ABSTRACTS OF LECTURES AND POSTERS

THE World Mycotoxin Forum®

13TH CONFERENCE

WMF meets Italy

16-18 MAY 2022

PARMA - ITALY

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Key to the abstracts of lectures and posters:

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

COMMITTEES

General Conference Chairs

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

Local conference chairs

Prof. Chiara Dall'Asta	University of Parma, Italy
Prof. Michele Suman	Barilla, Italy

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Dr Awanwee Petchkongkaew	Thammasat University, Thailand
Dr Lindy J. Rose	Stellenbosch University, South Africa
Dr Sheryl Tittlemier	Canadian Grain Commission, Canada

WELCOME TO PARMA

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 13th conference of the World Mycotoxin Forum® – **WMF*meets*ITALY** – 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
- workshops
- WMF Young Scientists Forum
- company pitches, case studies, and industry updates covering a wide range of topics
- 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

Local conference chairs
Chiara Dall'Asta
Michele Suman

UNIVERSITÀ DI PARMA

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ABOUT PARMA

Con il patrocinio

Comune di Parma

Located in northern Italy in Emilia-Romagna region, Parma is a wonderful destination for those who appreciate art, architecture, and Italian food. The city, located at the gateway to the area producing Parmigiano Reggiano cheese and balsamic vinegar, is probably most famous for Prosciutto di Parma. In addition to these Italian delicacies, there is plenty to do and see in Parma. The city has delightful streets, art museums, and a celebrated cathedral and baptistry.

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SOCIAL EVENTS

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

Sunday 15 May 2022
18:30 – 20:00

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The welcome reception – sponsored by R-Biopharm – will be held at the Palazzo Dalla Rosa Prati (TCafè), located just across the narrow streets of the Dom and the Baptistery on the Piazza Duomo. The welcome reception provides an excellent opportunity to network, meet old friends and colleagues as well as to make new contacts.

Delegates who joined the WMF*connects* online pre-conferences, may remember that they visited the Piazza Duomo already virtually. It will be a great to start the three-day in-person conference at the same spot.

TCafè at Palazzo Della Rosa Prati
Strada al Duomo 7

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GUIDED WALKING TOUR & CONFERENCE DINNER
(reservations only)

Tuesday 17 May 2022
19:15 – 22:30

Join the guided walking tour through the historic centre of Parma followed by a very special dinner with an unforgettable experience in the Circolo di Lettura di Parma. Dress code: business casual.

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

IMPORTANT NOTES

- Participants to the guided tour shall gather at **19:15 sharp** in front of the Monumento al Partigiano, Piazza della Pace.
- To take part, you must wear and show your name badge with the ticket.
- The tour ends at the Monumento al Partigiano, Piazza della Pace. From here, it is a few minutes walk to the Circolo di Lettura di Parma, Strada Macedonio Mellini 4, where the conference dinner takes place.

- Participants who do not want to join the guided tour but have registered for the conference dinner, shall gather at the Circolo di Lettura di Parma, Strada Macedonio Melloni 4, at **20:30 sharp**.

  Entrance only if you wear and show your name badge with the ticket.

PROGRAMME AT A GLANCE

SUNDAY 15 MAY 2022

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

MONDAY 16 MAY 2022

11:30 – 12:30	Registration and light lunch	EXHIBITION
12:30 – 15:00	PLENARY SESSION Addressing today's and tomorrow's challenges	
15:00 – 15:30	Networking break & poster viewing	
15:30 – 17:45	Company pitches* and Speed presentations**	
17:45 – 19:30	Wine tasting – sponsored by DSM and Romer Labs	

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

TUESDAY 17 MAY 2022

08:30 – 10:30	SESSION 1 Animal health and productivity	SESSION 2 Exposure assessment and human health	EXHIBITION
10:30 – 11:00	Networking break & poster viewing		
11:00 – 12:30	SESSION 3 Mitigating mycotoxin risks	SESSION 4 Update on EFSA activities in mycotoxin risk assessment	
12:30 – 14:00	Lunch break & poster viewing Workshops by Vicam and R-Biopharm/Trilogy Analytical Laboratory		
14:00 – 15:30	SESSION 5 Focus on mycotoxigenic fungi, plants, and soil	SESSION 6 Mycotoxin management along the food & feed chain	
15:30 – 16:00	Networking break & poster viewing		
16:00 – 17:30	SESSION 7 Managing mycotoxins in a sustainable future	SESSION 8 Smart solutions for advanced toxin control and mitigation (FFoQSI)	
17:30 – 18:30	WMF Young Scientists Forum sponsored by Trouw Nutrition		
19:15 – 22:30	Guided walking tour & Conference dinner (reservations only)		

WEDNESDAY 18 MAY 2022

08:30 – 10:15	SESSION 9 Update on (multi-)mycotoxin analysis	SESSION 10 Modelling strategies and digitalisation in mycotoxin management	EXHIBITION
10:15 – 10:45	Networking break & poster viewing		
10:45 – 12:40	PLENARY SESSION Looking further ahead		
12:40 – 12:50	Best Poster Award presentation		
12:50 – 13:00	**WMF*meets*ITALY** – Top Five Answers learned		
13:00	Closing of **WMF*meets*ITALY**		
13:30 – 16:30	Excursion to Barilla (reservations only)		

CONFERENCE PROGRAMME

MONDAY 16 MAY 2022

PLENARY SESSION
ADDRESSING TODAY'S AND TOMORROW'S CHALLENGES

Since its establishment in 2001, The World Mycotoxin Forum covers the latest issues in mycotoxin management targeting at everyone working in the mycotoxin space – researchers, food and feed industry, laboratories, policy makers, and enforcement agencies from around the world. The aim of this year's conference to elaborate further on key strategic issues looking forward, amid the current challenges.

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

Local Conference Chairs: Prof. Chiara Dall'Asta, Department of Food and Drug, University of Parma, Italy and Prof. Michele Suman, Barilla S.p.A., Italy

12:30 Welcome to Parma
- Prof. Paolo Martelli, Pro-Vice Rector, University of Parma
- Dr Rana Shehadeh, Chief RD&Q Officer, Barilla Group Research, Development and Quality

12:45 Opening of **WMF*meets*ITALY**
Prof. Chiara Dall'Asta and Prof. Michele Suman

13:00 20 years WMF: achievements, lessons learned and global challenges ahead
Prof. Rudolf Krska and Prof. Chris Elliott

13:15 Mycotoxins from the fungus´ perspective: when, how, where, and why?
Prof. Joseph Strauss, Department of Applied Genetics and Cell Biology, BOKU Vienna, Austria

13:35 The nexus between food safety and food security: the case of mycotoxins
Dr Cornelia Boesch, Food and Agriculture Organization of the United Nations (FAO), Italy

13:55 Toxigenic fungi, mycotoxins, and the Glasgow Climate Pact (COP26): what does the future hold?
Prof. Paolo Battilani, Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Italy

14:15 Finding the balance between safety and sustainability in a circular food system: the case of food processing by-products
Dr Madhura Rao, Food Claims Centre Venlo, Maastricht University, the Netherlands

14:35 Advancing the circular bionutrient economy to combat mycotoxins
Prof. Rebecca Nelson, Department of Global Development, Cornell University, USA

15:00 Networking break and poster viewing

MONDAY 16 MAY 2022

PLENARY SESSION
COMPANY PITCHES AND SPEED PRESENTATIONS

Short presentations by sponsors to inspire the audience to visit their booths (company pitches) and by selected poster presenters to provide an overview of their research (speed presentations)

Chairs: Prof. Chiara Dall'Asta, Department of Food and Drug, University of Parma, Italy and
Prof. Michele Suman, Barilla S.p.A., Italy

15:30 Company pitches
Short presentations (5-minutes) by sponsors to inspire the audience to visit their booths
R-Biopharm – Waters I Vicam – DSM/Romer Labs – Trouw Nutrition – Adisseo – Eurofins Technologies – Phileo - Libios – Alltech – EnviroLogix
For details, see pages 28-31.

16:25 Speed presentations (5-minutes) by selected poster presenters to provide an overview of their research
Octavian Augustin Mihalache (Italy) – Giorgia Del Favero (Austria) – Orphélie Lootens (Belgium) – Carolina Gómez-Albarrán (Spain)
For details, see page 27.

16:45 Company pitches (continued)
MiXscience – Impextraco – Cargill - ProGnosis Biotech – Agrimprove – Olmix – Charm Sciences – Neogen – Patent Co – Thermo Fisher Scientific – Bioeasy – SafeFood
For details, see pages 31-35

17:45 **WINE TASTING – SPONSORED BY DSM AND ROMER LABS**

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

TUESDAY 17 MAY 2022

SESSION 1
ANIMAL HEALTH AND PRODUCTIVITY

Knowing the effects that mycotoxins have on animal health and productivity is essential. In this session, contemporary issues will pass in review.

Chair: Prof. Isabelle Oswald, TOXALIM Research Centre in Food Toxicology, INRAE, France

08:30 Exploring knowledge gaps on the impact of mycotoxins on poultry health
Prof. Gunther Antonissen, Department of Pathology, Pharmacology and Zoological Medicine, Ghent University, Belgium

08:45 Intestinal toxicity of NX, a new type A trichothecene
Prof. Isabelle Oswald, TOXALIM Research Centre in Food Toxicology, INRAE, France

09:00 Aflatoxins in Kenya: A story of maize, milk, and money
Dr Johanna F. Lindahl, International Livestock Research Institute, Kenya

09:15 Development of a new model to assess the effect of mycotoxins on livestock
Dr Damien P. Prévéraud, Adisseo, France

09:30 Emerging mycotoxins and emergent effects
Prof. Francesca Caloni, Department of Environmental Science and Policy, University of Milan, Italy

09:45 Determination of AFM1 in raw milk to evaluate exposure to AFB1-contaminated feed and its control at farm level: a case study
Dr Mohammad H. Shojaee AliAbadi, Faroogh Life Sciences Research Laboratory, Iran and Boutros Kerbaje, Libios, France

10:00 Mycotoxin risk management: Why one-size-fits-all don't work?
Dr Swamy Haladi, Trouw Nutrition, India

10:15 *Winner of the Young Scientist Pitch Award 2021 'Animal Health':*
Mycotoxins in aquaculture: occurrence in feed ingredients and feed – effects on fish productivity and health
Paraskevi Koletsi, Aquaculture and Fisheries Group, Wageningen University & Research, the Netherlands

10:30 Networking break and poster viewing

TUESDAY 17 MAY 2022

SESSION 2
EXPOSURE ASSESSMENT AND HUMAN HEALTH

Developments and challenges in relation to mycotoxin exposure and health implications in humans will be presented

Chair: Prof. Marthe De Boevre, Centre of Excellence in Mycotoxicology and Public Health, Ghent University, Belgium

08:30 Dietary exposure assessment in sub-Saharan Africa: a practical case study
Dr Luc Ingenbleek, Nutrition and Food Safety Department, World Health Organization, Switzerland

08:45 You excrete what you eat – but is urinary deoxynivalenol a good biomarker of exposure?
Dr Gunnar Sundstøl Eriksen, Toxinology Research Group, Norwegian Veterinary Institute, Norway

09:00 Urinary excretion kinetics of zearalenone biomarkers over 72 hours after single oral administration in healthy adults
Dr Karsten Beekmann, Wageningen Food Safety Research, the Netherlands

09:15 The contributing role of deoxynivalenol and patulin in colorectal and hepatocellular carcinogenesis: the genomic perspective
Liesel Claeys, Centre of Excellence in Mycotoxicology and Public Health, Ghent University, Belgium and International Agency for Research on Cancer, France

09:30 The bidirectional relationship between *Alternaria* mycotoxins and the gut microbiota
Dr Francesco Crudo, Department of Food Chemistry and Toxicology, University of Vienna, Austria

09:45 Toxicology of mixtures: lessons from the mycotoxin combination studies
Dr Imourana Alassane-Kpembi, Department of Veterinary Biomedicine, University of Montreal, Canada

10:00 Evaluation of a tri-culture gut-on-chip model for long-term exposure studies
Dr Meike van der Zande, Wageningen Food Safety Research, the Netherlands

10:15 *Winner of the Young Scientist Pitch Award 2021 'Human Exposome':*
The epigenomic interplay of aflatoxins and a herpes virus towards childhood cancer
Thanos Michailidis, Centre of Excellence in Mycotoxicology and Public Health, Ghent University, Belgium

10:30 Networking break and poster viewing

TUESDAY 17 MAY 2022

SESSION 3
MITIGATING MYCOTOXIN RISKS

A look into a variety of strategies related to mitigating mycotoxin contamination in food and feed. What's up?

Chair: Dr Sheryl Tittlemier, Canadian Grain Commission, Canada

11:00 Effect of lactic acid bacteria in ochratoxin A and aflatoxin B1 reduction during bread fermentation
Dr Laura Escrivá, Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia, Spain

11:15 Detox your food – mycotoxin deactivation in food production using enzymes
Dr Nicolas Hardt, DSM, Austria

11:30 Ammonization of the R- and S-epimers of ergot alkaloids to assess detoxification potential
Jensen E. Cherewyk, Toxicology Center, University of Saskatchewan, Canada

11:45 Reduction of adverse effects of deoxynivalenol by selected yeast fractions
Dr Virginie Marquis, Phileo by Lesaffre, France

12:00 Safety of house fly larvae reared on mycotoxin-contaminated substrates
Kelly Niermans, Laboratory of Entomology, Wageningen University & Research, the Netherlands

12:15 Mitigation of mycotoxins by the use of magnetic nanostructured agents
Dr Jesús M. González Jartín, Departamento de Farmacología, Universidade de Santiago de Compostela, Spain

12:30 Lunch break
Exhibition and poster viewing

WORKSHOPS (for details, see page 21)
12:45 – 13:45

Workshop sponsored by VICAM
Solutions for mycotoxins and alkaloids detection from field to lab

Workshop sponsored by R-Biopharm and Trilogy Analytical Laboratory
QualiT™ – Sample preparation and quality assurance tools for mycotoxin analysis

TUESDAY 17 MAY 2022

SESSION 4
UPDATE ON EFSA ACTIVITIES IN MYCOTOXIN RISK ASSESSMENT
Session co-ordinated by the European Food Safety Authority (EFSA).

New approaches for risk assessment of mycotoxins, critical issues for assessing carcinogenic mycotoxins together with results from recent assessments will be presented. EFSAs initiatives in support of research activities in the field of mycotoxins will be introduced together with an update on regulatory follow-up of EFSA work.

Chair: Dr Marco Binaglia, Pesticides Peer Review Unit, EFSA, Italy

11:00 Methodological approaches developed by EFSA to include modified mycotoxins in risk assessments
Dr Hans Steinkellner, Feed and Contaminants Unit, EFSA, Italy

11:15 Specific outcomes of animal health risk assessments of mycotoxins including their modified forms
Prof. Isabelle Oswald, TOXALIM Research Centre in Food Toxicology, INRAE, France

11:30 Role of the mechanisms of genotoxicity in determining safe human exposure limits: Ochratoxin as a case study
Dr Margherita Bignami, Department of Environment and Health, Istituto Superiore di Sanità, Italy

11:50 The impact of EFSA collaboration in shaping methodologies in mycotoxin risk assessment
Prof. Chiara Dall'Asta, Department of Food and Drug, University of Parma, Italy

12:10 Regulatory follow up at EU level to EFSA opinions and future challenges
Frans Verstraete, Directorate-General for Health and Food Safety, European Commission, Belgium

12:30 Lunch break
Exhibition and poster viewing

WORKSHOPS (for details, see page 21)
12:45 – 13:45

Workshop sponsored by VICAM
Solutions for mycotoxins and alkaloids detection from field to lab

Workshop sponsored by R-Biopharm and Trilogy Analytical Laboratory
QualiT™ – Sample preparation and quality assurance tools for mycotoxin analysis

TUESDAY 17 MAY 2022

SESSION 5
FOCUS ON MYCOTOXIGENIC FUNGI, PLANTS, AND SOIL

In this session, we follow the fate of mycotoxins from a complex ecological perspective: fungus, plant, and soil, and their interactions.

Chair: Prof. Sarah De Saeger, Centre of Excellence in Mycotoxicology and Public Health, Ghent University, Belgium

14:00 Facial eczema caused by sporidesmin-producing strains of *Pseudopithomyces chartarum*: An important yet poorly understood disease of dairy cattle in South Africa
Dr Neriman Yilmaz Visagie, Department of Biochemistry, Genetics and Microbiology, University of Pretoria, South Africa

14:15 Key genes involved in mycotoxin biosynthetic pathways
Dr Oluwatobi Kolawole, Institute for Global Food Security, Queen's University Belfast, UK

14:30 Mapping resistance and susceptibility to *F. langsethiae* and T-2/HT-2 contamination in 190 spring oats varieties: what will happen with climate change?
Dr Angel Medina-Vaya, School of Water, Energy and Environment, Cranfield University, UK

14:45 Bioregulation of soil organisms – Suppression of pathogenic fungi and reduction of their mycotoxins in an agro-ecological context
Dr Friederike Meyer-Wolfarth, Institute for Plant Protection in Field Crops and Grassland, Julius Kühn-Institut, Germany

15:00 Sustainable cereal production reduces important *Fusarium* mycotoxins
Dr Aksel Bernhoft, Norwegian Veterinary Institute, Norway

15:15 Embracing the environmental complexity: from mycotoxins to multiple plant stressors
Dr Laura Righetti, Department of Food and Drug, University of Parma, Italy

15:30 Networking break and poster viewing

TUESDAY 17 MAY 2022

SESSION 6
MYCOTOXIN MANAGEMENT ALONG THE FOOD & FEED CHAIN

Where do we stand today? This session will introduce some options for the optimisation of processing and handling to manage mycotoxin contamination along the food & feed chain.

Chair: Prof. Michele Suman, Barilla S.p.A., Italy

14:00 Transformation of ochratoxin A during bread-making processes
Marta Modrzewska, Department of Food Safety and Chemical Analysis, Waclaw Dabrowski Institute of Agricultural and Food Biotechnology, Poland

14:15 Major ergot alkaloids mycotoxins, cereals, and cereal-derived food products: management and control strategies along the food chain
Dr Sofia Agriopoulou, Department of Food Science and Technology, University of the Peloponnese, Greece

14:30 Bioconversion of aflatoxin-contaminated agri-food by-products with fly larvae into animal feed
Dr Moritz Gold, Department of Health Science and Technology, ETH Zurich, Switzerland

14:45 Scrape the mould off the jam or toss the jar? - New answers to an old food safety dilemma
Dr Alexandra Malachova, Austrian Competence Centre for Feed and Food Quality, Safety and Innovation, Austria

15:00 Prevention of *Alternaria* emerging mycotoxins in the apple by-product process line
Dr María Agustina Pavicich, Centre of Excellence in Mycotoxicology and Public Health, Ghent University, Belgium

15:15 Understanding the potential for and control of aflatoxin development in almonds during storage and shipping
Tim Birmingham and Guangwei Huang, Almond Board of California, USA

15:30 Networking break and poster viewing

TUESDAY 17 MAY 2022

SESSION 7
MANAGING MYCOTOXINS IN A SUSTAINABLE FUTURE

An ideal and sustainable economy is one which provides for the greatest amount of general well-being with the least amount of resource use and environmental harm. How do we cope with naturally occurring contaminants, such as mycotoxins, in a sustainable future?

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

16:00 FoodSafety4EU – Sustainable food: how to keep it safe?
Dr Veronica Lattanzio, Institute of Sciences of Food Production (ISPA), National Research Council (CNR), Italy

16:15 Burgeoning sustainable aflatoxin management through a food convergence initiative
Dr Titilayo Falade, International Institute of Tropical Agriculture, Nigeria

16:30 Biostimulant microorganisms for mitigating the occurrence of *Alternaria spp.* and derived mycotoxins in tomatoes
Dr Giulia Leni, Department of Animal, Food and Nutrition Science, Università Cattolica del Sacro Cuore, Italy

16:45 Unravelling the mechanism of action of an environmentally friendly, defensin-based solution holding the promise to reduce mycotoxin contamination of wheat
Valentin Leannec-Rialland, Mycology and Food Safety, INRAE, France

17:00 Vegans vs. omnivores – a healthy diet with more mycotoxins?
Dr Benedikt Cramer, Institut für Lebensmittelchemie, Westfälische Wilhelms-Universität Münster, Germany

17:15 Young Scientist Forum: The challenge of mycotoxins in sustainable agriculture
Guan-Lin Wang, Trouw Nutrition, the Netherlands

WMF YOUNG SCIENTISTS FORUM (for details, see page 22)
17:30 – 18:30

The challenge of mycotoxins in sustainable agriculture
(sponsored by Trouw Nutrition)

GUIDED WALKING TOUR AND CONFERENCE DINNER (reservations only)
19:15 – 22:30

Join the guided walking tour through the historic centre of Parma followed by a very special dinner with an unforgettable experience in the Circolo di Lettura di Parma.
For details, see page 5.

TUESDAY 17 MAY 2022

SESSION 8
SMART SOLUTIONS FOR ADVANCED TOXIN CONTROL AND MITIGATION
Session co-ordinated by the Austrian Competence Centre for Feed and Food Quality, Safety and Innovation (FFoQSI).

The potential of novel and promising approaches, such as rapid screening of mycotoxins, effective monitoring of candidate detoxification genes, and microbiome analysis as developed by FFoQSI and its industrial partners from the food and feed industry, will be presented. The impact of mycotoxin contamination on food and feed waste and sustainable strategies to tackle the issue along supply chains in view of climate change will be also discussed.

Chairs: Prof. Rudolf Krska, Department IFA-Tulln, BOKU Vienna
Prof. Martin Wagner, FFoQSI and University of Veterinary Medicine, Austria

16:00 Impact of mycotoxins on food and feed waste, environmental sustainability, and loss of livestock productivity
Prof. Chris Elliott, Institute for Global Food Security, Queen's University Belfast, UK

16:15 The potential of IR spectroscopy for rapid mycotoxin screening
Dr Stephan Freitag, FFoQSI and Department IFA-Tulln, BOKU Vienna, Austria

16:30 Multi-year mycotoxins monitoring into cereals/cereals-based raw materials and food commodities, mirroring parallel origin/climate change information
Prof. Michele Suman, Barilla S.p.A., Italy

16:45 Microbiome analysis reveals fungi as a major driver in hard cheese ripening
Prof. Martin Wagner, FFoQSI and University of Veterinary Medicine, Austria

17:00 Genetic engineering of a fumonisin-sensitive yeast bioassay strain and its utilization to monitor candidate detoxification genes
Tamara Krska, FFoQSI, Austria

17:15 Fungi and fungal toxins in bakery plants and products
Dr Michael Sulyok, Department IFA-Tulln, BOKU Vienna, Austria

WMF YOUNG SCIENTISTS FORUM (for details, see page 22)
17:30 – 18:30

The challenge of mycotoxins in sustainable agriculture
(sponsored by Trouw Nutrition)

GUIDED WALKING TOUR AND CONFERENCE DINNER (reservations only)
19:15 – 22:30

Join the guided walking tour through the historic centre of Parma followed by a very special dinner with an unforgettable experience in the Circolo di Lettura di Parma.
For details, see page 5.

WEDNESDAY 18 MAY 2022

SESSION 9
UPDATE ON (MULTI-)MYCOTOXIN ANALYSIS

A small selection of recent research in the area of (multi-)mycotoxin analysis will be presented.

