses in 2023 and more than 400,000 in 2024. This extensive dataset enables detailed examination of mycotoxin contamination trends. For instance, in Europe (53,000 analyses in 2023 and 61,000 in 2024), levels of deoxynivalenol and fumonisin in feed ingredients were slightly lower for the first two-thirds of 2024 compared to 2023, while zearalenone levels remained relatively stable until fall 2024. A significant increase in fumonisin, deoxynivalenol, and zearalenone levels was observed starting in September-October 2024. Closer inspection reveals distinct regional trends. In northern and eastern Europe (20,000 analyses in 2023 and 22,000 in 2024), fumonisin levels were slightly lower in 2024 compared to 2023, with no increase observed at the end of 2024. Deoxynivalenol levels were significantly lower in 2024 compared to 2023, with no late-year increase, while zearalenone levels were similar in both years, but showing a slight increase from October 2024. Conversely, in southern Europe countries (33,000 analyses in 2023 and 39,000 in 2024), fumonisin levels were also lower in 2024 compared to 2023, but a substantial increase began in the summer of 2024. Deoxynivalenol levels were higher at the beginning of 2024, similar from April to September

compared to the same periods in 2023, with a sudden increase observed from October 2024. Zearalenone levels were similar in both years until a significant increase occurred from October 2024. These disparities underscore the importance of a sufficiently large analysis database, segmented by country and even by region within countries, to draw meaningful conclusions. By leveraging historical data, this database enables to identify local trends and patterns in mycotoxin contamination, crucial for anticipating risks and implementing effective preventive measures. Access to this comprehensive database allows to assess mycotoxin risk levels in raw materials, making informed decisions about sourcing and quality control. Combined with advanced analysis tools, this database provides actionable insights and guidelines for mycotoxin management, optimizing control plans by analysing recent contamination trends. This facilitates adjustments in the frequency of raw material batch analyses.

P21

Occurrence of multiple mycotoxins in various fibre sources

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Dietary fibre is commonly known as the part of the diet which is not broken down by the animal's digestive enzymes, but they are important for maintaining a healthy intestinal microflora. In animal feed, wheat bran, maize bran, rice bran, soy hulls, sugar beet pulp and lignocellulose (such as OptiCell® or FibreCell®) are commonly used as a fibre source. Among other sources, OptiCell®, a standardized, patented combination of natural dietary fibre derived from selected tree species and FibreCell® a natural product made of wood-based lignocellulose have gained increasing attention as a fibre source in animal feed. Animal feed materials including fibre sources can contain mycotoxins and other contaminants. These fibre samples for mycotoxins analysis were received from different regions of Argentina, Austria, Chile, Costa Rica, Ecuador, South Korea, Mexico, Peru, Philippines, Serbia, Taiwan and Turkey between June 2023 and March 2024. In this survey, approximately 500 samples were analysed for multiple mycotoxins. These samples were analysed by LC-MS/MS *triple quadrupole* (Agilent 6460 series) using a multi-mycotoxin method for quantification of all mycotoxins present. These included aflatoxins AFB1, AFB2, AFG1, AFG2, α -zearalenol (α -ZEL) β -zearalenol (β -ZEL), zearalanone (ZAN), zearalenone (ZEN), diacetoxyscirpenol (DAS), HT-2, T-2, 3-acetyl deoxynivalenol (3-ADON), 15-acetyl deoxynivalenol (15-ADON), deoxynivalenol (DON), nivalenol (NIV), fumonisin B1 (FB1), FB2, FB3, fusaric acid (FA), moniliformin (MON), ochratoxin A (OTA), beauvericin (BEA), enniatin A (ENA), enniatin A1 (ENA1), enniatin B (ENB) and enniatin B1 (ENB1) using LC-MS/MS. The main conclusions are: (i) OptiCell® and FibreCell® did not contain any mycotoxins and therefore can be used as a mycotoxin-free fiber source in animal nutrition; (ii) All fibre materials, i.e., maize bran, DDGS, rice bran, soyhull, wheat bran, and sunflower meal were contaminated with multiple mycotoxins. Among these maize bran, DDGS, rice bran and wheat bran were highly contaminated with 3-11 mycotoxins per sample; (iii) Maize bran samples (100%) were contaminated with more than 5 mycotoxins per sample. FB1, with a higher average of 4,790 ppb was the most dominant mycotoxin detected in maize bran samples. Among emerging mycotoxins, fusaric acid, BEA and enniatin group mycotoxins with higher occurrence were also detected in maize bran sample; (iv) DDGS (100%) samples were contaminated with more than 5 mycotoxins per sample. FB1, FB2 and FB3 were detected in 91% samples (high averages, for FB1: 1,080 ppb), AFB1 in 52%, and DON was detected in 78% DDGS samples. Fusaric acid, BEA and MON were also detected in DDGS samples; (v) The analysis of rice bran samples from Philippines, Taiwan, Korea, and Peru were found to contain more than 4 mycotoxins/sample. FB1/FB2/FB3, AFB1, OTA BEA, MON, DAS, and FA were the main mycotoxins detected in rice bran samples; (vi) The analysis of wheat bran samples between June 2023-March 2024 from Austria, Argentina, Chile, Costa Rica, Ecuador, Korea, Mexico, Peru, Philippines, and Serbia, showed that 100% samples were contaminated with 1-11 mycotoxins (FB1, FB2, AFB1, ZEN, OTA, DON, BEA, FA, ENNs) per sample. To conclude, the co-occurrence of more than one mycotoxin can lead to additive or synergistic effects when fed to sensitive animal species (e.g., swine and poultry amongst others). This suggests that technologies are required for the control of mixtures of such contaminants in feedstuffs predominantly based on maize to minimize impacts on animal development.

P22

Fumonisin production in onion (*Allium cepa*) inoculated with *Fusarium proliferatum*

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Fusarium proliferatum is one of the *Fusarium* species that cause *Fusarium* basal rot (FBR) in onion in Finland. It can also produce mycotoxins – fumonisins (B1, B2 and B3), beauvericin, and moniliformin – which are potentially harmful to humans and animals. To study the mycotoxin production, seven *F. proliferatum* isolates from Finnish onions, were inoculated in rice culture. Fumonisin were produced by all the seven *F. proliferatum* isolates studied, beauvericin was produced by six of them and moniliformin by four of them. Next, the ability of these isolates to produce mycotoxins, especially fumonisins, was studied in onion. Based on the previous results on virulence to onion and genetic features and the toxin production profile on rice culture, three *F. proliferatum* isolates (Fpr047, Fpr049, FUS16163) were chosen for onion inoculation study. After inoculation, onion samples were taken weekly for DNA, RNA and mycotoxin analysis during five weeks. The aims of the study were to determine the timepoint for the start of fumonisin production and to find out if there are differences between the isolates in the mycotoxin production. Five onions per each inoculation treatment were taken randomly at each timepoint from the onions inoculated with the three *Fusarium* isolates and from the control onions. The onions were cut into halves vertically from the point of inoculation to observe if there is any symptomatic area. The cut onions were photographed to record the symptoms. Three out of the five cut onions per isolate and controls at each timepoint were chosen for sample preparation for DNA and RNA extractions. The rest of each inoculated onion was divided into two separate samples for *Fusarium*-toxin analysis by UHPLC-MS/MS-quantification: symptomatic and non-symptomatic onion tissue. Three water-inoculated control onion samples were also taken at each sampling point. When growing inside the onion bulbs, all the three isolates studied caused visible disease symptoms and expressed the gene *FUM1*, required for fumonisin production. Fumonisin were not detected in the water-control samples at any timepoint but were detected in one non-symptomatic onion tissue sample. Fumonisin were already detected at the three-week timepoint after inoculation. The fumonisin concentrations showed a large variation between the three symptomatic onion samples analysed per each isolate. Mycotoxin concentrations were on average 1000-fold in the rice culture in comparison to the concentrations in the inoculated onions. Acknowledgements. The mycotoxin research was funded by Maiju ja Yrjö Rikalan puutarhasäätiö.

P23

Mycotoxin occurrence in European grains: An update on prevalence and economic impacts

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Mycotoxin contamination in grains is a serious issue as these toxic substances both threaten humans and animal health and cause economic losses. Despite the implementation of preventive measures to minimize mycotoxin contamination in grains, the presence of mycotoxin remains inevitable. EU regulations have set maximum limits for the presence of mycotoxins in food and feed. When mycotoxin concentrations in grains are above the respective EU maximum limits, grains can be downgraded from food to feed, or to biofuel. This study aims to understand the prevalence of mycotoxins in grains and estimate the economic losses due to downgrading in European countries. The presence of eight mycotoxins were analysed in more than 80,000 sample results from grains intended for food-use and feed-use (e.g., maize, wheat, barley, rye, oats, and triticale) from 33 European countries over a ten-year-period (2014-2024). While the limit of quantification (LOQ) indicated the presence of mycotoxins in crops (percent positive), the EU maximum limits for mycotoxins in food and feed were used as a threshold for estimating the percentage of exceedance. In this study, the estimation of economic losses focused on the losses due to food downgrading to feed and feed downgrading to fuel. Data analysis revealed that more than 80% of grain samples intended for food exceeded LOQ values, whereas approximately 25% of grain samples intended for feed-use were above LOQ. Around 11% and 0.5% of grain samples intended for food-use and feed-use, respectively were above their respective ML. Economic losses in the top five maize-producing countries (Ukraine, France, Russia, Romania, and Poland) due to mycotoxin contamination reached an average of 67 million euros. More comprehensive results regarding the mycotoxin occurrence per country and the associated economic losses will be presented at the conference. Analyses of over 80,000 samples results related to mycotoxin presence in

grains in Europe showed 80% prevalence, and 11% and 0.5% exceedance of legal limits for feed and food use respectively. Economic losses due to downgrading maize were estimated to be 67 million euros.

P24

Occurrence of regulated and non-regulated mycotoxins in plant-based foods from the UK by liquid chromatography coupled to mass spectrometry in tandem (UHPLC-MS/MS)

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Food consumption patterns are shifting toward dietary choices that are perceived by the consumers as healthier and more sustainable, leading to a growing demand for plant-based products. Within this trend, plant-based meat alternatives (PBMA) and plant-based beverages (PBB) represent the largest niche market of available plant-based products. Although the benefits of plant-based diets are well documented, the potential risk associated with mycotoxin exposure due to these food items remains largely unexplored. Moreover, currently there is not a regulatory framework for mycotoxins in PBMA and PBB, and only a few raw materials used in their manufacture are routinely monitored. The study aimed to determine 19 mycotoxins in PBMA (n=93) and PBB (n=121) obtained from the British market. An in-house validated method using liquid chromatography coupled to mass spectrometry in tandem (LC-MS/MS) was employed to cover the regulated mycotoxins aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1), and G2 (AFG2), ochratoxin A (OTA), zearalenone (ZEN), fumonisin B1 (FB1), and B2 (FB2), HT-2, T-2, deoxynivalenol (DON), as well as the emerging mycotoxins enniatin B (ENNB), B1 (ENNB1), A (ENNA), A1 (ENNA1), beauvericin (BEA), alternariol (AOH), alternariol monomethyl ether (AME), and tentoxin (TEN). The results show that all the samples analysed contained at least one mycotoxin. AFBs, OTA, ZEN, FBs, and HT-2 were prevalent across the PBMA, with overall incidence values of 80.6%, 62.0%, 71.7%, 74.2%, and 64.1%, respectively. The mean contamination values of these mycotoxins ranged from 0.57 µg/kg for AFB1 in cereal-based PBMA to 2.68 µg/kg for HT-2 in legume-based PBMA. Nevertheless, BEA was found in the highest percentage of samples (overall incidence 98.9%) at mean contamination values of 1.10 µg/kg. Regarding PBB, lower contamination values were found compared to the PBMA of the study. The contamination pattern varied according to the raw material used in the manufacture. Generally, high incidence of emerging mycotoxins such as ENNs (75.2%), BEA (100%), and *Alternaria* toxins (77.7%) were evidenced in the PBB, with contamination values ranging from 0.02 µg/l to 1.50 µg/l. Moreover, the co-occurrence of mycotoxins was frequently observed in both PBMA and PBB. Considering the reported prevalence of mycotoxins in plant-based products and the growing consumption of these products, additional monitoring studies are needed to better assess exposure and the potential health risks associated with the consumption of these food alternatives.

P25

Fumonisin B1 in cereals grain in Serbia

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Fumonisin contamination of wide commodities variety have mostly been reported in maize and maize-based foods and feeds. Just a few scientific researches were conducted to obtain results on natural contamination of wheat and barley with these mycotoxins. First report on the presence of fumonisins on naturally infected wheat in Serbia was recorded in 2009, which indicated the growing importance of these mycotoxins and the need to pay attention to it. This survey was conducted to evaluate fumonisin B1 contamination in wheat and barley grain in Serbia. A total of 100 wheat and 50 barley samples were obtained from different local warehouses between October 2023 and June 2024. Concentration of FB1 were analysed by the HPLC method. Positive results were found in 40.6% and 23.1%, wheat and barley samples, respectively. FB1 concentration varied from 750 to 2,300 µg/kg (mean levels 653.7 µg/kg in wheat and 568.2 µg/kg in barley grain). Mycotoxin contamination of cereals was affected by factors such as origin resistance, drought stress, and insect damage. Results showed that agroecological conditions in Serbia favoured the natural incidence of FB1, not only in maize, but also in grain of wheat and barley. This fact, as well as 10.1-17.8% of samples having a higher maximum level of FB1 than feed adopted

by the EC, point out that it is necessary to take measures for lowering concentrations of this mycotoxin in grain of cereals important for agriculture in Serbia.

P26

Insights on world emerging and masked mycotoxins 2024

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New emerging mycotoxins, lack regulation but their presence in feedstuffs is rising. Masked mycotoxins evade conventional detection. Their impacts on feed and food safety are becoming more understood. While over 1000 fungi-born toxins and metabolites exist, only a handful have legal or recommended maximum levels in most jurisdictions. The term 'emerging mycotoxins' is not clearly defined. Recently, emerging mycotoxins were defined as "mycotoxins, which are neither routinely determined nor legislatively regulated; however, the evidence of their incidence is rapidly increasing" (Vaclavikova *et al.*, 2013. Food Chemistry 136: 750). To test the occurrence of multiple mycotoxin metabolites such as emerging and masked toxins, a method based on liquid chromatography coupled with tandem mass spectrometry was used (LC-MS/MS method, Spectrum Top@50). In 2024, a total number of 2,608 finished feed samples, and 1,285 maize samples were analysed from over 60 countries around the world. Among the most common mycotoxins found in finished feed samples were the well-known *Fusarium* toxins deoxynivalenol and zearalenone, detected in 80% and 71% of all samples at an average of 325 ppb and 31 ppb, respectively. The emerging mycotoxins enniatin B, B1 and beauvericin occurred in 76% and 72%, showing maximum levels of 2,654 ppb and 537ppb, respectively. Like enniatins, beauvericin poses potential health risks when found in contaminated feed, as they both showing cytotoxic effects. The masked mycotoxin deoxynivalenol-3-glucoside was represented in 30% of all finished feed samples in 2024 associated with feed refusal and immune suppression. DON-3-glucoside is a masked form of deoxynivalenol, typically created when mycotoxins bind with plant components or other molecules. However, when the masked mycotoxin is ingested or undergo certain conditions, it can be released and become an active toxin again. By having a closer look on maize samples, deoxynivalenol and fumonisin B1 were the most occurring main mycotoxins, detected in 82% and 72% of tested samples at an average of 544 and 1,092 ppb, respectively. The emerging mycotoxin beauvericin occurred at 78% and moniliformin, produced by *F. proliferatum* or *F. verticillioides* was occurring in 71% of the tested maize samples. Both showing negative effects on immune system and cell function. Feed and animal protein producers face challenges from emerging and masked mycotoxins. Proactive measures are essential, employing innovative detection technologies like the LC-MS/MS-based Spectrum Top@ 50 programme. Continuous monitoring, collaboration with experts, and comprehensive strategies are crucial for maintaining product integrity, animal welfare, and feed/food supply chain safety in this evolving landscape.

P27

Uptake of beauvericin, deoxynivalenol, zearalenone and other mycotoxins by black soldier fly larvae growing on contaminated maize and market waste

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Mycotoxins are one of the major concerns affecting global food and feed safety. There is opportunity however for contaminated grains or market waste to re-enter the food chain through bioconversion. Black soldier fly (BSF) larvae - used as a rich protein source in animal feed – are stipulated to efficiently grow on contaminated feed without accumulation of mycotoxins, subsequently allowing the larvae to be used as animal feed with minimal food safety issues. To test this, a study was conducted in which BSF larvae were exposed to mixtures of clean and naturally mycotoxin contaminated maize as well as market waste in defined ratios. The insects were reared on different feed ratios, and at the end of the feeding experiment the larvae were separated from the frass. Extraction of mycotoxins from insects and feed was accomplished based on the modified methods of Camenzuli *et al.* (Toxins 2018, 10 :91) and DIN EN 17641:2022-12, respectively. Quantification was carried out using LC-MS/MS. Insect and feed

samples were screened for 16 mycotoxins which had been found in amounts above the limit of quantification in feed samples previously screened for 76 mycotoxins. Of the 16 mycotoxins measured via LC-MS/MS the 3 most abundant ones found in all feed ratios and all insect samples exposed to mycotoxin containing feed mixtures were beauvericin (BEA), deoxynivalenol (DON) and zearalenone (ZEN). Most abundant corresponding modified forms and metabolites were deoxynivalenol-3-acetate (DON-3-Ac), deoxynivalenol-15-acetate (DON-15-Ac), zearalenol-alpha (ZEL- α) and zearalenol-beta (ZEL- β). Hardly any mycotoxin contamination could be observed in clean maize and pure market waste, but contaminated maize mixed with market waste showed significantly higher mycotoxin concentrations than mixtures with clean maize. Thus, market waste might provide a substrate for fungal growth resulting in mycotoxin production, while the contaminated maize might cover various spores or fungi of the genera *Beauveria* and *Fusarium*. As key finding for DON, DON-3-Ac, DON-15-Ac and ZEN, BSF larvae showed a significant reduction of toxin content compared to the feed, whereas for the more lipophilic compound BEA an accumulation was observed. Interestingly a mixture of 75 % market waste and 25 % contaminated maize led to accumulation of ZEL- α and ZEL- β in BSF. These findings might be caused by metabolization of the corresponding mother compound in the insects or by transfer from the feed into the insects. When blending market waste with contaminated maize and subsequently feeding it to insects, accumulation of BEA and ZEN-metabolites in insects must be taken into account. Acknowledgements. This project is supported by the German Federal Ministry of Food and Agriculture, Grant No. 2819DOKA01.

P28

Aflatoxin contamination in maize from Bangladesh: A multi-mycotoxin analysis of harvested and stored grains

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Scarce data are available on mycotoxin contamination of maize from Bangladesh despite recent data suggesting contamination with aflatoxins. This research aims to provide current data on mycotoxin contamination of Bangladeshi maize at harvest and after storage. Maize samples were collected from 11 maize-producing areas in Bangladesh in 3 stages: Lot 1, 6 months stored grains from summer harvest, 2022; Lot 2, fresh summer harvest, 2023; Lot 3: 6 months stored grains summer harvest, 2023. The samples were collected after harvest and drying by the producers from each area. The mycotoxin concentrations were assessed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) system. Multi-mycotoxin analyses of the maize samples were performed. Aflatoxin B1 (AFB1) was detected in 45% of the samples from Lot 1 (2.0-225.3 $\mu\text{g}/\text{kg}$), 55 % from Lot 2 (1.6-3.3 $\mu\text{g}/\text{kg}$), and 91 % from Lot 3 (1.60-5.80 $\mu\text{g}/\text{kg}$). In total, 62% samples exceeded the limits in maize for AFB1 by EU legislation: 2 $\mu\text{g}/\text{kg}$. AFB2 was present in 27%, 9%, and 0% in Lot 1, 2, and 3, respectively in the range of 3.6-65 $\mu\text{g}/\text{kg}$, with 75% samples contributing to exceed the acceptable limit of for aflatoxins (sum of B1+B2+G1+G2): 4 $\mu\text{g}/\text{kg}$. Only one sample was found positive for AFG2 from Lot 1 (4.4 $\mu\text{g}/\text{kg}$). Only one sample from Lot 2 was positive for ochratoxin A (328.2 $\mu\text{g}/\text{kg}$) whereas the EU acceptance limit is 5 $\mu\text{g}/\text{kg}$. Fumonisin B1, B2, and B3 were found in 55% samples of Lot 1, and 24% equally in both Lot 2 and 3. The highest levels of fumonisin B1, B2, and B3 were found in Lot 1 with means of 115.0, 41.0, and 6.8 $\mu\text{g}/\text{kg}$, respectively. None of these samples exceeded EU limits for fumonisins (sum of B1+B2): 1000 $\mu\text{g}/\text{kg}$. Nivalenol was detected in 27% and 9% of the samples from Lot 1 and 3, respectively (5.6-63 $\mu\text{g}/\text{kg}$). AFG1, ochratoxin B, deoxynivalenol, and 3-acetyl-deoxynivalenol were not detected in any samples. This comprehensive study will serve as evidence for researchers to expand their research on mycotoxin-related issues and implement effective measures to guarantee the safety of maize and its derivatives from a Bangladeshi standpoint.

P29

Occurrence of deoxynivalenol and its conjugates in cereals harvested in Croatia

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Fusarium fungi are among the most abundant mycotoxigenic moulds capable of contaminating both food and feed. Mycotoxins produced by these moulds can be classified into three groups:

trichothecenes, fumonisins, and zearalenone. The representatives of group B trichothecenes include deoxynivalenol (DON) and its acetylated derivatives, 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON), as well as nivalenol (NIV) and fusarenon-X (FUS-X). The main fungal conjugates of DON, 3-ADON, and 15-ADON are directly secreted by the fungi and are biosynthetic precursors of DON. The primary plant conjugate of DON is 3-glucoside-DON (DON-3G), and its presence has been confirmed in various cereals such as wheat, maize, oats, and barley. Data on the occurrence of DON, especially its conjugates, in cereals grown in Croatia are still lacking, although several published studies have shown that *Fusarium graminearum* and *Fusarium culmorum* are the most prevalent moulds in Croatian crops. During the 2024 harvest season, a total of 142 samples (maize n=67, wheat n=30, barley n=18, triticale n=12, oat n=12, rye n=3) were collected immediately after harvest and analysed for the presence of DON and its conjugates using the liquid chromatography tandem mass spectrometry (LC-MS/MS) method. The highest prevalence of DON was found in triticale samples (91.7%), followed by wheat samples (70.0%), while the lowest prevalence was observed in maize samples (14.9%). The prevalence of acetylated conjugates 3-ADON and 15-ADON, as well as the plant conjugate DON-3G, followed the same pattern as that of DON. Overall, the prevalence of acetylated conjugates was below 30% in all tested cereals. Co-occurrence of DON with its conjugates was found in only six samples (4.2%). The peak concentration of DON was identified in a wheat sample (5652.08 ng/g), which also exhibited the highest concentrations of 3-ADON, 15-ADON, and DON-3G. Regarding legal limits, only four samples—one barley, one wheat, and two triticale samples—exceeded the maximum permitted level (MPL) for DON established by EU legislation. Although DON conjugates are not yet regulated in terms of MPL values, according to EFSA, there is a need to collect this data to establish guidance values. Results from this one-year survey revealed that triticale and wheat are more prone to DON contamination compared to maize, barley, and oats, and that the prevalence of DON conjugates followed the same pattern.

P30

European grain monitoring – An important risk management tool for the cereals industry using the example of enniatins and beauvericin

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Recognising risks and averting crises – the European grain monitoring (EGM) is the basis for the risk management of the entire cereals sector. In the last grain marketing year 2023/2024, 155 companies of the grain value chain have participated in the European monitoring for grain and grain products. The participants came from different industry branches with the largest group of 135 mills from Germany, Austria, Switzerland, Italy, and Poland. Contract partners from the trade, producers of bakery ingredients and bakeries participated as well. In total 3,223 samples were analysed. With 90 percent, most samples were grains and grain products intended for food use. In addition to other parameters such as heavy metals, pesticides and microbiological parameters, data on the following mycotoxins are collected as part of the EGM: aflatoxins B and G, ochratoxin A, deoxynivalenol, zearalenone, T-2/HT-2 toxins, nivalenol, ergot alkaloids, *Alternaria* toxins and fumonisins B1, B2. An extra budget is available for projects to improve the available data regarding current food safety issues or scheduled maximum residue levels ('Fund for more flexibility in risk communication'). Last year, this budget was used to obtain data and findings on the occurrence and content of enniatins and beauvericin in rye flour. These mycotoxins are categorised as 'emerging mycotoxins' as there is currently only little knowledge about them. As part of this project, 128 rye flour samples (74 wholemeal rye flours and 54 type 997 rye flours) were analysed for the presence of enniatins B, B1, A, A1 and beauvericin. Enniatins B and B1 were detected in up to approx. 60 percent of the samples. Enniatins A, A1 and beauvericin could not be quantified (with one exception for enniatin A1). The maximum content of 213 µg/kg for the sum of the four enniatins and beauvericin was measured in a sample from conventional cultivation. The investigations carried out have significantly improved the existing data on the content of enniatins and beauvericin in rye flours. Further research is necessary. The EGM provides a very good overview of the contaminants and residues in the raw materials used and forms an excellent data basis for the company's quality management. The data is also an important basis for the grain industry's communication with the authorities and politicians. Companies along the grain chain are invited to participate in the EGM.