Chair: Dr Monique de Nijs, Wageningen Food Safety Research, the Netherlands

08:30 Luminescent biosensing strategies for the analysis of biotoxins
Dr Elena Benito Peña, Department of Analytical Chemistry, Universidad Complutense de Madrid, Spain

08:45 Generation of high-affinity antibodies for patulin analysis
Dr Josep V. Mercader, Department of Preservation and Food Safety Technologies, Institute of Agrochemistry and Food Technology (IATA-CSIC), Spain

09:00 Validation of a screening method: a comparison of current guidelines and gaps for automation
Giulia Rosar, Eurofins Tecna, Italy

09:15 Strategies for the detection of multi-mycotoxins and alkaloids in food samples
Nicola Dreolin, Waters Corporation, UK

09:30 High resolution mass spectrometry: A promise or a blind alley in the routine analysis of multiple mycotoxins?
Lidija Kenjeric, Department IFA-Tulln, BOKU Vienna, Austria

09:45 Realization of the technical requirements of an ISO 17025 accredited LC-MS/MS multi-toxin method for feed and related matrices
Dr David Steiner, Analytical Service Department, Romer Labs Diagnostic GmbH (part of DSM), Austria

10:00 Occupational exposure to mycotoxins: A biomonitoring and airborne measurement approach
Dr Sophie Ndaw, Department Toxicology and Biometrology, INRS, France

10:15 Networking break and poster viewing

FINAL PLENARY SESSION
LOOKING FURTHER AHEAD
See page 20.

WEDNESDAY 18 MAY 2022

SESSION 10
MODELLING STRATEGIES AND DIGITALISATION IN MYCOTOXIN MANAGEMENT

The emergence of innovative solutions in mycotoxin management requires support from computational modelling and digitalisation. Recent developments will get through here.

Chair: Prof. Paola Battilani, Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Italy

08:30 Limiting mycotoxin exposure of livestock by monitoring and forecasting of contaminations in feed crops
Dr Wolfgang Schweiger, DSM, Austria

08:45 Take a byte of mycotoxins: why and how in silico analysis boosts knowledge and feeds mycotoxin research
Dr Luca Dellafiora, Department of Food and Drug, University of Parma, Italy

09:00 Utilizing computational tools to improve the biological detoxification of mycotoxins
Natalie Sandlin, Department of Biology, Boston College, USA

09:15 The design of effective monitoring for mycotoxins using machine learning and multiple criteria decision making
Prof. Ine van der Fels-Klerx, Department of Social Sciences, Wageningen University & Research, the Netherlands

09:30 Monitoring mycotoxins across scales: digital tools for smallholder farming systems
Will Stafstrom, Plant Breeding & Genetics Section, School of Integrative Plant Science, Cornell University, USA

09:45 A unique mycotoxin database: how it can be of help in the prioritization of mycotoxin toxicity assessment (EU project Agritox)
Dr Denis Habauzit, Toxicology of Contaminants Unit, French Agency for Food, Environmental and Occupational Health & Safety (ANSES), France

10:00 Can we predict aflatoxins occurrence in Africa? The APHLIS mycotoxins risk modelling – approach, lessons learned and re-orientation
Dr Monica Ermolli, European Commission, Joint Research Centre, Italy

10:15 Networking break and poster viewing

FINAL PLENARY SESSION
LOOKING FURTHER AHEAD
See page 20.

WEDNESDAY 18 MAY 2022

FINAL PLENARY SESSION
LOOKING FURTHER AHEAD

Take a step back, take a deep breath, and look forward. What can be expected?

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

10:45 Inclusion of antifungal resistance in One Health policy and dialogue
Dr Jomana Musmar, Office of the Assistant Secretary for Health, U.S. Department of Health and Human Services, USA

11:05 Expect the unexpected – Food trends influencing mycotoxin trends
Ronald Niemeijer, R-Biopharm AG, Germany

11:20 The European Human Biomonitoring Initiative (HBM4EU): lessons learned, looking forward
Dr Paula Alvito, Food and Nutrition Department, National Institute of Health Doutor Ricardo Jorge, Portugal

11:40 Analysing the chemical exposome: Delusion or next frontier?
Dr Benedikt Warth, Department of Food Chemistry and Toxicology, University of Vienna, Austria

12:00 The power of global networks for capacity building in mycotoxin research
Prof. Sarah De Saeger, Centre of Excellence in Mycotoxicology and Public Health, Ghent University, Belgium

12:20 An outline of the Food Safety Coalition project to address the challenges of aflatoxin contamination in raw materials
Prof. Chris Elliott, Institute for Global Food Security, Queen's University Belfast, UK

12:40 **BEST POSTER AWARD PRESENTATION**

12:50 Top Five Answers learned at **WMF*meets*ITALY**
Prof. Rudolf Krska and Prof. Chris Elliott

13:00 Closing of **WMF*meets*ITALY**
Take your packed lunch to eat along the way!

EXCURSION TO BARILLA (reservations only)
13:30 – 16:30
For details, see page 22.

MYCOTOXIN MANAGEMENT

IS NOT A BETTING GAME

IT'S A MATTER OF EXPERTISE

FORECAST CROP CONTAMINATION	SECURE STORAGE	SCREEN FINISHED FEED	PROTECT ANIMALS
MycoMan Harvest bulletin MycoMan Test (quick) MycoMan Predict	Mold-Nil®	MycoMan Test (lab) MycoMan® Mobile app	Unike® Plus Toxy-Nil® Plus Toxy-Nil®

Identify your risk and adopt the most efficient strategy

EXPERT SOLUTION ★★★★★

At Adisseo, we have developed a comprehensive approach to the management of mycotoxins. 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 mobile app.

Moreover, Adisseo has also developed a portfolio of products composed of Unike® Plus, Toxy-Nil® Plus and Toxy-Nil® in order to propose the best-suited solution to a specific challenge.

ADISSEO
A Bluestar Company

www.adisseo.com

Field-to-Lab Mycotoxin Testing Solutions

Rapid and precise detection tools to secure a safe food supply chain.

From harvest screening and raw material preventative monitoring to quality and compliance verification, quickly and confidently detect multi-mycotoxins in a wide range of settings and sample matrices.

- Lateral flow strip tests which reduce screening time and consumables use by 77%
- Simple to use IAC and HPLC combination for confirmatory testing
- Validated LC-MS/MS solutions for sensitive (sub-ppb) multi-toxin analysis in a single run

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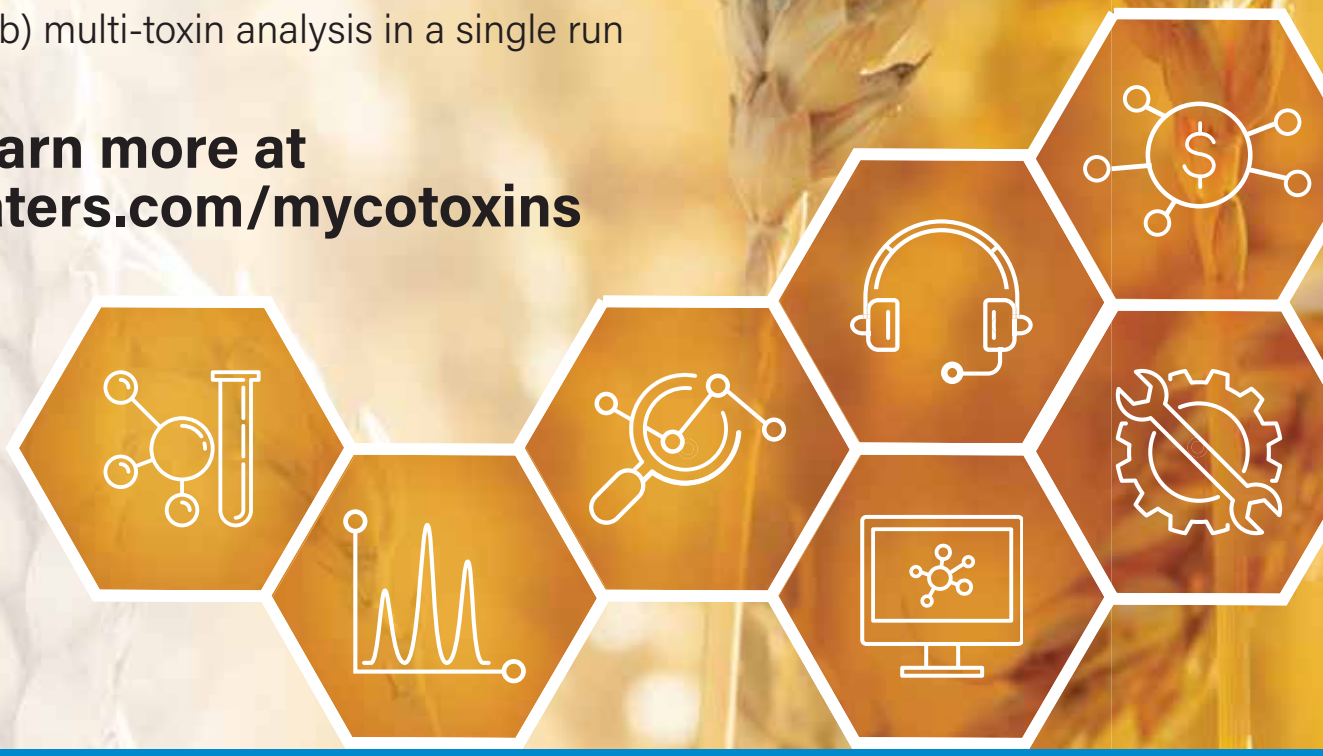

Waters™ | VICAM™

WORKSHOP PROGRAMME

TUESDAY 17 MAY 2022

12:45 – 13:45

SOLUTIONS FOR MYCOTOXINS AND ALKALOIDS DETECTION FROM FIELD TO LAB

SPONSORED BY VICAM

In our workshop we will take you through a 4-station process where we will showcase how to achieve results fast using our strip tests. For example, we will show you how you can reduce consumables, time, and waste up to 77% while obtaining precise results for up to six mycotoxins in less than ten minutes. We will introduce the latest news on our solutions for food testing laboratories needing to develop HPLC and LC-MS/MS confirmatory methods for mycotoxins and alkaloids. Finally, we will show you how to quickly process large sample batches and reduce data review time with the new state-of-the-art waters_connect for quantitation software.

TUESDAY 17 MAY 2022

12:45 – 13:45

QUALIT™ – SAMPLE PREPARATION AND QUALITY ASSURANCE TOOLS FOR MYCOTOXIN ANALYSIS

SPONSORED BY R-BIOPHARM AND TRILOGY ANALYTICAL LABORATORY

Mycotoxin contaminations of food and feed have a huge economic impact. Mycotoxin contaminations of crops are unavoidable, but mycotoxins can be managed. During the entire process from field to food or feed critical steps can be identified to monitor mycotoxins. It is well known that sampling plays an important role in the reliability of your result. Less well known is the influence of the sample preparation: sample grinding and sample size may have a big effect on the variabilities in your result. We will show results from different studies with cereal samples containing aflatoxin, deoxynivalenol, and zearalenone. To eliminate matrix effects a clean-up of the sample is necessary. For some samples a simple extraction is sufficient, but for other samples solid phase extraction/immunoaffinity extraction can make a big difference. But that is just one part of the story. You want to be sure you are making the correct decisions as well. QualiT™ is a toolbox developed by Trilogy Analytical Laboratory for quality control in mycotoxin analysis. QualiT™ offers (certified) reference materials, both as pure material and as well as naturally contaminated materials, quality control materials and analytical standards. Besides that, Trilogy offers additional useful tools for sample preparation and sample clean-up and knowledge database, collecting 20 years of experience as an (ISO 17025 accredited) food testing lab.

WMF YOUNG SCIENTISTS FORUM

TUESDAY 17 MAY 2022

17:30 – 18:30

THE CHALLENGE OF MYCOTOXINS IN SUSTAINABLE AGRICULTURE – HOW TO FIND OPPORTUNITIES IN EMERGING GLOBAL CHALLENGES THAT AFFECT OUR FUTURE

SPONSORED BY TROUW NUTRITION

Trouw Nutrition is hosting an open session for all young scientists, researchers, and students attending The World Mycotoxin Forum®. During this – very informal – discussion, we aim to connect and inspire the next generation of mycotoxin specialists and get input on various themes and statements. After a brief introduction to the discussion by Dr Paul Bruinenberg, we will gather for a brainstorm session and discussion, while enjoying some snacks and beverages. Besides gaining insights in current research and future developments, the session is also aimed to get to know each other and network. Together we will have small group discussions on topics such as climate change impact on mycotoxin prevalence, monitoring and prediction systems, and raising awareness on prevention and mitigation of mycotoxins. After the brainstorm, we look forward to getting your input and ideas during an Open Mic discussion. Like in any brainstorm, there are no wrong answers. Your input and ideas are valuable and appreciated, so don't miss this opportunity to make yourself heard.

VISIT BARILLA PLANT
(reservations only)

WEDNESDAY 18 MAY 2022

13:30 – 16:30

The World Mycotoxin Forum® would like to acknowledge the help from Barilla Plant responsibles and operators for their support into organizing and guiding the excursion

Please note: Participants who have registered for the excursion, shall gather at 13:30 sharp at the Auditorium Paganini parking space.

13:30	Departure by bus from the Auditorium Paganini parking space
13:50 – 14:10	Arrival and registration at the plant
14:10 – 14:30	Virtual tour at Barilla Research Labs Michele Suman, Food Safety & Authenticity Research Manager
14:30 – 14:50	Introduction on Barilla Plant Claudio Dallagiacoma, Barilla Pedrignano Plant Responsible
14:50 – 15:50	Guided tour at Barilla Pasta Plant
15:50 – 16:10	Refresh & greetings
16:30	Arrival back at the Auditorium Paganini parking space

MYCOTOXIN RISK MANAGEMENT

Selko®

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

Learn more, visit www.selko.com

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YOUR COMPLETE SOLUTION FOR MYCOTOXIN SCREENING

Eurofins Technologies provides different product lines to suit all needs for the **reliable and effective mycotoxins analysis** together with **automated applications** in cereals, feed, wine, coffee, spices as well as its internationally renowned ELISA kits for aflatoxin M1 in milk and dairy products.
The **new rapid patulin test kit** offers the quantitative and/or qualitative detection of patulin, a harmful mycotoxin found in fruits and fruit juices.

Multiple Mycotoxin Kits can be run on The Bolt™ Automated Platform

- I'screen product line
- Celer product line
- B ZERO product line

Other validated protocols are also available on The Bolt™

For more information, visit our booth.

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LECTURE ABSTRACTS

PLENARY SESSION
ADDRESSING TODAY'S AND TOMORROW'S CHALLENGES

Since its establishment in 2001, The World Mycotoxin Forum covers the latest issues in mycotoxin management targeting at everyone working in the mycotoxin space – researchers, food and feed industry, laboratories, policy makers, and enforcement agencies from around the world. The aim of this year's conference to elaborate further on key strategic issues looking forward, amid the current challenges.

MYCOTOXINS FROM THE FUNGUS' PERSPECTIVE: WHEN, HOW, WHERE, AND WHY?

Joseph Strauss

Department of Applied Genetics and Cell Biology, Institute for Microbial Genetics and Research Platform 'Bioactive Microbial Metabolites', BOKU Vienna, Austria

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Mycotoxins are metabolic products of fungi that accumulate in food and feed during their production or storage and which have acute or chronic toxicity already at very low concentrations. While it is obvious that such contaminations must be minimized and thoroughly monitored, it is less recognized that only a deep understanding of the process that triggers mycotoxin formation in the fungi can help us to suppress them. Mycotoxins, from a fungus' perspective, are metabolically costly substances and they are only produced upon a specific need. It must be 'clear' to the fungal cells that their biosynthesis will provide a competitive advantage under the given challenging condition. In this context, mycotoxins serve as virulence factors during pathogenesis, as growth inhibitors against bacterial or fungal competitors, as stress protectives or as signals for their own reproduction. For each of these challenges, it would be optimal to express only the specific 'useful' subset of bioactive substances from the whole armoury of literally hundreds of different metabolites that can theoretically be produced by a given species. But how do fungal cells judge which metabolite or which cocktail of mycotoxins to produce in a certain spatio-temporal context?

Extensive molecular genetic work by numerous groups, including ours, has by now revealed complex signalling networks that turn on subsets of specific biosynthetic gene clusters encoding the mycotoxin biosynthetic enzymes and regulators. Using surface receptors and nuclear relay stations, these systems decode external physical, chemical or biological signals and create an appropriate biochemical answer. Like that, a precisely targeted and energetically economized metabolic response is composed to a given environmental challenge. However, there is still much to learn on the details how specific signals are correctly decoded by the fungal cells and how the mycotoxins and other secondary metabolites are then synthesized accordingly, deposited, and employed. But definitely, this knowledge will be instrumental for us to device successful mycotoxin reduction strategies in food and feed production or storage.

THE NEXUS BETWEEN FOOD SAFETY AND FOOD SECURITY: THE CASE OF MYCOTOXINS

Cornelia Boesch and Catherine Bessy

Food and Agriculture Organization of the United Nations (FAO), Italy

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Fungal food spoilage and the detrimental effect of contaminated foods on the health of humans and animals are often perceived as distinct technical disciplines within policy discourses. While within academia and specialized public and private organizations the threat of mycotoxin contaminated staple foods to achieving food security is recognized and targeted approaches are proposed, the discourse at policy level is hampered due to the daunting challenges faced when aiming to design initiatives of preventive control. Consequently, approaches to mitigate the risk of fungal contamination within national food control systems often lack coherency and thus remain ineffective. The sustained research

undertaken by academia (often in collaboration with the business sector) continues to confirm the seriousness of the issue, especially in contexts of food insecurity. This growing body of research findings is a crucial resource for the development of guidance for regulatory frameworks that offer a pathway for countries to develop solutions for mycotoxin control adapted to their context. As the knowledge and methodologies to control mycotoxins in foods are known, the challenge remains to develop a narrative of feasible actions that policy makers can adopt. Given that a constant concern about hunger and malnutrition has been a main barrier to addressing the issue of mycotoxins, the current unprecedented challenges the food and agriculture sector is facing, indicate that challenging times lie ahead for programmes of effective mycotoxin control.

TOXIGENIC FUNGI, MYCOTOXINS, AND THE GLASGOW CLIMATE PACT (COP26): WHAT DOES THE FUTURE HOLD?

Paola Battilani

Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Italy

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Climate change is ongoing, it is a tangible issue and it is following a trend that, according to predictions, can lead to different scenarios, summarized with a supposed increase in temperature. The +2°C scenario is considered the most probable actually and Glasgow Climate Pact (COP26) agreed on taking actions to limit temperature increase to 1.5°C. A 0.5°C difference seems irrelevant, but at global level it can make the difference between a disaster and a manageable event. Moving from the summary number '+1.5°C' we consequently limit CO_2 increase, geographically and seasonally distribution of rainfall and the occurrence of extreme events, compared to '+2°C'.

Mycotoxin occurrence results from the interaction among producing fungi, host crops and the environment and the effect of changing the latter, due to climate change, was confirmed both on crops and fungi and on mycotoxin occurrence at large and small scale. Temperature increase shorten crop growing cycle and allow changes in crop growing areas. Fungal distribution and prevalence are confirmed as changing and the consolidated knowledge on global mycotoxin occurrence was undermined while the emerging issue of fungi/mycotoxin co-occurrence was stressed. Modified mycotoxins, in addition to the native forms, must be considered, resulting from the plant or the fungus actions, or from their crosstalk reaction, consistently affected by climate. In addition, some neglected mycotoxins are gaining relevance, and this cannot be disconnected by climate conditions.

Climate change matters policy makers, because of strategic decision to be taken, but it also influences farmers and crop chain stakeholders, in charge of tactic decisions. All countries are involved, even with differences; therefore, boosted efforts are needed to face the future. The outcome key words from COP26, that are leading the global action plan (mitigation, adaptation, collaboration, and finance) must be shared to achieve the objective of managing the future scenario of mycotoxin occurrence and deliver people healthy food while keep our planet healthy.

FINDING THE BALANCE BETWEEN SAFETY AND SUSTAINABILITY IN A CIRCULAR FOOD SYSTEM: THE CASE OF FOOD PROCESSING BY PRODUCTS

Madhura Rao

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Food businesses in the European Union (EU) are preparing for a carbon-neutral future by gradually transitioning to a circular way of operating. In the years to come, businesses will be expected to work with closed loops of resources to minimize waste and reduce the impact of their actions on the environment. In the context of food supply chains, closing the loop would mean making better use of

food waste, surplus, and by-products. It is estimated that around 88 million tons of food is wasted in Europe every year. While saving at least a part of this amount might seem like an irreproachable goal, some of the most attractive solutions may make food less safe for consumers. Against the background of a changing climate, food safety hazards such as mycotoxins are likely to become increasingly persistent in circular food supply chains. Current EU food safety legislation does not sufficiently accommodate this risk in the case of valorised food processing by-products. To enable a future where our food supply is safe, nutritious, and sustainable at the same time, food business operators and legislators must take cognizance of emerging risks associated with mycotoxin contamination and work towards effective management strategies.

ADVANCING THE CIRCULAR BIONUTRIENT ECONOMY TO COMBAT MYCOTOXINS

Rebecca Nelson

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Mycotoxin contamination of the food system is both an important problem and a symptom of wider stresses on the food system. In this presentation, the challenge of mycotoxin reduction will be considered through a 'One Health' lens, with a focus on the role of soil health in ensuring plant and human health. In this context, the recycling of carbon and nutrients from organic underutilized resources ('OURs'), including human excreta, into soils is proposed and progress toward the circular bionutrient economy (CBE) is reported.

Plant health depends, to a substantial extent, upon the health of soils. When soil health is low or compromised, plant stress makes crops vulnerable to colonization by mycotoxigenic fungi. Soils in many parts of the tropical world are inherently low in organic matter and farmers have difficulty maintaining adequate soil carbon and nutrients. In many parts of Africa where maize is a staple food, pervasive crop stress leads to widespread contamination of staple foods by aflatoxin and fumonsin, particularly when maize grown under drought-prone conditions. The lack of effective implementation of regulatory frameworks means that populations are exposed to high levels of mycotoxins, both chronically and episodically. Soil organic matter (SOM) is essential to soil health, playing a key role in holding water and cycling nutrients. SOM is inherently low in African soils, and the high temperatures make it difficult to build SOM. As fertilizer prices skyrocket, farmers' access to the crop nutrients in the form of conventional fertilizers becomes increasingly tenuous. The circular bionutrient economy offers potential opportunities for farmers, especially those struggling to grow safe food in low-resource contexts, to enhance their SOM and soil nutrients.

There are many potential OURs in a typical African context, including market food scraps, agricultural and agro-industrial by-products, and human excreta. Many apparent wastes are actually utilized in one way or another, but urine and faeces are only beginning to be considered as useful resources. Excreta are massive pollutants, particularly in urban and aquatic environments, causing ill health and harmful algal blooms. The potential fertilizer value of excreta is well established, but the practices for safe use are poorly developed and rarely implemented.

The Soil Factory Network (SFN) brings an incipient collaborative group together across the USA, Kenya, and India to advance the CBE. The SFN includes researchers, artists, artisans, community-based organizations, and others in an effort to develop better technologies for carbon and nutrient recovery, and to stimulate people to reconsider the ways in which their bodily functions can contribute to sustainable and healthy food systems. On a hyper-local basis, urine can be used as a garden fertilizer, in diverse contexts from urban to rural. On regional and global levels, transformation is required to produce concentrated, stable fertilizers. The presentation will note challenges and opportunities on socio-cultural, technical, and regulatory frontiers.

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 88-164).

P17
Natural toxins in plant commodities used in plant-based meat alternatives: A systematic review
Octavian Augustin Mihalache
Department of Food and Drug, University of Parma, Italy

P28
Combinatory effects of endocrine disruptive mycotoxins and foodborne xenoestrogens on breast cancer progression
Giorgia Del Favero[1,2]
[1]Department for Food Chemistry and Toxicology, University of Vienna, Austria; [2]Core Facility Multimodal Imaging, Faculty of Chemistry, University of Vienna, Austria

P31
Determination of pharmacokinetic parameters of efavirenz and aflatoxin B1: An approach to unravel possible interactions
Orphélie Lootens[1,2,3]
[1]Centre of Excellence in Mycotoxicology and Public Health, Department of Bioanalysis, Ghent University, Belgium; [2]Laboratory of Medical Biochemistry and Clinical Analysis, Department of Bioanalysis, Ghent University, Belgium; [3]MYTOX-SOUTH, International Thematic Network, Ghent University, Ghent, Belgium

P78
Gene editing of A*spergillus niger CBS 513.88* using a CRISPR-Cas9 based system
Carolina Gómez-Albarrán
Department of Genetics, Physiology and Microbiology, Complutense University of Madrid, Spain

PLENARY SESSION
COMPANY PITCHES

Short presentations (5-minutes) by sponsors to inspire the audience to visit their booths.

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

R-Biopharm is a leading developer of test solutions for clinical diagnostics and food and feed analysis. R-Biopharm's analytical tests offer certainty along the whole nutritional food chain – from safe animal feed and safe food to individualized nutrition and all the way to diagnosis of diseases. Founded in 1988, we have over 1,300 employees worldwide and are successfully represented in more than 120 countries by having 120 distributors and 28 subsidiaries throughout Germany, the USA, Great Britain, France, Italy, Spain, Belgium, Latin America, Brazil, Australia, India, China, and the Netherlands. Our extensive product range offers best solutions for reliable food and feed analyses. A variety of different test systems enables detection of mycotoxins, allergens or illegal residues and microbiological contamination. For mycotoxin testing we offer a very broad portfolio of tests, clean-up columns, quality control materials, standards, and analytical services. With innovative tools like smartphone-based measurements devices and app to evaluate result and share data real-time, R-Biopharm entered a new era of mycotoxin analysis. R-Biopharm is family-owned in second generation and combines development, production and sales under a single roof.

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https://www.vicam.com

Waters Corporation is the world's leading specialty measurement company, and has pioneered chromatography, mass spectrometry and thermal analysis innovations for more than 60 years. Waters delivers solutions for food and environmental, pharmaceutical and biopharmaceutical, material science industries as well as for clinical diagnostics, forensic toxicology, and biomedical research sectors. Our solutions for natural toxins and food safety include lateral flow strips and a range of targeted, multi-mycotoxins and alkaloids methods using liquid chromatography with mass spectrometry or fluorescence detection. Our VICAM brand has gained a worldwide reputation for leadership in the development of rapid diagnostic solutions. Today, VICAM is the trusted partner of the global food and agricultural industry. Customers in many countries depend on our reliable, cost-effective frequent monitoring solutions to achieve their quality, compliance, and business objectives. With a global workforce of more than 7,400 employees, Waters operates in 35 countries, including 14 manufacturing facilities and our products are available in more than 100 countries. Our diverse organization is well-positioned to deliver benefit through innovations that enhance human health and well-being.

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Royal DSM is a global, purpose-led, science-based company active in Nutrition, Health and Sustainable Living. DSM's purpose is to create brighter lives for all. DSM addresses with its products and solutions some of the world's biggest challenges while simultaneously creating economic, environmental, and societal value for all its stakeholders – customers, employees, shareholders, and society at large. DSM delivers innovative solutions for human nutrition, animal nutrition, personal care and aroma, medical devices, green products and applications, and new mobility and connectivity. DSM and its associated companies deliver annual net sales of about €10 billion with approximately 23,000 employees. The company was founded in 1902 and is listed on Euronext Amsterdam.

ROMER LABS – Making the World´s Food Safer®
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Romer Labs is a leading global supplier of diagnostic solutions for food and feed safety. With more than 35 years of experience in the area of mycotoxins, Romer Labs offers the most comprehensive portfolio of mycotoxin testing solutions on the market. This includes, among others, rapid tests, reference materials, clean-up columns and testing services in 4 accredited, full-service laboratories in Austria, US, China, and Singapore. Our key objective at Romer Labs is to provide scientifically sound, high-quality products and an exceptional service, in line with our mission – Making the World's Food Safer®.

TROUW NUTRITION
https://www.trouwnutrition.com

Trouw Nutrition is Nutreco's livestock feed business line and a global leader in feed, farm, and health. We have a 90-year history of developing innovative feed products and more sustainable ways of raising healthy farm animals and companion animals. With a presence in 105 countries and 22 manufacturing plants, we are a one-stop-shop for feed specialities, feed additives, premixes, nutritional models, and customised, integrated services. We are everywhere farmers and home-mixers, feed producers, integrators and distributors need us to be. Animal nutrition and good farm management have the power to transform our industry and even our planet. To solve the challenges facing our feed-to-food chain, Trouw Nutrition attracts the smartest minds. We have a dedicated team of 8,300 and a global network to help our customers feed the future. Our global brands include LifeStart, NutriOpt, Milkiwean, Gestawean, ChickCare, Maxcare, Sprayfo and Selko. Selko is the feed additive brand of Trouw Nutrition and aims to provide optimal feed additive solutions that are customised to customer's business needs. Selko offers science-based, innovative programmes and bespoke services that really make a difference for grain processors, feed producers, livestock and aquatic animal producers and the food- and beverage industry. These include solutions for feed safety, mycotoxin risk management, *Salmonella* control, gut health, antimicrobial resistance, trace mineral optimisation and food-by product preservation.

ADISSEO
https://www.adisseo.com

Adisseo is one of the world's leading experts in feed additives. The group relies on its 10 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 2,345 employees, it serves around 3,900 customers in over 110 different countries through its global distribution network. In 2020, Adisseo achieved a turnover of over 1.51 billion Euros. Adisseo is one of the main subsidiaries of China National BlueStar, leader in the Chinese chemical industry with nearly 20,800 employees and a turnover of 7,5 billion euros. Adisseo is listed on the Shanghai Stock Exchange. 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 mobile app. Moreover, Adisseo has also developed a portfolio of solutions composed of: Unike® Plus, Maximum protection against challenges posed by broad-spectrum mycotoxin contamination; Toxy-Nil® Plus, powerful protection against broad-spectrum mycotoxin contamination; and Toxy-Nil®, reliable protection against moderate-level mycotoxin contamination… in order to propose the best-suited solution to a specific challenge!

EUROFINS TECHNOLOGIES
https://www.eurofins-technologies.com

Eurofins Technologies 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, biopharma, and clinical industries. Eurofins Tecna, part of the Eurofins Technologies network, was founded in 1994 in Trieste, Italy, with a focus on the development of analytical kits for the research of mycotoxins and veterinary drug residues in food and animal feed. The company now provides a broad range of high-quality mycotoxin and VDR test kits and automation solutions to meet specific client needs. Based on our close field relationship with our partners, our R&D team is constantly working on new diagnostics solutions, responding to the real needs of those who must control every day the quality of their ingredients, semi-finished and finished products. Our goal is to supply the food and feed chain with reliable, affordable, robust, and flexible analytical solutions. We strive to provide the best service both in R&D and in technical education: our product specialists are available to train, support and teach companies how to select the best analytical solution for their needs. Since 2001 Eurofins Tecna has adopted an ISO 9001 Certified Quality Management System for 'Research, development, production, sale and trading of *in vitro* diagnostics for agriculture and food. Our experts are able to customize programs to serve specific needs with competitive pricing and high-quality standards. Contact us today and let us know how we can help you!

PHILEO
https://phileo-lesaffre.com

We daily support our partners, from global industry players to family farmers, in their transition towards sustainable good practices. With them, we develop tailor-made solutions for preventive care allowing a limited and responsible use of antibiotics by mastering microbiota and immunity. Our shared ambition is to raise healthy animals while sparing planet resources and energy. Our rich collection of proprietary probiotic strains allows us to develop evidence-based solutions taking into account animal species' diversity ranging from pets, livestock, poultry, fish to insects to overcome the increasing complexity of food production. *Let's act with nature for animal care.* Safglucan® is protecting farming animals against immunotoxicity and intestinal damages induced by DON. Safglucan® with premium purified 1.3 and 1.6 ß-glucans helps to mitigate the negative effects of DON by promoting a good intestinal health and therefore regulating susceptibility to infectious diseases and pathogen translocation. During the WMF pick up your copy of the white book 'How to mitigate risk associated with DON' at the Phileo booth or visit our website.

LIBIOS
https://libios.fr

LIBIOS, a French Biotech company, established in 2006 by Boutros Kerbaje. Founder and manager, he has extensive scientific and commercial experience in the field of tests and analysis as well as diagnostic solutions in food safety applications. LIBIOS is specialized in the development, production and worldwide marketing of kits and reagents for food/feed safety and traceability, especially for mycotoxin detection by HPLC and LC-MS users. Up to 20 LIB'UP® certified mycotoxins C13 uniformly and fully labelled internal standards solutions have been released for LC-MS equipped QC labs. LIB'UP®, LIBIOS trademark, is already a complete range of ready to use certified solutions for native and C13 fully labelled mycotoxins. Immunoaffinity, clean-up SPE columns and FARLIB® ECD Cells complete our range of appreciated tools for mycotoxins analysis on the market. Our expertise in production, extraction, and measurement of certified C13 stable isotope internal standards guarantees the food safety lab network reliable and safe function, compliant at national, European, and international levels. Today, reliable stable labelled isotope internal standards in quantitative analysis utilising LC-MS/MS techniques are an essential part of any reliable analytical approach. Our convenient packaging, relevant concentrations and choice of solvents are advantageous tools for both public and private laboratories and have been described as such by our customers. Discovery of new food safety hazards and progress in science and technology demands better analytical approaches. As mentioned above, at LIBIOS we focus our efforts on innovation to deliver such demands. We offer the industry practical solutions that ensure continuous improvement of analytical capabilities, which guarantees food safety for all. Our value is always on helping customers find appropriate technical solutions to ensure safe food for the world's growing population, and we frequently offer our two cents in numerous technical official committees.

ALLTECH – The Evolution of Mycotoxin Management Strategies and Beyond
https://www.knowmycotoxins.com

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 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 lltech, 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 programme provides a complete holistic solution to help producers take control of mycotoxin contamination. The programme 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 programme 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.

ENVIROLOGIX – Diagnostic Solutions, Built for the Grain Industry
https://www.envirologix.com

EnviroLogix has spent over twenty-five years dedicated to the development of rapid mycotoxin and GMO detection technology. This technology defines the nature of today's agricultural supply chain. That drive to pioneer continues today, as we evolve our portfolio with smart, simple diagnostic solutions built for the grain industry – in the field, in the lab, and in the cloud. From the beginning, our motivation has been the pursuit of innovative ways to help our customers solve their problems. We partner with businesses across the global agriculture industry, from leading life science companies working on new technologies, to farms, grain elevators and mills interested in making informed operational decisions. We take pride in delivering the point-of-need and laboratory test results that are helping our global customers. Our products determine greater degrees of mycotoxin contamination and deliver results on the most advanced diagnostic readers available. Quantitative mycotoxin test results are obtained using EnviroLogix Quick Scan, a system that combines digital imaging technology with advanced mathematical processing to provide rapid, objective, and quantitative results for a variety of mycotoxin and GMO test kits. Our TotalTox Comb is industry's fastest solution for the simultaneous extraction and reading of multiple mycotoxins. To serve the growing needs of our customers and our business, we value the diverse knowledge and experience of all team members. Our passionate commitment to research and development continues to drive this generation of grain testing innovation, and the next. It's the work that's put us where we intend to stay... one step ahead in the field.

MIXSCIENCE
https://www.mixscience.eu

MiXscience, an innovative company specialized in animal nutrition and production, develops differentiated products and services for sustainable agriculture:

- respectful of animal welfare and health;
- playing an active role in the protection of our environment; AND
- producing food of high sanitary and nutritional quality.

IMPEXTRACO
https://www.impextraco.com

Impextraco develops and produces feed ingredients that protect animal health and enhance productivity. Through the power of better nutrition, we improve the life of animals and people around the globe. 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. Our specialty additives provide functional feed ingredients which protect the animals as well as their feed, ensuring food safety and enhancing profitability. All made with the highest quality antioxidants, mould inhibitors, mycotoxin eliminators, salmonella inhibitors, acidifiers, enzymes, and prebiotics. Our single ingredients are a comprehensive range, including vitamins, micro minerals, amino acids, antioxidants, growth enhancers, anticoccidials, colouring agents and organic acid and their salts. All enhancing the nutritional value of the feed in order to bring superior performance to your animal production. To make sure you get the best feed ingredients possible, we strongly emphasise on R&D and quality control. Through advanced production facilities and experimental units, we deliver cost-effective, efficient, and sustainable solutions. The whole of our production, research, warehousing, and distribution facilities complies with the highest European Union and international quality control standards, such as GMP. They meet the most rigorous quality checks, physical inspections, manufacturing best practices and comprehensive traceability requirements. What and wherever your needs are, you can safely rely on Impextraco's full commitment to your success.

CARGILL
https://www.cargill.com

Cargill's 155,000 employees across 70 countries work relentlessly to achieve our purpose of nourishing the world in a safe, responsible, and sustainable way. Every day, we connect farmers with markets, customers with ingredients, and people and animals with the food they need to thrive. We combine 156 years of experience with new technologies and insights to serve as a trusted partner for food, agriculture, financial and industrial customers in more than 125 countries. Side-by-side, we are building a stronger, sustainable future for agriculture. Cargill Animal Nutrition provides feed additives designed to bring feed manufacturers, animal producers, and retailers innovative and impactful feed-additive solutions to boost profitability and address market challenges. Our additives put our feed industry leadership into your hands, helping you propel animal performance and business profitability to new levels. Our additives combine extensive research, feed-application insights, and global expertise in selecting, developing, and commercializing the most impactful additive products. This makes us a trusted advisor to customers around the globe. Our additives offering includes both multi-species lines and dedicated species solutions: Notox™, a range of solutions (both feeding programs and a unique online risk management tool (mycotoxins.com) that leverages the world's largest mycotoxin contamination pattern database) to support animal performance under mycotoxins challenge conditions; Valido™, portfolio of dedicated, sustainable solutions formulated specifically to support ruminant performance; Intella™, portfolio of dedicated, sustainable solutions formulated specifically to support poultry performance; Cinergy, a range of gut health solutions for swine, specifically selected for their targeted mode of action, which ultimately improves the animal performance; Proviox™ products are made of specific natural antioxidants blends that support an animal's performance throughout their whole lifecycle, especially during challenging production phases (reproduction, weaning, transportation, etc.); and Enzae® best in class enzymes solutions to optimize feed cost and consistently improve performance. For more information, visit the company's website.

PROGNOSIS BIOTECH – Accuracy through Innovation
https://www.prognosis-biotech.com

ProGnosis Biotech is an innovative biotechnology company, specialized in developing and manufacturing next-generation immunoassays for food & feed safety and clinical diagnostics. Based in Larissa, Greece, and also having offices in Spain, Serbia and China, ProGnosis Biotech is an export-oriented enterprise which, through a large distribution network, exports ELISA kits and Lateral-flow tests to more than 40 countries worldwide. In order to consistently provide top-quality products, our company participates on a monthly basis in Proficiency Tests in the UK, France, Germany, Italy, and the USA. Additionally, ProGnosis Biotech offers validated products from highly esteemed organizations worldwide. ProGnosis Biotech is certified with ISO 9001:2015 for all the R&D and production procedures as well as with ISO 13485:2016 for the development and manufacturing of *in vitro* diagnostic medical devices. Our mission is to provide innovative, accurate, and simple solutions to the challenges presented in the food safety and clinical diagnostics industries.

AGRIMPROVE
https://www.agrimprove.com

Agrimprove is the functional feed ingredients brand of Royal Agrifirm Group, a cooperative of over 10,000 Dutch farmers. In support of farmers all over the world, we develop new ideas in the field of animal health and nutrition and grow these ideas into tangible improvement strategies that add value throughout the agri-food chain. In close collaboration with stakeholders in farming, food and feed production, and retail, we deploy the power of science to continuously improve the way we produce food protein for our growing global population. For animal protein producers and farmers, we strengthen their bottom line by improving their animals' health and performance, with a higher value end product as the final result. For retailers, we enable the supply of qualitative food produce that meets the demands of today's critical consumers. For regulators, we support future-proof health and food safety standards. And for consumers, we safeguard the sustainable availability of responsibly produced food for future generations.

OLMIX
https://www.olmix.com/

Olmix is the innovative specialist in mycotoxin risk management in animal nutrition. A desire to provide natural alternatives to agricultural additives led to the creation of Olmix Group in Bréhan, at the heart of Brittany, in 1995. In more than 25 years, the company has become one of the major global specialists in supplying marine algae-based solutions for the animal feed industry. One of its missions is to make effective use of an abundant untapped resource to promote sustainable food. This approach guides the company's teams worldwide in their work of extracting value from green, red, and brown algae. Innovation is at the heart of the strategy and its R&D team comprise health and nutrition experts, along with specialists in algae, clay minerals and trace elements. Since 2004, year of the patent and launch, Olmix Algo-clay technology has gained notoriety worldwide as an innovative solution to manage mycotoxin risk in animal production. Its efficacy has been demonstrated many times from experimental units to on-farm conditions in all species. Many scientific and technical trials have been run worldwide, and Olmix is constantly looking for new opportunities to prove the efficacy of its products under diverse conditions. Recent publications have highlighted the potency of the technology to reduce the transfer of ZEA and DON from sows to piglets (Santos *et al.*, 2021. Frontiers in Veterinary Science / SFR, Holland) and to reduce the deposition of fumonisins in broiler tissues (Guerre *et al.*, 2021. Toxins / ENVT, France). To accompany its customers to make decisions and efficiently use its solutions, Olmix has also developed a deep expertise in mycotoxin risk and management, and a full set of tools from the risk evaluation to the optimal solution. It goes from the understanding of the mycotoxins issues (Myco'essentials), the risk evaluation on farm (Myco'Evaluator), the confirmation of the contamination with the suitable strategy for analytical method for both raw materials and feed (Myco'Screen package), and Myco'Calculator to take profit of mycotoxin analysis to adjust the toxin binder dosage depending on analysis results and animal's conditions. Lastly Olmix launched Myco'simulator to visually illustrate mycotoxins features and their potential interaction with usually used binders. Today, the unique and efficient technology of Olmix and the deep expertise on mycotoxin risk management, make Olmix a leading company in the feed industry.

CHARM SCIENCES
https://www.charm.com

Charm Sciences is a world leader in food safety diagnostics. Speed, simplicity, and sensitivity make Charm ROSA® (Rapid One Step Assay) the standard in mycotoxin detection. Detect multiple mycotoxins at once from the same extraction sample using Charm's Water Extraction Technology (WET®) tests. Our portfolio includes aflatoxin, DON, fumonisin, ochratoxin, T-2/HT-2, and zearalenone. Rely on Charm for excellence in quality, innovation, and sensitivity to protect your brand!

NEOGEN
https://www.neogen.com

At Neogen, we have over 30 years' experience as a trusted partner for mycotoxin testing solutions. We are on hand to help you face any potential mycotoxin problems and ensure the safety and quality of your crops. Our solutions are available for the detection of aflatoxin, deoxynivalenol (DON), fumonisin, ochratoxin, T-2 / HT-2 toxin, and zearalenone and are available in a variety of formats that provide rapid, accurate results. Easy to use onsite tests: Our Reveal® lateral flow tests provide quantitative results in minutes. Perfect for use onsite, the tests have unmatched accuracy and reproducibility and can be ran by staff of all skill levels for quick and convenient testing. Results from our Reveal Q+ MAX range are interpreted using our innovative Raptor® platform. ELISA Tests: For those with a laboratory set up, we also offer our Veratox® quantitative ELISA assays for accurate, low level results. These tests offer results comparable to analytical methods, such as HPLC, and are an efficient workflow with only minimal training required. It is not just about our products; our experienced R&D, technical support and customer service teams are on hand to offer guidance and support every step of the way, including through our LabLive remote training tool, webinars, proficiency testing, mycotoxin reference material and much more. For more information visit us at the World Mycotoxin Forum or visit the company's website.

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

Patent Co is a global player in animal nutrition, focused on multi-mycotoxin solutions and natural approaches towards some of the most practical problems in animal nutrition. The company offers pioneering products and expert support to the global animal feed industry. Since our establishment in 1993, we have grown to become a worldwide prevention solutions provider. Our headquarters are in the heart of South-East Europe, in Mišićevo, Serbia, but we have a global reach in all continents. At Patent Co, we are committed to research and development and have a growing portfolio of products for poultry, swine, ruminants, and aquaculture, which can be divided into three focused sections: (i) mycotoxin control; (ii) phytogenics – natural based solutions; and (iii) vitamin and mineral premixes. *Mycotoxin control*. After creating and patenting Minazel, a flagship clinoptilolite-based product for mycotoxin prevention, we then launched a newer generation of mycotoxin adsorbent – Minazel Plus. This offered a 360° total mycotoxin protection approach, with the product making effective inroads into various animal farms and premix facilities and becoming recognized worldwide. Recognizing the changes in the industry and understanding the need for even more effective and wide spectrum mycotoxin solutions, we recently developed a state-of-the-art mycotoxin binder: MycoRaid. This is one of the most effective mycotoxin solutions available, with the MycoRaid formulation having a positive influence on animal l health, ensuring mycotoxin biotransformation, while providing hepato- and immuno-protection. Through close collaboration with both customers and academics we are utilizing a broad foundation of know-how to the global market, identifying new opportunities for improving animal feed which translates into improved animal health and performance. Efficacy and safety of all our natural products are ensured by regular monitoring processes, from raw materials procurement, through production, to final control and distribution. All QC procedures are in accordance with implemented practice of FAMI QS, HACCP and ISO 2200.

THERMO FISHER SCIENTIFIC
https://www.thermofisher.com

At Thermo Fisher Scientific we are always looking ahead to help you meet the increasing analytical food and beverage testing demands – in your timeframe, and with the high degree of accuracy your consumers and clients expect. We offer complete end-to-end food testing solutions and educational resources so that you can keep your focus where it should be – delivering safe, high-quality food products. International trade regulations make testing food and animal feed for biotoxins and mycotoxins a critical step before export. When food producers think of mycotoxins, they often think of the big six: aflatoxin, deoxynivalenol (DON), T-2 toxin, fumonisin, ochratoxin and zearalenone. However, the presence of emerging or masked mycotoxins presents a risk because most of the 600+ known mycotoxins often go undetected today. Thermo Fisher Scientific can help your analytical laboratory comply with global testing requirements and ensure that products meet the necessary standards to ensure toxin-free food products. We can help you fine-tune testing strategies to address the perennial multiple variable mycotoxin identification problem while protecting your animals and end consumers. Our broad range of innovative chromatography and mass spectrometry instruments, data management and analysis software, sample preparation and separation consumables and general laboratory products will help you achieve your food safety analysis goals. Our LC-MS workflow solutions using triple quadrupole or high-resolution accurate mass along with sophisticated data analysis and well characterized spectral cloud-base libraries are ready to help you enable lower costs per test for multiple mycotoxins, identify emerging dangers, and meet your regional analytical performance criteria.

BIOEASY
http://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 addictive residues, serving clients all over the world in fields like dairy, meat and seafood, feed, grain and oil, food processing, etc.

SAFEFOOD
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SAFE FOOD is a trading company which started its business in 1998 and is VICAM distributor for the Italian, French and Romanian market. This year, SAFEFOOD has introduced on the market ALFA, the first Automatic Lateral Flow Analysis machine that, exploiting the lateral flow test, performs a fully automatic mycotoxin analysis in just a few minutes, from the extraction to the final result. ALFA can analyse all mycotoxins together (aflatoxin, ochratoxin, zearalenone, deoxynivalenol, fumonisin and T-2/HT-2) in just over 10 minutes or a single mycotoxin sample every 2’30” minutes. ALFA can work anywhere and the ALFA Software can be connected with any business management software for automatic data transfer.

SESSION 1
ANIMAL HEALTH AND PRODUCTIVITY

Knowing the effects that mycotoxins have on animal health and productivity is essential. In this session, contemporary issues will pass in review.