MYCOTOXINS IN ONE HEALTH PERSPECTIVE

P31

Exploring the effects of fumonisin B mycotoxins on rabbits: Cell membrane fatty acid composition and organ histopathology

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Fumonisin B series (FB) is known to have detrimental effects on mammals; however, species vary in their response. This study investigated the overall health integrity of rabbit kidneys and liver upon 65 days of exposure to 10 and 20 mg FB1+2+3 (FBs)/kg diet, relying on multiple endpoints, including the membrane total phospholipid fatty acid composition, oxidative stress, clinic-chemical parameters, and histological changes. This study involved 30 adult male rabbits (n=10/group) with the same age and nearly the same body weights. The data evaluation was performed with ANOVA with a Tukey post hoc test, using IBM SPSS 29; meanwhile, the discriminant analysis (sPLS-DA) was performed by R project version 4.1.2 (2017) and the mixOmics package (6.18.1.). For all tests, the significance threshold was set at a p-value of ≤ 0.05 . Despite the final body weight and feed intake remaining identical among groups, organ weights increased. Furthermore, membrane fatty acid composition of different organs exhibited marked proportional alterations, with greater tendency in the kidneys, primarily C14:0 (\downarrow), C22:0 (\downarrow), C20:5n3 (\uparrow), and C22:6n3 (\downarrow). The degree of EPA increase was more pronounced, with a correlation ($r = 0.702$) to the FBs dose, which resulted in an overall enrichment of sum omega-3 fatty acids and decreased omega-6:omega-3 ratio. Notably, renal C20:5n3 and C18:0 expressed a greater contribution to the 1st and 2nd loadings of sPLS-DA, respectively, which set the control group apart from the 20 FBs/kg groups. Although the liver was less responsive, C22:1n9 significantly increased in rabbits fed the highest dose of FBs (20 mg/kg). This highest dose also increased the serum total cholesterol concentration, alongside high-density lipoproteins and creatinine. With relation to antioxidant (glutathione and glutathione peroxidase) and lipid peroxidation (malonaldehyde) markers, no marked differences were noticed between FBs-fed groups and the control. In contrast, the average lesion score of both kidneys and livers from the 20 mg FBs/kg group significantly differed from the control group. In conclusion, the sensitivity to FBs varied across rabbit organs, with higher doses leading to stronger effects. The results indicate the rabbits' adaptability to FBs throughout the trial, as evidenced by their consistent growth performance. Acknowledgements. This work was partially funded by the Hungarian Academy of Sciences (HUN-REN-MATE, Mycotoxins in the Food Chain research group) and by the Hungarian National Laboratory project RRF-2.3.1-21-2022-00007, and further by the Flagship Research Groups Programme by the Hungarian University of Agriculture and Life Sciences.

P32

Contamination of feed silos as a source of mycotoxicological contamination – Impact on productivity and biochemical parameters of blood of domestic pigs

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Mycotoxins are secondary metabolites of fungi that often contaminate feed materials and complete feeds. They are responsible for causing mycotoxicoses, which manifest themselves in health disorders and a decrease in the productivity of farm animals. Developments in agricultural techniques and feed production technologies significantly contribute to reducing the occurrence of this threat. However, it is worth paying attention to the possibility of short-term subacute poisoning in individual production sectors. This phenomenon may be related to insufficient hygiene of storage silos and feed lines in livestock facilities. In the temperate climate zone, feed silos are exposed to significant daily temperature fluctuations, which favours the occurrence of the dew point phenomenon inside them. This phenomenon can lead to a local increase in humidity and thus the growth of fungi (especially on the walls of the silo).

This infected grain/feed can lead to local/accidental contamination of the feed of the selected production sector. To determine the effect of contaminated feed material from a silo with poor hygiene conditions, a feeding experiment was conducted on 16 weaners. The animals were divided into 2 groups (n=8) C (control) and E (experimental). During the experiment, two feeding rations were created, where the experimental rations used feed material from the contaminated silo. The experiment included 7 days of exposure to control and contaminated feed. During the experiment, body weight, growth and feed consumption were assessed. On the last day of the experiment, blood was collected for analysis of biochemical parameters. The conducted analyses allowed to find significant differences in body weight gain, feed consumption and feed conversion ratio. In addition, a significant decrease in serum iron (Fe) levels was observed in group E. The presented studies clearly indicate that short-term and accidental exposure to feed from contaminated silos can lead to significant losses in animal productivity. In addition, a reduced Fe level can be a factor influencing the immune status resulting in the occurrence of opportunistic infections. The presented data can be a valuable clue for veterinarians and zootechnicians in the field of diagnostics of non-obvious mycotoxicological problems.

P33

Blood levels of zearalenone, thyroid-stimulating hormone, and thyroid hormones in patients with colorectal cancer

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Mycotoxins are secondary metabolites produced by various species of fungi commonly found in plant materials. Zearalenone (ZEN) adversely affects the endocrine system. This study aimed to determine whether thyroid-stimulating hormone (TSH), prolactin (PRL), free triiodothyronine (fT3), and free thyroxine (fT4) levels are altered during natural zearalenone mycotoxicosis in patients diagnosed with sigmoid colon cancer (SCC) or colorectal cancer (CRC). A study was conducted on women and men diagnosed with SCC or CRC accompanied by the presence or absence (patients without ZEN – PWZ group) of ZEN in the blood. The PWZ group consisted of 17 patients with symptoms of SCC and CRC in whom ZEN and its metabolites were not detected in peripheral blood. The experimental (empirical) groups included a total of 16 SCC and CRC patients who tested positive for ZEN, but not its metabolites. TSH values in both sexes were within the upper limit of the reference range (0.27-4.2 μ IU/ml) adopted by the hospital laboratory and corresponded to the upper second tertile and the lower third tertile. PRL values demonstrated that SCC and CRC were accompanied by a systemic or local bacterial infection. All mean values of fT3 were in the middle of the reference range, and the mean values of fT4 were within the upper reference limit. The fT3/fT4 prognostic marker was somewhat above the cut-off point of 0.22. These results indicate that in postmenopausal women and andropausal men who were diagnosed with SCC and CRC and were exposed to food-borne ZEN, higher values of the prognostic marker (fT3/fT4) were associated with an unfavourable prognosis. The study also revealed that the more distal the neoplastic lesions in the colon, the higher the percentage of both thyroid hormones, regardless of the patient's sex. The presence of ZEN in the diet alters thyroid activity in patients diagnosed with SCC and CRC.

P34

Differential modulation of the lipid signature in *Zea mays* L. resistant and susceptible inbred lines following *F. verticillioides* infection

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Fusarium Ear Rot (FER) is one of the major diseases affecting maize worldwide, causing decreases in yield and fumonisins accumulation. Recent advances in omics have shed light on the intricate network of genes and signalling pathways involved in maize resistance to this pathogen. Among these, lipid signalling and metabolism have emerged as critical components of the plant's defence arsenal. To

deepen on the role that lipids could have on resistance to FER, a lipidomic study has been performed using resistant and susceptible maize recombinant inbred lines obtained from the same cross. Samples at 10 days after infection underwent untargeted UHPLC-HR-IMS analysis, leading to the putative annotation of 182 compounds significantly over- or under-accumulated in resistant inbred lines. Significant compounds were further investigated to better understand their biological role. Besides the involvement of well-described lipid classes such as oxylipins and phospholipids, this study pinpointed to the differential accumulation of phytoceramides and Amadori-glycated glycerophospho-ethanolamines in resistant compared to susceptible inbred lines. Taken altogether, our data demonstrated the complex interactions occurring at lipidome levels during plant-pathogen interaction.

P35

Impact of two probiotics (*Lactobacillus plantarum* and *Bifidobacterium animalis*) on productivity and selected parameters of blood morphology in swine fed with feed naturally contaminated with deoxynivalenol

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As mycotoxin remain as one of the most notorious problems of feed production industry, efficacy of different preventives is constantly tested. One of the promising ideas in this field is use of probiotics. Probiotics, microorganism know mostly from having positive effect on functions of gastrointestinal tract, can be applied to help animals fight off effects of mycotoxins. Pigs are often exposed to these toxic substances in feed and are susceptible to many of them, especially to deoxynivalenol. This makes these animals important experimental models, that are valuable in research on practical use of different preventive strategies against mycotoxins. The aim of this research was to evaluate effect of feed that was naturally contaminated with deoxynivalenol (1800 µg/kg of feed) and use of probiotics as preventives against mycotoxins, and their influence on productivity and health of pigs. In the research, pigs were divided into six groups: one was fed without any additives (C), one was fed with contaminated feed (E), two were given one probiotic each (E1, E2), and two were both given probiotics and fed with contaminated feed (E3, E4). The feeding experiment was conducted for 28 days. The following probiotics were used in the experiment: *Bifidobacterium animalis*, strain ATM30 and *Lactobacillus plantarum*, strain ATM14. Animals were weighted once a week and blood samples were taken to assess morphological and biochemical parameters to evaluate health status of animals. The results showed that contamination of feed and use of probiotics significantly influenced weight gain of animals. These factors did not influence the change in blood morphological parameters but caused a change in biochemical indicators (liver enzyme activity). In conclusion, the research suggests that presence of mycotoxin in feed and use of probiotics can profoundly impact productivity of pigs.

P36

Searching the mitigation silver-bullet by understanding aflatoxin contamination and drought stress in crops

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Drought stress has been shown to be an exacerbating factor contributing to aflatoxin contamination of crops like maize and peanut. Investigation into the relationship between drought stress and aflatoxin contamination has been an interesting and fruitful journey. We have conducted investigations in maize drought stress responses, which have shed light on possible reasons for the exacerbation of aflatoxin contamination under drought stress. Using high-throughput proteomics, developing kernels of drought tolerant and drought sensitive maize lines Lo964 and B73, respectively, were compared under drought and irrigated conditions. It was found that responses to oxidative stress resulting in detoxification of ROS over-accumulation were predominant among those observed in the drought tolerant line. These pathways were also linked to other metabolic pathways including carbohydrate and lipid metabolism which relate to stress responses, and to glutathione metabolism which possesses antioxidant activity using both proteomics and global metabolomics analyses. These studies also quantified ROS

accumulation and the activities of related antioxidant enzyme activities in seedling leaf tissues and in developing kernel tissues. In summary, the difference was observed between the lines with the drought tolerance accumulating significantly less ROS and exhibiting higher levels of antioxidant enzyme activity than the drought sensitive lines. The ROS compounds have also been shown to play a significant role in the regulation of aflatoxin production in *A. flavus* and *A. parasiticus*. Supplementation of culture medium in *in vitro* assays with ROS or ROS-producing compounds results in elevated aflatoxin production by toxigenic isolates of these fungi. These observations correlating ROS with both drought tolerance and aflatoxin production have led to the hypothesis that reducing ROS accumulation under drought stress may result in a reduction in aflatoxin contamination under drought stress.

P37

In ovo effect of single and combined aflatoxin B1 and B2 on growth performance, antioxidant systems, and energy metabolism genes of broiler chickens

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Aflatoxins are structurally intricate compounds produced by the fungus *Aspergillus flavus* or *parasiticus*. The *Aspergillus* typically contaminates cereal crops used to feed livestock and poultry, including chickens. The consumption of *Aspergillus*-aflatoxin B1 and B2 contaminated feed induces serious negative impacts in broiler chickens with *in ovo* exposure to the aflatoxins compromising bird embryos and their development. However, most broiler chickens *in ovo* toxicity studies have hitherto investigated the effects of individual aflatoxins, a scenario that does not mimic reality as mycotoxins occur in multiple combinations with synergistic mechanisms under natural conditions. Also, no *in ovo* studies have hitherto investigated long-term combined aflatoxin toxicity consequences into adulthood in modern birds. This study investigated the effects of *in ovo* administration of AFB1 and AFB2 on growth performance, antioxidant systems, carbohydrate and fatty acid metabolism genes in broiler chickens. Five concentrations (0, 2.5, 5, 10, and 20 µg/kg) of the aflatoxins, singly and combined, were injected into fertile broiler chicken eggs, which were then allowed to hatch, and the chicks reared for 42 days. Growth performance, serum systemic antioxidant activities, hepatic expressions of carbohydrate and fatty acid metabolic genes were measured. Significant differences were observed in embryo weight, hatchability, body weight gain, and feed intake ($p < 0.05$). In addition, at a molecular level AFB1 and AFB2 and their combination decreased catalase (dose dependent response), superoxide dismutase, and serum glutathione activities, with AFB2 having greater impacts. Also, AF downregulated the acetyl-CoA carboxylase, peroxisome proliferator-activated receptors and PPAR- γ and sterol regulatory element-binding proteins-1f genes. These results suggest that aflatoxin toxicity activates the reactive oxygen species by reducing the hepatic antioxidant functions and metabolism genes when the exposure level increases above 10 µg/kg.

P38

Unlocking diversity: Towards a comprehensive framework for physiologically based pharmacokinetic population qualification in Sub-Saharan Africa

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Physiologically based pharmacokinetic (PBPK) models are crucial for simulating diverse populations, particularly regarding the impact on the metabolism of mycotoxins involving CYP450 enzymes. This study explores CYP450 phenotype diversity across African countries to evaluate the feasibility of considering Sub-Saharan Africa (SSA) as a single population. A systematic review gathered CYP450 phenotype data for SSA, categorizing individuals into poor metabolizers (PM) or extensive metabolizers (EM) for various CYP450 enzymes. The aim was to check whether a significant difference in populations could be observed, leading to a different metabolism of mycotoxins. Populations where CYP450 phenotype abundancies are different, can have a different mycotoxins metabolism, leading to a higher or lower exposure to these toxic metabolites. Five populations (SSA, West, East; Central and South) were modelled using SimCYP, starting with the South African FW_Custom population and adjusting with

regional CYP450 frequency data. Statistical analysis including a pairwise proportion test with Bonferroni correction and Cohen's d test, was conducted on the phenotype frequency data. Simulations with 1,000 healthy volunteers (20-50 years old, 50% male) were performed for 30 days with five probe substrates: 600 mg efavirenz (CYP2B6), 10 mg warfarin (CYP2C9), 200 mg mephenytoin (CYP2C19), 22 mg dextromethorphan twice daily (CYP2D6) and 5 mg midazolam (CYP3A5). Significant differences in CYP450 enzyme phenotypes were found, particularly for CYP2B6, CYP2C19 and CYP2D6 across various regions. These differences were evident in the PBPK simulation bar charts, justifying the subdivision of SSA into West, East, Central and South Africa. Numerous studies have substantiated the presence of inter-ethnic diversity within SSA (Schuster *et al.*, 2010. *Nature* 463: 943; Tishkoff *et al.*, 2009. *Science* 324: 1035). This study supports the subdivision of SSA into West, East, Central and South Africa.

P39

Mycotoxin-induced dysbiosis: Effects of sub-chronical doses of ochratoxin a and aflatoxin B1 on beneficial and pathogenic gut microorganisms

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The gut microbiome is integral to human health, regulating immune function, metabolism, and gut barrier integrity. However, dietary contaminants such as mycotoxins represent a significant risk to this ecosystem. Despite extensive research on the teratogenic, mutagenic, and carcinogenic properties of mycotoxins, their sub-chronic effects on gut microbial composition and functionality remain poorly understood. This study assessed the impacts of sub-chronic doses of ochratoxin A (OTA) and aflatoxin B1 (AFB1) on beneficial bacteria (*Lactobacillus rhamnosus*, *Lactobacillus thermophilus*, *Lactococcus lactis*, and *Bacillus subtilis*) and pathogenic microorganisms (*Escherichia coli*, *Salmonella enterica*, and *Enterococcus* spp.). Kinetics of microbial growth was evaluated under anaerobic and aerobic conditions at 37°C using constant optical density measurements (OD600) during 10 h exposure. The cell survival was assessed with MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), while mycotoxin levels were quantified via high-performance liquid chromatography with fluorescence detection (HPLC-FLD) 24 h post-exposure. Preliminary results indicate that OTA concentrations <3.0 µM selectively inhibit the growth of beneficial bacteria potentially promoting the development of pathogenic species (*E. coli*). This imbalance suggests that OTA exposure could compromise microbial homeostasis and, by extension, host health. Notably, co-culture experiments revealed that *L. rhamnosus* partially reduced OTA levels, suggesting a capacity for toxin biotransformation. However, OTA exposure extended the lag phase indicating an interchange between detoxification activity and bacterial functionality. In contrast, AFB1 exposure resulted in dose-dependent inhibition of *B. subtilis* growth, with suppression observed at concentrations of 0.02 and 0.01 µM after 6 h. Despite reduced growth, MTT assays showed high cell viability (>90%), suggesting that AFB1 exerted minimal cytotoxic effects at sub-chronical concentrations. Ongoing investigation is focused on elucidating the interspecies interactions and potential detoxification mechanisms within the gut microbiome. A more detailed understanding of these interactions is essential for developing strategies to mitigate the adverse health effects of mycotoxin-induced dysbiosis. Acknowledgements. This research was supported by the Centre for Agriculture, Food and Environmental Management Research at University of Hertfordshire (CAFEM QR Grant 2024).

P40

The toxicokinetics of the mycotoxin T-2 toxin in human volunteers following a single oral exposure: Study design

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Mycotoxins can pose significant health risks for humans due to their prevalence in food and their toxicological potential. Human biomonitoring (HBM) can provide insights into the real-life integrated (all-route) exposure to multiple mycotoxins at once. It may also offer a direct link between exposure and health outcomes when biomarkers of effect are measured in the same individuals. Without toxicokinetic

data applicable for humans, HBM data are difficult to interpret in risk assessments. For several mycotoxins, including T-2 Toxin (T-2), limited toxicokinetic data are available and therefore, their behaviour in the human body is not well understood. We aim to generate high quality toxicokinetic data for T-2, through a controlled human intervention study, and this poster will present our study design. Twenty-four healthy adult volunteers will receive a single oral dose of T-2 following a flushing out period of two days (low mycotoxin diet). T-2 will be administered at its Tolerable Daily Intake (TDI) value set by the European Food Safety Authority at 0.02 µg/kg bw/day. Biological samples (i.e., urine, blood and faeces) will be collected from the participants throughout the course of the study and analysed for biomarkers of exposure and biomarkers of effect. The relationship between the dose administered and the amounts of parent compound and metabolites found in the biological samples will be investigated. This research will explore the relationship between a known external dose of T-2 and its concentration in blood, urine and faeces. With this information, we hope to gain a better understanding into the absorption, distribution, metabolism and excretion of T-2 in humans. Thereby improving the interpretation of future human biomonitoring data for risk assessment of T-2.

P41

Unraveling the impact of multiple mycotoxin exposures on post-kidney transplant outcomes through uniting epidemiological and multi-omics designs

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Mycotoxins are hazardous contaminants produced by moulds that can cause organ damage, immune dysfunction, and carcinogenesis. Global consumption of plant-based foods may lead to both acute and chronic exposure to these compounds, as mycotoxins can accumulate in ripening crops such as maize, cereals, soybeans, and peanuts, during growth, transport, and processing. This study investigates the impact of multi-mycotoxin exposure on post-kidney transplant morbidity and mortality, informed by dietary surveys from patients with kidney disease. Plasma levels of multiple mycotoxins were quantified in kidney transplant recipients (KTRs) (n=632) and a control group (n=392) using ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) with matrix-matched calibration. Clinical phenotypes were assessed through a multi-omics approach, incorporating untargeted metabolomics via UPLC-high-resolution mass spectrometry and functional profiling of gut microbiomes using shotgun metagenomic sequencing. Bayesian multinomial regression model, machine learning and deep learning techniques were applied for feature selection and integrative data analysis. Our preliminary findings indicated the presence of multiple mycotoxins – ochratoxin A (OTA), tenuazonic acid (TeA), enniatin B (EnnB), citrinin, deoxynivalenol, T-2 toxins, cyclopiazonic acid, and zearalenone – in plasma samples from the Dutch population. Notably, the levels of OTA, TeA and EnnB in KTRs were significantly higher than those in the control group (Mann-Whitney U test; p<0.01). Additionally, OTA exposure appeared correlated with significant alterations in gut microbial composition, marked by reduced faecal microbiota diversity; a decrease in the relative abundance of *Bifidobacterium adolescentis*, *Collinsella stercoris*, *Lachnospira pectinoschiza*, and an increase in the relative abundance of *Escherichia coli*. EnnB exposure was associated with an increase in *Clostridium clostridioforme*, *Hungatella hathewayi* as well as a decrease in *Gemmiger formicilis* and *Agathobaculum butyriciproducens*. TeA exposure led to a decrease in *Firmicutes bacterium CAG 94*, *Lawsonibacter asaccharolyticus*, *Roseburia hominis*, and *Anaeromassilibacillus sp An250* (FDR <0.05). KTRs with high OTA exposure had significantly lower survival rates than those with low or no exposure (Cox regression; hazard ratio for death, 2.63; 95% confidence interval, 1.07 to 6.42; p=0.034). Furthermore, gut microbial alterations associated with EnnB exposure significantly affected KTR survival (p=0.018). In conclusion, while OTA has long been suspected as a contributor to various human nephropathies, our study provides further evidence of OTA toxicity, especially in the patients with end-stage kidney disease. Additionally, the modulation of gut microbial composition by EnnB appears to significantly affect KTR survival. These findings underscore the critical impact of OTA and EnnB exposure on post-transplant

outcomes and highlight the need for tailored dietary strategies to minimize mycotoxin exposure, potentially improving the long-term prognosis for end-stage kidney disease and transplantation.

P42

Combined effects of arsenic and aflatoxin B1 present in infant foods: analysis in different target organs

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Among vulnerable population groups exposed to mycotoxins, newborns, toddlers and children and have attracted notable attention due to the cytotoxicity effects of certain mycotoxins, as well as the greater susceptibility of infants and young children to their toxic effects. Generally, the biotransformation capacity of xenobiotics in newborns is slower than in adults, leading to prolonged circulation of ingested chemicals. Consequently, infants, especially those under 12 months of age, are more vulnerable to the adverse effects of mycotoxins due to their underdeveloped detoxification system, smaller body mass, and higher metabolic rates. Given the importance of assessing mycotoxin exposure in children, the present study aimed to evaluate the combined toxicity of mycotoxins and heavy metals potentially present in infant foods. Using *in vitro* models, the study employed cell cultures representing organs, such as the intestine and liver, to investigate the impact of co-exposure on human health. Specifically, the study explored the combined effects of mycotoxins and heavy metals, such as arsenic (As) and aflatoxin B1 (AFB1), using dedicated software initially developed for drug interaction analysis to determine the interaction type (antagonistic, additive, or synergistic). The cell lines used in this study were Caco-2 for the intestine and HepG-2 for the liver. The tested doses of AFB1 and As were determined based on the ratio derived from the average upper and lower bound estimates established by EFSA for infants, toddlers, and children, corresponding to 1/150 and 1/400 for the lower and upper bounds, respectively. The combined toxic effects of AFB1 and As suggested a slightly additive to synergistic effect for both ratios in HepG-2 cells and an additive to slightly synergistic effect for both ratios in Caco-2 cells. In conclusion, these results suggest that co-exposure to AFB1 and As may have additive to synergistic toxic effects in intestinal and liver cells and emphasize the need for further research and regulatory measures to limit the presence of mycotoxins and heavy metals in this food matrix.

P43

Evaluating the impact of intermittent multi-mycotoxin exposure on layer breeder performance, egg quality, and hatchability: insights from an intervention study

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Mycotoxins, harmful fungal byproducts found in cereals, pose global threats to animal health. While in-feed interventions are known to effectively prevent mycotoxicosis, there is limited data on their performance during intermittent challenges, commonly encountered in commercial animal production. This study aimed to assess the impact of a natural intermittent challenge: 100 ppb aflatoxin B1 (AFB1) + 100 ppb ochratoxin A (OTA) on the performance and health of HyLine W36 layer breeders, including hatchery parameters. The study also evaluated the effectiveness of an in-feed intervention comprising bentonite, yeast cell wall components, and a blend of phytomolecules (IFI, EW Nutrition GmbH, Visbek, Germany). A total of 576 hens (18 replicates per diet, 8 hens each) and 58 roosters were randomly assigned to four diets at 28 weeks of age: 1-control (C), 2-control+IFI at 2 kg/ton of feed (CIFI), 3-intermittent mycotoxin challenge (IMC), and 4-IMC+IFI (IMCIFI). The 72-day experimental period included alternating 10-day challenges (ChI) and 21-day non-challenge intervals (NChI); with a total of three challenge and two non-challenge periods. The IMC tended to decrease overall egg production, egg mass, shell weight, and shell thickness. The initial 10-day challenge interval (ChI) did not impact production parameters, but from the first NChI, all parameters were lower for the IMC group. Productivity declines persisted after the first 21-day NChI period, continuing through subsequent ChI and NChI periods. Two incubation trials were conducted during the first ChI and third NChI periods, revealing a significant decrease in fertility and hatchability for IMC in both trials. Toward the study's conclusion, oxidative stress biomarkers in the blood serum of 15 hens per treatment indicated higher malondialdehyde (MDA) and lower glutathione peroxidase (GPx) and Superoxide dismutase (SOD) in

the IMC group compared to group C. The IFI effectively mitigated all adverse effects of IMC on productivity and biomarkers. Intermittent exposure to AFB1 and OTA detrimentally affected layer breeder productivity, egg quality, hatchability, and induced oxidative stress. These negative impacts persisted even after the withdrawal of contamination. This study underscores the effectiveness of in-feed interventions in mitigating the adverse effects of intermittent mycotoxin challenges on layer breeder health and productivity.

MANAGING AND MITIGATING MYCOTOXIN RISKS

P44

Synergistic antifungal effects of ammonium propionate and medium chain fatty acids

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Fungal contamination of animal feed leads to spoilage, reduced nutritional value, and the accumulation of harmful mycotoxins. *Aspergillus chevalieri*, a xerophilic feed spoilage fungus, produces mycotoxins, emodin and echinulin, leading to feed refusal by animals. While propionic acid is widely used as an antifungal preservative, its industrial application often involves ammonium neutralization, which compromises its efficacy. This study evaluates the synergistic antifungal effect of ammonium propionate in combination with medium chain fatty acids (MCFA) against *A. chevalieri* at different developmental stages. The antifungal activity of ammonium propionate and MCFA was tested against dormant conidia, germinating conidia, germ tubes, and hyphae of *A. chevalieri*. Treatments included propionic acid at 16 mM and 32 mM, and a 50:50 MCFA mixture (octanoic and decanoic acids) at 0.26 mM and 0.52 mM. Dormant conidia were incubated for 28–48 hours, and viability was assessed by colony formation. Conidial germination and hyphal damage were evaluated using real-time imaging, fluorescence microscopy (TOTO-1), and transmission electron microscopy (TEM). Dormant conidia were not significantly affected by ammonium propionate or MCFA alone, but their combination nearly eradicated germination and caused structural damage. At 16 mM ammonium propionate and 0.26 mM MCFA, conidial germination was significantly inhibited, while hyphal growth was further disrupted at 32 mM ammonium propionate and 0.52 mM MCFA. TEM analysis revealed mitochondrial abnormalities, vacuolization, and autophagy, indicating severe metabolic and structural stress. The synergistic combination of ammonium propionate and MCFA significantly enhances antifungal efficacy, effectively inhibiting *A. chevalieri* across different developmental stages compared to the negative control or the single components. Even dormant conidia are eradicated. These findings support the development of enhanced antifungal formulations to mitigate fungal spoilage and possible mycotoxin contamination in animal feed.

P45

Combination of physical and biological methods to mitigate aflatoxin M1 in milk

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Aflatoxin M1 (AFM1) is a mycotoxin derived from animal ingestion of aflatoxin B1 and it commonly found in milk. This toxin represents a global problem, especially in developing countries with inadequate regulatory control, posing a risk to consumers, especially children, due to their high milk consumption and their body weight. Several milk processing methods have been investigated for their effectiveness in reducing AFM1 contamination. Thermal treatments, such as boiling and sterilization, can reduce AFM1 content by up to 20%. In addition, non-thermal methods, such as high-pressure processing (HPP), have been studied as an alternative in the reduction of several contaminants, including mycotoxins. Although studies indicate the effectiveness of HPP on mycotoxins, there is little data available regarding AFM1. Alternatively, biological methods, such as lactic acid bacteria and enzymes, have demonstrated potential in the degradation of mycotoxins. Among these enzymes, peroxidase stands out for its ability to catalyze oxidative reactions that can lead to the degradation of these compounds. Therefore, this study aimed to evaluate the mitigation of AFM₁ by combining a physical method, HPP, and a biological

method, peroxidase enzyme. For this, 5 ml of commercially available milk was contaminated with AFM1 (15 ng/ml), added with peroxidase enzyme at 0.015 U/ml, and packaged in polyethylene bags that were sealed and treated by HPP. The processing conditions were 50 and 250 MPa for 5 and 20 min. Subsequently, the residual AFM1 was extracted from the milk by the QuEChERS method modified by Gonçalves (2018) and quantified in LC-FL. The results indicated that increasing the pressure from 50 to 250 MPa did not result in greater mycotoxin mitigation, 55.9% and 31.6%, respectively. However, increasing the processing time under high pressure from 5 to 20 min resulted in 17.5% greater mitigation. Among treatments tested, the greatest reduction of AFM1 was obtained with the combination of HPP and peroxidase (0.015 U/ml), resulting in 78.7% degradation when milk was processed at 50 MPa for 5 min. Therefore, the combination of HPP and peroxidase enzyme represents a promising approach to mitigating AFM1 in milk, being efficient under specific pressure and processing time conditions. This strategy can contribute to developing innovative technologies to ensure food safety while reducing human exposure to mycotoxins.

P46

Review of yeast cell wall mode of action and benefits on aflatoxin contamination

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This aims to review the effect of a yeast cell wall (YCW) (source of β -glucans) mode of action and benefits in aflatoxin-challenged models. The impact of mycotoxins on animal health and performance will depend on their concentration, interactions with other mycotoxins, animal age, nutritional levels in the diet, stress, etc. Several factors can worsen mycotoxin contamination, and many are difficult to control, including detecting contaminated grain. A meta-analysis by Campagnollo *et al.* (2020) showed that variables such as temperature, yeast, pH, and aflatoxin type could be considered influential factors in aflatoxin decontamination. In an *in vivo* trial (Gonçalves *et al.*, 2017), dairy cows were contaminated with 480 μ g aflatoxin B1 per day. After 3 days, the maximum levels of aflatoxin M1 (AFM1) were detected in the milk. At the end of the treatment period (4 to 6 days), the YCW reduced 78% of AFM1 from the milk. The aflatoxin contamination, besides causing liver damage (where they are bio-transformed into toxic metabolites that bind to the intracellular constituent and alter protein synthesis and liver function), also has negative impacts on immune responses. In broilers (non-published data), aflatoxin contamination at 2.5 ppm for 35 days adversely affected cellular responses. However, the supplementation of the YCW (at 0.5 kg/MT) demonstrated an immune modulation effect. The mycotoxins can affect the physical barrier of the epithelium, which is assisted by the trans-epithelial electrical resistance (TEER) in the cellular monolayer. Mycotoxins can reduce TEER, thus decreasing the number of proteins in the cellular tight junctions. Macrophages play a key role in the enrolment of inflammatory cells to defend the mucosa. Mycotoxins reduce the expression of these cells, making the animals more susceptible to bacterial and other infections. There is an influence on the humoral immunity (antibodies) that can be inferred from the diminished responses to vaccinations. Mycotoxins cause necrosis of crypt cells, causing atrophy of the villi and interfering in the differentiation of enterocytes and goblet cells. In summary, besides food safety alerts, aflatoxin causes tissue damage and can cause immune suppression, leading to extensive losses in animal health and performance. Through countless studies, YCW has successfully demonstrated its positive impact in binding aflatoxin and supporting animal's immune responses.

P47

Prediction of aflatoxin contamination outbreaks in Texas maize by using mechanistic and machine learning models

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Aflatoxins are carcinogenic and mutagenic mycotoxins produced by fungi that enter the food supply after growing on maize cobs in field or in grain during storage. We used an ensemble of models to predict aflatoxin contamination outbreaks of maize in fields in Texas, USA. We established three ensemble analysis pipelines to predict aflatoxin contamination events. Two pipelines used mechanistic models that included weekly aflatoxin risk indexes (ARI) as inputs and one used weather-dependent variables

only (temperature, precipitation, pressure, and relative humidity). For all three pipelines, input features included high-throughput dynamic geospatial data from remote sensing satellites, soil property maps, and barometric pressure data aggregated at county levels. The 1st stage of these pipelines was to prepare the inputs features, for the two mechanistic models we determined ARI using two approaches: (i) the AFLA-MAIZE mechanistic model and (ii) the Ratkowsky mechanistic model. For both ARI dependent models, the ARIs were weighted based on a maize phenological model that estimated the planting times of maize in the field per growing season. The 2nd stage of the pipelines was to train, test and validate gradient boosting and neural network models by using the inputs generated in the 1st stage. We determined that the AFLA-MAIZE and Ratkowsky mechanistic models had similar accuracy to predict aflatoxin outbreaks (72% and 76% respectively) while our weather only model had a higher accuracy compared to both mechanistic models (82%). These results led us to conclude that Texas has a wide geographical variability of ARI and ARI-hotspot locations due to differences in weather, planting times and maize temporal development among ecoregions in the state (mixed-dry, hot-dry, hot-humid, and mixed-humid ecoregions). For example, peak maize flowering time, which has been determined to be key in prediction of aflatoxin outbreaks, takes place 2-3 months earlier in southern Texas than northern Texas. Furthermore, we determined that depending on the ecoregion, there is a positive correlation between aflatoxin outbreaks and soil organic matter (Mixed-dry ecoregion), soil pH (hot-dry and hot-humid ecoregions), soil erodibility (hot-dry and mixed-dry ecoregion) and soil sodium adsorption ratio (hot-humid ecoregion), while a negative correlation between plant available water holding capacity (mixed-dry, mixed-humid and hot-dry ecoregions) and soil cation exchange capacity (mixed-dry and hot-humid ecoregions). These outcomes suggest that any implementation of prediction and prevention of mycotoxin outbreaks and pest management strategies needs to be tailored to specific geographical ecoregions in Texas.

P48

Exploring the potential of Ery 4 laccase-mediator system for aflatoxin B1 and zearalenone degradation: *In vitro* efficacy with mechanistic insights and application in contaminated maize
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Mycotoxins are secondary metabolites produced by filamentous fungi and are responsible for the reduction in crop quality and the toxic effects on humans and animals. Effective and environmentally friendly detoxification methods are required to ensure food and feed safety. In this context, enzyme-based approaches, such as the laccase mediator system (LMS) appears to be a powerful and green tool for detoxifying mycotoxins. Laccases (EC 1.10.3.2) are blue multi-copper oxidases that oxidize a variety of phenolic compounds, releasing water as a by-product. In an LMS, the phenol acts as a redox mediator as it is first oxidized by laccase to form phenoxy radicals, after which it can oxidize nonphenolic substrates via different mechanisms, such as electron transfer, radical hydrogen atom transfer or ionic oxidation. Although the mechanism of substrate oxidation has been extensively studied, the LMS are complex reaction systems whose reaction pathways are not yet fully understood. In the present work, the capability of Ery4 laccase to degrade aflatoxin B1 (AFB1) and zearalenone (ZEN) was investigated. In the case of ZEN, three different mediators representing three different oxidation mechanisms were tested *in vitro*: 2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) and acetosyringone (AS). The degradation products were also investigated *in vitro* by liquid chromatography (LC) coupled to high-resolution mass spectrometry (HRMS). AFB1 (0.1 µg/ml) was completely removed *in vitro* by Ery4 laccase (5 U/ml) using AS (10mM) as mediator. Several degradation products were detected and likely corresponded to aflatoxin Q1 (AFQ1), epi-AFQ1, AFB1-diol, or AFB1-dialdehyde, aflatoxin B2a, and aflatoxin M1. A reduction of 26% was obtained in artificially contaminated maize flour (50 µg/kg AFB1). In the case of ZEA degradation, AS was also found to be the most effective mediator compared to TEMPO and ABTS. The LC-HRMS analysis point out the formation of 15-OH-ZEN quinone and oxidation products of AS due to C-O coupling reactions when ZEA is treated with Ery4 laccase and AS. Further analyses are underway to fully characterise the profile of compounds obtained from the degradation reaction under the conditions studied. When applied to naturally contaminated maize, ZEN reduction reached 44%. This study demonstrates the potential of LMS to reduce mycotoxin contamination also in contaminated maize. Nonetheless, it highlights the need of a more holistic approach to fully understand the mechanism behind mycotoxin degradation and the specific removal pathways, clarify potential co-existing degradation and polymerization pathways.

P49

A comparative study of methods for determining minimum inhibitory concentrations in *Aspergillus* species

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Mycotoxins, contaminating crops, are a serious threat to humans, animals and plants, they cause global problems in agriculture, food industry and healthcare. Filamentous fungi of the genera *Aspergillus*, *Penicillium*, *Alternaria* and *Fusarium* typically cause their production. The genus *Aspergillus* includes more than 340 officially recognized species of filamentous fungi, which are among the most common and widespread fungi on Earth. This investigation focused on two species, *Aspergillus flavus* and *Aspergillus parasiticus*. Both groups play a significant role in producing one of the most dangerous mycotoxins, the carcinogen aflatoxin. When the use of different antimycotics is considered, the compound's efficacy on fungi, the possibility of resistance development, and the health impact of the chemical agents on the organism used or the short- and long-term consequences of its release into the environment when used as a spray. By finding the correct antifungal application rate, the ultimate goal is to inhibit toxin production and thereby reduce the total toxin level. Important aspect is to find a proper method for the determination of minimum inhibitory concentration (MIC), as treating the fungi with sub-inhibitory concentration can induce toxin-production. Aim of the study was to compare two widely used methods for the determination of the MIC: the CLSI microdilution and the agar dilution techniques. Two antifungal agents were applied, one of them is used in agricultural (metconazole) and the other in clinical (voriconazole) practice. In the case of *A. parasiticus* the CLSI microdilution technique showed significantly lower values, but the difference between the two techniques was much smaller in the case of *A. flavus*. An interesting result is that agar dilution showed the lower MIC values in the case of metconazole and *A. flavus*. All things considered the CLSI microdilution technique resulted lower MIC values with more easily reproducible results. Still, due to the heterogeneity of the mixture, the instrument often detects outliers when the light beam hits a denser micelle network. The agar dilution method is a less precise technique with a more complicated setup but showed more consistent inhibitory concentration values overall. It has the advantage that it can be easily performed by a non-expert and morphological changes can be also observed. To consider the advantages and disadvantages of the two protocols, the authors plan to develop a uniform, well-represented hybrid procedure that can be incorporated into research and agricultural practice. To develop a standardised method, additional *Aspergillus* species would be included in the future.

P50

Biocontrol potential of yeast-produced volatile organic compounds against *Aspergillus carbonarius*
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The contamination of agricultural products by *Aspergillus carbonarius* poses significant economic and health risks. Yeast antagonists are prevalent biocontrol agents against fungal infections in agricultural products, producing volatile organic compounds (VOCs), a key mechanism for inhibiting fungal growth. This study aimed to evaluate the efficacy of VOCs from antagonistic yeasts – *Wickerhamomyces anomalus* MSCU 0652, *Saccharomyces cerevisiae* MSCU 0654, and *Kluyveromyces marxianus* MSCU 0655 – on the growth and ochratoxin (OTA) production of *A. carbonarius* *in vitro* and real food samples and to identify the primary VOCs produced by yeasts. The results showed that all yeasts produced VOCs, effectively inhibiting the fungal growth and its ochratoxin production *in vitro* as well as in coffee beans and maize kernels with growth inhibition ranging from 60-100% and more than 99% of OTA reduction. The main VOCs produced by these yeasts included ethyl acetate, 1-propanol, 2-methyl-, 1-butanol, 3-methyl-, acetate, and 1-butanol, 3-methyl.

P51

MYTOX-SOUTH®, a sustainable scientific network for global food safety.

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MYTOX-SOUTH® is a scientific collaboration initiative established at Ghent University (2017-2026; funded by Global Minds UGent/VLIR-UOS) with the mission to combat mycotoxin contamination in food. The network currently consists of more than 48 sustainable partners worldwide and is actively expanding. Mycotoxins pose a significant global public health threat, disproportionately affecting vulnerable populations in developing countries. While strict food safety regulations are enforced in Europe, low-income countries often lack such controls, leading to severe consequences for food security, health, and economic stability. Additionally, climate change is increasing mycotoxin pressure on European crops, further amplifying the urgency of global mitigation efforts. MYTOX-SOUTH® integrates scientific excellence with social engagement through three core objectives: (i) capacity building through training and co-creation – training students and researchers to become experts in food safety and mycotoxicology; (ii) awareness and knowledge sharing – educating policymakers, farmers, food producers, and scientists on the risks of mycotoxins and potential interventions through workshops and collaborations; and (iii) innovative research for sustainable solutions – Developing technologies and strategies to reduce mycotoxin production and exposure, tailored to local conditions. Furthermore, in collaboration with our industrial partner Patent CO, we conduct mycotoxin analyses on various food matrices from our network. Over the past year, we have analysed samples of rice, maize, sunflower, and spices such as red pepper powder, contributing to a deeper understanding of mycotoxin contamination patterns and mitigation strategies. MYTOX-SOUTH® continues to expand its impact, fostering international collaboration to enhance food safety and protect global health.

P52

Biocatalysts against the zearalenone mycotoxin

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Animal feed frequently contain mycotoxins, especially zearalenone (ZEN). The mycotoxin, primarily produced by *Fusarium sp.*, has an estrogen-like molecular structure, disrupting hormone homeostasis leading to reproductive health problems upon acute and chronic dietary exposure, mostly in gilts and swine. Currently discarding the grains or the use of adsorbents are the most commonly used strategies to deal with the mycotoxin contamination. Enzymatic degradation of ZEN enables cereals initially contaminated with mycotoxins to be safely used for animal feed and protect the animals for any negative effects of ZEN in the feed (Alexander and Wallace, 2017. EFSA Journal 15: 4851). Our research focuses on the discovery of novel enzymes for the biotechnological inactivation of ZEN. Lactone-hydrolases have attracted considerable attention because they can degrade ZEN to a non-toxic form by hydrolyzing the lactone bond in the molecule (Fruhauf and Moll, 2024. ACS Catalysis Journal 14: 3392). This research focused on finding new zearalenone degrading enzymes with K_M values comparable to the environmental contamination levels, and high k_{cat} values to enable the very effective degradation of ZEN. Zearalenone hydrolase was expressed in a recombinant, antibiotic marker-free *pichia* expression system, that is highly efficient for pure enzyme production, without the risk of contamination from the production host. Several formulations were tested to improve the storage and applicability of the enzyme in the food and feed industry. In conclusion, our study showed a formulation for possible application of ZEN degrading enzyme in food and feed industry.

P53

Knowledge centre for global food and nutrition security (KC-FNS)

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Since 2018, the European Commission has considered it relevant to monitor food crises and mycotoxin contaminations, especially in African countries, through its Knowledge Centre for Global Food and Nutrition Security (KC-FNS). This is because mycotoxin contamination monitoring remains a priority in Africa. Mycotoxins are known to have a direct impact on food and nutrition security and are an important element of the food environment that influences the sustainability of food systems. For these reasons, the KC-FNS maintains a strong interest in this scientific area and intends to follow its developments. With particular interest it will follow those related to the digitisation of food system management aimed at reducing the presence of mycotoxins as well as those achieved through the implementation of targeted regulatory frameworks that improve traceability and risk management, significantly increasing overall food safety.

P54

Food safety risks (mycotoxins and heavy metal contamination) associated with ginger and aflatoxin decontamination via probiotics

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Ginger (*Zingiber officinale* Roscoe) is an important food and cash crop in Nigeria used as spices and for pharmaceutical purposes. However, it is susceptible to contamination by mycotoxigenic fungi and mycotoxins and other food safety hazards. This study investigated the incidence of aflatoxins and heavy metals along the value chain from the farm to the informal market. Additionally, the use of probiotics was explored for aflatoxin decontamination to address contamination risks. Ninety-five (95) samples of fresh and processed ginger samples from multiple value chain points (i.e. harvest, drying, storage and sale points) were obtained in Kaduna state and Oyo State, Nigeria. Macroscopic and microscopic features of contaminating mycotoxigenic fungi were identified on potato dextrose agar and via compound microscope respectively. Aflatoxin concentrations were determined via thin layer chromatography. Reference probiotic strains were used in decontamination studies on spiked fresh ginger samples. Mycotoxigenic fungi *Aspergillus* spp. (38.5%), *Fusarium* spp. (36.3%), and *Penicillium* spp. (25.1%) were isolated. Total aflatoxin concentrations (aflatoxins A1, B2, G1 and G2) ranged from 3 to 185 ppb in fresh, split dried and milled ginger. Co-contamination with heavy metals was also recorded, indicating a combined risks to multiple food hazards. In decontamination assessments, live reference lactic acid bacteria strains, *Lactiplantibacillus plantarum* ATCC8014 and *Lactobacillus helveticus* ATCC15009 reduced aflatoxin concentration levels from 50 ppb similarly by 34% to 52% after 48 h to 120 h treatments with *Lactiplantibacillus plantarum* ATCC8014 and by 36% to 52% from 48 h to 120 h treatments with strain *Lactobacillus helveticus*. In conclusion, food hazards such as toxigenic fungi, aflatoxin, co-occur with other food hazards like heavy metals posing combined food safety risks. Probiotics hold promises for aflatoxin decontamination.

P55

Effectiveness of monitoring aflatoxins along the feed and dairy supply chain

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Feed ingredients may be contaminated with aflatoxin B1 (AFB1) which may lead to contamination of the dairy cow's milk with aflatoxin M1 (AFM1). To safeguard human health, monitoring of AFB1 and AFM1 is performed at various stages in the feed and dairy supply chain. This study aimed to assess the effectiveness of monitoring aflatoxin B1 during feed production for dairy cows. First, a simulation model was developed to simulate AFB1 contamination throughout the dairy chain, from feed ingredients to the produced dairy milk. Historical monitoring data from feed companies were used to define the AFB1 contamination in feed materials used for compound feed production for dairy cows. Two monitoring points for AFB1 were considered: in feed ingredients, and in compound feed for dairy cows. Monitoring

consisted of collection of feed samples and performing AFB1 analyses. The effectiveness of monitoring was estimated using Monte Carlo simulation modelling with fixed numbers of samples per monitoring point (based on historical data). Monitoring effectiveness was expressed as the probability that milk is contaminated with AFM1 at the milk processing step of the dairy supply chain and has thus surpassed current monitoring activities for AFB1 at the feed ingredient and compound feed points of the chain. Historical AFM1 data in milk were used to validate the model calculations. Results showed that no contaminated batches were detected with current monitoring activities. The reason is the low presence of AFB1 in feed ingredients and compound feed. In a worst-case scenario in which contaminated maize (15 batches with AFB1 > 0.02mg/kg) entered the dairy chain, 34% of the contaminated batches were detected. It is concluded that the current monitoring plan can help to reduce the AFB1 contamination in feed ingredients and compound feed, and finally AFM1 concentration in dairy milk. Stakeholders can apply the developed approach to the effectiveness evaluation of their monitoring plan and support the decision-making on designing new monitoring plans.

P56

Mycotoxin predictions for cereal and maize worldwide extension

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Mycotoxins may be analysed and sometimes found at high levels in harvested crops. Syngenta has collected a big number of cereal and maize samples over the past 25 years, with datasets linking location, weather and agronomy to mycotoxin analysis results. This is the most extensive database of field sample analyses in Europe for recommending Good Agricultural Practices, such as variety tolerance, residue management, and crop protection. Leveraging Syngenta's digital expertise and big data mining with new machine learning techniques, we have developed robust and reliable mycotoxin prediction models for pre-harvest assessment, called Qualimetre. Initially designed in France, the tool is now integrated into our Cropwise digital offer and operational across Europe. Further geographical extensions are being tested for wider application. Mycotoxins pose a challenge for food and feed safety, and risk prediction before crop harvest is a key management contribution.