EXPLORING KNOWLEDGE GAPS ON THE IMPACT OF MYCOTOXINS ON POULTRY HEALTH

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The poultry sector, which has an extremely important place in terms of food safety and nutrition, is the fastest growing agricultural sub-sector, especially in developing countries. Despite substantial advancements in different aspects of the poultry industry, the sector will continue to face numerous challenges on a global basis, such as increased feed cost and continuous efforts to get a better understanding of available, more sustainable, alternative feed ingredients; antimicrobial resistance; poultry welfare-related issues; nutrition-related environmental issues; food safety; and the emergence and re-emergence of diseases. The presence of mycotoxins in feed represents a severe threat for animal health and welfare and poses relevant research challenges in the field of feed toxicology. Poultry species are considered to be less sensitive to mycotoxins, particularly *Fusarium* toxins, compared to other species, such as pigs. Although symptoms of clinical mycotoxicosis are less frequently observed, recent research clearly demonstrated that even low to moderate concentrations of mycotoxins negatively affect intestinal health, immune function and/or animal susceptibility to infectious diseases.

Research has focused in the past mainly on exposure to a single mycotoxin such as aflatoxins, ochratoxin A, deoxynivalenol, T-2 toxin, fumonisins, and zearalenone. In contrast, mycotoxin co-occurrence is a common feature. The toxic effect of the mycotoxin mixture can be more severe than when the mycotoxins occur alone. Besides, toxicological data about the frequently occurring modified mycotoxins and emerging mycotoxins is rather limited. In addition to the direct effects of mycotoxins on poultry health, more research is also necessary to explore if mycotoxins also have an indirect health impact as predisposing factor in the pathogenesis of viral poultry diseases, in vaccine and therapy failure, in food safety problems such as *Salmonella* contamination, and welfare issues associated with for example bacterial chondronecrosis with osteomyelitis. Finally, also the impact of mycotoxins on stress resilience and gut-brain communication are interesting fields for future research.

Energy and protein sources constitute a significant part of the cost in poultry feeds. New feedstuffs may offer tantalizing potential to meet nutritional and environmental goals, but also come with challenges and risks. Therefore, besides exploring the nutritional and technical aspects, it will be of major importance to also evaluate mycotoxin occurrence in these novel feed ingredients, and even bioconverting capacity when studying insects. Furthermore, alternative chicken production systems (e.g., organic, slow-growing breeds) are on the increase in many EU countries. More data is needed, to evaluate the impact of mycotoxins on poultry health in these new production systems. Finally, the poultry industry in developing countries is an essential subsector of agriculture, providing food, employment, and other economic resources for these regions. Mycotoxin research in developing countries have mainly focused on mycotoxin occurrence in poultry feed, but data on the impact of mycotoxins on animal health and low-cost mitigation strategies in these production systems is limited.

INTESTINAL TOXICITY OF NX, A NEW TYPE A TRICHOTHECENE

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NX is a recently discovered type A trichothecene produced by *Fusarium graminearum* with limited information on its toxicity. NX is structurally similar to deoxynivalenol (DON), only differing by the keto group at C8. Because of the structural similarity of the two toxins as well as their potential co-occurrence in food and feed, it is of interest to determine the toxicity of this new compound. A similar decrease in viability of human intestinal Caco-2 cells upon 24 h of exposure to 3 µM NX or DON. Histological observations in porcine jejunal explants exposed for 4 h to 10 µM of the toxins showed interstitial oedema and cellular debris. Explants exposed to NX also displayed cell vacuolization, a broken epithelial barrier and high loss of villi. Whole transcriptome profiling revealed that NX and DON modulated 369 and 146 genes, respectively. Functional analyses indicated that the two toxins regulate the same gene networks and signalling pathways mainly: cell proliferation, differentiation, apoptosis and growth, and particularly immune and pro-inflammatory responses. Greater transcriptional impacts were observed with NX than with DON.

The protein composition of the extracellular media of pig intestinal explants (secretome) exposed to 10 µM of toxins were also compared. Two complementary quantitative proteomic approaches (a gel-based and a gel-free approach) identified 18 and 23 differentially abundant proteins (DAPs) for DON and NX, respectively, compared to controls. Functional analysis suggested that, whereas DON toxicity was associated with decreased cell viability and cell destruction, NX toxicity was associated with an enrichment of mitochondrial proteins in the secretome. The presence of these proteins may be associated with the already known ability of NX to induce an intestinal inflammation.

In conclusion, our data revealed that DON and NX target the intestine. Histological and transcriptomic analysis indicates a greater effect of NX compared to DON. Analysis of the extracellular proteome revealed an - increased leakage/secretion of mitochondrial proteins by NX, which may be a feature of the toxicity of this new toxin. Thus, what seems to be a minor structural change from DON, the elimination of the carbonyl moiety at the C8-position, has an important impact on its toxicity.

AFLATOXINS IN KENYA: A STORY OF MAIZE, MILK, AND MONEY

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Aflatoxins are carcinogenic substances produced by some Aspergillus spp. moulds and are widespread in tropical and sub-tropical areas including Kenya. Here, aflatoxin B1 (AFB1) is found in many staple foods and feeds and the metabolite aflatoxin M1 (AFM1) is frequently detected in milk. Reducing human exposure to aflatoxins is a priority in the country. This can be done through legislation and building capacity for better controls throughout the value chain, but because of limited testing, enforcement, and affordable aflatoxin control options, progress has been limited. In addition, animal consumption of

aflatoxins contributes to reduced production and animal disease, thus leading to reduced cost effectiveness of livestock production, impairing food security and poverty as well as animal welfare. In a series of research projects focusing on the maize and dairy value chains in Kenya, the International Livestock Research Institute (ILRI) and International Food Policy Research Institute (IFPRI), together with partners, have explored different ways to mitigate the burden of aflatoxins, as well as their cost effectiveness. Evidence from various sources spanning back to 1960 shows that aflatoxin contamination in maize, the primary staple food in Kenya, is high. The proportion of maize testing over the regulatory limit ranges between 7.5% and 83%, depending on the year, with average level of contamination as high as double the allowable level. We add to these findings with new evidence on high prevalence of aflatoxin contamination in milk and milk products, with up to 100% of the raw milk present in low-income households contaminated, though at lower levels than found in maize. Even dairy products in the formal market were contaminated, but to a lesser degree. Contamination occurs already at farm level, with highly varying levels of contamination in the feed, but there is also a high variation of AFM1 levels in Kenyan cows fed the same diet. Even though AFM1 contamination was common, our risk assessment estimated a very low contribution of cancer cases from AFM1 exposure (0.004 cases per 100,000). The health burden of exposure through maize is higher, leading to an estimated 1.07 cases of cancer per 100,000. As both cow's milk and maize are important to the diets of infants and young children in Kenya, there may be an additional burden depending on whether and how much aflatoxins contribute to stunting and immunosuppression. The best way to mitigate aflatoxins in food and feed is to stop the production of aflatoxins in the crop. Our research shows that farmers are often willing to implement control measures for maize they will consume themselves. However, the absence of a price premium for food and feed that meets the aflatoxin standard, and limited enforcement of associated regulations, means there is little incentive to invest effort or money to control aflatoxin in crops produced for sale. One option is to provide farmers with training and simple tools for aflatoxin control in regions with the highest levels of contamination. Another option for reducing aflatoxins in cow milk is the use of mycotoxin binders in animal feeds. In selected urban and peri-urban areas of Kenya, 9 different mycotoxin binder types were used. The binders were sold by 8% of agrovets and 33%. Inclusion of binders in animal feeds is not mandatory and there are no specific standards governing their use in Kenya. In our trials conducted in Kenyan cows and among smallholder dairy farmers, levels of aflatoxins were reduced and milk production increased in the trial farms compared to the control farms. Using binders in individual Kenyan dairy cows, fed naturally contaminated commercial feed, reduced the AFM1 levels, but even with high levels of binders, milk did not fall below 50 ppt, which means it would still not comply with European standards. While farmers were interested in purchasing the binder used in the trial, they could not find it in affordable volumes on the market, and they were sceptical about purchasing from local agrovets due to concerns about possible adulteration.

In conclusion, our results show that while there are options for reducing aflatoxins in Kenya, benefits to 7farmers are critical for motivating their use. As premium prices for aflatoxin-safe products are generally absent in this context, other levers for behaviour change, such as ease of post-harvest crop processing and increased milk volumes, can be used.

DEVELOPMENT OF A NEW MODEL TO ASSESS THE EFFECT OF MYCOTOXINS ON LIVESTOCK

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Quantifying the productive impacts of mycotoxins and their effects on the efficiency by which animals convert their feed is essential to understand further this complex health challenge. In this context, a systematic review followed by a meta-analytical study was performed to investigate the effects of mycotoxins on the productive performance of broilers and growing pigs.

The current study was developed using updated databases (PubMed, Scopus, and Web of Science) as a complementary approach to previously published meta-analysis (Andretta *et al.*, 2011, 2012, 2016; Kipper *et al.*, 2020). The selected studies aimed to report experimental challenges of pigs by mycotoxins

during any growth phase. The first search reached 3,464 records, which were all evaluated for duplicity and later for adherence to the research criteria. After applying our inclusion criteria, 104 articles were included in the database. The selected studies included 13,184 pigs distributed in 772 treatments. Of these, 51% were developed using mixed groups (females and males), 30% used males, 10% used females, and 9% did not describe this trait. Deoxynivalenol was the most studied mycotoxin in the database with 39 papers (considering only single contamination). Fumonisins were tested in 17 studies and aflatoxins in 19 papers, while other 29 studies reported challenges with pooled mycotoxins. Statistical analyses were performed using Minitab (Minitab for Windows, v. 19). The effect of the dietary mycotoxin concentration on the animal productive performance was studied using prediction equations with 4 models: linear, polynomial, logistic, exponential.

Most mycotoxin challenges impaired several performance responses compared to control groups. Feed intake reduction ($P < 0.05$) was observed in pigs that consumed diets containing aflatoxins ($\Delta = -13\%$) and deoxynivalenol ($\Delta = -9\%$). The challenge impact on growth responses was even higher, with weight gain reduction ($P < 0.05$) observed in pigs receiving diets containing aflatoxins ($\Delta = -15\%$) and deoxynivalenol ($\Delta = -11\%$). The feed efficiency ratio was also affected by the challenges. Thus, pigs fed diets containing aflatoxins ($\Delta = -5\%$; $P < 0.05$), deoxynivalenol ($\Delta = -6\%$; $P < 0.05$), and fumonisins ($\Delta = -8\%$; $P = 0.053$) showed lower feed efficiency than the non-challenged groups. Dietary concentrations of aflatoxins, deoxynivalenol, and fumonisins also showed a negative correlation with the variation of performance parameters with different patterns.

The implication of these findings is to predict the mycotoxin loss on productivity, and then to adapt the best strategy to mitigate the risk for animal producers.

EMERGING MYCOTOXINS AND EMERGENT EFFECTS

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Beuvericin (BEA) and enniatins (ENNs), are natural contaminants of food and feed. These emerging fusariotoxins were defined as ‘mycotoxins, which are neither routinely determined, nor legislatively regulated’ and data on toxicological risk are still required. Research studies, *in vitro* and *in vivo,* demonstrated an implication of BEA and ENNs on reproductive effects in farm animals.

BEA shows a dramatic inhibitory effect, as well as ENNs, on bovine granulosa cell steroidogenesis; furthermore, they exert a detrimental action on embryo development, ovarian function, and testicular function of animals. Considering the co-occurrence of emerging mycotoxins, further studies are necessary, in order to clarify the mechanisms of action of BEA and ENNs, alone and in mixture, and set up a correct risk assessment.

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DETERMINATION OF AFM1 IN RAW MILK TO EVALUATE EXPOSURE TO AFB1-CONTAMINTED FEED AND ITS CONTROL AT FARM LEVEL: A CASE STUDY

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Aflatoxin M1 (AFM1), a monohydroxylated metabolite of aflatoxin B1 in raw milk as a biomarker of AFB1 exposure was studied to evaluate feed contamination in Iran for a 12- year period, i.e., 2010-2021. Due to a sudden and drastic devaluation of the local currency during this period, an increase in aflatoxin M1 was noticed. This was probably due to using less toxin binders as a result of their increase in price. Further investigations of milk from selected farms revealed that with a change of toxin binder strategy, from mixing with Total Mixed Ration to mixing with cottonseed, a significant reduction of aflatoxin M1 levels in raw milk occurred, while quitting cottonseed resolved the problem at all.

MYCOTOXIN RISK MANAGEMENT: WHY ONE-SIZE-FITS-ALL DON'T WORK?

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Understanding the mycotoxin challenges in animals is never easy. This complexity stems from the fact that many moulds can produce several mycotoxins in raw materials and then several raw materials are used to make up the complete feed. On top of this, there are significant differences in how different species of animals respond to the mycotoxin exposure. For practical reasons, we accept the rapid analysis of 'Big 6' mycotoxins as the total mycotoxin toxicity of the feed, but we all know the existence of huge numbers of masked and emerging mycotoxins. These mycotoxins interact with 'Big 6' mycotoxins and increase the toxicity further. Due to the above reasons, mycotoxin risk management programme must be integrated. It must start with providing right conditions for crop growth and then extend on to the optimal management of crop harvesting, storage, transportation, feed production and feeding to the animals. The greater the deviations in these steps, the greater will be the risk and animal performance losses.

At the animal level, different mycotoxins have different effects on animals. Let us take the example of zearalenone. Pigs are very sensitive to this toxin while poultry can tolerate quite a high level. On the other hand, poultry and pig are sensitive to ochratoxin A (OTA) whereas ruminant animals can manage OTA quite well. Taking the example of deoxynivalenol, it can reduce feed intake and growth of pigs while the main effect in poultry is on the gut and immune systems. Some mycotoxins such as aflatoxins and ergot toxins can be bound very well inside the gastrointestinal tract of animals while there is low to moderate binding to other mycotoxins. Such observations make it clear to us that mycotoxin mitigation strategies within the body of animals should vary based on the species of animal and mycotoxin/s in question. In the last decade or so, there has been greater interest to understand the negative impact of mycotoxins on gut health and immune system of animals. Understanding such mycotoxin-specific effects will help us to formulate a feed strategy that can not only bind certain mycotoxins but also effectively manage the negative effects of mycotoxins on such systems. We have published quite a bit of research in this area and more work is in progress. As compared to monogastric animals, antioxidant system of ruminant animals, especially in dairy cows seems to be under great threat from mycotoxins. Mitigation strategy in this species must look at this key area more closely. Moreover, silages contribute additional mycotoxins to the toal mixed ration (TMR) of dairy cows. Such understandings need a closer collaboration with universities and scientific institutions across the world.

Although animal toxicity studies are limited in relation to emerging and masked mycotoxins, the feed industry is encouraged to investigate these toxins more seriously as unfolding their toxicity will explain much of the 'mycotoxin mystery'. We are conducting quite a few studies to bind these emerging

mycotoxins. Understanding the mycotoxin presence in the raw materials and feeds in the shortest time possible, helps animal industry to manage mycotoxins effectively. In addition to analysis, simple animal toxicity interpretation tools are necessary to employ proper preventive and control procedures.

MYCOTOXINS IN AQUACULTURE: OCCURRENCE IN FEED INGREDIENTS AND FEED - EFFECTS ON FISH PRODUCTIVITY AND HEALTH

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In the last decade, the aquafeed industry has made significant attempts to develop sustainable fish feeds by replacing traditionally used marine ingredients with novel ingredients derived from crops and their by-products. However, crops are susceptible to fungal species and their secondary metabolites, mycotoxins. Fungal growth is climate-dependent, and with the undergoing climate change, the quantity of mycotoxin-producing fungi in the environment and fungal community structure alter, raising the concerns in the aquaculture industry for mycotoxin contamination in the fish feeds. Firstly, we aimed to identify mycotoxin contamination patterns from a large data pool derived from wheat (n = 857), maize (n = 725), soybean meal (n = 139), and fish feed (n = 44) samples in European countries and based on sample analyses by liquid chromatography/tandem mass spectrometry (LC-MS/MS) in the period between 2012-2019.

Our results showed that a *Fusarium* mycotoxin, namely deoxynivalenol (DON), was present in maize (in 47% of the samples) > wheat (41%) > soybean meal (11%), and in fish feeds (48%). The next step was to explore the impact of DON on fish performance and health. Firstly, we employed a meta-analysis to estimate to which extent DON affects feed intake and growth performance in fish. Prediction equations showed that each additional mg/kg of DON in fish feed would reduce feed intake and growth exponentially by 13.2% and 16.5%, respectively. Responses were more severe for rainbow trout (18.8% and 20.0%), and thus it was characterised as the most sensitive fish species, among others. Secondly, we carried out an *in vivo* experiment using rainbow trout fingerlings (8 g) as a model species to investigate the impact of realistic DON doses (up to 1.6 mg/kg) on fish. Results showed that restrictive exposure to DON for six weeks reduced retained protein in trout treated with the highest DON levels. By feeding DON-contaminated diets for two more weeks *ad libitum*, we also reported suppressed body weight gain and altered feed efficiency. Our histological assessment revealed severe hepatic damage, which was alleviated overtime during restrictive exposure and aggravated after *ad libitum* exposure. Overall, DON is an underrated and highly present feed contaminant that threatens rainbow trout productivity and health at levels even below the current EC recommended limit (5,000 mg/kg).

SESSION 2
EXPOSURE ASSESSMENT AND HUMAN HEALTH

Developments and challenges in relation to mycotoxin exposure and health implications in humans will be presented.

DIETARY EXPOSURE ASSESSMENT TO MYCOTOXINS IN SUB-SAHARAN AFRICA: A PRACTICAL CASE STUDY

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A total diet study (TDS) is a cost-effective tool, which helps in prioritizing risk management approaches by narrowing down potential health concerns and identifying the main contributors. National Food Safety Authorities in Benin, Cameroon, Mali, and Nigeria supported by the Food and Agriculture Organization of the United Nations and the World Health Organization (WHO) conducted a TDS (2014-2018) to assess exposure levels of households to chemical hazards including mycotoxins, covering more than 90% of their diet. The analysis of a large spectrum of mycotoxins and other fungal, bacterial and plant secondary metabolites by liquid chromatography, coupled with tandem mass spectrometry was performed by the Department IFA-Tuln, BOKU Vienna, Austria.

Exposure to aflatoxin B1 (AFB1), fumonisins (FB_{tot}), sterigmatocystin, ochratoxin A, citrinin (CIT) was identified as a public health concern in some of the locations investigated. Considering the respective AFB1 exposure as well as national prevalence of hepatitis B virus infection, the impact on liver cancer was estimated. Extrapolating to the country populations, several thousands of additional cases of liver cancer are likely to occur in Benin, Cameroon, and Nigeria every year. Where our estimates exceeded 20 additional cases of liver cancer per 100 000 inhabitants per year, maize was the main contributor (>80%), followed by peanut or peanut oil (>10%). In the same locations, our estimates further showed that about 75% of households were exposed in excess of the FAO/WHO Joint Expert Committee on Food Additives (JECFA) Group Provisional Maximum Tolerable Daily Intake (PMTDI) of 2 µg/kg bw/d, for FB_{tot}. Maize almost exclusively contributed to the FB_{tot} exposure (>97%). Moreover, we estimated that the majority of the households living in these centres are also exposed to CIT above the EFSA PMTDI for nephrotoxicity of 0,2 µg/kg bw/d, due to the contribution of maize (>96%). In addition, the African TDS highlighted further health concerns in relation to co-exposure to lead, aluminium (nephrotoxic), polycyclic aromatic hydrocarbons (genotoxic carcinogen) and one organophosphate pesticides (chlorpyrifos, acethylcholine esterase inhibitor).

These data were uploaded into the WHO GEMS/Food database, and may, in conjunction with additional considerations pertaining to the mode of action, contribute to identifying which mixtures of chemical hazards need assessment.

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YOU EXCRETE WHAT YOU EAT – BUT IS URINARY DEOXYNIVALENOL A GOOD BIOMARKER OF EXPOSURE?

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The aims of this study were to compare the urinary concentrations of the mycotoxin deoxynivalenol (DON) in different age and population groups in Norway and to evaluate urinary DON as a biomarker of dietary exposure. Participants registered food intake for 24 h using an open food diary and collected spot urine samples. All urinary samples were analysed for DON and metabolites. Urinary concentrations of DON and metabolites were determined using high resolution LC–high/resolution MS/MS with appropriate LOQs. Dietary intake of DON was estimated based on food consumption and a database of concentrations of DON in food. Finally, we estimated the intake of DON based on urinary DON concentrations or creatinine adjusted DON-concentrations combined with estimated daily creatinine excretion. Practically all individuals excreted DON in the urine. Children had higher total DON and DON-glucuronides DON concentrations than adults, probably reflecting the higher food intake on a body weight basis. The estimated intakes indicate that children ingest DON in the range of and exceeding the TDI. The estimated DON intake based on food consumption correlated well with DON intake estimated from urinary DON concentrations. Finally, we developed a model to predict the daily dietary intake of DON based on the urinary concentration of the metabolite DON-15 glucuronide, collected over 24 h.

In conclusion, practically all individuals in the studies were exposed to DON. Children have a higher intake of DON than adults, probably reflecting the higher food intake on a body weight basis. The daily intake of DON can be estimated from the excretion of DON-15-glucuronide. There are uncertainties related to all methods to estimate the dietary intake and the data did not allow an evaluation of the precision of these estimates.

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URINARY EXCRETION KINETICS OF ZEARALENONE BIOMARKERS OVER 72 HOURS AFTER SINGLE ORAL ADMINISTRATION IN HEALTHY ADULTS

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To be able to infer exposure to mycotoxins based on the excretion of biomarkers of exposure it is necessary to quantitatively understand the relationship between ingested mycotoxins and excreted biomarkers. In a previous study we quantified zearalenone (ZEN) in 24-hour duplicate diet samples and matching 24-hour urine samples from 35 subjects. The ratio of urinary excretion of ZEN biomarker vs. dietary intake showed a high variance, supporting the hypothesis that excretion of ZEN may take longer than 12-24 h.

To further study the excretion kinetics of urinary biomarkers of ZEN, 19 subjects ingested ZEN at 0.19 µg/kg bw (i.e., 75% of the current TDI), and urine was collected as individual voids for the following 72 h (ethical approval was obtained through the METC of Wageningen University). Subjects refrained from consuming food items with a high likelihood of being contaminated with ZEN two days before dosing and until the end of the study period. Urine was analysed using dedicated methods involving enzymatic deconjugation and immunoaffinity clean-up to reach appropriate LOQs (<0.02 ng/ml for ZEN biomarkers). Urinary excretion of ZEN had a very rapid onset after dosing, and the potent estrogenic metabolite α-ZEL was formed very efficiently, albeit underlying interindividual differences. The largest fraction of biomarkers was excreted in the first 24 h but excretion continued until 48-72 h. Ratios of ZEN

biomarker excretion vs. ZEN intake showed substantial interindividual differences and ranged from 14% to 63% (median 30%), implying lower internal exposure in some subjects.

The presented data provide valuable information on the absorption, distrivution, metabolism and elimination (ADME) characteristics of ZEN in humans. These data can further be used to support the establishment of urinary biomonitoring equivalents for ZEN for human biomonitoring.

MECHANISTIC INVESTIGATION OF THE CONTRIBUTING ROLE OF DEOXYNIVALENOL AND PATULIN IN COLORECTAL AND HEPATOCELLULAR CARCINOGENESIS

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Given the ubiquitous nature of human exposure to multiple mycotoxins, there is an urgent need to understand these toxins' mechanistic contributions to tumorigenesis. The European Prospective Investigation into Cancer and Nutrition (EPIC) study revealed significant associations of exposure to deoxynivalenol (DON) and patulin (PAT) with hepatocellular cancer risk, whereas exposure to DON, PAT and *Fusarium* toxins may increase colorectal cancer risk. Chronic multi-mycotoxin exposures were associated with both hepatocellular and colorectal carcinogenesis. Building on the epidemiological results, we designed experimental studies analogous to our work on aflatoxin B1 to better understand the mechanistic roles of DON and PAT in colorectal and hepatocellular carcinogenesis. Previous experimental *in vitro* and *in vivo* exposure studies using aflatoxin B1 and ochratoxin A provided a proof-of-principle for characteristic mycotoxin-induced mutation spectra associated with specific DNA adducts on guanines and with oxidative DNA damage, respectively. Here, analogous effects of DON, PAT and their combination on epithelial liver and colon cancer cell lines (HepG2 and HT-29) and primary mouse embryonic fibroblasts (MEF) were investigated. The cyto- and genotoxicity was evaluated using, respectively, the colorimetric MTT assay and γH2Ax immunostaining. Clones amenable to whole-genome sequencing analyses were generated before and after chronically exposing the two human cell lines to the mycotoxins (for 6-8 weeks), whereas MEF-derived clones were obtained upon exposure, senescence bypass and clonal outgrowth. The independent exposures to DON and PAT induced cytotoxicity and genotoxicity in both, HepG2 and HT-29 cells, and in MEFs, suggesting potential exposure-specific mutagenic effects. Co-exposure to a mixture of DON and PAT consistently induced augmented cytotoxic and genotoxic effects in all cell systems. Genome-scale sequencing of the extracted DNA from the chronically single and co-exposed cell lines has been carried out to identify potential exposure-specific mutational signatures that can be assessed in human cancers by computationally screening the pan-cancer whole-genome sequencing data. In addition, RNA-sequencing upon short-term exposure has been performed to address non-genotoxic immediate-early effects of DON and PAT exposure on the human colon cells.

Our findings address important, not yet investigated mechanistic insights, potentially applicable to a targeted reduction of relevant dietary mycotoxin exposures and associated cancer-risk control in policy development and regulations at (inter)national level.

THE BIDIRECTIONAL RELATIONSHIP BETWEEN *ALTERNARIA* MYCOTOXINS AND THE GUT MICROBIOTA

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Species belonging to the genus *Alternaria* produce several mycotoxins, some of which are speculated to pose a threat for human health. However, this class of food contaminants is still not regulated due to the limited toxicological and occurrence data. In this context, the possible role of the gut microbiota in modulating the toxic effects of this class of mycotoxins and, vice versa, the ability of the latter to target the gut microbiota are rarely investigated. This contribution will summarize recent findings on *Alternaria* mycotoxins and their interaction with the human gut microbiota. In particular, the impact of short-term faecal incubations on the *in vitro* DNA-damaging effects exerted by a complex extract of *Alternaria* mycotoxins (containing eleven chemically characterized mycotoxins) was investigated. A particular attention was drawn to the role of the various faecal fractions (microorganisms, undigested food constituents and soluble substances) in the modification of the bioavailability and genotoxicity of the extract. In addition, the *in vitro* effects of different concentrations of the same *Alternaria* extract on human gut bacterial strains belonging to five of the most dominant human gut microbial phyla, as well as the ability of the gut bacterial strains to adsorb or metabolize the mycotoxins of the extract, were also investigated. Results of these studies clearly showed the existence of a bidirectional relationship between *Alternaria* mycotoxins and the gut microbiota. The DNA strand break properties of the extract were found to be almost completely quenched by the contact with the various faecal fractions (including gut microorganisms). However, the potency to induce formamidopyrimidine DNA glycosylase-sensitive sites in the comet assay was only slightly reduced. The suppression of the genotoxic properties was found to be a direct consequence of the reduction of mycotoxin concentrations in samples analysed by LC-MS/MS. Further investigations revealed the ability of human gut bacterial strains (especially the gram-negative ones) to adsorb the most lipophilic *Alternaria* mycotoxins (i.e., alternariol, alternariol monomethyl ether, altersetin, altertoxin-I and alterperylenol), thus potentially reducing the free absorbable proportion of mycotoxins. On the other hand, the *Alternaria* mycotoxin extract was found to affect the growth of several bacterial strains and their ability to produce biofilms.

Taken together, these findings highlight the potential role of mycotoxins in impacting the gut microbial community, as well as the importance of the latter in modifying the systemic bioavailability of this class of food contaminants.

TOXICOLOGY OF MIXTURES: LESSONS FROM THE MYCOTOXIN COMBINATION STUDIES

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Most of the published toxicological data concern the effects of chemical contaminants when present alone. Fungi produce several mycotoxins simultaneously. Moreover, food and feed can be contaminated by several fungi species at the same time or in a quick succession. Therefore, humans and animals are generally exposed to a cocktail of mycotoxins. The emerging exposome studies also depict co-occurrence of mycotoxins with environmental chemicals including heavy metals, which highlights the interest of characterizing the effect of complex mixtures involving mycotoxins.

Deoxynivalenol (DON) is of public health concern owing to the narrow margin between exposure and tolerable daily intake (TDI). However, the implications of the combined effects of DON, and its co-occurring congeners, or other food contaminants have not received adequate attention. In fact, the combined effect of a mixture in terms of additivity, antagonism or synergy cannot be predicted based on the individual toxicity of its components. We have investigated the mixture toxicity of DON and other type B trichothecene (TCTB) mycotoxins, and DON and the widespread toxic heavy metal cadmium (Cd). On the one hand, proliferating human and porcine intestinal cells, as well as fully differentiated porcine jejunal explants, have been used to analyse the combined effect of TCTBs on intestinal epithelium renewing and gut inflammation. On the other hand, the organ toxicity of the DON-Cd mixture has been analysed on different human cell lines. The toxicological interactions were assessed by means of the isobologram-combination index method. We have reported that low doses of TCTB clearly below the TDIs synergistically decrease the epithelial cells renewing and increased the expression of pro-inflammatory genes in the intestine, with magnitude of synergy ranging from 2 to 10. Regarding the DON-Cd mixture, the interactions ranged from nearly additive to antagonism or synergy, depending on the tested organ.

All together, the results indicate that very low doses of mycotoxins can synergistically impair the intestinal health, and the consequences of combined exposure to environmental and food contaminants are specific to the target organ. Considering the frequent co-occurrence of mycotoxins and other contaminants in diet, and the concentrations of toxins to which consumers are exposed, there is a need for a re-evaluation of the health risk associated to these contaminants taking into account the most likely exposure patterns and the most sensitive endpoints.

EVALUATION OF A TRI-CULTURE GUT-ON-CHIP MODEL FOR LONG-TERM EXPOSURE STUDIES

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Caco-2 based models are commonly used *in vitro* models for the gut barrier, but they are not suitable for long term exposure studies. The aim of this study was to improve the longevity by incorporating endothelial cells to improve the homeostasis and by using dynamic (flow) culture conditions.

A tri-culture of Caco-2 epithelial cells, HT29-MTX goblet cells and hMVEC endothelial cells was cultured in an organ-on-chip device for 6 weeks (3 weeks until full differentiation, 3 weeks in fully differentiated state) and was characterized and compared to a monoculture (Caco-2) and di-culture (Caco-2/HT29-MTX) in the chip device. Next, as a case study, the tri-culture model was exposed daily (2h/day) to three different concentrations of deoxynivalenol (DON) and a vehicle control for 21 days. Confocal microscopy analysis showed confluent cell layers for all models. DNA and ALP assays showed a constant cell number and differentiation after ~1 and ~3 weeks, respectively. BrdU staining demonstrated steady proliferation in the tri-culture up to 6 weeks of culture, which decreased after 4 weeks in the mono and di-cultures. Lucifer yellow (LY) transport indicated constant barrier integrity in the tri-culture model, which also decreased in the mono-and di-culture modes in time. After daily exposure to DON for 21 days, the weekly LDH and LY transport measurements indicated good viability and barrier integrity except for the highest dose of DON, which showed a decrease in barrier integrity in the first week of exposure. RNA sequencing gene expression profiles, after three weeks of exposure, are currently being evaluated. In summary, the tri-culture gut-on-chip model showed good integrity and homeostasis up to 6 weeks of culture and could be daily exposed to DON for a period of three weeks, which makes this a very promising model for long term studies as an alternative to animal models.

THE EPIGENETIC INTERPLAY OF AFLATOXINS AND A HERPES VIRUS TOWARDS CHILDHOOD CANCER

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Populations in low- and middle-income countries face environmental challenges that include the exposure to mycotoxins and infection by endemic oncogenic viruses. Chronic intake of multiple mycotoxins is hypothesized to interact with oncogenic viruses enhancing the risk of developing several carcinomas. For example, infection by a herpes virus called Epstein Barr virus (EBV) is linked to a form of childhood non-Hodgkin lymphoma; Burkitt lymphoma (BL), endemic in parts of Africa (lymphoma belt), where chronic mycotoxin exposure co-exists. It was recently demonstrated that aflatoxin B1 (AFB1) can modify the DNA methylome and promote EBV infection in B-cells. In addition, both AFB1 and EBV may alter DNA methylation levels and deregulate the expression of cancer-related genes. For a long time, research focused almost exclusively on two types of epigenetic modifications: DNA methylation and non-coding RNAs. The role of histones in epigenetics, in contrast, has remained largely fragmented, until recently. Histones are proteins present in the nucleus of a eukaryotic cell, which typically possess an N-terminal tail that is a template for a plethora of histone post-translational modifications (hPTMs). In particular, hPTMs are known to interfere with the histone-DNA interplay. The aim of this study is to assess the epigenetic interplay between aflatoxins and EBV (and other infections) in children in affected populations and validate the underlying mechanisms using *in vitro* and animal models. In this context, an *in vitro* mass spectrometry-based screening method to identify the epigenetic modifications caused by aflatoxin exposure was performed. In the future, accurate exposure assessments of mycotoxins and EBV in a cohort of African infants and children will be conducted. Furthermore, the cancer-related gene profile will be integrated with epigenetic mechanisms of the co-exposure in the cohort, as well as in cell lines and humanized mice.

The outcome of this research will elucidate the mechanistic pathway of environmentally induced cancer. Given the omnipresence of mycotoxins and viruses, it is imperative to assess their contribution to cancer. Furthermore, understanding the mechanisms by which mycotoxins and viruses interact to induce tumours will provide insights to researchers and public health officials on how to overcome this challenge.

SESSION 3
MITIGATING MYCOTOXIN RISKS

A look into a variety of strategies related to mitigating mycotoxin contamination in food and feed. What's up?

EFFECT OF LACTIC ACID BACTERIA IN OCHRATOXIN A AND AFLATOXIN B1 REDUCTION DURING BREAD FERMENTATION

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Aflatoxin B1 (AFB1) and ochratoxin A (OTA) are considered the most important mycotoxins with common presence in bread and bakery products. Biological detoxification of mould food spoilage and mycotoxin contamination by lactic acid bacteria (LAB) exhibits high potential on a cost-effective and large scale. The aim of the present study was to evaluate the potential effect of several LAB isolated from milk whey on reducing AFB1 and OTA concentration during bread making process. A screening of twelve LAB strains (B1, B2, B3, B4, B5, B6, B7, B9, B10, BS4, BS6, BS7) was performed by evaluating OTA and AFB1 reduction after 72 h incubation in MRS broth (37ºC). The most effective LAB were lyophilized and added as ingredient in bread formulation partially (50%) or totally (100%) substituting yeast, with five tested conditions: (i) control, (ii) yeast-B10, (iii) yeast-B3, (iv) B10, and (v) B3. Mycotoxins concentration was evaluated in both dough and bread after 4, 8 and 24 h of LAB fermentation by HPLC-qTOF/MS analysis. All conditions, as well as the control containing only yeast, were prepared in triplicate. Nine LAB reduced OTA (12-40%) in MRS broth with B3, B10, BS4 and BS7 as the most active ones. AFB1 was reduced by five LAB (11-35%), highlighting B3 activity. OTA was significative reduced in dough by yeast-B10 (22-29%), yeast-B3 (9-34%) and B3 (17-28%) fermentation compared to the control; while AFB1 was reduced up to 11% with yeast-B3. OTA showed significative reductions in bread after yeast-B10 (8-25%), yeast-B3 (14%), and B3 (19-26%) fermentation; while AFB1 reductions were observed in bread with yeast-B3 (35-52%) and B3 (32-55%) fermentation. For both mycotoxins, the highest reductions were obtained after 8 h fermentation with yeast-B3. The selected BAL showed OTA and AFB1 reduction during bread fermentation when added as ingredient alone or in combination with yeast, pointing to a potential biocontrol strategy for mycotoxins reduction in bakery products.

Acknowledgements
Spanish Ministry of Science and Innovation (PID2019-108070RB-I00-ALI).

DETOX YOUR FOOD – MYCOTOXIN DEACTIVATION IN FOOD PRODUCTION USING ENZYMES

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Mycotoxins are commonly present in food and pose a health risk to consumers. Moreover, they can act synergistically, potentiating each other's effects. The major mycotoxins, such as aflatoxins, fumonisins (FUM), deoxynivalenol (DON), ochratoxin A, and zearalenone (ZEN) are regulated in the European Union (EU), and we very rarely observe commercially available food products exceeding these legal limits. Nevertheless, mycotoxins are toxic and can still exert chronic effects even below the legal limit. Furthermore, legal limits are not only based on human nutrition but also on technical feasibility. For instance, the EU allows higher legal limits for zearalenone for maize-based breakfast cereals (100 ppb) than for breakfast cereals of other cereal sources (50 ppb). The allowed content in maize oil is even higher (400 ppb).

Although proper management strategies are recommended and applied, complete avoidance of mycotoxins in food is challenging. We often observe, e.g., FUM, DON and ZEN, in maize-derived products or DON in malted barley. Biotransformation can be an an effective strategy for deactivation of these mycotoxins. In this talk, we will show process examples on using a fumonisin esterase for FUM deactivation and a zearalenone hydrolase for ZEN deactivation. The focus will be on cereal-based processes with an emphasis on maize. Process conditions, such as temperature and pH, will not only affect the fate of mycotoxins during processing but also affect enzyme activity. The goal is to completely degrade FUM and ZEN during processing or to reduce mycotoxin levels to values as low as reasonably achievable (ALARA).

AMMONIZATION OF THE *R* AND *S*-EPIMERS OF ERGOT ALKALOIDS TO ASSESS DETOXIFICATION POTENTIAL

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The fungus, *Claviceps purpurea,* infects cereal crops intended for human and animal consumption. The fungus forms a dark hard kernel on the head of the plant known as an ergot sclerotium. The ergot sclerotium contains chemical compounds known as ergot alkaloids. Ergot alkaloids exist in two conformational forms, the *R* and *S*-epimer. These alkaloids exhibit toxic effects when consumed by humans and animals. Specifically, livestock can be exposed to ergot through their feed. Multiple methods of detoxifying ergot contaminated feed have been assessed. Ammonia has been utilized to detoxify ergot. However, previous studies that assessed detoxification of ergot by ammonia did not investigate the *S*-epimers. Ammonia is also routinely used in the agriculture industry. Detoxification of ergot-contaminated feed by ammonia could be a potential practical application. To assess the effects of ammonia on ergot alkaloids (*R* and *S*-epimers), natural ergot contaminated wheat was ammoniated and analysed using ultrahigh-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS). A decrease in the total concentration of ergot epimers (*R* and *S*) at 2% and 5% ammonia concentrations, compared to the control group, at various time points (1, 2 and 3 weeks) were observed (8-29% reduction) (Generalized Estimating Equation, Multiple Pairwise Comparison, Sequential Sidak Correction, $P<0.05$). Separately, the total *R*-epimer concentration decreased (40-66%), whereas the total *S*-epimer concentration increased (21-81%), after ammonization, at the time points evaluated (Generalized Estimating Equation, Multiple Pairwise Comparison, Sequential Sidak Correction, $P<0.05$). Specific ergot alkaloids, *R* and *S*-epimer pairs, were comparatively more affected by the ammonia treatment. To assess ergot epimer-ammonia adducts, ergot epimer standards were ammoniated resulting in degradation products and epimerization. Ammonization alters the *R* and *S*-epimers of ergot alkaloids, which may lead to a potential practical detoxification process of ergot-contaminated feed.

REDUCTION OF ADVERSE EFFECTS OF DEOXYNIVALENOL BY SELECTED YEAST FRACTIONS

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Mycotoxins are the most frequently occurring natural contaminants in human and animal diet. Among them, deoxynivalenol (DON), produced by *Fusarium* species, is one of the most prevalent mycotoxins and occurs worldwide in feed. DON-contaminated feed may be responsible for emesis and anorexia, alteration of intestinal and immune functions, reduced absorption of the nutrients as well as increased susceptibility to infection and chronic diseases, with a major impact on animal production. Therefore, strategies are needed to reduce its risk for health of the livestock and to minimize its economic impact on production. To assess the efficacy of an immune-modulating feed additive based on yeast fraction in reducing DON toxicity in animal, several studies have been conducted *in vitro*, *ex vivo* and *in vivo*. Considering that DON interacts initially with the intestinal epithelium to mediate intestinal damage through different mechanisms including inhibition of protein synthesis, altered cytokines production, apoptosis, oxidative stress and cellular damages, models focusing on intestinal epithelium integrity and immune function were developed.

The first study, *in vitro*, conducted on a porcine intestinal epithelium cell line named IPEC-J2 aimed to investigate the potential effects of the yeast fraction on trans-epithelial electrical resistance (TEER) and on inflammatory cytokines gene expression in conditions of DON challenge. The second model concentrated on an *ex vivo* study on pig intestinal explants stimulated with DON to explore the benefice of the product. Finally, to complete the results obtained in the lab, an *in vivo* challenge was performed on broilers using diet naturally contaminated by DON. Notably, the yeast fraction supplementation alleviated the adverse effects of DON on intestinal epithelium integrity by mitigating TEER reduction induced by DON in IPEC-J2 cells and by reducing effects of DON on histomorphology of the intestine tissue observed *ex vivo* and *in vivo*. *In vivo* in broiler chicken, the yeast fraction treatment reduced the liver and intestinal lesions. In the three models, DON induced inflammation as seen by induction of *il8* gene expression which was counteracted by the immune-modulating compound.

In conclusion, these results suggested that the immune-modulating yeast fraction effectively relieved DON-induced inflammation in IPEC-J2, intestinal swine explants and intestinal broiler tissue, as well as improved intestinal barrier function and reduced liver lesions in broilers. These results indicate that supplementation with the selected yeast fraction increases broilers resilience to a subclinical challenge with the mycotoxin.

SAFETY OF HOUSE FLY LARVAE REARED ON MYCOTOXIN CONTAMINATED SUBSTRATES

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Novel protein sources are urgently needed for feed and food production, and the use of fly larvae as feed ingredient is receiving increasing attention. Fly larvae can be reared on a variety of organic residues, however, such organic residues potentially contain contaminants which could accumulate in the insect body. Mycotoxins are natural contaminants and due to climate change, mycotoxin contamination in agricultural crops has been increasing over the past decades. Mycotoxin contamination is considered as one of the most important food/feed safety challenges in the food and feed industry. Recent studies show that the use of mycotoxin contaminated organic residual streams as substrates for insect rearing seem to provide a promising approach for the future of circular economy and mycotoxin remediation. Studies are available for a variety of insect species, however, data for housefly larvae *Musca domestica* L. (Diptera: Muscidae) are lacking.

In this study, *M. domestica* larvae were exposed to mycotoxin contaminated diets. Larval feed was spiked with the mycotoxins aflatoxin B_1, deoxynivalenol, fumonisin B_1, ochratoxin A or zearalenone at a concentration of 1 and 10 times the maximum limit or the guidance value set for feed materials by the European Commission. The effect of mycotoxin exposure on survival and growth over five days was examined, showing no effects on both growth and survival at the concentrations examined. Possible accumulation of the toxins in the larvae was determined by an LC-MS/MS-based method to analyse the concentrations of the mycotoxins and several metabolites in the feed offered, the larvae and residual feed material. Furthermore, it was determined how much of the initially fed mycotoxins can be recovered in the larval body and the residual materials. Results of mycotoxin analyses showed that the tested mycotoxins do not seems to accumulate in the insect body. Additionally, for aflatoxin B_1, fumonisin B_1 and zearalenone no full recovery of the initial amount of mycotoxin present in the diets was achieved, indicating mycotoxin metabolism. It can be concluded that the absence of mycotoxin accumulation indicates the possible safe use of *M. domestica* larvae as food- and/or feed when reared on mycotoxin contaminated residual streams.

MITIGATION OF MYCOTOXINS BY THE USE OF MAGNETIC NANOSTRUCTURED AGENTS

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Mycotoxin contamination of foods and feeds represents a worldwide public health issue. Although good agronomical practices and control strategies have been developed along the food/feed production chain, mycotoxin accumulation in agricultural commodities cannot be completely avoided leading to economic losses. Therefore, there is a stringent need to look for new strategies to mitigate mycotoxin contamination. Magnetic nanostructured agents composed of different mixtures of magnetite and traditional adsorbents, such as clays, active carbon or alginate, provide a new generation of toxin mitigation agents that can be separated from the matrix by magnetic extraction, which is considered a green technique. Recently, we have described the use of different magnetic nanostructured particles to remove up to 80% of mycotoxins from contaminated aqueous solutions. In this work, a new set of magnetic nanostructured materials, including TEMPO-mediated oxidized cellulose nanofibers, was studied for the extraction of mycotoxins. Their application in beverages such as beer or milk and in distiller's dried grains with solubles (DDGs) was explored. With these new particles, mycotoxin elimination was highly improved, deoxynivalenol was 30% removed while zearalenone, fumonisins, aflatoxins and ochratoxin A were almost completely eliminated from contaminated solutions. Similar results were obtained in beer and milk. In addition, it was observed that these materials can be applied to reduce contamination levels of DDGs. Therefore, magnetic nanomaterials are promising adsorbents for mycotoxin mitigation in commodities and beverages.

SESSION 4
UPDATE ON EFSA ACTIVITIES IN MYCOTOXIN RISK ASSESSMENT

New approaches for risk assessment of mycotoxins, critical issues for assessing carcinogenic mycotoxins together with results from recent assessments will be presented. efsa's initiatives in support of research activities in the field of mycotoxins will be introduced together with an update on regulatory follow-up of efsa work. This session is co-ordinated by the European Food Safety Authority (EFSA).

METHODOLOGICAL APPROACHES DEVELOPED BY EFSA TO INCLUDE MODIFIED MYCOTOXINS IN RISK ASSESSMENTS

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In 2014, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) published its first scientific Opinion in which modified forms of fusarium toxins were explicitly the subject of the analysis and evaluation. This Opinion concluded that modified fusarium toxins might constitute a risk both regarding their toxicity and their occurrence in food and feed [1]. Since then, another seven scientific Opinions [2-8] followed, in which the (toxicological) relevance of modified *Fusarium* toxins was evaluated. A general and major challenge of the toxicological assessment of modified *Fusarium* toxins is the scarcity of (appropriate) data.