P57

Potential of the yeast *Hyphopichia burtonii* for the decontamination of mycotoxins

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Among the methods for mycotoxin detoxification in food products, biological approaches are considered the most adequate, as they efficiently decontaminate these compounds while preserving their organoleptic properties. Although numerous methodologies exist to assess the ability of microorganism to detoxify mycotoxins, a particularly promising strategy involves evaluating whether the microorganism can utilize the mycotoxin as a sole carbon source, achieving detoxification without compromising product quality. Recent studies have demonstrated the ability of the insect *Tenebrio molitor* to detoxify certain mycotoxins present in its diet, a capability believed to be mediated by its microbiota. Building on these findings, our research group aimed to analyse microorganisms isolated from the microbiota of *T. molitor* to evaluate their potential for mycotoxin detoxification. In this study, we focused on assessing the genetic variability of 15 isolates of *Hyphopichia burtonii*, a yeast obtained from the insect's microbiota since mycotoxin detoxification capacity is generally strain-dependent. Genetic diversity among the isolates was characterized using two typification techniques: phylogenetic analysis using various informative sequences from the ITS region derived from the primer sets ITS1-ITS4, ITS1-NL2 and ITS1-LR1 and fingerprinting using the (GAG)₅ primer. The results revealed substantial genetic diversity among the isolates, with phylogenetic analysis demonstrating greater resolution by delineating four distinct groups, compared to fingerprinting, which identified only two. Subsequently, we evaluated the ability of four isolates, which represented the four supposed strains obtained from the phylogenetic analysis, to degrade aflatoxin B1, ochratoxin A, fumonisin B1, and deoxynivalenol by examining their potential to utilize the mycotoxins as a carbon source under three different glucose concentrations: 0%, 0.1% and 2%. This allowed us to distinguish between mycotoxin metabolism as a sole carbon source versus co-metabolism. Growth assays revealed significant increases in biomass when fumonisin B1 or deoxynivalenol were included in minimal media with a glucose concentration 0.1%. Conversely, no significant growth differences were observed for aflatoxin B1 between media with or without the toxin

whereas the strains were not able to grow without glucose in the media. Ochratoxin A exhibited pronounced toxicity, as evidenced by a marked reduction in yeast growth in its presence. These findings suggest that certain *H. burtonii* isolates could serve as promising candidates for application in the food industry as biological detoxification agents of fumonisin B1 and deoxynivalenol. Further studies are required to discard their potential to eliminate mycotoxins by other mechanisms.

P58

The use of mycotoxin binders: A sustainable strategy to support the feed and dairy industry
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The intestinal absorption of aflatoxin B1 (AFB1) in dairy cows is rapid and its conversion into aflatoxin M1 (AFM1), which is excreted in milk, represents a food safety issue because of the harmful effects of these toxins (both are classified carcinogenic for human according to the IARC classification) but also has a significant economic impact mainly due to the disposal of contaminated food and feed. According to the Regulation (EC) No 386/2009, in this study, two feed additives were developed and evaluated *both in vitro* and *in vivo* to improve the safety of mycotoxin-contaminated feed while safeguarding animal welfare and the safety of animal products. These feed preparations were obtained by combining materials already authorised in the feed industry such as smectite and lignin (binder 1) or humic acid and natural extracts (binder 2). *In vitro* experiments showed, for both binders, a high *in vitro* binding efficacy ($\geq 70\%$) towards some of the regulated mycotoxins with the highest prevalence in feed, i.e., aflatoxin B1, zearalenone, fumonisin B1, and ochratoxin A. The binding efficacy of both mycotoxin binders were also tested in lactating cows which were fed for 14 days with a TMR (Total Mixed Ration) diet contaminated with aflatoxins (AFB1+AFB2) at levels below the limit set by Directive 2002/32/EC (5 $\mu\text{g}/\text{kg}$). The *in vivo* study showed that, despite the low levels of feed contamination, AFM1 excreted in milk exceeded the limit of 50 ng/Kg fixed in the EU legislation 2023/915. However, the addition of the binders to the contaminated TMR, by decreasing the bioavailability of the aflatoxins, reduced the carry-over rate of AFB1 by almost 30%, resulting below 2%. Thus, the presence of the binders guaranteed a significant reduction of the AFM1 levels in milk and related dairy products (~30%), i.e., grana-type and mozzarella cheese without, albeit, altering their quality. In conclusion, the results of this study demonstrate that the maximum level of AFB1 in the compound feed for dairy cattle established by the EU Directive 2002/32/EC may not be sufficient to ensure AFM1 levels in milk below 50 ng/kg. Therefore, to safeguard the economy of the feed and dairy supply chain a careful selection of feed additives, like those examined in this study, could be the ideal strategy to preserve (or improve) the health of lactating cows without compromising milk production and cheese yield as well as the quality of milk and dairy products. Acknowledgements. This work was carried out within the framework of the project Ager Farm-level interventions supporting dairy industry innovation (FARM-INN) – Grant 2017-1130.

P59

Prediction of emerging mycotoxins: Comparison of six machine learning models

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Mycotoxin contamination in grains can affect human and animal health. Many previous studies have been conducted in predicting if mycotoxin contamination will occur, and in some instances, also mycotoxin concentrations. These predictive models can be simple with a mechanistic and or empirical basis, or much more complex machine learning models. One major similarity of previous predictive models is their focus on the major mycotoxins, such as aflatoxin and deoxynivalenol. In this research we focus on lesser known and emerging mycotoxins: *Alternaria* toxins, ergot alkaloids, enniatins and beauvericin. Emerging mycotoxins which are mostly unregulated are known to occur frequently in food. While often less toxic than their well-known counterparts, they remain a source for concern. Mycotoxin survey data on the three emerging mycotoxins in grains (wheat, oat, barley, rye and maize) in the period 2014-2023 was obtained from DSM. Data include grain type, European country location, testing method and concentration of the toxin in the sample. The data is cleaned and linked to weather data. The dataset

is split into training and internal validation set (80/20) and used to train six different machine learning models (Naïve Bayes, Gaussian Process Classification, Decision Trees, Adaboost, Random Forest and K Nearest Neighbors), which are then cross validated with K-fold for robustness. The objective is to compare the six models on their performance in predicting the probability of the presence of one of the three emerging mycotoxins in the specific grain and country. Results of the machine learning models show large variation in model performance with Random Forest scoring highest on accuracy (0.970696). Gradient Boosting Trees scores best overall with accuracy of 0.969, sensitivity of 0.986 and specificity of 0.606. Each model has its benefits, for instance Adaboost has high specificity which can be useful to identify true positives, which is especially important to avoid unidentified contamination risks. The mycotoxin prediction models could be integrated to combine each of its strengths, which in turn will contribute to food safety management in face of lesser known and emerging mycotoxins. In future research, data with more specific location information can be used to get a more specific prediction and could be combined with satellite image data.

P60

Differential *in vitro* capabilities of mycotoxin binders to mitigate DON damage in IPEC-J2 cells

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Deoxynivalenol (DON) is a common environmental and food contaminant that widely contaminates food and feed. The intestinal epithelium is the first barrier against food contaminants and is highly sensitive to DON, which is absorbed mainly in the small intestine and can damage the integrity and function of the intestine, causing intestinal inflammation. Products containing yeast materials have potential to adsorb mycotoxins due to the physical mycotoxin adsorbent properties of the yeast cell wall. Some yeast materials may also improve the health of pigs through their prebiotic properties, which in turn can protect gut health, reduce inflammation, and improve performance. The objective of this study was to assess the impact of several commercially available mycotoxin mitigation products on Deoxynivalenol (DON) contamination of intestinal porcine epithelial cells (IPEC-J2) *in vitro*. Differentiated IPEC-J2 were supplemented with six different products, M, MA, B, C, D, E, which had been digested using a simulated porcine digestion model. The cells were exposed to 0.9ppm of DON for 2 h after which time the cells were washed and allowed to recover. Trans electrical epithelial resistance (TEER) readings were taken after exposure to DON as an indicator of intestinal barrier function. Cells treated with product M or MA had significantly higher TEER readings than the DON only treated control ($p \leq 0.05$), or cells treated with other products. Lower levels of the inflammatory marker TNF- α in terms of both protein and gene expression were noted in product M ($p \leq 0.05$) and MA ($p \leq 0.001$) supplemented cells compared to the DON control or other products studied. Glucose uptake by the cells was negatively impacted by DON exposure only, with a 40 % decrease in glucose utilisation. There was no observable impact on glucose uptake by cells supplemented with products M, MA and E following DON exposure ($p \leq 0.05$). The reduction in inflammation in these cells may have positively impacted nutrient utilisation by the cells following DON exposure. DON exposure in IPEC-J2 cells elicited negative cellular effects and the mitigation capabilities of differing mycotoxin adsorbents were noted in this study to be product specific. These results indicate that whilst mycotoxin adsorbents may have quite similar compositions, their functionality differs. Both product M and MA improved barrier resistance, reduced inflammation and increased glucose utilisation in intestinal cells following DON exposure. This contrasted with products B, C, D and E which did not mitigate or significantly lessen the cellular impacts associated with DON contamination.

P61

Understanding the molecular mechanism of a fumonisin esterase by kinetic and structural studies
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Fumonisin esterase (FE) is a tricarballic acid (TCA) units of fumonisin B1 (FB1; accounting for 70% of fumonisin contamination) can be removed by fumonisin B1 esterase (FE, EC 3.1.1.87) providing a biotechnological FB1 detoxification possibility. Here, we report the regioselective cleavage of the TCA ester at C6 in the first step of FB1 hydrolysis and kinetic characterization for FE2. The low K_M values are comparable to concentrations of environmental contaminations, and the high catalytic efficiencies are promising for practical applications. The X-ray structure of FE2 enabled the understanding of the FB1 hydrolysis at molecular level and revealed an arginine binding pocket and the catalytic role of the glutamate preceding the catalytic serine. Computations showed that FE2 is likely capable of detoxifying any fumonisin indicating its potential applicability in food and feed products. The *in silico* molecular docking results were validated through co-crystallization of FE2 with FB1 or its hydrolysis intermediate, partially hydrolysed FB1 (pHFB1), followed by X-ray structure determination. *In vitro* experiments also confirmed that FE2 can hydrolyse other fumonisins in the FB family, including FB2, FB3, and FB4.

P62

Mitigation of negative effects of enniatin in creep feed and post-weaning diets in piglets

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Presence of enniatins (ENNs), produced by *Fusarium* species, in pig feed may have a negative effect on performance especially in young pigs in which the gut is not yet matured. Mycotoxin research often focusses on weaned piglets, ENNs in creep feed, an essential phase for gut maturity and smooth weaning process, is not studied yet. The objective was to investigate the effects of ENNs in piglets via creep feed and post-weaning diets. Litters of 16 sows were included from birth and followed up until 35 days post-weaning. Four dietary treatments were tested in creep feed and post-weaning diets: 1) low mycotoxin contaminated control (CON), 2) natural ENNs contaminated diet (ENNs), 3) ENNs diet with test product 1 (TOXO-XL, Selko, Tilburg, the Netherlands) (ENNs+TP1), 4) ENNs diet with test product 2 (TOXO-XL plus, Selko, Tilburg, the Netherlands) (ENNs+TP2). Creep feed contained a blue colour marker to identify the eaters. Growth performance was measured at day 0, day 3 of age, day 28 of age (weaning) and weekly thereafter. Gut leakage was measured by FITC-d levels in serum after oral gavage at weaning and 7 days post-weaning. During creep feed phase, ENNs had the least eaters (3 out of 60), while CON had the most (30 out of 60) with ENNs+TP1 (20 out of 59) and ENNs+TP2 (26 out of 54) in between (no statistical analysis), which explains the numerically lower feed intake ($p=0.43$). At weaning, ENNs had the lowest body weight (BW), and average daily gain (ADG) compared to CON (BW=7.1 kg, ADG=208 g/pig/day and BW=7.9 kg, ADG=238 g/pig/day for ENNs and CON respectively), with test product groups in between. Post-weaning BW remained highest ($p<0.01$) for ENN+TP1 and ENN+TP2 compared to CON and ENNs throughout the entire nursery period. Overall feed intake (FI) was lowest for ENNs and highest for ENNs+TP2 with the other groups in between ($p<0.001$). Post-weaning feed conversion ratio (FCR) was lower ($p<0.001$) for ENNs (1.284), ENNs+TP1 (1.302) and ENNs+TP2 (1.323) compared to CON (1.388). Although numerical differences, future research should confirm that ENNs predisposes to gut leakage (21.2 μg FITC-d/ml serum, day 7 post-weaning) and the mitigation by test products (16.8 and 17.9 μg FITC-d/ml serum for ENN+TP1 and ENN+TP2 day 7 post-weaning, respectively). In conclusion, ENNs negatively affected feed intake and performance in young piglets, which was mitigated by the test products. Future studies should focus on the mode of action, including effects on gut level.

P63

Comparison of the effects of commercially available fungicides and a novel compound on mycotoxin-producing *Fusarium species*

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The main mycotoxin-producing fungi are *Aspergillus*, *Fusarium* and *Penicillium species*. Once fungi start to produce mycotoxins it is practically impossible to remove them from contaminated crops. Therefore, targeting the toxin-producing fungi prior to the appearance of the toxins might be the best preventative step in mycotoxin management. There are already some substances which are used in agriculture as fungicides, such as propionic acid (PROP) and azoles, like metconazole (MET) as the most common ones. This work proposes a novel, chlorine-containing substance that could be used against filamentous fungi. To find the best benchmarking, we compared the clinically applied Clinical Laboratory Standard Institute (CLSI) M38-A2 protocol to the poisoned food method, which is usually used in agricultural studies. We observed a higher minimal inhibitory concentration (MIC) with the CLSI method. Treating the fungi with a sub-inhibitory concentration could induce toxin production, therefore this study used the CLSI method. Some modifications were applied as the evaluation was performed not only at 48 h but also at 8 days. We determined the growth inhibition effect of chlorine-containing substance on mycotoxin-producing *Fusarium species*: the T2- producing *F. sporotrichioides* var. *minus* (DSM62425), the DON (deoxynivalenol)-producing *F. graminearum* (FZL Fg2022/17) and the fumonisin producer *F. verticillioides* (FZL Fv2022/1) from Fumizol Ltd., Szeged. As the protocol required, we included *A. flavus* ATCC 204304 strain and voriconazole (VOR) as reference experiments. The MET MIC values were 0.01-0.87 µg/ml after 48 h and 0.23-1.73 µg/ml after 8 days. The PROP MIC values were 109.42-394.25 µg/ml for 48 h and 210.71-515.05 µg/mL for 8 days. In the case of the chlorine-containing substance, MIC values were between 0.91 µg/ml and 1.62 µg/ml after 48 h and 0.91 µg/ml and 1.81 µg/mL after 8 days. Two out of three observed *Fusarium* spp. consistently showed the same MIC values over time, indicating that it eradicates all the colony-forming units simultaneously. While MET can be applied at lower concentrations,azole resistance remains a significant issue. Contrary to this resistance cannot occur against the novel substance, as it acts rapidly. We observed inhibitory effects for all the species studied. Therefore, the chlorine-containing substance is a promising compound for further research in the application during mycotoxin management. However, additional studies are needed on other mycotoxin-producing filamentous fungi and the potential method of application for the chlorine-containing substance as well.

P64

Adsorption of zearalenone by smectite-based materials of different surface chemistry

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This research focuses on the challenge of adsorptive removal of zearalenone (ZEN). ZEN is a non-steroidal, oestrogen-mimicking mycotoxin found in food and feed. It shows high stability throughout the food processing treatments, which leads to its entry into living organisms, subsequently causing harmful toxic effects. The experiments were designed to study the affinity of various smectite-based materials for ZEN immobilization with initial concentration (Cp) of 4.0, 1.0 and 0.1 ppm, adsorbent dosage in the range of 20-500 mg/l, and pH 2, 6 and 8. The materials were based on 3 smectite-rich samples purchased from the Clay Minerals Society repository: SW (SWy-2, Wyoming smectite), ST (STx-1b, Texas smectite), and SA (SAz-2, Arizona smectite). The smectites differed in chemistry, textural properties as well as cation exchange capacity (CEC). Several modifications which altered surface chemistry were tested including: (i) cation exchange with Na⁺, Mg²⁺ and Li⁺, (ii) calcination at 300, 500 and 700°C, and (iii) intercalation with cationic surfactants: HDTMA-Br (C16) and ethyl lauroyl arginate (LAE®). The raw, cation-exchanged as well as calcined smectites did not show satisfactory ZEN removal which was lower than 10% regardless of ZEN Cp. The results showed that ZEN removal was favoured by the surfactant-treated materials, which indicated the predominance of hydrophobic interactions. For example, a 75% removal efficiency was achieved when ZEN Cp was equal to 1 ppm, and adsorbent

dosage was set to 200 mg/l. The ZEN removal increased along with the surfactant content determined for the smectites which correlated with their CEC: SA > ST > SW. Moreover, the removal also correlated with LAE[®] content in the smectites' structure. For the LAE[®] intercalates, the low pH (~2.3) only slightly affected the removal efficiency as compared to pH of ~6.0. However, at the pH ~8.0, the removal was visibly lowered due to LAE[®] hydrolysis. In general, the C16 intercalated materials removed ZEN more efficiently than the LAE[®] intercalates, however the latter surfactant is considered as safe for application in food industry. Acknowledgements. The research project was supported by the programme 'Excellence initiative – research university' for the AGH University of Krakow, no. 9793 (501.696.7996/L34).

P65

Smectite-based modified materials for adsorption of emerging mycotoxins: Alternariol, enniatin B1, and beauvericin

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Emerging mycotoxins are compounds that are not currently included in standard monitoring or regulations, yet they are frequently detected and exhibit a rapidly growing prevalence. Chronic exposure to these toxins poses notable health concerns, underscoring the need for new mitigation strategies. Hence, this research focused on the synthesis and evaluation of materials with different surface properties derived from the smectite-rich sample. Smectites are renowned for their excellent adsorption properties, largely due to their high cation exchange capacity (CEC) and well-developed specific surface area. However, they are not sufficiently effective against low-polarity or nonpolar contaminants. In this study, we addressed this limitation by modifying Texas smectite STx-1b (ST) purchased from the Clay Mineral Society repository. These modifications aimed to alter the surface chemistry and thereby enhance the materials' affinity for mycotoxins. The synthesis procedures involved 3 types of modifications: (i) cation exchange with Na⁺, Mg²⁺, and Li⁺, (ii) calcination at 300, 500, and 700°C, and (iii) intercalation with cationic surfactants – hexadecyltrimethylammonium bromide (HDTMA-Br) and ethyl lauroyl arginate (LAE[®]), the latter of which was obtained from Lamirsa (Vedeqsa). LAE[®] intercalation was performed at 0.25, 0.50, 0.75, and 1.0 of the cation exchange capacity (CEC) of the ST sample. The experiments, conducted at an initial concentration of 1 ppm for alternariol (AOH), with a material dosage of 40-200 mg/l, demonstrated the significance of hydrophobic interactions, as evidenced by the higher adsorption of AOH by the surfactant-intercalated materials. The highest AOH removal efficiency of ~95%, with 200 mg/L adsorbent dosage, was achieved by the HDTMA-Br-containing smectite, whereas LAE[®]-containing samples showed lower removal efficiency (10-35%). Nearly all of the tested materials adsorbed over 50% of BEA, which indicates complex interaction mechanisms most likely including hydrophobic interactions involving its aromatic rings. Similar observations were found for the ENN B1 with the lowest removal efficiency below ~30% for all the surfactant-containing materials, which might be attributed to the lack of aromatic rings in the ENN B1 structure. Acknowledgements. The research project was supported by the program 'Excellence initiative – research university' for the AGH University of Krakow, no. 9793 (501.696.7996/L34).

P66

Mycotoxins and predictive models in Africa.

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Africa, a major producer, consumer, and trader of cereals, faces significant challenges due to mycotoxin contamination. These fungal metabolites, primarily produced by *Aspergillus* and *Fusarium* species, pose serious threats to food safety, security, and public health. Factors such as geolocation, climate uncertainties, and socioeconomic disadvantages exacerbate the problem, particularly affecting small-scale farmers. Despite ongoing efforts to develop diagnostic, monitoring, and management tools for mycotoxin control in Africa, many methods have proven labour-intensive and cost prohibitive. Predictive modelling has emerged as a potential solution, with successful applications in developed countries and adaptations for African contexts. This review systematically analyses research articles published from 2014 to 2024 on machine learning-based and mechanistic predictive models for mycotoxin

contamination in maize, millet, and sorghum during pre- and post-harvest stages in Africa. It aims to understand frequently used models, important features, data sources, preparation techniques, and model accuracy in the African context. The preliminary results show that the majority of studies focused on Eastern Africa, particularly Tanzania and Kenya, with an emphasis on aflatoxin detection in maize. Half of the studies validated their models using real mycotoxin experiments, while others utilized spectral and image data. Many studies leveraged freely available climate and GIS data from FAO databases, requiring rigorous preprocessing. Key predictive variables included soil organic carbon content, soil water supply to demand ratio, pre-flowering NDVI, soil pH, and growing season length. Future research should comprehensively address relevant mycotoxins across all African sub-continent and incorporate agronomic practices in modelling processes. Combining historical and climate variables could enhance prediction accuracy. Additionally, developing user-friendly mobile applications for real-time prediction and risk assessment of mycotoxin contamination in maize, millet, and sorghum across African countries would improve accessibility and practical application of these models. To advance the field, researchers should focus on expanding the scope to include multiple mycotoxins and crops, integrate diverse data sources, validating models for different African regions, establishing collaborative networks to share data and improve model accuracy. These efforts will contribute to more effective mycotoxin management strategies and enhance food safety across the African continent.

P67

Bioprospecting and valorisation of citrus crops to obtain preharvest and post-harvest biocontrol agents against mycotoxigenic fungi

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Citrus fruits are among the most widely grown fruits globally. In 2022, the Food and Agriculture Organization estimated their production at 158.5 million tons, with about one-third of the produce lost to fungal rot. In addition, 20-30% of the inedible parts of these fruits generate significant food waste. We investigated the microbiome of citrus fruits and crops and selected and characterized microbes for developing natural alternatives through microbial fermentations using citrus residues. The objective was to prevent fungal contamination, reduce the presence of mycotoxins and economic losses in the field and during storage. Strains of lactic acid bacteria (LAB) and fungi from different citrus crops were isolated. An initial test determined the antifungal activity of the LAB strains. Selected strains were used to ferment a medium with citrus residues. Antifungal metabolites were studied, and *in vitro* tests were performed against pathogenic fungi isolated. The most effective treatment was applied as a biocoating on fruits to test its biopreservation capacity. The metabolome of the fermented medium was studied to determine its biostimulant and biocontrol capacity, aiming to develop dual purpose products that enhance citrus crop productivity and prevent pathogenic fungal infections. Strains N3B1 showed the best antifungal activity, with MIC/MFC values between 1.8-250 g/l. These culture media had a presence of antifungal metabolites, such as lactic, acetic, DL-3-phenyllactic, 3-4-dihydroxyhydrocinnamic, salicylic, and vanillic acids. Tests carried out on oranges showed that the treatment reduced the proportion of contaminated oranges by 90% after 10 days of storage, decreased the prevalence of fungi by 4log₁₀ spore units/g of citrus and the presence of mycotoxins in the fruits. In conclusion, the results demonstrate the potential of the fermented medium with citrus residues as biocontrol agent for the development of natural products in the field and storage, exemplifying a circular economy model.

P68

Inhibitive effect of *Urginea epigea* methanolic extract and silver/zinc oxide nanoparticles on *Aspergillus* and aflatoxin production

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Food crops contaminated with *Aspergillus flavus* due to aflatoxins can be hazardous for humans and animals, hence endeavours are being explored to find natural antifungals to combat the contamination and mycotoxin issue. The current study used the agar dilution method to assess the effect of *Urginea epigea* methanolic extract and biosynthesized silver-zinc oxide nanoparticles on toxigenic strains of *Aspergillus*. In the experiment, an aflatoxin-producing strain was used, and potato dextrose agar was

diluted with methanolic extract from *U. epigea* and silver/zinc oxide nanoparticles at concentrations of 0, 6.5, 12, 25, and 50 mg/ml, respectively. Mycelia growth diameters were measured to test inhibitory activity. A significant decrease in fungal growth was observed at different concentrations ($p < 0,05$) when compared to the control. At 50 mg/ml, the extract of *U. epigea* significantly reduced the growth of *A. flavus* by 100% as compared to the control. *U. epigea* and Ag/ZnO nanoparticles downregulated *AflR* and *AflD* expression, however there was less expression by nanoparticles, as evidenced by the sequence alignment. *Aspergillus flavus* growth and aflatoxin B1 production were both considerably suppressed by *U. epigea* methanolic extract, thus has the potential to be employed as an alternative antifungal agent to control aflatoxigenic fungus.