For the parent *Fusarium* toxin zearalenone (ZEN), the CONTAM Panel established a tolerable daily intake (TDI) of 0.25 µg/kg bw based on effects on the female reproductive system. Modified forms of ZEN identified were ZEN glucosides and sulphates, and α-ZEL (α-zearalenol), β-ZEL (β-zearalenol), α-ZAN (α-zearalanone), α-ZAL (α-zearalanol), β-ZAL (β-zearalanol), cis-ZEN (cis-zearalenone), cis-α-ZEL (cis-α-zearalenol), cis-β-ZEL (cis-β-zearalenol) and their glucosides, sulphates and glucuronides. Neither of these were sufficiently characterised toxicologically. To overcome this situation and because *in vitro* studies showed that the modified forms of ZEN may act via the same mode of action as ZEN (i.e., oestrogenicity) leading likely to the same effects, results from *in vivo* uterotrophic assays were used to establish relative potency factors (RPFs) to ZEN, making it possible to include modified forms in the hazard assessment. For T-2/HT-2 toxins (T2/HT2), a TDI for the sum of T2/HT2 was set at 0.02 µg/kg bw based on haematological effects. An acute reference dose (ARfD) of 0.3 µg/kg bw was also established based on emesis seen in different animal species. Several modified T2/HT2 forms were identified (phase I metabolites formed by hydrolytic cleavage, phase II metabolites formed by conjugation with glucose, modified glucose, sulphate, feruloyl and acetyl groups). As for ZEN metabolites, there were insufficient data to characterise the hazard of these metabolites. But as *in vivo/in vitro* studies showed that they act via a similar mode of action (i.e., protein synthesis inhibition, leading to haematotoxicity), their hazards can be characterised applying RPFs reflecting their toxic potencies in comparison to the parent compounds. For nivalenol (NIV), a TDI of 1.2 µg/kg bw based on immunotoxicity and an ARfD of 14 µg/kg bw based on emetic effects were set. Regarding its modified forms, only NIV-3-glucoside was identified as a relevant metabolite. There were no toxicity data for the compound available but because it can be assumed that it is cleaved in the intestinal tract releasing NIV, the Panel concluded that the same reference levels can be applied. A TDI of 1.0 µg/kg bw based on liver toxicity was set for fumonisin B1 (FB_1) which is also applicable for FB_2, FB_3, and FB_4. A series of modified forms of fumonisins B (FB) were identified (i.e., hydrolysed $FB_{1–4}$ ($HFB_{1–4}$), partially hydrolysed $FB_{1–2}$ ($pHFB_{1–2}$), N-(carboxymethyl)-$FB_{1–3}$ (NCM-$FB_{1–3}$), N-(1-deoxy-D-fructos-1-yl)-FB1 (NDF-FB1), O-fatty acyl FB_1, N-fatty acyl FB_1 and N-palmitoyl-HFB_1. HFB_1, $pHFB_1$, NCM-FB_1 and NDF-FB_1. Overall, the data available suggest a similar toxicological profile as the parent compounds but the data were too limited and inconsistent to assess their relative potencies in quantitative terms.

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SPECIFIC OUTCOMES OF ANIMAL HEALTH RISK ASSESSMENTS OF MYCOTOXINS INCLUDING THEIR MODIFIED FORMS

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Contaminants have been evaluated by the European Food Safety Authority (EFSA) since its foundation in 2002 and the risk of mycotoxins from their occurrence of mycotoxins in food and feed was since then characterized in about 25 opinions and statement by the Panel on Contaminant in the Food Chain (CONTAM) for humans, farm and companion animals. Recently three opinions were adopted concerning the risk assessment of three mycotoxins (fumonisin, deoxynivalenol and zearalenone) and they modified forms with specific emphasis on animals.

For fumonisins, the EFSA Panel on Contaminants in the Food Chain (CONTAM) considered the sum fumonisin B1, FB2 and FB2 as well as their hidden forms. It identified no-observed-adverse-effect levels (NOAELs) for cattle, pig, poultry (chicken, ducks, and turkeys), horse, and lowest-observed-adverse-effect levels (LOAELs) for fish (extrapolated from carp) and rabbits. No reference points could be identified for sheep, goats, dogs, cats, and mink. Based on mean exposure estimates, the risk of adverse health effects of feeds containing FB1, FB2 and FB3 was considered very low for ruminants, low for poultry, horse, rabbits, fish and of potential concern for pigs. The same conclusions apply to the sum of FB1, FB2 and FB3 and their hidden forms, except for pigs for which the risk of adverse health effect was considered of concern. For deoxynivalenol (DON), the panel considered the parent toxin as well as its acetylated forms (3-Ac-DON and 15-Ac-DON) and the DON-3-glucoside. The Panel established no observed adverse effect levels (NOAELs) for cattle (dairy cows, heifer, and steers), sheep, goats, pigs, poultry (broiler chickens, laying hens, ducks, and turkeys), horses, farmed rabbits, and farmed fish (extrapolated from carp), farmed minks, dogs, and cats. Based on estimated mean dietary concentrations in ruminants, poultry, rabbits, dogs and cats, most farmed fish species and horses, adverse effects are not expected. At the high dietary concentrations, there is a potential risk for chronic adverse effects in pigs and fish and for acute adverse effects in cats and farmed mink. For zearalenone (ZEN), Modified forms occurring in feed include phase I metabolites a-zearalenol (a-ZEL), b-zearalenol (b-ZEL), a-zearalanol (a-ZAL), b-zearalanol (b-ZAL), zearalanone (ZAN) and phase II conjugates. ZEN has oestrogenic activity that differs considerably in the modified forms. The Panel established no observed adverse effect levels (NOAELs) for pig (piglets and gilts), poultry (chicken and fattening turkeys), sheep and fish (extrapolated from carp) and lowest observed effect level (LOAEL) for dogs. No reference points could be established for cattle, ducks, goats, horses, rabbits, mink, and cats. For modified forms, no reference points could be established for any animal species and relative potency factors previously established from rodents were used. Based on exposure estimates, the risk of adverse health effects of feed containing ZEN was considered extremely low for poultry and low for sheep, dog, pig, and fish. The same conclusions also apply to the sum of ZEN and its modified forms.

ROLE OF THE MECHANISMS OF GENOTOXICITY IN DETERMINING SAFE HUMAN EXPOSURE LIMITS: OCHRATOXIN A AS A CASE STUDY

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In 2020, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) published an update of the 2006 opinion on ochratoxin A (OTA) in food because new toxicity studies had become available. This mycotoxin, produced by *Aspergillus* and *Penicillium* fungi, is found as a contaminant in various foods. OTA exerts adverse effects in repeated dose studies in several species (mice, rats, rabbits, and pigs). At high doses these adverse effects include nephrotoxicity, immunotoxicity, neurotoxicity and developmental effects. Kidney tumours have been observed in both sexes in rats and mice, with tumours in mice (kidney and liver) occurring at higher doses in comparison to rat renal tumours. OTA is genotoxic both *in vitro* and *in vivo*. OTA is a weak mutagen *in vivo*. Gene mutations occurring in rats and mice are restricted to the cancer target site (the outer medulla of the kidney) with large deletions and base substitutions being the main mutagenic events.

The molecular mechanisms underlying OTA genotoxicity remain however unclear. This is due to a controversy on a) the formation of OTA specific DNA adducts (low levels with no chemical characterization of DNA adducts) and b) the nature of chromosomal damage (damage to the proteins of the mitotic spindle or presence of DNA double strand breaks occurring in mitosis). In addition, since exposure to OTA increases the levels of reactive oxygen species (ROS) and 8-hydroxyguanine in DNA, some of the reported genotoxic effects may be secondary to oxidative stress. However, a distinction between direct and indirect genotoxic modes of action which might each contribute to tumour formation could not be established. Since these recent studies have raised uncertainty regarding the mode of action for kidney carcinogenicity, with a possible role of genotoxicity in OTA carcinogenicity not being excluded, a health-based guidance value (HBGV) and a margin of exposure (MOE) approach was applied. The estimation of chronic dietary exposure ranged from 0.6 to 17.8 (mean) and 2.4 to 51. 7 (95th percentile) ng/kg bw per day. Median OTA exposures in breastfed infants ranged from 1.7 to 2.6 ng/kg bw per day, 95th percentile exposures from 5.6 to 8.5 ng/kg bw per day in average/high breast milk consuming infants, respectively. For the characterisation of non-neoplastic effects, a BMDL10 of 4.73 ug/kg bw per day was calculated from pig kidney lesions. Comparison of exposures with the BMDL10 resulted in MOEs of more than 200 in most consumer groups (low health concern), with the exception of MOEs for high consumers in the younger age groups (possible health concern). For characterisation of neoplastic effects, a BMDL10 of 14.5 ug/kg bw per day was calculated from rat kidney tumours. When compared with the BMDL10 based on the neoplastic endpoint, MOEs were lower than 10,000 for almost all exposure scenarios, including breastfed infants. This would indicate a possible health concern if genotoxicity is *via* direct interaction of OTA with DNA. Uncertainty in this assessment is high and risk may be overestimated.

THE IMPACT OF EFSA COLLABORATION IN SHAPING METHODOLOGIES IN MYCOTOXIN RISK ASSESSMENT

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Over the last decade, the European Food Safety Authority has played a fundamental role in inspiring and driving the research in the field of mycotoxins, moving the focus from the regulated to the yet unregulated ones. More recently, EFSA has promoted the development of methodologies for the risk assessment of multiple chemicals, and more specifically for the understanding of combined effects of co-occurring mycotoxins. Besides the crucial role of the Authority for scientific advisory and risk assessment, it represents a crucial player for networking, capacity building and promotion of collaborative research.

In this communication, the latest initiatives promoted by EFSA to support collaboration in the field of mycotoxins will be described, and the main outcome together with gaps and limitations will be discussed. In addition, an overview of the ongoing EFSA funded projects related to innovative methodologies for the risk assessment of multiple chemicals will be given to promote networking and knowledge sharing.

REGULATORY FOLLOW UP AT EU LEVEL TO EFSA OPINIONS AND FUTURE CHALLENGES

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Taking into account the possible animal and public health concern related to the presence of mycotoxins in feed and food, the mycotoxins are regulated at EU level in feed and food to ensure a high level of animal and human health protection. Based on the outcome of EFSA opinions, providing the scientific basis for EU regulatory measures in feed and food, strengthening of existing maximum levels of mycotoxins in food (such as for ochratoxin A and deoxynivalenol) or the setting of new maximum levels (such as for T-2 and HT-2 toxin and ergot alkaloids) are discussed to continue to ensure a high level of human health protection. Also, attention is paid to mycotoxins for which no maximum levels have been set such as *Alternaria* toxins. Also, the approach to regulate mycotoxins in feed is under discussions to be strengthened to ensure a high level of animal health protection. The presence of mycotoxins in feed and food has been increasing in recent years also due to changing weather conditions. Given the high influence of weather conditions on the presence of mycotoxins in feed and food, there is a high year-to-year and geographical variation in the occurrence of mycotoxins in feed and food as the presence cannot be fully controlled by good agricultural practices. This results in particular challenges from a regulatory point of view. For the setting of maximum levels is based on the available data in the EFSA database and taking into account the year-to-year variation and geographical variation as reflected in the EFSA occurrence database, thereby also taking into account the information provided by the stakeholders.

In the presentation, more details will be provided how the outcome of EFSA opinions is followed up in EU regulation using recent examples.

SESSION 5
FOCUS ON MYCOTOXIGENIC FUNGI, PLANTS, AND SOIL

In this session, we follow the fate of mycotoxins from a complex ecological perspective: fungus, plant, and soil, and their interactions.

FACIAL ECZEMA CAUSED BY SPORIDESMIN-PRODUCING STRAINS OF *PSEUDOPITHOMYCES CHARTARUM*: AN IMPORTANT YET POORLY UNDERSTOOD DISEASE OF DAIRY CATTLE IN SOUTH AFRICA

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Mycotoxins are toxic secondary metabolites produced by fungi that cause an undesirable effect (mycotoxicosis) when animals are exposed. Facial eczema (FE) which is also known as pithomycotoxicosis is caused by the ingestion of fungal spores produced by *Pseudopithomyces chartarum* which contains the toxin sporidesmin. Sporidesmin damages the liver and bile which can then lead to photosensitisation mostly in sheep and cattle. Facial eczema is generally not fatal but can negatively affect the production and welfare of dairy cattle. Facial eczema was first reported in New Zealand in 1894 and occurs frequently there, but is also reported in Argentina, Australia, France, the Netherlands, Portugal, South Africa, Spain, Turkey, the United States, and Uruguay. In South Africa, FE is increasingly recognized as a problem in the dairy industry. After a recent outbreak in and around Humansdorp, mixed-grass samples were collected from these areas and the fungi were isolated by using different media. In total 738 strains were isolated which represented 57 genera, but only 12 of them had more than ten isolates. Three different species of *Pseudopithomyces* were isolated from the pasture samples together with many other mycotoxigenic genera including *Fusarium* and *Aspergillus*. The results illustrate a paucity of knowledge regarding the identification of *Pseudopithomyce*s spp. and the role that they, as well as other fungi, play in the health of cattle in South Africa. These questions will be considered in future studies.

KEY GENES INVOLVED IN MYCOTOXIN BIOSYNTHETIC PATHWAYS

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Fungal contamination of agricultural commodities, particularly by mycotoxigenic fungi, represents an enormous concern for global food security in terms of feeding the world's growing population with sufficient and safe food. Not only do they reduce crop yield and quality, but they also produce substantial numbers of mycotoxins, which pose serious adverse health effects in human and animals. As the genome of most mycotoxigenic species have been sequenced, the gene clusters involved in the biosynthesis of important mycotoxins including aflatoxins, fumonisins, ochratoxins, zearalenone and trichothecenes, have been largely identified and characterised, with their roles elucidated by many researchers. This paper provides a comprehensive overview of the current knowledge of genes involved in the biosynthetic pathways of mycotoxins, with a special focus on aflatoxins.

MAPPING RESISTANCE AND SUSCEPTIBILITY TO *FUSARIUM LANGSETHIAE* AND T2/HT2 CONTAMINATION IN 190 SPRING OATS VARIETIES: WHAT WILL HAPPEN WITH CLIMATE CHANGE?

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Fusarium langsethiae is a symptomless pathogen of oat panicles that produces T-2 and HT-2 mycotoxins, two of the most potent trichothecenes produced by *Fusarium* fungi in cereals. In the last few years, the levels of these mycotoxin in oat grain have increased and the European commission have already recommended a maximum level for of 1000 µg/kg for unprocessed oat for human consumption. The optimal and most sustainable way of combating infection and mycotoxin contamination is by releasing resistant oat varieties. Here, the objective was to determine if we could identify any genomic loci associated with either the accumulation of *F. langsethiae* DNA or mycotoxins in the grain. In each of two years, field trials were conducted wherein 190 spring oat varieties were inoculated with a mixture of three isolate of the pathogen. Furthermore, since climate change is expected to influence fungal colonisation and associated mycotoxin production by *F. langsethiae* we examined the effect of acclimatisation for up to 10 generations of strains of *F. langsethiae* (seven strains under diurnal temperature conditions in either 400 or 1000 ppm CO_2 and tested the effect of acclimatisation after different generations when exposed to elevated CO_2.

Mycotoxins were quantified using liquid chromatography–tandem mass spectrometry. Varieties were genotyped using 16,863 genotyping by sequencing markers. Genome- wide association studies associated 5 SNPs in the linkage group Mr06 with T-2 + HT-2 mycotoxin accumulation. Markers were highly correlated, and a single QTL was identified. The marker *avgbs_6K_95238.1* mapped within genes showing similarity to lipase, lipase-like or lipase precursor mRNA sequences and zinc-finger proteins. These regions have previously been shown to confer a significant increase in resistance to *Fusarium.* The acclimatisation results showed that some *F. langsethiae* strains had an increased lag phase at generations 1, 7 and 10 and the growth rate were generally increased after exposure to elevated CO_2 levels. The production of T-2 and HT-2 was stimulated at generations 7 and 10 for 3 out of the 7 *F. langsethiae* strains tested.

BIOREGULATION OF SOIL ORGANISMS – SUPPRESSION OF PATHOGENIC FUNGI AND REDUCTION OF THEIR MYCOTOXINS IN AN AGRO-ECOLOGICAL CONTEXT

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Due to probable increasing occurrence of plant pathogenic fungi such as *Fusarium* sp. and their mycotoxins in crop products and crop residues, a rise of yield losses can be expected in the future. Fungivorous representatives of soil organisms act as biological regulators. The group of earthworms plays an important role in this regard. It is known that different earthworm species are able to control significantly different plant diseases, including *Fusarium* diseases and reduce their mycotoxins. Against this background, the bioregulatory capacity of different common earthworm species (*Lumbricus terrestris* and *Aporrectodea caliginosa)* was assessed within the BiodivERsA project SoilMan. The studies focused on the question if the introduced earthworms are successful in suppressing economically relevant *Fusarium* species (*F. graminearum*, *F. culmorum* and *F. verticillioides*) and

reducing their main mycotoxins deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-AcDON) and zearalenone (ZEN) in maize residues. For this purpose, several field and laboratory experiments were carried. In general, the reduction of the mycotoxins DON and 3-ADON by earthworms could be verified in all experiments. In contrast, the bioregulatory capacity of earthworms on Fusarium fungi turned out to be species specific: *F. graminearum* was suppressed, *F. culmorum* was not affected and *F. verticillioides* was slightly promoted.

In conclusion, the primary decomposer (*Lumbricus terrestris)* within the earthworm community make an important contribution to the bioregulation of plant pathogens. Due to their digging and feeding activity, infected plant material is incorporated from the surface of the soil into the soil and by using phytopathogenic fungi or fungal infected plant residues as food source, earthworms perform to naturally pathogen regulation. The synergy of such natural bioregulatory bottom-up effects and anthropogenic top-down effects (agricultural management) can, within the framework of adapted agricultural management, help to ensure long-term sustainable agricultural production on healthy soils.

SUSTAINABLE CEREAL PRODUCTION REDUCES IMPORTANT *FUSARIUM* MYCOTOXINS

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Fusarium mycotoxins in cereals constitute major problems for animal and human health worldwide. A range of plant pathogenic *Fusarium* species that can infect cereal plants in the field are considered the most important source of mycotoxins such as deoxynivalenol (DON), zearalenone (ZEA), T-2 toxin and HT-2 toxin in small grain cereal crops in temperate climates. We have critically reviewed the available knowledge on the impact of contrasting production system (organic versus conventional), and specific agronomic parameters on the occurrence and concentrations of DON, ZEA and T-2/HT-2 in small grain cereals (wheat, oats, barley, and rye). Furthermore, the *Fusarium* mycotoxin risks are discussed in the context of the need to develop more sustainable cereal production systems. Overall, the available evidence from studies of acceptable scientific quality suggests that the incidence and concentrations of *Fusarium* mycotoxin are lower in organic compared with conventional cereals. Specifically, 24 comparisons showed lower mycotoxin level in organic production, 16 detected no significant difference, whereas only two showed higher level in organic production. When the mean concentrations from all studies were compared, conventionally produced cereals had 62, 110 and 180 % higher concentrations of DON, ZEA and T-2/HT-2 respectively, than organic cereals. The published studies on effects of specific agronomic practices on mycotoxin levels suggest that diverse crop rotations, high soil organic matter content/biological activity are associated with a lower risk of *Fusarium* mycotoxin contamination, whereas high mineral nitrogen fertiliser inputs, some fungicides and herbicides, and minimum or no tillage may increase the risks of *Fusarium* mycotoxin contamination in cereals. The management of *Fusarium* head blight and mycotoxins therefore requires a preventative, integrated, holistic agronomic approach.

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EMBRACING THE ENVIRONMENTAL COMPLEXITY: FROM MYCOTOXINS TO MULTIPLE PLANT STRESSORS

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Among cereal crops, increasing wheat yield is vital to meeting future global food demand. It has been estimated that we will need to produce 60% more wheat by 2050]1] and this is going to be a challenge in the facing of climate change and environmental contamination. Concerns about environmental and soil are exponentially growing as reported in 2021 by FAO technical reports [2]. The matter is critical, as about 95% of the food we eat comes from it. Indeed, due to their sessile lifestyle, plants are continuously exposed to a large set of natural (i.e., mycotoxins) and man-made contaminants (i.e., pesticides, veterinary drugs, pharmaceuticals, perfluoroalkyl substances). So far, an individual-contaminant approach has been employed to study wheat response to most xenobiotics. As an example, metabolomic strategies have compared control wheat samples vs. samples treated with deoxynivalenol (DON) [3] to decipher the plant chemical defence employed to counteract mycotoxins accumulation. This strategy is useful to gain knowledge on the mechanism of action, but it is not representative of the open field scenario, in which wheat plants are simultaneously exposed combined stressors.

In this study, we aimed at investigating the wheat metabolic response to combined abiotic stressors, including mycotoxins (DON) and pesticides (glyphosate). Experiments were conducted in greenhouse, spiking mycotoxins and pesticide in the sterile soil. Healthy wheat plants were first exposed to a single contaminant and then to both, to better simulate the open-field environment. Further open-field experiments were conducted, applying glyphosate in pre-sowing and avoiding any fungicide treatments. Our preliminary results revealed the effect of xenobiotic-xenobiotic interaction on plant metabolism and on the plant capability to metabolize xenobiotics. Therefore, we suggest the need for a paradigm shift, from multi-mycotoxins to multiple-chemicals, expanding the spectra of plant stressors to be considered to get the full picture of the defence mechanism, useful to guide breading improvement.

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SESSION 6
MYCOTOXIN MANAGEMENT ALONG THE FOOD & FEED CHAIN

Where do we stand today? This session will introduce some options for the optimisation of processing and handling to manage mycotoxin contamination along the food & feed chain.

TRANSFORMATION OF OCHRATOXIN A DURING BREAD-MAKING PROCESSES

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Cereal-based foods can very often be contaminated with ochratoxin A (OTA). Heat treatment of food can reduce the level of OTA content, but also its racemization, during which 2'R-ochratoxin A (2'R-OTA) can be formed. In this study, the stability of OTA during rye bread production processes and pizza wheat base production processes (including dough kneading, dough fermentation, and baking) was investigated. The bread was prepared using rye flour naturally contaminated with OTA (concentration 6.41 ± 0.52 µg/kg) and yeast. The bread was baked for 40 min at 180°C, or for 70 min at 240°C. The pizza bases were baked from a dough made of wheat flour with artificially added OTA in the amount of 8.0 and 18.0 µg/kg. The baking was carried out at various temperatures (180°C, 40 min and 240°C, 70 min for rye bread and 320°C, 8 min and 370°C, 6 min for pizza bases). The research showed that the process of kneading and fermenting the dough did not change the OTA content. The OTA content in the crumb of rye bread and pizza generally did not differ significantly from the OTA content in the fermented doughs. It was found that the OTA content was reduced in the crust of rye bread and pizza crust with respect to the OTA content in the dough after fermentation. In the case of rye bread, the degree of OTA reduction was similar in both baking variants and amounted to 25.6% for baking conducted at 180°C and 23.0% for baking at 240°C. In the case of baking pizza bases, the degree of OTA reduction ranged from 8.0% to 25.4%, depending on the OTA content in the dough, and was higher in the case of baking at higher temperatures. A small concentration of 2'R-OTA was recorded in the crust of rye bread baked at 240°C (0.18 µg/kg, i.e., 3.5% OTA content). On the other hand, the content of this isomer in the crust of the pizza ranged from 0.13 µg/kg (i.e., 2.2% of OTA content) to 1.57 µg/kg (i.e., 10.1% of OTA content) and increased with the baking temperature.

MAJOR ERGOT ALKALOIDS MYCOTOXINS AND CEREALS AND CEREAL-DERIVED FOOD PRODUCTS: MANAGEMENT AND CONTROL STRATEGIES ALONG THE FOOD CHAIN

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Cereals represent a food product with huge impact on human and livestock diet, providing a significant amount of protein globally and, it is expected that their production will be expanded up to 13% till 2027. Ergot alkaloids (EAs) mycotoxins are mainly produced by several species of fungi of the genus *Claviceps*, among which *Claviceps purpurea* is the major producer of EAs and the most widespread in Europe, that infect more than 400 species of monocotyledonous plants, among them the weedy grasses. Crops and especially cereals can be infected in both pre-harvest and post-harvest stage by EAs; this species has been mainly found in many economically important cereal grains, such as rye, barley, wheat, millet, oats, and triticale and cereal-derived food products. During *C. purpurea* infection, healthy

kernels are replaced by purple-black-coloured sclerotia (also known as ergots, or ergot bodies) that contain high concentrations of various EAs. Environmental conditions, such as low temperatures and humid weather before and during flowering, influence contamination agricultural products by EAs, contributing to the appearance of outbreak after the consumption of contaminated products. Fungal strains, geographic regions, host plants and regional/local weather conditions are also involved in its occurrence. Cool, damp weather favours ergot by enhancing the germination of sclerotia, the alkaloid wintering body.

All these cereals represent an important part of the daily human diet and are widely consumed by the population, including infants, children, adolescents, and the elderly, and therefore have to be safe. Control of EA mycotoxins in cereals should focus on two main stages, namely, pre-harvest and post-harvest. Good agricultural practices related to crop rotation, weed control, and herbicide application, fertilizers for nutrient enrichment, could contribute to controlling EA mycotoxins by helping to maintain a robust and therefore non-vulnerable crop. Although the number of published works on the biological control of EAs is limited, *Pseudomonas aureofaciens* and the fungi *Trichoderma lignorum* and *Fusarium roseum* have been tested for biological control against EAs. Decontamination by heating is a physical way to convert the toxic forms of EAs (ergotamine) to less toxic forms of EAs (ergotaminine). Grain processing is an approach by which EAs could be possibly eliminate, although common processing techniques, such as boiling, cooking, baking, frying, and pasteurization, fail to reduce most mycotoxins that are considered stable chemical compounds. From the limited published data, related to EAs decontamination, most of them are focused on rye and rye products, with differences in results. The different rate of degradation that occurs is attributed to the different nature of ergot contamination. Moreover, ergot sclerotia can be removed from cereals by grain cleaning machines based on photocells but this is not standard in all countries and milling companies. An integrated system management approach that includes all individual control strategies is important to mitigate EAs in cereals and cereal-based foods.

BIOCONVERSION OF AFLATOXIN-CONTAMINATED AGRI-FOOD BY-PRODUCTS WITH FLY LARVAE INTO ANIMAL FEED

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Globally, large amounts of food crops such as maize are lost due to contamination with mycotoxins. In addition, urban food wastes that can contain mycotoxins are frequently unmanaged jeopardizing public and environmental health. The black soldier fly (*Hermetia illucens*) is a tropical fly whose immature larval life stage grows on a large variety of organic wastes/side-streams. The larvae convert the organic material into valuable fertilizer and animal feed products. In two case studies, we assessed the larval bioconversion performance and transfer of aflatoxin-contaminated wastes/side-streams into bioconversion products with the aim to establishing a safe reuse option for mycotoxin contaminated wastes/agri-food side streams. In Kigali, Rwanda (ongoing, completed by May 2022), we study feed safety and bioconversion efficiency of naturally and highly aflatoxin-contaminated maize. Aflatoxin

contaminated maize will be separated from clean maize with an automatic sorting machine. The maize will be then milled and mixed with organic waste. Using typical commercial procedures, all diets will be fed to larvae in controlled feeding experiments. Larval weight as well as environmental parameters will be recorded, and larvae and frass (i.e., larval excretions and undigested residue after larval feeding) analysed for nutrient composition. The safety of the larvae will be assessed by comparing analyses results of aflatoxins and degradation products to animal feed standards. In Surabaya, Indonesia, and Zurich, Switzerland, we studied the transfer of selected contaminants to larvae and poultry-derived food. 4 poultry diets were formulated from 4 partially defatted meals produced at 2 different facilities. In Indonesia, larvae were reared on food waste containing animal by-products spiked with environmentally relevant concentrations of Cd, Pb and aflatoxin B1, next to a non-contaminated control. As an additional control, in Switzerland, larvae were reared on substrates approved in the EU. Defatted larvae were included in late-laying hens diets and fed for 4 weeks. Only the diet including larvae reared on Cd contaminated substrate exceeded the EU maximum level for Cd for complete feed. No diet affected laying performance or egg quality. Feeding heavy metal contaminated larval-based diet doubled Cd concentrations in breast meat and elevated Cd concentrations in kidneys and liver compared to the control. However, all respective poultry products and tissues (except kidneys) ranged below permitted limits for food. Our results show that, under certain conditions, even contaminated food can provide a suitable substrate to produce larval for use as feeds for poultry nutrition.

SCRAPE THE MOULD OFF THE JAM OR TOSS THE JAR? – NEW ANSWERS TO AN OLD FOOD SAFETY DILEMMA

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Scrapping the mould off a jar and salvage the rest of the jam beneath it used to be a common habit of our grand mamas when a fuzzy white-and-blue mould appear on the top after a lid unscrewing. Although, the official recommendation of the food safety and health risk authorities is to discard a mouldy fruit jam or jelly immediately due to potential health hazard [1], the initiatives in lowering the global food waste opened that question lately again. The presence of moulds can be identified by their fuzzy appearance and musty odour, whereas their toxic metabolites, mycotoxins, are odourless and tasteless. Mycotoxins can penetrate from the mycelium surface to the deeper layer of a jam jar, hence even the removal of the visible fungal mycelia does not have to help to reduce the potential health risk when consuming a mouldy jam [2].

To obtain broader knowledge about the spectrum of mycotoxins occurring in mouldy fruit jams, a total number of 75 naturally infected jam samples were collected and analysed by an in house-validated LC-MS/MS method covering >500 mycotoxins. The analysis was performed in triplicate from three different sampling spots - mycelium and 2 cm- and 4 cm-distance from a mycelium spot. In addition, identification of the fungi was made by the combination of phenotypical traits (macro-and micromorphology) and sequencing of the internal transcribed spacer region (ITS). *Penicillium crustosum, Penicillium brevicompactum*, *Aspergillus versicolor* and *Penicillium expansum* were the species found with the highest incidence in 21%, 15%, 11% and 7% of the mouldy jams, respectively. A broad spectrum of mycotoxins corresponding with a respective producer, *P. crustosum* (e.g., penitrem A, paxillin, viridicatol, roquefortine C, viridicatin, paspalin), *P. brevicompactum* (e.g., mycophenolic acid, mycophenolic acid IV, andrastin A, andrastin B, quinolactacin A, citreohybridinol), *A. versicolor* (e.g., versicolorin C, 8-O-methylaverufin, averufin, emodin) and *P. expansum* (e.g., citrinin, dihydrocitrinone) was found at the highest levels at the mycelium spot. The level of mycotoxins penetration from the mycelium spot to the deeper layers of jam depended on the type of jam and physico-chemical properties of the respective toxin.

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UNTARGETED METABOLOMICS ANALYSIS FOR THE DETECTION OF *ALTERNARIA* MOULDY CORE APPLES

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Apples are susceptible to contamination with toxigenic fungi. *Alternaria,* a major one, causes two diseases: external contamination and mouldy core, which develops in the centre of the fruit. When fruits are destined to industrialization, visual inspection commonly performed in fruit processing industries does not prevent the incorporation of *Alternaria*-contaminated apples into the process line, and high incidence of *Alternaria* mycotoxins in apple by-products has been detected. The aim of this study was to develop a control strategy based on LC-high resolution mass spectrometry (HRMS) to detect batches of apples destined to industrialization affected with *Alternaria*. Three *Alternaria* strains isolated from apples were inoculated in the interior and the exterior of non-contaminated fruits separately and incubated at 25°C and 4°C for 1 and 9 months, respectively, to simulate retail and storage conditions. Each assay was done in triplicate, and 2 g of each fruit were extracted with 10 mL of MeOH/H_2O/AA (79:20:1, v/v/v) for 30 minutes in a shaker. The samples where then centrifuged at 4,000 g for 15 min and a 1 mL aliquot was taken from the supernatant and evaporated till dryness under a N_2 steam. Samples were resuspended in the injection solvent H_2O/ACN (70:30, v/v) and filtered through PTFE filters. An aliquot of 5 µl was injected into an ACQUITY UPLC system coupled to a Synapt G2−Si High-Definition instrument (Waters Corporation). The column was a HSS T3 (1.8 µm, 2.1 × 100 mm) held at 40°C. A linear gradient elution program with solvent A (H_2O:FA, v/v) and B (ACN:FA, v/v) was applied with a flow rate of 0.35 mL/min. The instrument was operated in resolution mode, the data type was continuum and acquired in MS^E mode on ESI^+ and ESI^- in separate runs in the scan range m/z 50 to 1,200 Da. Progenesis QI (Waters Corporation) was used to analyse the data using retention time alignment and peak picking. Composite ion maps which contained 21,804 and 21,506 compounds in ESI^+ and ESI^- mode, respectively, were built. Metabolites were filtered according to the ANOVA p-value <0.05, which decreased the number to 9,308 and 10,505 metabolites in positive and negative mode, respectively. This data reduction allowed to focus on the metabolites that clearly discriminate contaminated from non-contaminated samples by principal component analysis. An HRMS model was proposed for the detection of contaminated apple batches to prevent their processing and consequent mycotoxin accumulation in apple by-products.

UNDERSTANDING THE POTENTIAL FOR AND CONTROL OF AFLATOXIN DEVELOPMENT IN ALMONDS DURING STORAGE AND SHIPPING

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Although aflatoxin in nuts, such as almonds and other low moisture foods, continues to present challenges in markets around the world, much progress has been made in the areas of prevention and control. Research efforts in California almonds over the past 15 years has identified a strong correlation with level of insect damage and prevalence of aflatoxin. This understanding has led to development of practices by growers and processors to minimize insect pressure and utilize sorting techniques to maintain low levels of aflatoxin. Work has also been done to understand the properties of almonds such as moisture and water activity in relationship to environmental conditions encountered during storage and shipping, and the potential for aflatoxin development. Multiple transit studies at different times of the year from California to ports in Asia, Europe and India have been conducted to collect temperature and humidity data. In addition to sharing data on aflatoxin prevention, control and removal in almonds, this session provide insight into the temperature and humidity conditions collected during shipping and detail how such conditions relate to potential *Aspergillus flavus* or *A. parasiticus* mould growth. Attendees will gain a clear understanding of whether aflatoxin production during shipping is of concern.

SESSION 7
MANAGING MYCOTOXINS IN A SUSTAINABLE FUTURE

An ideal and sustainable economy is one which provides for the greatest amount of general well-being with the least amount of resource use and environmental harm. How do we cope with naturally occurring contaminants, such as mycotoxins, in a sustainable future?

FOODSAFETY4EU – SUSTAINABLE FOOD: HOW TO KEEP IT SAFE?

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Big and interconnected challenges, such as natural resource scarcity, climate change, and population growth, are affecting the food systems globally. Addressing these challenges requires a transformation towards more sustainable food systems, ensuring healthy diets and nutrition security for all. Through its Farm to Fork strategy and the broader European Green Deal policy, the European Commission is strongly committed to drive and accelerate the food system transformation towards increased sustainability. Food safety, being an integral part of food and nutrition security, is crucial for sustainable development. However, due to the complexity of underlying science, intensifying cooperation between food safety ecosystem actors has become a prerequisite to deliver future-proof solutions. Within this framework and as specifically foreseen in the Food 2030 pathway for action 'food safety system of the future', a multi-stakeholder collaborative platform is expected to support a safe transition towards a more sustainable food system. This is FoodSafety4EU: a Horizon 2020 collaborative action intended to drive the transition of the existing European food safety system towards a comprehensive and constantly updated framework.

As a final and main output of the project, the European Food Safety Forum will be launched in December 2023. However, thanks to the vibrant ecosystem already established during the first year, the first stone of the path towards a long-term and self-sustainable forum was laid in December 2021, when the first edition of the pre-forum took place. The event was designed to elicit the discussion and collect opinions, hopes and appreciations about the current and future European food sustainability issues and food safety system. The audience identified on the top of their priority scale the future food safety issues related to 'circular food economy: waste recycling, reuse'. The pre-forum outcome was definitely in line with the results of an in-depth survey among EU food safety authorities that recognized circularity related issues as relevant for the food safety system of the future. Among them, mycotoxins were identified as (near) future food safety issue posing relevant challenges in risk analysis. Needs for harmonization and integration of risk assessment of emerging mycotoxins is now the focus of one out of four Food Safety Operational Labs (social labs), where a multi-actor group is addressing this issue at systemic level, by proposing and testing novel approaches to define and incorporate the data needed to include sustainability (climate change, consumption data) in current risk assessments.

Acknowledgements
This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No 101000613.

BURGEONING SUSTAINABLE AFLATOXIN MANAGEMENT THROUGH A FOOD CONVERGENCE INNOVATION (FCI) INITIATIVE

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Food safety in sub-Saharan Africa (SSA) requires critical attention and sustainable solutions that converge efforts. In SSA, acute and chronic exposure to aflatoxins in the food and feed value chains are common. Chronic dietary exposure is associated with elevated liver cancer rates, especially in regions where hepatitis B virus is endemic, such as SSA. Whereas in Nigeria, awareness is slowly growing for the need to manage aflatoxins, efforts to address the toxins are siloed, consequently are sluggish and unsustainable. An initiative to create and implement convergence between system-level technical, socio-cultural, and institutional solutions through a digital backbone called the Food Convergence Innovation (FCI) is being developed with several partners to premier a system of catalysed and sustainable aflatoxin management in maize that can be expanded to other food value chain systems in Nigeria and other countries in Africa. Through a use case, we illustrate how supporting the production and flow of aflatoxin-safe grains in the maize value chain contribute towards ensuring the provision of traceable aflatoxin-safe produce for trading and regulatory purposes. This system promises to contribute to sustainable aflatoxin management, catalyse awareness on aflatoxin management, and serve as entry point for building convergence economy throughout Africa. Multi-stakeholder involvement including producers, private sector, public sector, and development partners is growing and holds great promise to address the challenges associated with overcoming siloed aflatoxin management and limitations in production practices, infrastructural deficits, shortfalls in regulatory monitoring and compliance, and other weaknesses in the food value chain. The FCI is the burgeoning of sustainable aflatoxin management in Nigeria.

BIOSTIMULANT MICRORGANISMS FOR MITIGATING THE OCCURRENCE OF *ALTERNARIA* SPP. AND DERIVED MYCOTOXINS IN TOMATOES

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Alternaria fungal species produce more than 70 toxins, but only a small number of them have been chemically characterized and reported as toxic for human and livestock. In the European Union maximum levels for a number of mycotoxins in food and feed are in force, however *Alternaria* toxins are not included in this list but are nowadays candidates for regulation. The European Food Safety Authority (EFSA) published a first risk assessment on *Alternaria* toxins in 2011 and after five years updated the dietary exposure to *Alternaria* toxins in the European population. Between the different *Alternaria* toxins, tenuazonic acid reported the highest level in food commodities, tomato-based products included. This is not of a secondary importance if it is considered that in European countries the dietary intake of tomato-based products is often daily, thus even a low level of *Alternaria* toxin contamination could potentially cause health concerns for consumers, especially toddlers and vegetarians. To control and mitigate the occurrence of *Alternaria* in the field, different cultural practices have been proposed, including both chemical and biological techniques. Among the latter group, microbiological stimulant represents a sustainable strategy for minimizing potentially harmful chemical inputs. In particular, microbiological stimulants are microorganism, or substances produced by them, which can be directly applied to plants, seeds, and soil to improve plant growth, increase crop yield, but also reduce plant stress. In this way, they act as plant stimulators and enhancers of resistance to biotic and abiotic stress, fungi contamination included.

In the present work, different biostimulant microorganisms with inhibitory and/or mitigatory properties have been tested and compared by *in vitro* analysis against the occurrence of *Alternaria solani* in tomato seedlings. Furthermore, the efficacy of these biostimulants in reducing the production of *Alternaria* toxins in tomatoes was evaluated as well with UPLC-MS/MS.

Acknowledgements
This research was funded by Regione Lombardia (Italy) project PROBIOPOM (Biostimulant micro-organisms for the biological protection of tomatoes from insects, fungi and mycotoxins), Grant No. 32 dds, 5 March 2020, n.2995.

COMBINING TRANSCRIPTOMIC AND METABOLOMIC TO UNRAVEL THE MECHANISM OF ACTION OF THE TICK PEPTIDE TC3, AN EFFICIENT INHIBITOR OF *FUSARIUM GRAMINEARUM* GROWTH AND DEOXYNIVALENOL PRODUCTION

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Fusarium head blight (FHB), mainly caused by the fungus *Fusarium graminearum*, is a devastating disease affecting cereal crops that leads to significant yield losses and a reduced grain quality. Indeed, *F. graminearum* produces type B trichothecene mycotoxins (TCTB) which are detrimental to the health of humans and livestock. Recently, a peptide referred to as TickCore3 (TC3), the γ-core of the tick defensin DefMT3, was demonstrated to be a potent antifungal against *F. graminearum* and an efficient inhibitor of the production of TCTB [1-2]. Furthermore, the structural and physico-chemical determinants required for the peptide efficacy were clarified [2]. TC3 appears a promising candidate for new eco-friendly plant protection solutions. To go further and unravel the mechanism of action of TC3, a strategy combining transcriptomic (RNA seq) and metabolomic (LC-MS and HRMN) has been implemented and the resulting data integrated. Significant changes in *F. graminearum* transcriptome such as in its secondary and primary metabolome were induced by TC3 exposure evidencing a multi-faceted mechanism. These data will be discussed within this presentation.

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VEGANS VS. OMNIVORES – A HEALTHY DIET WITH MORE MYCOTOXINS?

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Veganism, the practice of only eating food not derived from animal origin, is becoming increasingly popular in the Western World. In Germany, the group of people seeing themselves as vegans or persons who largely abstain from animal products increased between 2016 and 2021 by 75% to now 1.41 million [1]. Compared to omnivores, vegans need to access alternatives to animal protein and animal based nutrient sources for a healthy diet. This is typically done by consumption of higher portions of legumes, nuts, fruits, and cereals, which are plant-based foods but also important contributors to mycotoxin exposure. So far, this assumed increase of exposure to mycotoxins in a vegan diet has not been investigated in detail. Comprehensive data on internal mycotoxin exposure among vegans, especially in comparison to omnivores is lacking.

The cross-sectional Risks and Benefits of a Vegan Diet (RBVD) study comprised the collection of 24-h urine and serum samples of 36 vegans and 36 omnivores (aged 30-60 years, 50% females). Samples were analysed for 28 mycotoxins and mycotoxin metabolites by validated multi-mycotoxin methods (HPLC-MS/MS) which included aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2, AFM_1), *Alternaria* toxins (ALT, AOH, AME), beauvericin and enniatins (EnA, EnA_1, EnB, EnB_1), citrinin and dihydrocitrinone (CIT, DH-CIT), deoxynivalenol and its glucuronide (DON, DON-GlcA), fumonisin B_1, ochratoxin A and related mycotoxins (OTA, OTα, 10-OH-OTA, 2'R-OTA), T-2 and related mycotoxins (T-2, HT-2, HT-2-GlcA), zearalanone and zearalenone. OTA, 2'R-OTA, and EnB in serum and DON-GlcA in 24-h urine were quantified in 100, 85, 90, and 57% of the samples, respectively. Among the other analysed mycotoxins, OTA, DON, CIT, DH-CIT, and AFB2 were detected in few 24-h urine samples, and citrinin in few serum samples, whereas all remaining mycotoxins were non-detectable. Serum OTA levels were twofold higher among vegans compared to omnivores (median 0.24 vs. 0.12 ng/ml; $P<0.0001$) while levels of other mycotoxins did not differ significantly between vegans and omnivores. Serum OTA levels were associated with dietary intake of 'vegan products' ($r = 0.50$, $P< 0.0001$) and 'pasta & rice' ($r = 0.33$, $P = 0.006$). Yet, sensitivity analyses of the association with 'vegan products' in subgroups of 'vegan products'-consumers ($r = 0.33$, $P = 0.025$, $n = 48$) or vegans ($r = -0.03$, $P = 0.9$, $n = 36$) yield inconsistent results and call for careful interpretation. Beyond that, serum levels of 2'R-OTA were associated with dietary intake of 'coffee' ($r = 0.64$, $P< 0.0001$) and serum levels of EnB with dietary intake of 'cereals & cereal products' ($r = 0.28$, $P = 0.019$). No significant associations were observed between 24-h urine levels of DON-GlcA and dietary intake of any food group.

In conclusion, our data suggest that vegans are indeed challenged with a higher exposure to OTA, but not to other mycotoxins. Further, our data indicate a relevant contribution of dietary intake of vegan products and pasta and rice to OTA, coffee to 2'R-OTA, and cereals to EnB exposure. Additional larger studies with repeated measurements of mycotoxin levels in blood and 24-h urine will help to improve internal exposure evaluation [2].

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YOUNG SCIENTIST FORUM: THE CHALLENGE OF MYCOTOXINS IN SUSTAINABLE AGRICULTURE

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The global population is predicted to further grow and may end up at 10 billion people in 2050. To meet the futural surging demand in food, we will need to produce 60% more food than we do today but with 75% less emissions within planetary boundaries – a momentous challenge for our agriculture industry. The rising mycotoxin prevalence worldwide, unfortunately, has put extra pressure across the food value chain to make this challenge even bigger. During the Young Scientist Forum, we look forward to connecting to students, scientists, and researchers to discuss various topics within the challenge of mycotoxins in sustainable agriculture. For instance, grains contaminated by mycotoxins are widely considered as inferior quality ones, leading to their restricted usage in both food and feed production. Mycotoxin footprint, however, is expanding rapidly and globally, while mycotoxin profile is getting more and more dynamic per region. If the situation continues, the volatility of raw material markets and the trade barrier are predictably becoming higher in the future, both of which may struggle the global supply chain to the next level.

Tremendous efforts have been made by multiple institutes to build up a rapid alert system on focused mycotoxins, but the speed of progress is different among regions or grain varieties. On the other hand, the list of newly identified mycotoxins grows all the time, while the sound research on the toxicity of each and the potential interaction with one another is usually organized at a far lower speed. Obviously, some mycotoxins might be 'emerging' sooner or later in front of us, up to the speed of both the scientific dossier substantiation as well as the development of analytical toolkits. How to accommodate emerging mycotoxins into the existing alert system? More importantly, how to tackle emerging mycotoxins in a

cost-effective way in reality? Furthermore, mycotoxin exposure could be a risk factor for health or welfare of both human beings and animals. Acute toxicity can be expected when a certain mycotoxin is present at high concentration in diet. Co-occurrence of multiple mycotoxins, however, is more popular in the field nowadays, where each mycotoxin is present at a moderate level. Animal production is often confronted by this situation. Instead of showing any clinical poisoning symptom, production efficiently could be significantly compromised, indicating a suboptimal health status. Considering the potential interaction between mycotoxins and other environmental challenges, e.g., pathogens, the diagnosis to confirm mycotoxins' contribution to the whole picture then becomes a challenge in the practice. Some animal producers may choose a prevention measure by using a less expensive commercial adsorbent for the whole production cycle, while other peers are interested in a premium mycotoxin-mitigating agent and only apply it during some occasions. What are pros and cons of each option or strategy? What else can we recommend to animal producers to enable a more sustainable practice?

Join Trouw Nutrition's Young Scientist Forum to share your insights, learnings, and expectations for the future.

SESSION 8
SMART SOLUTIONS FOR ADVANCED TOXIN CONTROL AND MITIGATION
Session co-ordinated by the Austrian Competence Centre for Feed and Food Quality, Safety and Innovation (FFoQSI).

The potential of novel and promising approaches, such as rapid screening of mycotoxins, effective monitoring of candidate detoxification genes, and microbiome analysis as developed by FFoQSI and its industrial partners from the food and feed industry, will be presented. The impact of mycotoxin contamination on food and feed waste and sustainable strategies to tackle the issue along supply chains in view of climate change will be also discussed.

IMPACT OF MYCOTOXINS ON FOOD AND FEED WASTE, ENVIRONMENTAL SUSTAINABILITY, AND LOSS OF LIVESTOCK PRODUCTIVITY

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The contamination of food and feed with mycotoxins continue to present a huge challenge in terms of their profound negative impacts on human and animal health as well as on the economies of countries. The changing climate and agronomic practices are expected to exacerbate and increase mycotoxin incidences and concentrations in the food and feed supply chains, with a significant change in the geographic distribution of mycotoxins. The elevated mycotoxin levels imply that food and feed are wasted due to non-compliance with regulatory requirements. Furthermore, exposure of livestock animals to mycotoxins can result in substantial feed waste and production of additional feed for energy maintenance due to reduced feed consumption and low feed utilization. Therefore, it is of vital importance to establish the impact and scale of mycotoxin contamination of food and feed on environmental sustainability. Using a life cycle sustainability assessment tool and a statistical analysis that combines the results of multiple scientific studies, this paper evaluates the impact of mycotoxins on livestock performance and greenhouse gas emissions generated because of mycotoxin contamination of food and feed crops.