P69

The investigation of effectiveness of different concentrations of the mycotoxin detoxification agent added to broiler feed, in the presence of T-2 toxin, on performance, organ mass and the residues T-2 toxin and its metabolites in the broiler tissues

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The experiment was performed on a total of 99-day-old broilers, which were divided into 9 equal groups. Broilers of the E-I group were fed 0.25 mg T-2 toxin/kg feed; E-II and E-III groups were fed 0.25 mg T-2 toxin/kg feed with the addition of 1 kg/t and 3 kg/t of the mycotoxin detoxification agent MDA, respectively. The E-IV group received 1 mg T-2 toxin/kg of feed, and the broilers of E-V and E-VI groups received 1 mg T-2 toxin/kg of feed with the addition of 1 kg/t and 3 kg/t of the MDA detoxification preparation, respectively. The E-VII group received commercial feed without toxins and additives, the E-VIII and E-IX groups received feed with 1 kg/t and 3 kg/t of the MDA detoxification preparation. The trial lasted 42 days. Observing the results obtained on the 42nd day of the experiment, we conclude that the change in absolute mass of the spleen occurred in broilers of the E-IV group (1.66 ± 0.14 g), which was statistically significantly lower compared to the broilers of E-V and E-VI groups (2.58 ± 0.15 and 2.68 ± 0.23 g). Heart mass was significantly lower in broilers of group E-IV (9.1 ± 0.38 g) compared to broilers of groups E-V and E-VI (12.23 ± 0.5 and 11.43 ± 0.51 g). It can be concluded that the broilers that received 1 kg/t and 3 kg/t of the detoxification preparation had an absolute mass of organs within physiological limits. Broilers of the E-IV group achieved the lowest BM during the experiment (on the 42nd day of the experiment, $1,879 \pm 52.73$ g), they were significantly statistically lower than the BW of broilers of all experimental groups. This trend is observed from the beginning to the end of the experiment. The protective effect of the detoxification preparation can be seen in broilers of the E-V group, that had a significantly statistically higher BM on the 42nd day of the experiment (2225 ± 58.81 g) compared to broilers of group E-IV. Broilers of E-VIII group (2452 ± 46.71 g), which received commercial feed with the addition of 1 kg/t MDA preparation, had the highest BMI at the end of the experiment. At the end of the trial on the 42nd day, blood samples were collected from broilers of the experimental groups that received T-2 toxin and MR detoxification preparations in different concentrations. Also, liver and breast musculature samples were collected for testing for the presence and content of T-2 toxin, HT-2 toxin, T-2 tetraol and T-2 triol. Due to very rapid elimination from the blood, no residues of T-2 toxin and its metabolites were detected in the blood of broilers of groups E-I to E-VI. In the breast muscles, T-2 toxin residues below $LOQ < 0.2$ $\mu\text{g}/\text{kg}$ were detected in all groups that received T-2 toxin in food, the highest value was recorded in the E-IV group (0.122 $\mu\text{g}/\text{kg}$) and the lowest in E-VI group (0.096 $\mu\text{g}/\text{kg}$). No T-2 toxin residues were detected in the liver. Remnants of HT-2 were detected in the breast muscles and livers of broilers from E-IV, E-V and E-VI groups, $LOQ < 1$ $\mu\text{g}/\text{kg}$; for the breast muscles: 0.054 , 0.044 and 0.041 $\mu\text{g}/\text{kg}$, and for the liver: 0.473 , 0.231 and 0.185 $\mu\text{g}/\text{kg}$. Summing up all the results, a partial protective effect of the detoxification preparation, added to food in the amount of 1 kg/t, can be seen.

P70

Gut microbiota maturation and diversity in broilers: the impact of deoxynivalenol contamination

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The intestinal microbiota plays a crucial role in the immune system, nutrient absorption, and overall growth performance of broilers. It undergoes significant changes with age, starting from less diverse to

a more stable and mature state by late growth. However, mycotoxins can disrupt this maturation process, negatively impacting the stability and diversity of the microbiota, and consequently, the health and performance of the host. An *in vivo* trial was conducted by Cargill Animal Nutrition and Health to investigate the effects of deoxynivalenol (DON) and an innovative anti-mycotoxins agent (AMA) on the gut microbiota of broilers. Animals were fed either an uncontaminated feed, a DON-contaminated feed (8 ppm), or a DON-contaminated feed (8 ppm) supplemented with an AMA (2.5 kg/mT). The intestinal microbiota was analysed at 7 and 28 days of age using Cargill's proprietary tool, Galleon. The results demonstrated that broilers fed the uncontaminated diet exhibited significant changes in their gut microbiota between 7 and 28 days of age, indicating a healthy maturation process. In contrast, broilers fed a DON-contaminated diet showed minimal changes, suggesting that DON negatively affects the maturation of the gut microbiota. The addition of AMA to DON-contaminated feed counteracted DON's negative effects, allowing the microbiota to mature similarly to the uncontaminated group. Specifically, between 7 and 28 days, *Clostridium botulinum* levels decreased in broilers fed uncontaminated feed but remained stable in those fed DON-contaminated feed. The addition of AMA decreased *C. botulinum* levels despite the presence of DON. *Clostridiales*, *Yersinia enterocolitica*, *Campylobacter jejuni*, *Salmonella*, and *Lactobacillus* decreased in broilers fed uncontaminated feed but stagnated or increased with DON. AMA supplementation restored these bacteria to levels similar to the uncontaminated group. *Lactobacillus crispatus* decreased more in the group fed the DON-contaminated diet than with the uncontaminated feed, but AMA mitigated this effect. Conversely, *Lactobacillus salivarius* and *Prevotella* increased less in the DON group than in the uncontaminated group, but AMA again counteracted DON's negative impact. The trial confirms that DON impacts the maturation process of the gut microbiota. This effect can be attributed to DON either inhibiting the growth of beneficial bacteria, thereby creating an environment conducive to the proliferation of harmful bacteria or directly stimulating the growth of harmful bacteria restraining the development of beneficial bacterial. The innovative AMA effectively mitigates these adverse effects, promoting a mature and diverse microbiota essential for better performance and health.

P71

Efficacy of bentonite-based toxin binder in mitigating the effects of aflatoxin B1 on the growth performance of tilapia

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Mycotoxin contamination poses a significant risk to fish health and productivity in aquaculture. These toxic compounds can infiltrate feed ingredients, and when consumed, adversely affect the growth, immunity, and health of farmed aquatic species. To combat these effects, toxin binders are commonly added to aquaculture feeds to adsorb and neutralize aflatoxins within the digestive system, reducing their bioavailability and mitigating toxicity. This study investigates the efficacy of a bentonite-based toxin binder (Toxo-MX, Trouw Nutrition, the Netherlands) on the growth performance of tilapia challenged with aflatoxin B1 (AFB1). A total of 320 juvenile tilapia (11.33±0.420 g) were randomly assigned to four treatment groups, each with four replications of 20 fish: a negative control (NC) group receiving a basal diet, a positive control (PC) group receiving a basal diet with 250 ppb AFB1, and two treatment groups with the PC supplemented with Toxo-MX at doses of 1 kg/ton (TMX1) and 2 kg/ton (TMX2). Fish growth performance parameters were recorded over a 42-day challenge period, and the hepatosomatic index was evaluated at the end of the study. Results indicated that feeding AFB1 diets (PC) significantly impaired feed intake (-11.1%), final body weight (-13.6%), ADG (-15.8%), specific growth rate (-7.3%), and FCR (+5.8%) compared to fish fed NC diets. The addition of toxin binders effectively mitigated some of the adverse effects of aflatoxins, with the efficacy being more pronounced in the group receiving the higher dose of toxin binder (TMX2). Compared to the PC group, TMX2 supplementation significantly increased final body weight (+7.0%) and ADG (+7.9%). However, these improvements did not fully restore the growth rate to levels comparable to those observed in the NC group. Additionally, TMX2 improved feed intake (+1.7%), specific growth rate (+3.2%), FCR (-5.8%), and hepatosomatic index (+1.2%), although these differences were not statistically significant compared to the PC group. In contrast, the TMX1 group, which received a lower dose of toxin binder, exhibited performance metrics similar to those of the PC group (p>0.05). In conclusion, these findings suggest that toxin binders can effectively mitigate the negative effects of AFB1, improving growth rates and feed efficiency of fish under AFB1 challenge conditions. The higher dose of toxin binder (TMX2) showed more pronounced benefits, suggesting a dose-dependent response.

P72

A mycotoxin binder containing bentonite and yeast cell wall fractions improves the performance of laying hens exposed to multiple mycotoxins

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The co-occurrence of multiple mycotoxins in poultry feed is a global concern that presents a significant challenge to the poultry industry. Mycotoxin binders like bentonites are usually added to animal feed as a strategy to reduce the adverse effects of these toxins on bird health, productivity, and the overall profitability of layer operations. However, bentonites have limitations and can't bind multiple mycotoxins present in the layer feeds to the same extent, thus creating the need for mycotoxin mitigation products with multiple modes of action. This study aimed to evaluate the efficacy of a broad-spectrum mycotoxin binder, Toxo-XL, containing bentonite, yeast cell wall fraction, and activated β -glucans, on the production performance of laying hens challenged with aflatoxin (AF), T2 toxin (T2), ochratoxin (OTA), and deoxynivalenol (DON). White Leghorn BV300 layer hens (n=420, 24 weeks old) were allocated to five treatment groups with 14 replicates of six birds each (2 cages of 3 birds). The treatments included: (i) a negative control (NC, basal diet without mycotoxins); (ii) a high mycotoxin diet (PCH, NC + 150 ppb AF, 100 ppb T2, 120 ppb OTA, 1,500 ppb DON); (iii) PCH + 2 kg/T Toxo-XL (TXH); (iv) a low mycotoxin diet (PCL, NC + 100 ppb AF, 50 ppb T2, 50 ppb OTA, 1000 ppb DON); and (v) PCL + 1 kg/T Toxo-XL (TXL). The diets were fed for 12 weeks, and production performance was recorded. Data were analysed using the MIXED procedure in SAS. Exposure to both low and high levels of multiple mycotoxins compromised hen performance and resulted in a significant drop (6-8%) in egg production (HDEP) and a 3-4% increase in FCR compared to non-challenged layers fed the NC diet. Feeding diets supplemented with toxin binders significantly alleviated these effects. Among the challenged groups, TXL and TXH improved ($p < 0.05$) HDEP by 2-4%, egg mass by 4%, and FCR by 3-4% compared to PCL and PCH. Additionally, feed intake was higher ($p < 0.05$) in hens supplemented with TXL and TXH compared to the PCH group, but similar ($p > 0.05$) to that of the PCL group. These findings suggest that Toxo-XL supplementation can effectively mitigate the detrimental effects of multiple mycotoxins on commercial layer performance, improving egg production and FCR by 3-4% in laying hens.

P73

Remediation of emerging mycotoxins using a premium mycotoxin remediation product

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Mycotoxins are the secondary metabolites produced by various fungal species on food and feed materials. Most mycotoxins are produced by *Aspergillus*, *Fusarium* and *Penicillium* sp. Commonly, most people think of main six mycotoxins that contaminate feed: aflatoxins, deoxynivalenol (DON), T-2 toxin, fumonisins, ochratoxin A and zearalenone. Testing and reporting on the prevalence of mycotoxins in feed has increased the awareness about emerging mycotoxins which are also commonly produced by various *Fusarium* moulds. Recently, maize survey conducted by PATENT CO DOO., Serbia in 2022, also demonstrated higher occurrence of emerging mycotoxins in maize (<https://mycotoxinsite.com/higher-prevalence-fumonisin-fusaric-acid-2022-harvested-corn/?lang=en>). MycoRaid is a premium mycotoxin remediation product developed by PATENT CO for bioremediation of polar and non-polar mycotoxins. In this report MycoRaid has been tested for adsorption of emerging mycotoxins at pH 3.0 for desorption at pH 6.5. Weighed 100 mg of mycotoxin remediation product (MycoRaid) into duplicate 15 ml falcon tubes and added 10 ml of 0.1 M citrate buffer (pH 3.0) containing 2 ppm moniliform (MON), fusaric acid (FA), enniatin (ENN) B, beauvericin (BEA), ENNB1, ENNA and ENNA1. To measure loss due to non-specific binding and to eliminate exogenous peaks, a control was prepared. The tubes were placed on an orbital shaker for 30 min at 37 °C. After incubation, centrifuged samples (test and control sample) at 4,200 rpm for 5 min, removed 100 μ l supernatant to glass vial and added 900 μ l of dilution solvent (50% acetonitrile:50% water and 0.1% formic acid). An aliquot of the original pH 3 buffered mycotoxin test solution was used as standard for each mycotoxin. Samples and controls were analysed by LC-MS/MS. The supernatant was removed from the remaining sample and control Falcon tubes and the mycotoxin remediation product pellet was resuspended in 4 ml of 0.1 M phosphate buffer (pH 6.5). The tubes were shaken, and sample was prepared as shown above. Samples and controls were analysed by LC-MS/MS. Overall efficacy was calculated using adsorption and desorption data. The results show that MycoRaid can effectively remove 56% MON, 89% BEA and more than 95% fusaric

acid and enniatins. In conclusion, due to the lack of information on toxicity and prevalence of emerging mycotoxins, it is difficult to assess the role of these undetected toxins on animal health and performance. However, emerging toxins are likely to co-occur with major mycotoxins that contaminate grains used in livestock production and therefore a remediation strategy should be used to reduce the carryover of mycotoxins from animals to humans.

P74

Degradation of ochratoxin A by the strain *Stenotrophomonas acidaminiphila* PAFO/6
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Ochratoxin A (OTA) mycotoxin is produced by several *Penicillium* and *Aspergillus* species of moulds. OTA presents a major contaminant of wide variety of food and feed, mainly contaminating cereal-based and fruit-based products. As such, OTA is considered one of the most economically significant mycotoxins. Food and animal feed contaminated with OTA present a health risk for both humans and livestock. Biological degradation is one of many remediation methods for reduction of mycotoxins including OTA. In this research work, a soil sample was collected from a parking lot of a popular tourist spot. From a soil contaminated with fuel residue, a microbe was isolated by enrichment culture procedure using mineral salts medium (MSM) and 1 ppm of OTA as a sole carbon source. The bacterium PAFO/6, was identified by NCIMB, UK using 16S rRNA sequencing, as *Stenotrophomonas acidaminiphila*. The microbe was able to degrade 94.1% of 1 ppm of OTA within 6 h, under aerobic conditions at 40°C and neutral pH. The microbe was able to reduce OTA at pH levels ranging from 5.0-9.0, optimum being at 7.0-9.0, and temperatures ranging from 30-40°C, optimum being at 40°C. OTA reduction level was quantified using LC-MS/MS. This research demonstrated the ability of *S. acidaminiphila* to degrade ochratoxin A and utilize it as the sole carbon source. Under optimum conditions, *S. acidaminiphila* will degrade 98.1% of 10 ppm of ochratoxin A in 6 h in MSM. This indicates the potential use of *S. acidaminiphila* for bioremediation of ochratoxin A contaminated crops. Degradation of OTA resulted in creation of OTα, a less toxic metabolite. This research can provide a potentially new practical way for reduction of ochratoxin A in an OTA-contaminated environment.

P75

Stenocarpella maydis ear rot and diplodiatoxin in maize grain production areas of South Africa
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Stenocarpella maydis (*Diplodia*) ear rots have long been a major concern in maize production areas of South Africa, causing yield and grain quality losses to the entire industry. The recent isolation and characterisation of diplodiatoxin allows researchers to now study the toxin and not just the fungus producing the toxin. The aim of this study is to give an overview of recent *S. maydis* research advances and the occurrence of *S. maydis* ear rots and diplodiatoxin in maize grain production areas of South Africa. Fifty-six and 77 maize samples were collected from conservation agriculture commercial- (Free-State and North-West Provinces) and-smallholder farms in Kwa-Zulu Natal), respectively, during the 2019-2023 seasons. During the 2023/24 season, 200 samples were collected from commercial conventional farms as well as smallholder farms from the Free-State and North-West, Eastern Cape, Kwa-Zulu Natal and Limpopo provinces. Individual maize grain samples (2019-2024 samples) were subjected to surface sterilization and plated out to determine the severity of *S. maydis* infections. Sub-samples were subjected to LC/MS (liquid chromatography-mass spectrometry) to quantify diplodiatoxin levels from the 2019-2023 samples. Diplodiatoxin of the 2023/24 grain samples are being quantified. During the 2019-2023 seasons, *S. maydis* severity was low in commercially produced grain from the northern and eastern Free-State Provinces (8% and 4% respectively, no-till) and medium in the North-West Province (32%). During the 2020-2021 season, *S. maydis* severity was high in smallholder farmer-produced grain in Bergville (72%, maize monoculture, no-till) and low in the Midlands (4%). During 2020/21, *S. maydis* severity was high in Eswathini (69%). During the 2023/24 season, *S. maydis* severity was low in the North-West (15%) and Northern-Cape (25%), medium in the Free-State (46%) and Limpopo (40%) and the highest in the Eastern-Cape (80%, smallholder farms). None to trace quantities of diplodiatoxin were observed in the 2019-2023 seasons in commercial farm grain, except for Ottosdal during the 2019/20 season. A grain sorghum/maize rotation (no-till) and a monoculture maize field (no-till) yielded 1,662 µg/kg and 1,527 µg/kg diplodiatoxin, respectively. Diplodiatoxin quantification varied

from not detected to 15,736 µg/kg from smallholder farm grain in Kwa-Zulu Natal (2019-2023). Irrespective of season, diplodiatoxin were mainly present in monoculture maize fields (no-till). *S. maydis* infections from monoculture maize plots (especially those of smallholder farmers) had the highest levels of diplodiatoxin. This is because maize is the only host crop for this diplodiatoxin producing pathogen. This study emphasises the importance of choosing the correct crop to be used in a rotation/intercrop system to reduce primary *S. maydis* inoculum and subsequent infections. The paucity of information regarding possible human and animal health effects due to diplodiatoxin ingestion warrants urgent investigations.

P76

The effect of conservation cropping systems used by small-scale farmers in KwaZulu-Natal, South-Africa, on mycotoxigenic fungi and mycotoxin incidence in maize grain

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Conservation agriculture (CA) systems conserve and improve soil health by means of maintenance of a permanent soil surface cover (crop stubble), minimum soil disturbance and diversification of plant species. On the other hand, mycotoxin producing pathogens such as *Fusarium verticillioides*, *Fusarium graminearum* and *Stenocarpella maydis* spores can survive on crop stubble, thereby infecting plants in the following season. The aim of this study was to investigate the effect of CA practices on maize ear rot incidence and mycotoxin contamination of grain from small scale farmers' fields. A total of 76 individual maize grain samples were collected from 13 small scale farmers' at on-farm experimental plots from KwaZulu-Natal (2019/20-2022/23). Farmers used differing CA practices (monoculture maize served as controls). *F. verticillioides*, *F. graminearum* and multiple mycotoxins were quantified (qPCR and LC-MS/MS). *F. graminearum* and *F. verticillioides* infections were greater in maize grain collected from maize-cowpea intercrop systems (685.70 pg/20 µl and 99.80 pg/20 µl, respectively) compared to the maize monoculture systems (513.78 pg/20 µl and 26.56 pg/20 µl, respectively). Maize grain from the maize-dry bean intercrop system had the lowest *F. graminearum* and *F. verticillioides* infections. Maize grain from the monoculture maize systems had high mean levels of deoxynivalenol (2,894 µg/kg) and diplodiatoxin (1,401 µg/kg). This is expected because maize is the only host to *S. maydis*. Due to the paucity in research regarding the mycotoxins produced by *S. maydis*, there are no regulations for these mycotoxins. Maize grain from the maize-cowpea intercrop systems had slightly higher zearalenone levels (808.45 µg/kg) when compared to the monoculture maize systems (724.20 µg/kg). With this study we wanted to elucidate the effect of conservation cropping systems on ear rot diseases and resultant mycotoxin production in maize grain. Maize grain intercropped with cowpea had unexpected higher levels of *F. graminearum* and *F. verticillioides* ear rot as well as zearalenone when compared with maize grain from monoculture plots from smallholder farmers in KwaZulu-Natal. It is speculated that cowpea and babala produce minimal crop residues and that elevated *F. graminearum* ear rot infections come from maize residues. Cowpea could be a possible host for this fungus, and this warrants further investigation. Grain from monoculture maize plots had higher levels of fumonisins, deoxynivalenol and diplodiatoxin. This study emphasises the importance of choosing the correct crop to be used in a rotation/intercrop system. Primary inoculum and weather conditions are suspected to play a critical role in the presence or absence of mycotoxins in this study. Acknowledgements. Agricultural Research Council-Grain Crops for facilities and Maize Trust for funding.

P77

Evaluation of an anti-mycotoxin agent on mycotoxin binding and ruminal chemical profiles in ruminants: an *in vitro* study

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It is well established that ruminal microbiota plays a pivotal role in degrading and deactivating certain mycotoxins in ruminants' daily diet. However, this process can be impaired by several factors (e.g., low ruminal pH; ruminal dysbiosis; high-producing cows), reducing the rumen's ability to mitigate mycotoxins effects. Ruminants' exposure to mycotoxins is responsible for a decline in milk quality and quantity, reduced ruminal fermentation efficiency and an increased incidence of reproductive disorders. To

counteract these detrimental effects, nutritional strategies have been implemented, such as the inclusion of anti-mycotoxin agents. This study evaluated the *in vitro* ruminal initial sequestration (weak binding) and subsequent desorption (strong binding) of an anti-mycotoxin agent that included adsorbing material, turmeric (curcumin) and milk thistle (silymarin) extracts, and yeast-based components. The agent targeted aflatoxins (AFs), fumonisins B1 and B2 (FBs), deoxynivalenol (DON), T-2 and HT-2 toxins, and zearalenone (ZEN). Two doses were tested *in vitro*: dose 1 and 2, simulating *in vivo* dosages of 30 mg/cow/day or 90 mg/cow/day, respectively. Weak and strong bindings were analysed at 1, 4 and 24 h of buffered rumen fluid incubations. Ultra-high-performance liquid chromatography coupled with high-resolution mass spectrometry (UHPLC-HRMS) followed by multivariate data analyses was used for mycotoxin quantification and dose-dependent reductions reveal, with statistical validations indicating significant changes in mycotoxin levels across dose and time. Statistical analysis was performed with the general linear model (GLM) procedure using R software. Results indicated that the agent effectively bound AFB1, T2 and HT-2 toxins in both doses, since the concentrations were always below the detection limit ($p < 0.05$). The ZEN, DON and FBs adsorption values reached 78.5%, 41.5% and 45.5% at dose 1; and 74.7%, 43.3% and 53% at dose 2, respectively ($p < 0.05$). Metabolomics based on UHPLC-HRMS identified over 1,500 mass features in rumen samples, highlighting the anti-mycotoxin agent's impact on the untargeted chemical profiles. Unsupervised hierarchical clustering analysis (HCA) revealed significant shifts in untargeted metabolomic profiles after 24 h of treatment. 44 discriminant compounds were identified in the strong binding model and 16 in the weak binding model, with silibinin, a flavolignan with antioxidant and anti-inflammatory properties, strongly characterizing the weak binding group. These findings suggest that the anti-mycotoxin agent, even at low dose, potentially enhances mycotoxin binding and positively influences ruminal chemical profiles. Results from *in vitro* rumen simulating method should be validated *in vivo* to confirm their validity.

P78

Ex vivo efficacy trial of an anti-mycotoxin agent in counteracting the detrimental effects of *Fusarium* mycotoxins in porcine ileal organoid monolayers

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Deoxynivalenol (DON) and T-2 toxin are mycotoxins generated by *Fusarium* fungi that have been proven to damage the intestinal epithelial cell layer integrity, increasing animal susceptibility to pathogens and other toxic compounds (Antonissen *et al.*, 2014. *Toxins* 6: 430). In this context, natural plant extracts have received special attention due to their wide range of beneficial health properties. Therefore, the aim of the present study was to evaluate the capacity of an anti-mycotoxin agent that contains a combination of inorganic and organic adsorbents and polyphenolic compounds from turmeric (*Curcuma longa*) and milk thistle (*Silybum marianum*) extracts, to reduce the negative effects induced by DON and T-2 exposure in ileal porcine organoid (IPO) monolayers in an *ex vivo* model. IPOs were kept in 3D culture embedded in Matrigel droplets in 24-well plates and were fed daily with organoid growth medium (OGM). After they reached the correct size and confluence, they were dissociated with TrypLE for 5 min at 37 °C and placed into 24-well inserts treated with 0.5% Matrigel. 2D cultures were kept at 37 °C and 5% CO₂ for 2 days in OGM, and then the medium was changed to organoid differentiation medium (ODM) for 3 days. IPOs were treated with DON (1 µM) and T-2 toxin (0.1 µM), the anti-mycotoxin agent (0.5 mg/ml), and FITC-dextran (0.5 mg/ml) for 18 h. Afterwards, 100 µl of the cultured material were collected from the inserts for the mitochondrial activity and FITC-dextran permeability reading. The experimental data was analysed with ANOVA (GenStat Version 21.1). The anti-mycotoxin agent increased the mitochondrial activity, augmenting the cell viability in comparison to the control group ($p \leq 0.05$). Besides, the exposure of IPOs to the combination of DON and T-2 toxin significantly increased the FITC-dextran permeability ($p \leq 0.05$), indicating leakage. The medium supplementation with the anti-mycotoxin agent in the presence of the mycotoxins resulted in a significant reduction of leakage by 44% ($p \leq 0.05$). Hereby, the anti-mycotoxin agent containing inorganic and organic adsorbents and phytochemicals from turmeric (*Curcuma longa*) and milk thistle (*Silybum marianum*) natural extracts was effective to counteract the detrimental effects induced by *Fusarium* mycotoxins in ileal porcine organoid monolayers.

P79

The effect of curcumin and silymarin in mitigating the oxidative stress induced by deoxynivalenol in hepatic cells

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Oxidative stress is an important mechanism of deoxynivalenol (DON) toxicity. DON mycotoxin generates free radicals that disrupt the redox balance and induce DNA damage and apoptosis in the liver (Wu et al., 2017). In this context, natural plant extracts have received a great deal of attention due to their powerful antioxidant capacity, among a wide range of beneficial-health properties. Therefore, the aim of the present study was to evaluate the *in vitro* capacity of an anti-mycotoxin agent that contains a combination of polyphenolic compounds from turmeric (*Curcuma longa*) and milk thistle (*Silybum marianum*) extracts to reduce the oxidative stress induced in hepatic cells by DON. The main ingredient of milk thistle extract is silymarin, which is a standardized mixture of flavonolignans, and in regard to turmeric extract, curcumin is the principal compound. The *in vitro* antioxidant capacity of both natural extracts was tested by the ferric reducing antioxidant powder assay according to Trujillo Hernández (2019). Briefly, the capacity to reduce Fe³⁺ ion to Fe²⁺ ion was quantified by spectrophotometry at 593 nm and compared to butylated hydroxytoluene (BHT). Additionally, the thermostability of the active ingredients (curcumin and silymarin) was evaluated based on their recovery under controlled temperature treatment simulating feed manufacturing processes (from 75 to 135 °C at 5 min) by HPLC-DAD. To alleviate DON induced-oxidative stress by the anti-mycotoxin agent, an *in vitro* study was carried out in HepG2 cells using the H2-DCFDA assay. The reactive oxygen species (ROS) were determined upon different DON concentrations (3, 6, 12 µM) and two levels of the anti-mycotoxin agent (500 and 1000 mg/l) under controlled exposure for 24 h. Turmeric and milk thistle extracts antioxidant activity was greater than the BHT standard antioxidant capacity (149.4 and 340.7%, respectively). Concerning the thermostability, the anti-mycotoxins product has shown recoveries above 80% for both active ingredients tested, up to 135 °C for 5 min. The use of the anti-mycotoxin agent, containing curcumin and silymarin, reduced DON-induced oxidative stress by reducing the ROS levels by more than 41% after 5 min of exposure and the values remained stable for 24 h. In conclusion, the combination of curcumin and silymarin is a thermostable combination of natural extracts that provide effective antioxidant activity to alleviate the oxidative stress induced by DON in hepatic cells.

P80

Evaluation of the immunomodulatory capacity of an anti-mycotoxin agent for aquatic species containing phytochemicals and emulsifiers in gastrointestinal cells

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Mycotoxins are prevalent contaminants in feed worldwide that can cause severe detrimental effects on the health and profitability of animal productions, including aquaculture. One of the most widely known effects of mycotoxin exposure is the immunotoxicity, which has been reported in several studies in various terrestrial and aquatic species. In this scenario, phytochemicals from plant extracts have been in the spotlight due to their wide range of benefits, such as anti-inflammatory effects. For the assessment of innovative therapeutic treatments, the *in vitro* evaluation of the immune response in different cell models can be conducted by the analysis of the cytokines released. Therefore, the objective of the present study was to evaluate the *in vitro* capacity of an anti-mycotoxin agent for aquatic species that contains inorganic and organic adsorbents, bioactive compounds from turmeric (*Curcuma longa*) and milk thistle (*Silybum marianum*) extracts, and lysolecithin as an emulsifier, to mitigate the inflammatory response in a gastrointestinal cell co-culture model. In order to evaluate the immunomodulatory activity of the test product, the release of cytokines (IL-6, IL-10 and TNF-α) at intestinal level was conducted in a Caco-2/THP-1 cell co-culture. The cells were differentiated and were cultured in different conditions: negative control (NC), positive control (PC), product (P), preventive treatment (PT) and curative treatment (CT). For the production of the inflammation, LPS and IFN-γ were added to the medium at 10 ng/ml in PC and CT at time 0, and in PT after 24 h. The anti-mycotoxin agent was added at 50 mg/ml dose in P and PT at time 0, and in CT after 24 h. The analysis of the cytokines released was performed

with the cytokine kit MACSplex and flow cytometry (Miltenyi Biotech, Germany). The anti-mycotoxin agent decreased the concentration of the proinflammatory cytokines IL-6 and TNF- α and increased the expression of the anti-inflammatory cytokine IL-10 in comparison to the inflammation control. This effect was observed in both preventive and curative treatments, which proves that the anti-mycotoxin agent for aquatic species, containing phytochemicals and lysolecithin, has a potential implication in the reduction of the inflammatory response at a gastrointestinal level, thus preventing the incidence of different intestinal pathologies.

P81

The impact of deoxynivalenol and enniatins on brain and gut of weaned piglets, and the effect of an algoclay-based decontaminant in counteracting the negative effect of these mycotoxins

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This study evaluated the effects of dietary deoxynivalenol (DON, 0.7 mg/kg) and enniatins (ENNs, 0.8 mg/kg) on weaned piglets' growth and mRNA expression of markers related to inflammation, oxidative stress, and tight junctions in the gut and brain. Additionally, an algoclay-based mycotoxin decontaminant (TP) was tested (1.5 g/kg diet). Naturally contaminated diets used maize and barley as sources of DON and ENNs, respectively. Piglets (60 per treatment; divided into 10 pens/replicates with 6 piglets each) were fed the experimental diets for 14 days and subsequently fed a marginally contaminated diet until day 35. The experimental diets consisted of a control diet, either or not supplemented with the TP, and a diet contaminated with both DON and ENN, either or not supplemented with the TP. The performance of the piglets was evaluated (D0-14, D14-35, and D0-35). The mRNA expression analysis of the intestinal and brain tissues was performed directly after six days of dietary exposure. Compared to the control, DON+ENN piglets received 8 times more antibiotics. TP supplementation of the DON+ENN diet reduced antibiotic dosages by 31%. Dietary contamination with DON+ENNs tended to decrease average daily gain (ADG) and increase feed conversion ratio (FCR) from D0-14 ($p=0.09$), but not when supplemented with TP. Feeding the piglets given a marginally contaminated diet from D14-35 did not recover ADG ($p<0.03$). The relative expression of ZO1 in the jejunum muscular layer was downregulated ($p<0.01$), whereas IL-8 was upregulated in the mucosa layer of the jejunum ($p<0.001$) after exposure to DON+ENNs, and these effects were absent when the TP was added to the diet. IL-1b was upregulated in the colon of the piglets exposed to DON+ENNs ($p<0.01$). The hippocampus was the most affected brain tissue, where exposure to DON+ENNs resulted in the downregulation of serotonin transporter and occludin ($p<0.02$). In conclusion, exposure to 0.7 mg/kg DON and 0.8 mg/kg ENNs negatively impacts the growth performance of piglets and probably increases their susceptibility to bacterial infections. Losses in growth performance are not fully recovered after feeding the piglets a marginally contaminated diet, and the algoclay-based decontaminant was able to support the jejunum integrity and decrease inflammation caused by DON+ENN, by maintaining the mRNA expression of ZO-1 and IL-8 similar to the control group. Exposure to DON+ENN also impaired hippocampal serotonin transporter and occludin expression. Future research that focuses on these mycotoxins on gut-brain-microbiota axis could enhance the comprehension of their mechanism of action.

P82

Association of preharvest practices with multimycotoxin contamination in sorghum (*Sorghum bicolor*) in Northwest Ethiopia

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Mycotoxins are toxic metabolites produced by certain fungal species that can cause animal and human health problems and can contaminate food crops at any stage of the value chain. Sorghum is one of the important food crops in Ethiopia, where the occurrence of mycotoxins in this grain has been frequently reported. However, information on the relationships between the specific local sorghum production practices and mycotoxin contamination is rarely available. In this study, the occurrence of multiple mycotoxins in newly harvested sorghum samples was determined, as well as potential relationships between preharvest practices for sorghum growing and mycotoxin contamination. In 2022, 120 farmers in Northwest Ethiopia were asked about preharvest practices of sorghum fields, and sorghum samples were collected right after harvest. Samples were analysed using UPLC-MS/MS for a total of 33 different mycotoxins. About 75% of the samples were contaminated with at least one specific mycotoxin. The detected mycotoxins belong to one of the four mycotoxin categories, produced by *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp. and *Alternaria* spp. The concentrations of regulated mycotoxins were below the EU regulatory limits except for ochratoxin A, which was found in a concentration above the EU regulatory limit for unprocessed cereal grain in four percent of the samples. Several of the investigated preharvest practices showed a significant relationship ($p < 0.05$) with either one or more specific mycotoxins and/or with one of the mycotoxin categories. Among these practices, sowing method and type of fertilizer were the most positively related to multiple mycotoxin contamination. In addition, seed treatment practice before sowing contributed to the low mycotoxin presence. The practices of seed treatment, sowing, and fertilizer application can be further researched to develop a sustainable mycotoxin prevention strategy.

P83

Innovative enzyme solutions: advancing ochratoxin a detoxification in broilers

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Ochratoxin A (OTA) is a highly potent nephrotoxin produced by *Aspergillus* and *Penicillium* species. Because of its widespread occurrence in agricultural crops and processed foods it plays a critical role in the evaluation of feed and food safety. OTA's prolonged half-life and significant potential for bioaccumulation through dietary exposure exacerbate its risk (Malir *et al.*, 2016. Toxins 8: 191). The European Food Safety Authority (EFSA) recently proposed 0.03 mg OTA/kg feed as a new reference threshold for adverse health effects in growing chickens and hens (CONTAM, 2023. EFSA Journal 21: 8375). This broiler study evaluated the effectiveness of an OTA amidohydrolase (OAH; OCHRAzyme®) as a feed additive to detoxify OTA by converting it into the non-toxic metabolites ochratoxin α (OT α) and phenylalanine within the digestive tract of broilers. A 36-day feeding trial was conducted using 192 one-day-old broiler chickens, randomly allocated to one of four dietary treatments: (i) a control diet, (ii) a diet containing 1 mg/kg OTA, (iii) a diet with 1 mg/kg OTA supplemented with 15 U/kg OAH, and (iv) a diet with 1 mg/kg OTA supplemented with 75 U/kg OAH. Growth performance was tracked throughout the trial, and plasma and kidney samples were collected at the end of the study to measure OTA and OT α concentrations as exposure biomarkers. Supplementation with OAH led to significant reductions in OTA concentrations ($p < 0.0001$) in both, plasma and kidney samples, alongside a marked increase in the non-toxic metabolite OT α ($p < 0.0001$). Additionally, the negative effects of OTA on final body weight and feed intake were significantly alleviated by both tested enzyme concentrations ($p < 0.01$). These results demonstrate the potential of OAH as a feed additive to mitigate OTA-related health and performance issues in poultry.

P84

Effects of bacteria-based anti-biotoxins supplementation on post-weaning gilt performances, blood parameters, gut health and microbiome, in the presence of feed contaminated with deoxynivalenol and zearalenone

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Deoxynivalenol (DON) and zearalenone (ZEA) are mycotoxins that frequently contaminate maize and grain cereals. This study aims to assess the effect of a bacteria-based anti-biotoxins solution to alleviate the effect of post-weaned gilts exposed to artificially contaminated diet with DON (~1000 ppb) and ZEA (~500 ppb). A total of 180 gilts, randomly divided in 3 groups of 60 animals were used. The negative control (T1) has a feed without mycotoxin contaminations and without supplementation. T2 (supplemented with anti-biotoxins solution at 0.15%) and T3 (positive control) received contaminated feed. The experimental period lasted 42 days. Contamination with mycotoxins occurred from 7 to 29 days. Growth performance, blood, gut health and microbiome were evaluated. From d7 to d29, no differences were observed between the 3 groups for ADFI, ADG and FCR. From d29 to 42, ADG of T2 (0.551) was significantly different from T1 (0.474) and intermediate for T3 (0.532). From d0-d42, FCR were significantly different for groups T2 (1.568) and T3 (1.571) compared to T1 (1.678). The performance results indicate that the contamination induced with a mix of mycotoxins, was not enough to induce a challenge that would significantly compromise the performance of the animals but that the supplementation help to the performance recovery of the animal after contamination. Until d35, T2 and T3 groups behaved similarly, with greater vulvar volumes than T1 group. At d42, the T2 group presented a vulvar volume (3.136 cm³) greater than the negative control (2.464), but less than the positive control (3.615), indicating a beneficial action in the recovery of the animals after contamination. At d28, the weight of the reproductive tract was significantly lower for T2 (15.890 g) compared to T3 (17.420 g). No statistically significant effects were observed on liver and kidneys weights. In contaminated diets, no differences were observed for mycotoxins content in bile, whereas the addition of the anti-biotoxins solution significantly reduced serum DON concentration from 5.82 to 4.18 µg/l (p<0.05). The histomorphology results showed smaller crypt depth and goblet cell count for T2, and comfort that intestinal damage was less pronounced than the positive control. This finding was coherent with the results of pro-inflammatory cytokine IL-6, found in ileal mucosal scrapings, where the concentration in pg/ml was 88 times higher in T3 compared to T2. Analysis of microbiome for T2 and T3 has shown higher *Firmicute/Bacteroidota* ratio for T3. This last result deserves to be further explored.

P85

Mitigation of mycotoxin effects on broiler performance and intestinal health using a *Bacillus*-based anti-biotoxins solution

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Poultry industry in sub-Saharan Africa is a growing sector facing many challenges, including the feed contamination by mycotoxins. To mitigate the effects of mycotoxins on animals and limit their presence in the food chain, traditional control methods such as sorting, crushing, extrusion are no longer sufficient, due to the increasing risks: climate changes, emerging mycotoxins or their synergistic effects. Functional feed solutions with adsorption properties or with combining strategy such as degradation and bioprotection has been considered as effective measures to protect the animals. The objective of the study was to evaluate the impact of an innovative anti-biotoxins solution based on bacteria on the health and productivity of Arbor Acres broiler chickens in Côte d'Ivoire.

For this purpose, 900 day-old chickens were randomly divided into three groups (10 pens/group, 30 animals/pen) and fed with a diet naturally containing mycotoxins (CON, control group) or with a CON diet + a bacteria-based anti-biotoxins solution (SBB) or with a CON diet + a enzyme-based anti-mycotoxins solution (SBE, a reference in the market), for 42 days, subdivided into three periods (starter, grower, finisher). Feed analysis revealed the co-occurrence of mycotoxins, mainly aflatoxins, fumonisins (B1 and B2) and deoxynivalenol, at levels reaching 0.123, 2.35 and 0.03 mg/Kg of finished feed, respectively. Histological sections of ileum taken at 23 days of age indicated that SBB and SBE had significantly increased the number of goblet cells compared to CON (p < 0.05) unlike the lengths of villi

and depths of crypts which were not different ($p > 0.05$). At the end of the trial, SBB and SBE supplementation increased the yields of thighs and drumsticks, respectively, compared to the CON group ($p < 0.05$), and no significant effects were observed on weight, feed conversion, mortality, visceral organ lesions and relative weight and yields of carcasses and breasts ($p > 0.05$). These results show that supplementation with anti-biotoxins solution based on bacteria improves meat yields and can have a beneficial effect on the protection of the intestinal mucosa of broilers subjected to a natural challenge with multiple mycotoxins.

P86

A global strategy using ingredients with complementary mode of actions to limit mycotoxins adverse effects on animals

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Feed contamination by mycotoxins remains a big issue worldwide. With the new challenges facing the feed industry, it is crucial to integrate an integrated approach to address this key topic in a performant, effective and sustainable way. The impacts of mycotoxins on animal health and performance are diverse; from chronic syndromes, with reduced productivity, impaired gut function or increased predisposition to infectious diseases and, less frequently, acute toxicoses with severe illness and death. The degree of toxicity that these compounds exert on the animal's body is complex and depends primarily on the type of mycotoxin, their amounts, the duration of exposure, the presence of multi-contaminants, the overall health of the animal, sex, age, race, and many other factors. Due to hazardous, ubiquitous nature of mycotoxins contamination and their variable effects, different strategies are usually used to alleviate their impacts on animals. First, the most known strategy is to reduce the exposure to mycotoxins by decreasing their bioavailability. Several compounds can also be used such as adsorbing agents, mainly clays (bentonites, montmorillonites, zeolite...) or carbon-based organic polymers such as some complex indigestible carbohydrates (cellulose, polysaccharides from the cell walls of yeasts or bacteria). Their main mode of action is to bind the mycotoxins in contaminated feed and the elimination of the complex through faeces. Other mode of action can be the mycotoxins metabolization or degradation using specific microorganisms or enzymes. Secondly, bioprotection strategies are key levers to alleviate mycotoxins impacts on animal. Bioprotection can be defined as all physiological action mechanisms that can support animal global health from mycotoxins negative effects. It can be antioxidant, immunostimulatory agents or specific amino acids or other molecules that can allow liver, gastro-intestinal tract, kidneys, and other organs to counteract mycotoxins effects and keep their functionalities. It can also involve compounds that strengthen the intestinal barrier and tight junctions, thus limiting the transfer of endotoxins or (emerging) mycotoxins into the organism. Finally, the best strategy to counteract the adverse effects mycotoxins, but also other biotoxins on animals is to combine complementary mode of action from the association of ingredients specifically selected to mitigate these various effects. The other key to consider facing to mycotoxins in feed sector is all the management around this topic with the risk evaluation, the analysis or the upstream means of prevention.

P87

Biocontrol potential of VOCs-producing *Hanseniaspora uvarum* L793 and *Metschnikowia pulcherrima* L672 against *Aspergillus flavus* M114 isolated from fig (*Ficus Carica* L.)

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Aflatoxins are highly toxic secondary metabolites present in a wide variety of food commodities that pose significant health and safety hazards for humans and livestock due to their carcinogenic effects. In this context, a major concern in dried fig (*Ficus Carica* L.) production is the presence of these toxins, mainly produced under warm and humid conditions by *Aspergillus flavus*. In recent years, biocontrol agents (BCA), particularly antagonistic yeasts, have been evaluated as a promising strategy to control the development of toxigenic fungi in food as an alternative to synthetic fungicides.

In the present study, two fig yeast isolates (*Metschnikowia pulcherrima*, L672; *Hanseniaspora uvarum*, L793) were tested by *in vitro* co-culture against the pathogen *A. flavus* M114 on dried fig-based medium.

After 8 days of incubation, a reduction in mycelial weight of 94.7% and 94.1% was observed for *A. flavus* M114 when confronted with *M. pulcherrima* L672 and *H. uvarum* L793 yeasts, respectively. In addition, both yeasts were able to reduce mycotoxin synthesis. Specifically, *M. pulcherrima* L672 reduced the synthesis of AFB1 and AFB2 by 94.0% and 88.4%, respectively, while *H. uvarum* L793 inhibited the production of any aflatoxin. In order to investigate the antifungal properties of the isolated yeasts, the volatile profile was evaluated by gas chromatography coupled to mass spectrometry (GC-MS) combined with headspace solid-phase microextraction (HS-SPME). Both yeasts presented characteristic volatile organic compounds (VOCs), and their production was increased when confronted with *A. flavus* M114. A total of 66 VOCs were tentatively identified, including 10 carboxylic acids, 17 esters, 9 aldehydes, 18 alcohols, 7 ketones, and 5 miscellaneous compounds. The subsequent partial least squares regression-discriminant analysis (PLS-DA) showed significant differences in the VOCs profile produced by each BCA against *A. flavus* M114. Moreover, the variable importance in projection (VIP) scores was used to identify discriminant molecules potentially responsible for the antifungal activity. Common key VOCs included ethanol, 1-butanol-3-methyl, benzoic acid, 2-furan methanol, 1-propanol-2-methyl, phenyl ethyl alcohol, and acetoin. Although antifungal VOCs were identified in the co-culture, other possible mechanisms underlying the biocontrol potential of the studied yeasts are the competition for nutrients and space and the secretion of enzymes, which should be further evaluated. Overall, both VOCs-producing yeasts have shown promising results in controlling *A. flavus* M114 development and mycotoxin production in dried figs.

P88

Ambrosia (2024-2027): Bridging knowledge, communication, and action for food safety in a changing climate

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The Ambrosia project aim to transform the European approach to food safety by addressing the increasing challenges posed by climate change. The project adopts a holistic, systemic methodology to assess and mitigate food safety risks across the entire supply chain, from primary production to consumption. By leveraging advanced digital technologies, such as artificial intelligence (AI) and predictive analytics, AMBROSIA integrates climate change projections with food safety hazard models to address emerging and cumulative risks. A core focus of the project lies in understanding and mitigating the risks posed by *Fusarium* mycotoxins, a significant hazard in grains like wheat, oats, barley, and maize, and their derived products. Using predictive modelling, AMBROSIA will analyse spatio-temporal climate projections to anticipate how changing climate variables – such as heatwaves, extreme rainfall, or prolonged droughts – might influence the prevalence and spread of mycotoxins in different biogeographical regions across Europe (Atlantic, Boreal, Continental, and Mediterranean). Furthermore, AMBROSIA extends this predictive framework to fresh produce, examining risks associated with enteric pathogens like *Salmonella* and *E. coli*, which are also expected to evolve due to climatic shifts. By integrating data from diverse sources, including real-time climate models, microbiological data, and agrifood supply chain analytics, the project will develop robust predictive tools to forecast food safety risks at both regional and EU-wide scales. Acknowledgments. This research is funded by the European Union (GA 101181300).

P89

Two mycotoxin-mitigating products improve the reproductive performance of sows exposed to *Fusarium* mycotoxins

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Grain ingredients are widely used for sow diet but often contaminated by *Fusarium* mycotoxins, fumonisins (FUM), deoxynivalenol (DON) and zearalenone (ZEN) being the most common. The co-

occurrence of these dietary mycotoxins presents a huge threat to sow production and therefore requires effective mitigation products. This study aimed to evaluate the efficacy of two mycotoxin-mitigation products (Toxo[®]-XL and Toxo[®]-HP) in sow diets contaminated with a combination of FUM, DON and ZEA. A randomized block design was used with four treatments and 10 replicates per treatment totalling 40 sows. The treatments included a negative control (T1), a positive control with diets containing 250 ppb ZEA, 5ppm FUM, and 1ppm DON (T2), a treatment with the addition of 2 kg/t of Toxo-XL to T2 diet (T3), and a treatment with the addition of 2 kg/t of Toxo-HP to T2 diet (T4). The experimental period lasted 53 days and was divided into gestation (D0–D26 of the trial), pre-farrowing (D26–D31 of the trial), and lactation (D31–D53 of the trial). The results showed no significant differences in sow performance and body condition, including feed intake, weight gain, and backfat thickness among treatments ($p>0.10$). On the other hand, T2 showed a significantly smaller litter size (= the number of born alive piglet) than T1 ($p<0.10$). Compared to T2, T3 increased the litter size by 4.3% ($p>0.10$), while T4 significantly increased it by 25.0% ($p<0.10$). Regarding the litter weight (=number of born alive piglet * birth weight of each piglet), both T3 and T4 increased it numerically by 4.8% and 8.6%, respectively, compared to T2 ($p>0.10$). In conclusion, dietary contamination of *Fusarium* mycotoxins posed a significant challenge to the reproductive performance of sows, especially the litter size. Two products mitigated some of these negative effects wherein Toxo-HP showed a higher efficacy. The higher efficacy of Toxo-HP can be attributed to the inclusion of a specific enzyme to detoxify fumonisins. Findings highlight the necessity of dietary mycotoxin-mitigating products to optimize sow productivity.

P90

Development of a feed additive (zearalenone detoxifier) using yeast enzymes

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Zearalenone (ZEN) is a mycotoxin produced by fungi of the genus *Fusarium*, which poses a serious threat to human and animal health due to its estrogenic properties. ZEN contamination of feed is a global problem, especially in regions with warm and humid climates that favor fungal growth. High concentrations of ZEN in feed can lead to serious health problems in livestock, such as decreased fertility, hormonal disorders, and reduced production efficiency. Therefore, there is a need to develop effective methods for detoxifying ZEN in feed. In this study, the ability of selected yeasts from the class *Saccharomycetes* to biodegrade ZEN was evaluated. Several species of yeast were used, including *Saccharomyces cerevisiae*, which are commonly used in the food industries. They are also known for their ability to survive in various environmental conditions, which increases their potential in biotechnological applications, making them an attractive candidate for this process. In the experiment, prepared microorganisms were suspended in a buffer mixture with a specific pH value with the addition of a zearalenone solution. Incubation at approximately 37°C was carried out for 12 h. The concentration of the mycotoxin in the solutions was measured at time points 0, 1, 3, 6, and 12 h to determine the level of mycotoxin biodegradation and the kinetics of this reaction over time. The analysis of ZEN degradation was performed using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The results showed that two of the tested strains – *Metschnikowia pulcherrima* KKP 1368 and *Hanseniaspora uvarum* KKP 3969 – exhibited maximum zearalenone degradation with efficiencies of 59% (*M. pulcherrima*) and 42% (*H. uvarum*), suggesting their potential in biodegradation processes. The results of this study indicate the high efficiency of yeasts in biodegrading zearalenone, opening up new possibilities for using these microorganisms to reduce the impact of mycotoxin contamination. The practical application of yeasts can contribute to improving feed safety and livestock health, as well as protecting the environment from the harmful effects of mycotoxin contamination. Further research in this area may lead to the development of new, more effective methods for feed detoxification, which is crucial for agriculture and the food industry worldwide.

P91

A novel yeast cell wall based preparation with improved mitigation efficacy toward deoxynivalenol and fusaric acid

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The ubiquitous nature of deoxynivalenol (DON) has been reported in feed ingredient surveys worldwide. Significant co-occurrence of DON with other *Fusarium* toxins and fusaric acid (FA) was found (Weaver *et al.*, 2021. *Toxins* 13: 516). This latter has only rarely been reported but was found to interact negatively with DON on animal health (Smith *et al.*, 1997. *Journal of Animal Science* 75: 2184). To gain insight into the extended applicability of a novel yeast cell wall-based preparation (YCWP, Alltech, Inc.) at augmenting the detoxification characteristics toward DON and FA alongside 60 other mycotoxins, we conducted *in vitro* measurements of the detoxification potential using chemical and cell culture assays. Adsorption studies were conducted to assess the isothermal adsorption kinetics of YCWP. Differential quantification of the free toxin comparing YCWP-treated versus non-treated toxin mixtures was performed. Six concentrations (0.5 to 5.0 µg/ml) of DON and FA mycotoxin mixtures were prepared at pH 3.0 or pH 6.0. Each concentration was treated with a slurry of YCWP in a Pierce Spin Column cassette incubated for 60 min at 37°C under agitation. Cassettes were then centrifuged, and the supernatant was transferred to silanized chromatography vials. Mycotoxin quantification utilized a matrix-matched calibration curve on a UPLC-high-resolution hybrid mass spectrometer (Vion™, Waters Corp.) with electrospray ionization in positive mode. *In vitro* evaluations extended to iso-mixtures of over 60 different mycotoxins, ranging from 0.5 to 5 µg/ml. These included aflatoxins, trichothecenes (groups A and B), other *Fusarium* toxins such as macrocyclic trichothecenes, fumonisins, zearalenone, ochratoxins, *Aspergillus* and *Penicillium* mycotoxins, *Pithomyces* toxins like sporidesmin, and ergot alkaloids from *Claviceps*. Additionally, the effects of DON and FA were investigated on porcine intestinal epithelial cells (IPECJ2) to assess YCWP's remediation potential. Transepithelial resistance and permeability were also evaluated. For both pH conditions tested, the adsorption of DON and FA followed a dose-dependent pattern, achieving rates of 66.8% and 96.5%, respectively, while maintaining 97.0% aflatoxin B1 adsorption rate. T-2 toxins and fumonisins showed maximal adsorption rates of 63.9% and 57.4%, respectively. Of particular note, positive interactions were observed for the first time with sporidesmin (52.6%) and phomopsis A (73.5%) among other toxins. The adsorption rate for *Penicillium* and emerging toxins averaged 50%. In cell culture, we observed a dose-dependent logarithmic decreased response of IPECJ2 viability. The DON insult predominantly contributed to viability loss, but at concentrations below 1 ppm, FA further increased this effect by 40%. The use of YCWP at 0.2% (w/v) partially restored cell viability in DON-treated cells alone and a combination of [DON+FA] by an average of 37.5% and 52.0% ($p < 0.01$), respectively.

This study further demonstrated the favourable properties of YCWP toward the adsorption of DON and FA as well as mycotoxins from all family groups.

SAMPLING AND ANALYSIS

P92

Linking *Fusarium* damaged kernels and deoxynivalenol contamination in wheat: A synchrotron-based X-ray imaging approach

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Fusarium head blight (FHB), primarily caused by *Fusarium graminearum* (Fg), is a major fungal disease affecting wheat worldwide. FHB disrupts kernel development, leading to morphological alterations and an increased risk of mycotoxin contamination, particularly deoxynivalenol (DON). Infected kernels often appear shrivelled, lightweight, and chalky white, sometimes covered with white or pink mycelia. Accurately identifying damaged kernels is essential, as they are often associated with DON contamination. Traditional methods like visual assessment are the most commonly used, however, they are subjective, time-consuming, and some studies have reported high DON levels in visually asymptomatic kernels. Therefore, new advanced detection techniques would be of benefit to the food and feed industries. In this study, we employed synchrotron-based X-ray phase-contrast computed

tomography (CT) technology to assess morphological parameters on Fg inoculated wheat kernels and correlate it with DON. Thirty-four spikes from five bread and four durum wheat varieties were scanned using the X-ray CT machine at the Canadian Light Source (CLS). After image reconstruction, the individual kernels from each spike image were segmented and converted into a 3D image. In total, 725 segmented kernels were rendered in 3D to assess kernel volume, area, mean attenuation, length, width, thickness, shapeVA3D, and grey mass. Meanwhile, the infection status of each individual kernel was determined by quantitative real-time PCR (qPCR) and HPLC-MS/MS, respectively. Wheat kernels were then classified into three groups: group 1 ('no Fg infection') – no DON and Ct>28.5; group 2 ('Fg infection without DON production') – no DON and Ct≤28.5; and group 3 ('Fg infection') – DON present and Ct≤28.5. A generalized linear model (GLM) was fitted to determine if there were significant differences in the image features among the groups. Estimated marginal means were used to make pairwise comparisons. Among the eight 3D image parameters analysed, only for length was there no statistical difference among groups (p=0.262). Volume, area, mean attenuation, width, thickness, and grey mass were greater in group 1 and 2 compared to group 3. As expected, kernels from group 1 and group 2 had a lower shapeVA3D than those from group 3. There was no difference between group 1 and 2 in any of the image parameters analysed. Our results suggest that the presence of DON changes the morphology of wheat kernels. Therefore, CT imaging provides a powerful and advanced approach for accurately measuring key morphological indicators of *Fusarium* infection.

P93

Analysis of multi-mycotoxins including emerging mycotoxins in cereals, finished feeds and food matrices by liquid chromatography-tandem quadrupole mass spectrometry with lower limits of detection

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This method development and study objective was to quantitatively detect multiple mycotoxins in cereals, finished feed and food products at lower limits of detection. As mycotoxin regulations have been lowered in some countries around the world and emerging mycotoxins detected, a method that could extract and detect these at lower levels was developed. Many studies have been performed in more recent years that are showing that mycotoxins are occurring in areas of the world that historically have not had contamination such as aflatoxin in wheat in Europe. Mycotoxins are also occurring due to storage conditions from shipping containers that are held up for months. Advances in technology have also proven that it is common to have multiple mycotoxins occur in samples. The matrices chosen were from basic cereals to finished animal feeds including some of the novel foods on the market and alternative protein sources. The extraction used was 80/20 acetonitrile/water and a tandem SPE purification was applied using QP1000 and QP1100 (QualiT Pure SPE columns from Trilogy Analytical Laboratory, Washington, MO USA). Samples were analysed using various matrix calibrations and sample fortifications for method recovery.

P94

Use of flexible scope accreditation under ISO/IEC 17025 for the analysis of mycotoxins in new food matrices

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Imports in the European Union have increased in the last years, including many countries of origin and different types of foodstuffs available for population. Nowadays European population enjoy a great variety of nourishing goods in their shopping basket. These facts obligate public health laboratories, as a part of their task, to develop strategies to control the harmless of foodstuff. Laboratories must give results with the maximum confidence, due to the legal consequences for consumers as well as for food producers. In order to assure food safety, the flexible scope of accreditation has become a basic tool, as well as the latest instrumental novelties. The flexible scope accreditation, as outlined in ENAC's (Spanish national accreditation body) Technical Note NT-18 and in accordance with ISO/IEC 17025, provides laboratories with the ability to adapt their testing capabilities to emerging needs without undergoing a full re-accreditation process. This approach is particularly beneficial in the dynamic field of food safety, where the detection and quantification of mycotoxins are critical. Mycotoxins, such as aflatoxins, ochratoxin A, zearalenone, fumonisins, etc, pose significant health risks to humans and

animals, necessitating rigorous monitoring and analysis. Under the flexible scope framework, laboratories accredited by ENAC can extend their testing methods to include new mycotoxins or matrices as long as they demonstrate competence through internal validation and proficiency testing. This flexibility ensures that laboratories can promptly respond to new regulatory requirements or contamination incidents, without waiting for next audit to achieve the accreditation for those new matrices or mycotoxins that were not yet included in the accredited scope. This work presents flow charts, checks to be carried out and acceptance criteria to be used in the face of requests for official control analysis of new analytes or matrices, not previously studied, and which require accredited results under ISO17025 in a very short time.

P95

Improving internal laboratory processes and sustainability using rapid online automation for aflatoxin analysis

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Automation is becoming an increasingly important tool for laboratories to manage growing sample numbers and to maintain the highest quality results. Increased sample analysis for mycotoxins has created a bigger demand on staff resources, consumable and solvent costs. Following a review of various automation options the Chronos Symbiosis Rida@Crest was selected for mycotoxin analysis due to consistency of results with a wide variety of matrices. The re-usability of the Immunoprep® Online immunoaffinity cartridge required less solvent, reduced storage and transport costs helping achieve sustainability. As part of the system and method validation, several FAPAS samples were analysed achieving good z-scores. In addition, a variety of spiked matrices were tested, and the methods measured for specificity, precision, trueness, linearity, and limit of quantitation. In this study LOQ of <10 % of the maximum regulatory limits were achieved in foodstuff (except for infant formula) using the automated Immunoprep® Online Aflatoxin method greatly exceeding the new EU 2023/2782 requirements. At spiking levels of 0.2 ppb, recoveries ranged from 87%-118% with an RSD of between 0.99%-7.75%. At spiking levels of 5 ppb, recoveries ranged from 85%-105% with an RSD of between 0.91%-3.32%. Therefore, complying with the new regulations. The Chronos Symbiosis Rida@Crest was easy to use, providing faster results, reducing manual handling whilst improving quality and consistency of results.

P96

Leveraging the full potential of LC-MS/MS to study the impact of climate change on mycotoxin occurrence

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Climate change challenges our food system. Due to global warming, not only crop yields shift, but the occurrence of contaminants such as mycotoxins also changes. Currently, the influence of climate change on mycotoxin occurrence is studied, focusing on a few regulated mycotoxins on a large, often global scale (Casu *et al.*, 2024. Comprehensive Reviews in Food Science and Food Safety 23: e13323). In the last two decades, liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) has developed into the gold standard for mycotoxin analysis, as it enables selective and sensitive multi-analyte screening. The simultaneous determination of several hundred secondary fungal metabolites is feasible using this technique Sulyok *et al.*, 2024. npj | Science of Food 8: 49). We have shown that not only regulated mycotoxins but also other secondary fungal metabolites are influenced by regional and environmental weather differences already on a regional scale Freitag *et al.*, 2024. Journal of the Science of Food and Agriculture 104: 77880). We screened wheat samples from selected sites in Austria using LC-MS/MS, and the obtained occurrence data were analysed using chemometric tools such as principal component analysis (PCA) and analysis of variance simultaneous component analysis (ASCA) to identify regional and yearly trends within the dataset. Our findings show that not only regulated mycotoxins but also usually neglected secondary fungal metabolites were significantly influenced by

weather conditions. This underlines that maximizing the information obtained by LC-MS/MS analysis for a wide range of secondary fungal metabolites is beneficial to understand the environmental impact on fungal pathogens of crops. Therefore, increasing the number of analytes included in routine LC-MS/MS-based surveys holds the potential to improve the resilience of our food system in a changing climate.

P97

LC-MS matrix effect and correction using ^{13}C stable isotope internal standards

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Matrix effects present a significant challenge in liquid chromatography-mass spectrometry (LC-MS) analysis, particularly when analysing complex matrices such as animal feed. These effects, caused by co-eluting compounds that suppress or enhance analyte signals, can lead to significant inaccuracies in quantification. This study evaluated the use of ^{13}C stable isotope-labelled (SIL) internal standards to correct the matrix effects during the analysis of mycotoxins in animal feed. A variety of animal feed samples, including maize, wheat, pet food, and compound feed were extracted and analysed by LC-MS. Analyte concentrations were quantified both with and without SIL correction and compared to certified reference values. The results showed a significant improvement in analyte recovery for deoxynivalenol, zearalenone, and aflatoxin B₁, when using SIL internal standards ($p < 0.001$). Notably, zearalenone recovery was underestimated by over 80% without correction. Additionally, SIL correction significantly reduced data variance for fumonisin B₂, T-2 and HT-2 toxin recoveries ($p < 0.05$), showing improved analytical precision. The findings show the power of SIL internal standards in overcoming matrix effects, ensuring both accuracy and reproducibility in LC-MS analyses of complex feed matrices. Future work will focus on expanding sample types and quantities to better understand the variability in matrix effects across different sample types.

P98

Advanced tools for rapid and on-site detection of ochratoxin in the food chain from farm to fork (the OTASens project)

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Climate change, driven by global warming, has a significant impact on agriculture and food security by affecting crop production and supply. The increasing occurrence of fungi and their metabolites in unexpected geographical areas has direct and important repercussions on human health. Humans are exposed to mycotoxins because of the consumption of agri-foods and food derived from animals that were fed with contaminated feed. In this perspective, research and innovation in food safety, particularly in mycotoxin risk assessment, are essential for creating food systems that are more sustainable, resilient, inclusive, and healthy. The OTASens project has demonstrated and validated the use of a DNA-based biosensor (OTA biosensor) as a rapid testing system for monitoring the mycotoxin Ochratoxin A (OTA) in the pork meat-producing chain with a farm-to-fork approach. This innovative detection system was implemented as a useful tool to promote the safety of the swine production chain, via ease of use, affordability, and shorter time-to-results compared to the traditional LC-MS/FLD methods used to assess mycotoxin contamination and the compliance with current EU limits/recommendations for feeds and foods. In OTASens we extended the knowledge acquired on the use of the OTA biosensor initially developed and successfully in-house validated for testing OTA in urine. To further improve the portability of the OTA biosensor, an eco-friendly protocol for OTA analysis in feed and feed ingredients was developed and optimized using natural and common deep eutectic solvents (DES) which extracted up to 90% of OTA from artificially contaminated feed. The LC analyses revealed that these extraction methods using DES, effectively detect and quantify low OTA levels in complete pig feeds since they showed no endogenous interferences, good calibration linearity ($R^2 \geq 0.99$), high sensitivity as demonstrated by low LOD (0.2 ng/ml) and LOQ (0.7 ng/ml) values, acceptable recoveries (80–102%) and precision (RSDr: 2-7%). This eco-friendly OTA extraction protocol, combined with the OTA biosensor, will be practically applied for the detection and quantification of OTA in feed samples, their components, and blood samples collected from pigs on farms and feed mills in Southern Italy. The data obtained will contribute to a more accurate risk assessment of OTA exposure in livestock, supporting the development of targeted mitigation strategies to ensure feed and

animal health safety. Acknowledgements. The OTASens project (PRIN2022 PNRR, P20224NLZZ) is funded by the European Union – Next Generation EU, Piano Nazionale di Ripresa e Resilienza (PNRR), Missione 4 'Istruzione e Ricerca' – Componente C2, Investimento 1.1, Fondo per il Programma Nazionale di Ricerca e Progetti di Rilevante Interesse Nazionale (PRIN), CUP B53D23031940001.

P99

Development of an ultra-fast, green and sustainable LC-MS/MS method for the analysis of multiple mycotoxins in feed and food

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Due to climate change and resulting environmental stresses, mycotoxigenic fungi and their secondary metabolites (mycotoxins) have become more prevalent and are an ongoing global concern. Mycotoxin contamination of food and animal feedstuffs is considered unavoidable and unpredictable, even with good agricultural, storage, and processing practices, posing challenges to food safety. Apart from the threat to human and animal health, exposure to low doses of mycotoxins below EU guidance limits, as well as the co-occurrence of emerging mycotoxins, impacts an animal's feed conversion efficiency. This leads to increases in production costs and environmental emissions, having a negative effect on the sustainability of global food and feed systems. Furthermore, Green Analytical Chemistry (GAC) has emerged as an important metric regarding sustainability. Initially centred around the (industrial) chemical industry, this concept has migrated into analytical laboratories, with the aim of reducing the negative impact of chemical analyses on the environment and to enable implementation of sustainable development principles to analytical laboratories. As the control of mycotoxins is important in the food and feed industries, samples are routinely tested for mycotoxins which have regulatory/guideline values as set by EFSA and the EU. Additionally, the co-occurrence of emerging mycotoxins has a negative effect on animal performance and sustainability. Therefore, it would be beneficial that samples are analysed not only for regulated mycotoxins, but additionally, those emerging mycotoxins that appear to contribute to the negative effects, including the enniatins (ENNs), diacetoxyscirpenol (DAS) and beauvericin (BEV) to name a few. However, although the costs associated with LC-MS/MS has reduced over time, they can still be expensive, especially to smaller companies. The aim of this research was to develop a rapid screening (LC-MS/MS) method to detect and semi-quantitate regulated, masked and emerging mycotoxins in under 5 minutes. After which, any samples breaching specific cut-off values would be analysed using a fully validated quantitative method with a run time of 14 min. To facilitate this, an LC-MS/MS multiclass method was developed with 30 mycotoxins, with analysis time markedly reduced from 14 to 4 min. This innovative workflow enables a reduction of samples requiring 'full' analysis, reduction in costs, is more environmentally friendly and increases the methods greenness. Furthermore, real-world feed samples analysed by both methodologies indicates that the ultra-fast method may be fully quantitative. This study, conducted in collaboration with Agilent and their Intelligent Reflex function could provide a fast, robust, and reliable screening method that could be used across the feed and food industry.

P100

Using aflatoxin spiked maize kernels for testing sample preparation

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An automated mycotoxin testing solution (MycoSens™) is extracting mycotoxins from 5 g of a ground maize sample. To ensure this sub-sample is representative of the entire sample, it is required to grind the entire sample fine so that >95% of the particles pass through a 20-mesh sieve and mix it properly. This study examines if a 5 g sub-sample is sufficient for obtaining consistent results using artificial worst-case maize samples. Single kernels of maize have been reported to contain up to 200 ppm aflatoxin (Shotwell et al., 1974. Cereal Chemistry 51: 492). This means a highly contaminated kernel could potentially increase the average aflatoxin concentration of a 1kg maize sample to >20ppb. To simulate a worst-case sample, 1 aflatoxin spiked maize kernel was mixed with 550 g of sound maize kernels and ground. The simulated worst-case samples were ground so that >60% and >95% of the particles passed through a 20-mesh sieve, respectively. From each, 5 g portions were measured both with and without

mixing between sampling sub-portions. Only a minor effect was observed between the particle sizes, but a clear effect of the mixing between sampling sub-portions was seen. When measuring 5 g portions of a sample, where >95% of the particles pass through a 20-mesh sieve and where the sample was properly mixed between sampling sub-portions, an RSD of 19% was seen. Based on this, we conclude that fine grinding and mixing of maize samples accounts for sample inhomogeneity even in the worst-case scenarios and measuring 5 g samples is sufficient to obtain an acceptable result.

P101

Multi-mycotoxin analyses by UPLC-MS/MS in wheat: Situation in Wallonia in 2023 and 2024

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The present research is part of a Walloon project 'ValCerWal' (funded by 'Plan de relance de la Wallonie') conducted by the Walloon Agricultural Research Centre (CRA-W) in Belgium. This project aims to increase the valorisation of cereals such as wheat, spelt, etc. for local production to reach industrial and craft quality requirement. One of its specific goals is to do an overview of the mycotoxins observed on cereals in Wallonia and link the observation with the climatic conditions. Mycotoxins pose a significant threat to food safety and human health. This study focuses on the analysis of wheat aiming to detect and quantify 20 different mycotoxins, including both emerging and conventional ones. This presentation proposes a comparison of the data from two years (2023 and 2024) and advances hypotheses to explain the observed differences. Potential contributing factors include variations in weather conditions and agricultural practices. In summer 2023, a total of 114 samples of wheat flour coming from 13 different locations from conventional and organic farming were analysed by UPLC-MS/MS. For 2024 harvest, the same number of samples should be collected and analysed. In 2023, the analyses revealed an absence of mycotoxin concerns in the studied samples. Indeed, over the 114 samples studied only 24 samples contain mycotoxins at levels below the maximum allowable limits fixed by the European Commission. Mycotoxins detected were deoxynivalenol, zearalenone, HT-2 toxin, T-2 toxin, alternariol-methyl-ether, enniatins B and B1, and sterigmatocystin. The data for 2024 are not yet acquired as the harvest will happen in July and August. However, preliminary indications point to an increased risk of mycotoxin contamination, attributed to unfavourable climatic and environmental conditions due to high levels of precipitation during the wheat flowering stage. In conclusion, this research provides a comprehensive analysis of mycotoxin contamination in wheat samples from various locations in Wallonia (Belgium). The findings from 2023 indicated minimal mycotoxin presence, with detected levels below the maximum allowed limits. This data highlights the importance of continuous monitoring and analysis, this enables the anticipation of risks, the warning of the sector, and the limitation of contaminated batches entering the processing chains.

P102

Development of analytical method for multi-mycotoxins using rapid sample preparation technique for food safety

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There are regulations for 11 mycotoxins: aflatoxins (B1, B2, G1, G2, M1), fumonisins (B1, B2), ochratoxin A, zearalenone, deoxynivalenol, patulin in Korea. In this study, we developed the simultaneous analytical method for determination of 11 mycotoxins using high-performance liquid chromatography (HPLC) coupled with tandem mass spectrometry (MS/MS). The developed method could simplify process to determine 11 mycotoxins by application of the rapid sample preparation technique (QuEChERS) and addition of internal standard (¹³C labelled). The internal standard addition method demonstrated satisfactory accuracy and precision within the range of Codex guidelines for validation in the selected representative materials: rice, instant noodles, and milk.

P103

Targeted analysis of 1000 fungal metabolites using UPLC-Orbitrap-HRMS: Method transfer and optimization challenges

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High resolution mass spectrometry (HRMS) is increasingly being used for targeted analysis and quantification; a domain previously dominated by triple-quadrupole MS/MS instruments. The transition to Orbitrap HRMS presents both opportunities and challenges. While HRMS offers advantages such as retrospective data mining, its lack of established validation guidelines poses a substantial drawback. Our current routine method employs a triple-quadrupole LC-MS/MS system with single-polarity operation (20 min total runtime) in positive and negative mode. It utilizes two product ions per precursor ion, yielding 12-15 data points per peak for quantitative analysis. Current validation guidelines for conventional targeted LC-MS/MS measurements specify that identification requires two product ions (fragment ions) derived from the same precursor ion, with 12-15 data points per peak generally sufficient for quantitative methods. For HRMS guidance criteria, a fragment ion is mandatory. The most intense adducts are accepted as the molecular/adduct ion, and 6-9 data points per peak are commonly considered sufficient. Despite the growing adoption of HRMS, the absence of established validation guidelines introduces uncertainty. To evaluate HRMS for routine targeted analysis, full-scan mode was initially tested on a Thermo Scientific IQ-X Orbitrap instrument using fast polarity switching and chromatographic conditions comparable to our existing LC-MS/MS method. Over 500 analytes were simultaneously measured. Based on initial results, we also tested ddSIM and tSIM scan modes. An inclusion list was created, and the number of data points per peak was closely monitored. Despite these optimizations, the HRMS approach still yielded insufficient data points per peak for accurate analysis. In highly complex regions of the chromatograms, data point acquisition remained low-ranging from just 3 to 6 data points per peak when full-scan mode was combined with these scan types. Recognizing the value of full-scan mode for retrospective data mining and untargeted analysis, we decided to retain it. To improve data point acquisition, we explored switching to single-polarity measurements, as in the LC-MS/MS method, and combining full-scan mode with tSIM scans. While this approach appears promising, it has yet to be fully tested. This work highlights the complexities of method transfer to HRMS for targeted analysis, particularly concerning data point requirements and the role of adduct ions in validation.

P104

High-throughput, fully automated sample preparation for contaminant analysis

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Comprehensive manual sample preparation is a challenge when analysing contaminants in food and feed. Contract laboratories require trained personnel to perform the time-consuming steps of sample extraction, purification, evaporation and dilution. Therefore, labour costs contribute the most to the cost of testing, and working hours limit the time of sample preparation. In recent years, more and more automation solutions have been developed. Initially, semi-automated systems focussed on the handling of liquids or the integration of preparation steps into measuring systems. However, the trend is moving towards fully automated robotic systems. One of the biggest advantages of these systems is a sample processing of 24 h a day, 7 days a week, even without personnel. A new robotic system has been set up at Eurofins WEJ Contaminants GmbH in Hamburg for analysing contaminants in food and animal feed samples. The system consists of multiple different sample clean-up modules, three 6-axis ABB robots and is capable to process up to 400 samples per day. It was developed based on an earlier, simpler but also fully automated model that has been successfully used in the analysis of mycotoxins and veterinary drugs. From spiking with standards, multiple extractions, salt dosing, centrifugation, phase separation, evaporation and filtration to the preparation of dilutions and the filling of the final solution into a vial and its labelling, every step is fully automated. The only remaining manual steps are placing the aliquots of the samples on the input conveyor belt and removing the prepared vials for LC-MS/MS measurement. Comprehensive software makes it possible to run several different methods in

parallel. The system covers various methods for mycotoxin (e.g., *Alternaria* toxins, aflatoxin, ochratoxin, patulin, fumonisins), process contaminants and adulterants, and veterinary drug residue analyses. Matrix-dependent individual sample preparation and thus new approaches for the development of robust and cost-efficient methods are also possible. Acknowledgements. We would like to thank the manufacturer of the system, Elbatron GmbH, for the joint development, the trusting, intensive collaboration, the many years of cooperation and the realisation of our wishes in this system.

P105

Developing of a MS-eNose tool for the early detection of ochratoxigenic *Aspergillus westerdijkiae* on traditional Italian caciocavallo during ripening process

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A rapid mass spectrometry-based electronic nose (MS-eNose) method, in combination with chemometrics, was exploited aiming to early detection of *Aspergillus westerdijkiae*, an ochratoxin A (OTA) producing fungus, on caciocavallo cheeses during ripening process. Caciocavallo samples were inoculated with ochratoxin A (OTA) non-producing species and with *A. westerdijkiae* and analysed with MS-eNose. To discriminate cheese samples based on their contamination with toxigenic or non-toxigenic fungal species two supervised models were applied, i.e., PLS-DA and PC-LDA. Accuracy values of 87-100% and 86-100%, in calibration and validation were obtained, respectively, with best results obtained at 15-ripening days. Finally, potential volatile markers of the presence of *A. westerdijkiae* were identified by using GC-MS analysis.

P106

Increase performance whilst reducing operating costs: Combining immunoaffinity clean-up with automation

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The automation of immunoaffinity clean-up processes has revolutionised sample preparation in quantitative mycotoxin analysis. Traditionally performed manually, immunoaffinity column clean-up is a critical step for isolating and concentrating analytes from complex food matrices. The Gilson ASPEC® platform, incorporating Trilution® LH software and positive pressure technology, provides a fully automated solution that enhances reproducibility, efficiency, and throughput. This poster highlights the capabilities of the Gilson ASPEC® GX-271 and GX-274 systems and benefits offered to analysts when used with immunoaffinity clean-up. The systems support precise liquid handling and unattended sample processing and are optimised for seamless integration with LC systems, allowing for high-throughput workflows and reduced hands-on time. Key features include mobile rack technology for automated column positioning, customisable elution and flow rate control, and advanced software enabling rapid protocol development. By leveraging these innovations, laboratories can achieve superior recoveries and %RSD, significantly improving quality of results. Collaboration between R-Biopharm Rhône and Gilson has further advanced the automation of immunoaffinity column methods, with the development of optimised application notes and customisable automation kits. These advancements cater to the evolving needs of scientists seeking scalable, reliable, and cost-effective solutions for mycotoxin analysis. This poster provides insights into the core technologies, and transformative potential of automated immunoaffinity clean-up, showcasing its value in streamlining workflows and meeting rigorous analytical standards.

P107

Validation of single extraction method for the simultaneous analysis of mycotoxins using immunoaffinity columns

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This validation evaluated the 11+Myco MS Prep® immunoaffinity column with LC-MS/MS for the simultaneous determination and confirmation of aflatoxins B1, B2, G1, G2, and M1; deoxynivalenol, fumonisins B1, B2, and B3; ochratoxin A; T-2; HT-2; and zearalenone from a single sample. Matrices tested included maize, wheat, cereal-based baby food, paprika, chili powder and animal feed and an independent laboratory verified the method performance on maize and animal feed. 11+Myco MS PREP® uses a single extraction method that requires no pH adjustment and can be applied to a range of matrices providing excellent clean-up. Solvent based standards were used for calibration removing the need for expensive isotopic standards and offering a more acceptable and cost-effective solution. The ability of 11+Myco MS PREP® to analyse 11+ mycotoxins simultaneously is highly advantageous as it saves significant time, reagents and consumables without compromising on results. Once extracted the sample was analysed using LC-MS/MS with electrospray ionization mode scheduled multiple reaction monitoring in positive polarity. Data was analysed for recovery, repeatability precision, LOD, LOQ, and method selectivity. When testing for acceptable bias by comparing a matrix calibration curve using the above method to a standard calibration curve, no matrix effects were found for any of the commodity types tested removing the need for matrix matched standards or isotopic labelled standards. Selectivity results demonstrated the ability of the 11+Myco MS-Prep® method to react to all variants of the analytes and to exclude similar compounds or other mycotoxins that could be encountered in the claimed matrixes. In addition, no significant positive or negative interferences were observed from the challenges. In conclusion, validation of the 11+Myco MS-Prep® method for determination of aflatoxins, fumonisins, deoxynivalenol, ochratoxin A, zearalenone, HT-2, and T-2 toxins in cereals, baby food, spices, and animal feed by immunoaffinity column with LC-MS/MS has been certified as an AOAC Performance Tested MethodSM 112401.

P108

New lower EU legislative levels for *Fusarium* toxins: Improving limits of detection with baby food using a multi-toxin immunoaffinity column for sample clean-up

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The legislative requirements for mycotoxins in food products, particularly baby food, necessitate highly sensitive and efficient analytical methods to detect and quantify contaminants like deoxynivalenol (DON), zearalenone (ZON), T-2, and HT-2 toxins. DZT MS-PrepP® was developed as an advanced immunoaffinity-based sample preparation method enabling simultaneous determination of these four *Fusarium* mycotoxins in baby food matrices using LC-MS/MS. The method features streamlined extraction, selective toxin isolation, and improved chromatographic performance, facilitating cleaner eluates and lower detection limits. Validation studies demonstrated robust performance with mean recoveries between 84-106 % and relative standard deviations (RSDs) below 5%, meeting European Commission criteria. The use of a single immunoaffinity column reduced preparation time to under 20 min per sample, significantly improving throughput while minimizing reagent and consumable use. Moreover, the high specificity of monoclonal antibodies in the DZT MS-Prep® columns simplified clean-up and reduced the need for matrix-matched standards and LC-MS/MS maintenance. This innovative approach ensures efficient mycotoxin detection, fulfilling stringent regulatory limits for baby food while offering economic and operational advantages for routine laboratory analysis.

P109

Quantum Ochratoxin Green is a rapid and novel lateral flow device for quantification of ochratoxin in grains, cereals and nuts: Identification of toxin-free samples in just 2 min

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Ochratoxins are a group of mycotoxins produced by certain fungi, particularly species of *Aspergillus* and *Penicillium*. Ochratoxin A (OTA) is the most prevalent and relevant fungal toxin of this group, while ochratoxins B and C are of lesser importance. OTA is a potent nephrotoxin and causes both acute and chronic effects in the kidneys of all mammalian species tested. Most controlling government agencies worldwide have regulations regarding the amount of ochratoxin allowable in human and animal foodstuffs. Accurate and rapid determination of ochratoxin presence in commodities is of paramount importance. Quantum Ochratoxin Green cassettes are based on the competitive format immunoassay principle. A capture line of ochratoxin is placed below the control line. The detection system consists of specific antibodies against the toxin ochratoxin, conjugated to colloidal gold. Within the scope of this study, ochratoxin-free samples were chosen and spiked with a known amount of OTA standard solution according to the United States Department of Agriculture, Agricultural Marketing Service, GIPSA's Federal Grain Inspection Service (FGIS) protocol. The samples and reference materials were extracted, analysed and their recoveries were evaluated. The determination of the ochratoxin levels in the spiked samples of the matrices of interest, showed that the recovery levels were acceptable. The results were also confirmed by analysing the reference material. The coefficient of variation (CV) of all samples was also within the acceptable range. Prognosis Biotech S.A. demonstrates a new innovative, simple, rapid, highly sensitive lateral flow method, Quantum Ochratoxin Green G60, for accurate detection and quantification of OTA in grain, cereal and nut samples. Due to its fast scan technology, samples free of ochratoxin can be detected in 2 min. The total time of the reaction takes 4 min. This technology gives acceptable recovery and CV levels, high sensitivity and accuracy.

P110

development of a high-sensitivity ELISA method for aflatoxin b1 detection in human blood serum

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Aflatoxin B1 (AFB1) is a potent mycotoxin produced by *Aspergillus flavus* and *A. parasiticus*, commonly contaminating foodstuffs such as grains, nuts, and spices. It is classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC) due to its strong association with hepatocellular carcinoma (HCC). Chronic exposure to AFB1 through dietary intake poses significant health risks, necessitating sensitive and reliable biomonitoring tools to detect and quantify exposure levels. The study aimed to develop a competitive enzyme-linked immunosorbent assay (ELISA) method for the detection of AFB1 in human serum, with a focus on achieving high sensitivity, specificity, and repeatability while maintaining practicality for routine use. A competitive ELISA was optimized for AFB1 detection in human serum. The assay features a straightforward sample preparation process requiring only a 5-fold dilution of serum to minimize matrix effects. Key parameters, including incubation times and antibody concentrations, were optimized to enhance assay performance. The method's sensitivity, specificity, and repeatability were evaluated using standard curves, intra-assay coefficients of variation (CVs), and cross-reactivity studies with other aflatoxins. The ELISA method achieved a limit of detection (LOD) of 0.007 ppb and a limit of quantification (LOQ) of 0.02 ppb, demonstrating exceptional sensitivity. The standard curve showed a reliable, consistent decrease in B/B0 (%) as AFB1 concentration increased, achieving an IC50 of 0.141 ppb. This highlights the assay's precision and sensitivity within the quantifiable range of 0-2 ppb. Specificity tests revealed minimal cross-reactivity with AFB2 (45.2%), AFG1 (38.8%), and AFG2 (3.4%). Intra-assay CVs were consistently below 6%, ensuring robust repeatability. The assay requires 90 min after sample preparation, making it efficient for high-throughput applications. This ELISA method provides a robust and practical solution for monitoring AFB1 exposure in human populations. The simple sample preparation, high sensitivity, and specificity make it ideal for early detection, risk assessment, and supporting preventive strategies against aflatoxin-related diseases. Future work will focus on expanding validation to diverse sample sets, field testing under real-world conditions, and automating the process to enhance throughput and consistency.

P111

A quantitative analysis of aflatoxin B1, B2, G1, G2 and ochratoxin A in food samples via 2D-LC-MS/MS

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Common analytical methods for analysing aflatoxin B1, B2, G1 and G2 and ochratoxin A in food or feed are based on a costly and time-consuming sample clean-up. To reduce interfering signals or matrix effects including a sample clean-up with solid phase extraction (SPE) or immunoaffinity columns (IAC) prior measurement is necessary. The subsequent measurement is proceeded with high performance liquid chromatography (HPLC) coupled with a fluorescence detector (FLD) or a mass spectrometer (MS). One opportunity to reduce the effort for sample preparation and saving time and costs while retaining comparable quality in result, is two-dimensional liquid chromatography (2D-LC) coupled with a MS/MS. A 2D-LC-System enables the separation of one sample with two chromatographic systems under different/orthogonal chromatographic conditions – two dimensions. After the analyte eluted from the first dimension, an aliquot containing the analyte will be stored in a capillary, called loop. After a certain time, the liquid inside the loop is transferred to the second dimension, separated again, and analysed by MS/MS. The whole procedure can be repeated for plenty analytes in numerous loops. As part of the here presented method Agilent's 1290 Infinity II 2D-LC system with Masshunter 12 software and a 6495 MS/MS is used. The multiple heartcutting of Masshunter 12 enables an intuitive "cutting" of the first dimension to transfer the 'peak' into one of the loops. Different food samples of the groups grains, cereals and dried fruits are extracted with a simple QuEChERS oriented approach. Aim of the method is to pass a validation that fulfils the standards of an accredited high throughput laboratory following the EU commission regulation (EU) 2023/915 and implementing regulation (EU) 2023/2782. Another benefit in reduction of the complexity in sample preparation is the ability to implement the method on an automatic robotic system. System-based additional flexible capacities, 24/7 availability, and a high quality with excellent precision and reproducibility increase lab productivity.

P112

Quantitation of mycotoxins and tropane alkaloids in food using triple quadrupole and QTRAP™ technology

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Mycotoxins are compounds that are naturally produced by fungi, many of which grow on foodstuffs such as cereals, dried fruits, nuts and spices. Several of these mycotoxins have gained attention due to their toxicity towards human health, with reported adverse health effects including gastrointestinal and kidney disorders, immune deficiency and cancer. Humidity and temperature are important factors in fungal growth, and it is anticipated that climate change may impact the presence of these mycotoxins. This makes the low-level quantitation of mycotoxins in foodstuffs a high priority for food producers. There are several EU regulations that control the prescribed limits of mycotoxins and other plant toxins in food. Regulation EU 2023/27824 and 2023/27835 control the methods for sampling and analysis for the control of mycotoxins and plant toxins in food. Regulation EU 2023/9151 and EU 2024/10382 and Recommendation EU 2022/5533 stipulate the MRL values for mycotoxins and certain plant toxins in a variety of different food matrices. Mycotoxin analysis must be comprehensive and able to deliver accurate and consistent results across a wide range of matrices. Highly sensitive instruments provide the possibility to minimize sample preparation steps and still obtain high quality, reliable quantitative results to meet the requirements of different regulations. In this poster, we will demonstrate a highly sensitive and robust LC/MS method by using SCIEX 7500+ system to quantify EU regulated mycotoxin compounds in different matrices, including baby food. Additional MRM3 quantitation method is employed to reduce the interference peaks and noise. Good chromatographic separation was achieved for all target mycotoxins over the 15-min gradient method that allowed for the accurate quantitation down to the LOQ values reported here, with the LOQ defined by S/N of the quantifier ion >10, as well as an ion ratio of ±20% of the average, and accuracy 80-120%. Linearity (1/x weighting, r²>0.99) was achieved for the calibration curves of all mycotoxins in all four matrices without the need for labelled internal standards LOQ values achieved for all compounds in baby food, almond, grape juice and wine. Average accuracy and precision were assessed for 5 replicate injections of the extracted baby food LOQ standard

and found to be within acceptable validation requirements (accuracy $\pm 20\%$ and peak area $\%CV < 15\%$). A simplified sample preparation method, without lyophilization, is used here. And all EU regulated MRL could be achieved. Furthermore, a single MRM3 experiment can be implemented with a scan time of only 60 ms, so that up to two MRM3 scans can be undertaken, alongside the full mycotoxin screen using positive and negative MRMs with polarity switching, without any drop in sensitivity.

P113

Hydrolysis of T-2 toxin in aqueous maize extracts

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T-2 is one of the most toxic members of the trichothecene family of mycotoxins. It is produced when *Fusarium* moulds contaminate grain samples in the field or due to improper storage conditions. The closely related toxin HT-2 is produced by the hydrolysis of T-2 and is often found concurrently in T-2 contaminated samples. In this work it was found that when naturally contaminated or spiked maize samples are extracted with water, the T-2 toxin present is rapidly hydrolysed to HT-2. Maize samples spiked at 100 ppb, 200 ppb, and 300 ppb, T-2 and HT-2 toxin were extracted in 70% methanol and analysed by LC/MSMS, their calculated values were within 2% of the expected values. However, when the same samples were extracted in water, they showed zero recovery for T-2 toxin and values for HT-2 that were 56.93% to 75.50% higher than expected. The increase in HT-2 above the expected value is due to hydrolysis of the T-2 toxin that is present. Spiking T-2 and HT-2 toxin at 250 ppb in water without matrix present did not show any increase in HT-2 over a similar time frame. These results suggest that the hydrolysis of T-2 is not due to the solvent, but some component contained in the maize.

P114

Multiyear inter-laboratory HPLC and LCMS results for analysis of naturally incurred aflatoxin containing reference materials in ground maize

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Ground maize reference material with known amounts of naturally incurred aflatoxin (B1, B2, G1 and G2) was divided and double-blind paired samples were provided to several analytical laboratories for analysis in triplicate over multiple years. Standard solutions of aflatoxin B1 were also provided to evaluate precision and accuracy of the analytical methods removing the effects of sample extraction from the results. Samples were analysed by HPLC-FLD, HPLC-UV or UPLC-MS depending on the laboratory. Statistical analysis including ANOVA was completed using Minitab 18 (Minitab, LLC). Accuracy, precision, and consistency of results on paired samples from the same lot over multiple years will be presented. The comparison of multi-year results can assist with identification of outlying results. In addition, results with greater variability and trends in long-term performance can be identified.

P115

Approval of the AgraStrip® Pro Total Aflatoxin WATEX® test kit for FGIS Certification

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Aflatoxins (B1, B2, G1, G2) pose significant risks to food safety and trade, necessitating accurate and reliable detection in maize and its derivatives. This study presents the successful approval of the AgraStrip® Pro Total Aflatoxin WATEX® test kit with the AgraVision™ Pro reader by the Federal Grain Inspection Service (FGIS). Experiments performed demonstrated robustness, selectivity, product consistency, and stability across a quantitative range of 0-460 ppb. Updated procedures enhanced usability and reduced complexity while improving LOD/LOQ. Results from 63 measurements met acceptance criteria, confirming the test kit's reliability for routine aflatoxin screening, supporting compliance with USDA standards and international trade regulations.

P116

Validation of a lateral flow device for ochratoxin A detection in roasted coffee

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Ochratoxin A (OTA), a heat-stable mycotoxin produced by certain fungi, frequently contaminates coffee beans, posing health risks if regulatory limits are exceeded. This study validates the AgraStrip® Pro Ochratoxin A WATEX® test kit, paired with the AgraVision™ Pro reader, as a reliable and rapid screening tool for OTA in roasted coffee. Validation included correlation analysis with a reference LC-MS/MS method, achieving an $R^2 > 0.9$. Recovery and precision align with Codex Alimentarius standards. Results confirm the LFD method as suitable for routine screening, offering an efficient alternative for ensuring regulatory compliance and consumer safety in international coffee trade.

P117

Accelerated and parallel mycotoxin clean-up by thermal elution – higher sensitivity and faster processing

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Mycotoxin testing demands higher sample throughput and gain of sensitivity, as the maximum tolerated levels of mycotoxins (aflatoxin B/G, M1 or ochratoxin A) are strongly regulated (EU 2023/915; FDA compliance policy guide Sec.555.400). The high occurrence of aflatoxin and ochratoxin in certain commodities and their corresponding matrix interferences need a stringent sample clean-up procedure to reduce those interferences during analysis and to improve sensitivity. The SMART immunoaffinity cartridges allow an accelerated sample clean-up, in combination with a manual thermal elution by the so-called ThermElute light system. The unique process is a water-based mycotoxin elution from immunoaffinity cartridges by high temperatures, allowing a high-volume injection into the chromatographic system, which leads to a higher analytical sensitivity. Sample clean-up is processed according to the extraction and clean-up procedures of the Smart cartridges AflaClean Sart, AflaClean M1 Smart or OtaClean Smart. The resulting eluates could be analysed by HPLC-FLD or LC-MS/MS. An increase of injection volume in analysis was investigated in comparison to solvent-based elution strategies to allow higher analytical sensitivity. In a comparative study a peak broadening as observed for toxins that were eluted with organic solvents could not be observed. This facilitates an increase of analytical sensitivity and accelerated sample processing. The technology was tested for aflatoxins B/G and M1 in various food and feed products. Beside aflatoxins, ochratoxin A in various matrices was investigated and showed similar results – acceleration of sample clean-up and gain of sensitivity. Both was achieved just by the higher injection volume fulfilling regulation and performance criteria. The technology is suitable for isocratic as well as for gradient chromatography and not restricted only to LC-MS/MS but could be used for LC-MS as well as for HPLC-FLD chromatography. For fluorescence analysis, a post column derivatization by UV light (UVE®) was used. The results show a high consistency and linearity over a wide range of contamination levels. The top advantages using thermal elution for mycotoxins is the increase of injection volume leading to higher analytical sensitivity without the drawback regarding peak broadening or retention time shift observed with a similar injection of organic eluents. The benefits of antibody-based analyte concentration and efficient matrix removal could be projected as this is a kind of gold standard for single mycotoxin analysis in difficult commodities.

P118

Rapid analysis of ochratoxin A in cocoa powder (cocoa cake) using Ochra-V™ Max method

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Ochratoxin A (OTA), a mycotoxin produced by various *Aspergillus* and *Penicillium* species, is commonly found in agricultural commodities, including cocoa beans. OTA is known for its nephrotoxic, immunotoxic, and carcinogenic effects on humans and animals, leading to stringent regulations in many countries. The European Union's Regulation (EU) 2022/1370 mandates a maximum OTA limit of 3 µg/kg in cocoa powder. Cocoa powder is a complex matrix for analytical analysis due to the various types of cocoa ingredients and process procedures. OTA in cocoa product is usually analysed by instrumental

methods, such as high-performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC/MS). The cocoa industry seeks a quick screening tool to reduce the risk of OTA contamination. The Ochra-V Max method, combining immunoaffinity column with lateral flow strip test, was developed to achieve rapid, sensitive and accurate OTA analysis in cocoa samples. The cocoa samples are extracted with an aqueous solution and then the supernatant is passed through an immunoaffinity column (OchraTest™ 100 column). After a water wash, the column was incubated at 90 °C for 5 min and then eluted with an aqueous solution. The eluate was then added to a strip test which was interpreted by a Vertu™ Touch reader after a 5 min incubation. Natural or alkalized cocoa powder and cocoa cake samples with various fat contents were evaluated with the Ochra-V Max method. The results showed that Ochra-V Max method has a high degree of linearity ($r^2=0.999$) for ochratoxin in the range of 0 to 10 ppb with a limit of detection of 0.50 ppb. Precision study indicates that Ochra-V Max method results correlate very well with the results of HPLC. Ochra-V Max method utilizes advanced immunoassay technology to provide precise, sensitive, and rapid results for OTA detection across all cocoa powder (cocoa cake) sample types.

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