Acknowledgements
This work was created within a research project of the Austrian Competence Centre for Feed and Food Quality, Safety and Innovation (FFoQSI). The COMET-K1 competence centre FFoQSI is funded by the Austrian federal ministries BMK, BMDW and the Austrian provinces Lower Austria, Upper Austria and Vienna within the scope of COMET - Competence Centers for Excellent Technologies. The programme COMET is handled by the Austrian Research Promotion Agency FFG.

THE POTENTIAL OF IR SPECTROSCOPY FOR RAPID MYCOTOXIN SCREENING

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Infrared (IR) spectroscopy is increasingly used to analyse food crops in a rapid, non-destructive, label-free, and eco-friendly manner. The lack of sensitivity and the overlapping absorption characteristics of major matrix components, however, do hardly allow for the direct determination of food contaminants at trace levels. Nevertheless, by tracking fungal induced matrix changes with near IR and mid IR spectroscopy as well as hyperspectral imaging the indirect determination of mycotoxins in food crops

has been demonstrated. Recent studies underline that such IR spectroscopic platforms have great potential for the analysis of mycotoxins along the food and feed supply chain. However, there are no reports on fully validated IR methods and publications demonstrating their applicability in a routine analytical set-up are scarce. Therefore, we provide a summary of the current state-of-the-art highlighting the potential of IR spectroscopic methods for the determination of mycotoxins in food crops. We critically reflect on the applicability and limitations of IR spectroscopy in routine analysis and provide guidance for experts from the food and feed sector considering implementation of IR spectroscopy for rapid mycotoxin screening. In the end, an outlook on trends and possible fields of applications will be given.

MULTI-YEAR MYCOTOXINS MONITORING INTO CEREALS/CEREALS-BASED RAW MATERIALS AND FOOD COMMODITIES

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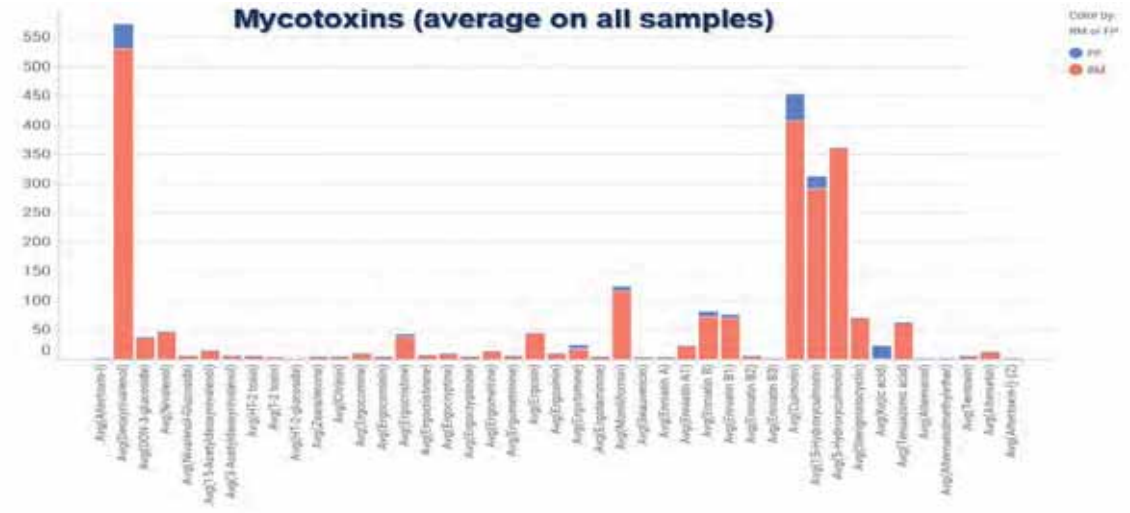
A comprehensive validated multi-mycotoxin LC-MS-MS based method has been successfully exploited to collect valuable and reliable data related to durum wheat - common wheat - rye in several European countries and different yearly campaigns. The main aspects that have been inferred from this study put in evidence:

- the clear prevalence only of specific categories of mycotoxins, within a defined raw material, in a quite independent way with respect to the harvesting campaign; and
- the general predominance of deoxynivalenol (and conjugated forms), ergot alkaloids, moniliformin, culmorins, sterigmatocystin and enniatins.

A similar figure has been collected also for pasta / bakery products, industrialized through exploitation of the above cited raw materials. In this way, potential correlations were explored in terms of average mycotoxin type/content in the main cereal ingredient with respect to the residual detectable amount found in the finished products. The obtained outcomes can be considered strategically relevant for mycotoxins risk assessment of cereals food chains, also having the possibility to gain important information for the dialogue with authorities in view of future new/revised legal limits.



Acknowledgements
This project has received funding from Austrian Competence Centre for Feed and Food Quality, Safety and Innovation FFoQSI GmbH, 2017-2020 Cooperation Agreement CP FFoQSI – Barilla V1 171117.

MICROBIOME ANALYSIS REVEALS FUNGI AS A MAJOR DRIVER IN HARD CHEESE RIPENING

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Vorarlberger Bergkaese (VB) is an artisanal Austrian washed-rind hard cheese produced from alpine bovine raw milk without the addition of ripening cultures. Its protected designation of origin and the standardized, traditional manufacturing method makes it an exceptional traceable system for exploring microbial succession. In a first step, we used a quantitative PCR approach and a targeted amplicon sequencing strategy for samples taken during VB ripening to understand the dynamics of previously described abundant bacterial and fungal taxa. In the fresh products, VB rinds were dominated by *Staphylococcus equorum* and *Candida*-associated fungi. At early ripening times (14–30 days), *Psychrobacter* and *Debaryomyces* flourished, whereas at the latest ripening times (90–160 days), rinds were dominated again by *S. equorum,* as well as by *Brevibacterium*, *Corynebacterium*, and *Scopulariopsis* strains. Dependent on the ripening period, dynamic correlation patterns could be identified for bacteria and fungi. In a next step, we used an integrative approach leveraging microbial metagenomes and metatranscriptomes (by using shotgun high-throughput sequencing) to unravel microbial function throughout ripening. We found that in 30-day old cheeses, degradation of residual lactose, lactate, proteolysis and lipolysis pathways were more transcribed and mainly associated with *Staphylococcus* species. After 90 days of ripening, genes associated with metabolizing smaller compounds derived from this initial degradation process, e.g., fatty acids and amino acids were significantly upregulated and associated mainly to *Brevibacterium* and *Corynebacterium*. These late metabolic activities included end products that are important for cheese flavour and aroma, e.g., methanethiol and 2,3-butanediol. Towards this, we have isolated a number of co-existing fungi, among them a highly abundant *Scopulariopsis* species. The compounds of the *Scopulariopsis* pure culture were separated with HPLC. Co-cultivation experiments with purified fractions could identify bioactivity against *Staphylococcus* and *Brevibacterium* species. This study deepens our understanding of microbial succession during ripening and it is the starting point for a targeted modelling approach, which will aim to enhance defined organoleptic properties. Furthermore, metabolites from the active *Scopulariopsis* fraction will be characterized in an ongoing project and might be used as biocatalysts or medicine in the future.

FUNGI AND FUNGAL TOXINS IN BAKERY PLANTS AND PRODUCTS

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Temperature and humidity in bakeries are optimized to allow for ideal proliferation of the Baker´s yeast. At the same time, these environmental conditions also enable the growth of mould species that can contaminate the final product directly or indirectly via released mycotoxins. However, occurrence data on mycotoxins in finished products from both small- as well as large-scale bakery plants seems to be less comprehensive compared to data sets obtained on raw grains. In this work, we have validated a previously developed ‘dilute and shoot’ method for four matrices of processed cereal products, i.e.,

noodles, biscuits, crackers, and muesli and applied it to environmental and flour samples from a local bakery as well as to various sample types deriving from industrial baking. Citrinin and dihydrocitrinone were identified in the mouldy bakery baskets (confirming the related identification of *Penicillium citrinum* using a PCR-based method) but were not detected in the related flour samples. The samples from industrial-scale baking exhibited very low levels mainly of type A- and B-trichothecenes, ergot alkaloids and tenuazonic acid.

SESSION 9
UPDATE ON (MULTI-)MYCOTOXIN ANALYSIS

A small selection of recent research in the area of (multi-)mycotoxin analysis will be presented.

LUMINESCENT BIOSENSING STRATEGIES FOR THE ANALYSIS OF BIOTOXINS

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Biotoxins are toxic compounds found in nature produced by microorganisms, plants or animals. They can be metabolites of living organisms, products of the decomposition of dead organisms or substances that become toxic through their metabolic activity. Biotoxins have a detrimental impact on human and animal health as well as in the environment and the economy worldwide. Within this group, mycotoxins are secondary metabolites produced by fungi that can cause acute or chronic diseases, known as mycotoxicosis, and there is a great interest in the development of simple, fast and robust analytical methods that can be applied for the analysis of these compounds at low cost. The use of antibody-based methods is especially attractive for sensitive and selective detection of mycotoxins in food matrices. However, these methods conventionally require conjugation of the toxin molecule, either to a carrier protein or to a label, and this step can be time-consuming, costly, and challenging because it may result in conjugates showing low affinity for the antibody.

To overcome such limitations, we have focused on the application of peptidomimetics (mimopeptides), and recombinant antibody fragments (rAb) selected by phage display techniques for the analysis of micotoxins of particular incidence in Mediterranean countries such as zearalenone (ZON), HT-2 toxin and an emerging mycotoxin, mycophenolic acid (MPA). The functionality of the novel binders has been confirmed in phage-based ELISAs. After identifying the specific sequence coding for the novel peptide or rAb, they have been fused by genetic engineering to luminescent proteins, using different approaches, providing simple and cost-effective alternatives to the traditional immunoassays using labelled secondary antibodies for the analysis of the selected mycotoxins. The assay has been implemented on antibody-coupled magnetic beads, and the bioluminescent sensors enabled the determination of ZON, MPA and HT-2 toxin with a detection limit of 4.2 ng/ml (IC_{50} value of 11.0 ng/ml), 0.26 ng/ml (IC_{50} value of 2.9 ng/ml) and 0.24 ng/ml (IC_{50} value of 4.8 ng/ml), respectively. The biosensors showed an excellent selectivity to the selected mycotoxins and have been applied to the analysis of the target compounds in food samples, followed by validation by LC-MS/MS or HPLC-DAD.

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This work has been funded by the Ministry of Science, Innovation and Universities (RTI2018-096410-B-C21).

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GENERATION OF HIGH-AFFINITY ANTIBODIES FOR PATULIN ANALYSIS

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Rapid immunoanalytical methods greatly contribute to strengthening the safety of our food supply by efficiently monitoring chemical contaminants, so high-affinity and specific antibodies have been generated for almost all the internationally regulated mycotoxins. The only exception is patulin, a mycotoxin mainly produced by *Penicillium expansum* for which such a goal has not yet been achieved. Accordingly, no point-of-need tests are commercially available for patulin immunodiagnostics in food. The first reported attempt to produce anti-patulin antibodies dates back to 1986; however, low antibody titres were obtained and poor recognition of free patulin was observed [1] The same approach was further followed by other authors, with similar disappointing results [2-4]. In 2007, De Champdoré *et al.* [5] reported a different strategy, but the ability of the so-obtained antibodies to recognize free patulin in solution by standard competitive ELISA was not demonstrated. Accordingly, it remains controversial whether the approaches described to date for the generation of anti-patulin antibodies may result in specific, high-affinity binders suitable for the development of rapid test for food immunoanalysis. Moreover, the lack of commercial immunoassays for patulin most likely reflects the inherent difficulties in raising antibodies for this relevant mycotoxin.

In the present study, three functionalized derivatives conforming to generally accepted rules in hapten design were firstly tested in order to generate suitable antibodies for the sensitive immunodetection of patulin. However, these conventional bioconjugates were unable to elicit the desired immune response, so an alternative strategy that takes advantage of the high electrophilic reactivity of patulin was explored. The obtained adduct was used to produce antibodies with nanomolar affinity values. These results demonstrated for the first time that targeting the adduct resulting from the reaction of patulin with a thiol-containing compound is a promising approach for developing user-friendly immunoanalytical techniques for this elusive mycotoxin.

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VALIDATION OF A SCREENING METHOD: A COMPARISON OF CURRENT GUIDELINES AND GAPS FOR AUTOMATION

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Screening test kits for mycotoxin analysis, based on immunochemistry, are widely used in the food and feed industries for on-site control, and could also be useful for managing high analytical volumes or analysing perishable matrices in public and service laboratories. Before test kits can be used in a laboratory process, their performances must be verified and validated with a well-defined, fit-for-purpose, and affordable protocol. But how are the most relevant parameters to a validation process chosen? There are several recognised sets of guidelines available that are issued by international bodies and certification entities. Depending on the source, different approaches are followed: some approaches focus on rating the ability of a test to distinguish whether the samples are below or above a certain target concentration. They can also set the criteria for accuracy and precision in a given

measuring range or demonstrate the reliability and robustness of commercial kits. Depending on the approach, a higher degree of attention may be given to specific indicators or aspects of the test, such as the matrix effect, the false-positive and false-compliant ratio, the repeatability and intermediate reproducibility, the correlation to instrumental analysis, or the lot-to-lot consistency and ruggedness. Therefore, the material effort, in terms of experiments, time, sample availability and kits consumed, is consequentially different. Besides specific prescriptions for validating manually run assays, what should be tested and investigated when a routine method is shifted to an automatic analyser? As the extraction efficiency is not impacted by the assay execution, which parameters should be evaluated and monitored during automatic implementation?

In this communication, a comparison of the most widely used validation guidelines will be presented through practical examples, followed by a presentation of a proposed approach for the validation of automatic ELISA methods.

STRATEGIES FOR THE DETERMINATION OF MULTI-MYCOTOXINS AND ALKALOIDS IN FOOD SAMPLES

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Herein we present a series of quantitative methods based on LC-MS/MS that constitute a 'toolkit' for the determination of multi-mycotoxins and alkaloids in food and feed commodities. The most widely used dilute-and-shoot approach will be discussed, while reporting a comprehensive study of the qualitative and financial benefits led by the addition of an appropriate SPE pass-through step for extract clean-up prior to mass spec analysis. The use of immunoaffinity chromatography coupled to HPLC with fluorescence detection can be used for compliance testing of targeted mycotoxins, and it represents a valuable alternative to the more expensive mass spectrometry systems, while providing the highest level of selectivity. Finally, a validated method for the determination of 35 pyrrolizidine alkaloids in botanicals and honey samples will be presented. Method performance fulfil the requirements set by the European legislation.

HIGH RESOLUTION MASS SPECTROMETRY: A PROMISE OR A BLIND ALLEY IN THE ROUTINE ANALYSIS OF MULTIPLE MYCOTOXINS?

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There is a constant need for multi-methods that can measure as many analytes as possible in a short time and to overcome obstacles such as matrix effects in routine analysis. Due to that high-resolution mass spectrometry emerges as a potential solution to all these challenges. Given the possibility of retrospective data mining, shortening measurement time and non-targeted data analysis. This study aimed to compare the suitability of the LC-MS/MS and LC-HRMS approach in the targeted analysis of > 500 mycotoxins using a dilute-and-shoot samples preparation protocol. The current LC-MS/MS QTrap 5500 (Sciex, Foster City, CA, USA) used 'dilute and shoot method' routinely in mycotoxin analysis [1] has been transferred to an LC-Q Exactive HF high-resolution MS. The chromatographic conditions by

Sulyok *et al.* [1] remained identical for both approaches. Primarily, QTrap 5500 MS/MS data were acquired in scheduled MRM mode in two individual chromatographic runs, in positive and negative ionization mode, respectively [1]. Afterward, the HR-MS data were acquired in the following full scan modes: (i) fast polarity switching positive(ii) and negative ionization(iii) mode, and all three of them with two resolving powers (i, ii, iii) of 120,000 FWHM (at m/z 200) and (i, ii, iii) 240,000 FWHM (at m/z 200). For most analytes, the quality of obtained data did not suffer (the number of points per peak ranged on average from 12 to 15) when fast polarity switching was used in comparison to individual negative/positive modes. Moreover, no significant difference in quantification limits was observed between those two modes. Detection limits were even slightly improved at higher resolving power. By comparing the linear range between fast polarity switching mode on high and low resolution, it can be noted that in both cases >90% of analytes have a determination coefficient (R^2) higher than 0,99. Matrix effects were expressed as signal suppression/enhancement (SSE) and calculated as the slope of matrix-matched calibration divided by the slope of external neat solvent calibration multiplied by 100. Fast polarity switching mode at lower resolution showed to be the best setup since 87% of analytes were in the SSE range of 80-120%. Contrary to expectations, individual ionization modes had a lower percentage (80% of analytes) in this range of SSE. While in the case of higher resolution, in fast polarity switching mode, only 70% of the analyte fell in the SSE of 80-120%. Thus, the fast polarity switching mode at lower resolution improved the matrix effects the most. The preliminary results showed that the application of LC-HRMS in multiple mycotoxin analysis with the option of retrospective data mining proved to be a competitive technique to LC-MS/MS. In addition, fast polarity switching allows to shorten the measurement time by half.

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REALIZATION OF THE TECHNICAL REQUIREMENTS OF AN ISO 17025 ACCREDITED LC-MS/MS MULTI-TOXIN METHOD FOR FEED AND RELATED MATRICES

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To provide confidence in the operation of laboratories in the field of confirmatory assessment, the International Organization for Standardization (ISO) has developed requirements which are summarized in the ISO norm 17025. The fulfilment of these requirements enables laboratories to demonstrate that they operate competently and are able to generate valid results. In this contribution an LC-ESI-MS/MS method, covering 90 secondary fungal and plant metabolites, was developed following a stable isotope dilution assay. The approach was successfully validated in thirteen complex feed materials, including pet food and related matrices, based on the recommendation of SANTE/12682/2019 which obtained an accredited status for the entire commodity group of animal feed. Complex matrices including cattle, chicken and swine feed were artificially prepared in-house by modelling five individual lots for each matrix group, to challenge the protocol of extraction and provide a better picture of real-world conditions. More than 60% of all analyte matrix combinations complied with the target performance criteria for recovery (70–120%) and precision (<20%). High matrix effects, especially absolute rather than relative, emerged as the main obstacle to the overall analytical performance. Low extraction efficiencies were obtained for compounds with an alkaline functional group, indicating the need to perform the extraction process at low pH, considering the majority of target analytes contain an acidic moiety. To optimize the standard management protocol and gain important information about the preparation of individual standard mix solutions, a long-term stability study was concomitantly performed. Results obtained for eleven intermediate solutions, each including between three to ten compounds, revealed a stability of the reference standard mixtures for at least six months. Method authenticity was tested by performing an Interlaboratory Comparison Study in four different feed matrices with eight international laboratories specialized in mycotoxin analysis.

In summary, the work presents a confirmatory method for 90 biotoxins based on LC-MS/MS for feed at the highest metrological level, fully compliant with the high technical requirements from ISO 17025.

OCCUPATIONAL EXPOSURE TO MYCOTOXINS: A BIOMONITORING AND AIRBORNE MEASUREMENT APPROACH

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It is now recognized that additional exposure to mycotoxins may occur through inhalation of contaminated dust at workplace. The aim of this study was to characterize the exposure to multi-mycotoxins of French grain elevator workers using biomonitoring and airborne measurements. A multi-class mycotoxin method was developed in urine for the determining aflatoxin B1 (AFB1), aflatoxin M1 (AFM1), ochratoxin A (OTA), ochratoxin α (OT α), deoxynivalenol (DON), zearalenone (ZEN), α-zearalenol (α-ZEL), β-zearalenol (β-ZEL), fumonisin B1 (FB1), HT2-toxin and T2-toxin. Analysis was based on liquid chromatography–high resolution mass spectrometry. Sample pre-treatments included enzymatic digestion and an online or offline sample clean-up step. The method was validated according to the European Medicines Agency guidance procedures. To estimate external exposure, air samples were collected with a Capteur Individuel de Particules 10 personal dust sampler. Up to ten mycotoxins, including aflatoxins, ochratoxin A, deoxynivalenol, zearalenone, fumonisin B1 and HT-2 toxin and T-2 toxin were quantified in the samples. The biomonitoring and airborne mycotoxin measurements showed good analytical performances.

They were applied in a pilot study to assess mycotoxin contamination in eighteen grain elevator workers. Workers provided multiple urine samples including pre-shift, post-shift and first morning urine samples or 24 h urine samples. Grain elevator workers were highly exposed to organic airborne dust (median 4.92 $mg.m^3$). DON, ZEN and FB1 were frequent contaminants in air samples. The mycotoxin biomarkers quantified were DON (98%), ZEN (99%), α-ZEL (52%), β-ZEL (33%), OTA (76%), T-2 (4%) and HT-2 (4%). The urinary DON concentrations were significantly higher in post-shift than in pre-shift-samples (9.9 and 22.1 µg/L, respectively). ZEN and its metabolites concentrations did not vary according to the sampling time. However, the levels of α-/β-ZEL were consistent with an additional occupational exposure. These data provide valuable information on grain worker exposure to mycotoxins. They also highlight the usefulness of multi-mycotoxin methods in assessing external and internal exposures, which shed light on the extent and pathways of exposure occurring in occupational settings.

SESSION 10
MODELLING STRATEGIES AND DIGITALISATION IN MYCOTOXIN MANAGEMENT

The emergence of innovative solutions in mycotoxin management requires support from computational modelling and digitalisation. Recent developments will get through here.

LIMITING MYCOTOXIN EXPOSURE OF LIFESTOCK BY MONITORING AND FORECASTING OF CONTAMINATIONS IN FEED CROPS

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Mycotoxins are prevalent fungal contaminants in animal feed crops. They negatively affect animal health and consequently performance. Minimizing mycotoxins improves the animal's development but also reduces crop waste or need for blending below harmful levels. Occurrence in crops may vary strongly depending on growing region, agronomic factors, and climatic conditions. Measures aiming to reduce contamination in feed need to be based on prior knowledge on toxin prevalence and contamination levels in a certain area. DSM's mycotoxin survey is an extensive database that aggregates global mycotoxin occurrence data from over 130,000 samples collected on the field, from various crop-based commodities as well as finished feed. It provides users with a sound basis for estimating their local mycotoxin risk. This service is now expanded to prediction of mycotoxins in upcoming harvests by using weather as main predictor. Users can either access field-based custom predictions for deoxynivalenol on wheat via the adapted MyToolBox platform or access weekly updated regional predictions for maize and wheat in combination with four regulated mycotoxins. Increased regional risk, may warrant targeted toxin-specific measures, ranging from changed agronomic practices, to additional testing of grains at risk or treatment of finished feed with mycotoxin degrading products, such as the Mycofix product line.

TAKE A BYTE OF MYCOTOXINS: WHY AND HOW *IN SILICO* ANALYSIS BOOSTS KNOWLEDGE AND FEEDS MYCOTOXIN RESEARCH

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Mycotoxins are a class of chemicals with members posing toxicological and food safety concerns due to their effect on human and animal health. Mycotoxins may also affect plant kingdom raising agronomical issues, and they have an ultimate impact on the environment in line with the One Health paradigm. In turn, all this may eventually affect economy, society and politic at a national or international scale with potential consequences on global health.

Almost the entire biology of mycotoxins, including the capability to harm living organisms, depends on their chemistry, and adverse stimuli are likely elicited whenever mycotoxin chemistry allows targeting certain biological macromolecules and structures. However, many pieces of information are still missing in that sense preventing the full comprehension of mycotoxin biology in spite of the wealth of data collected over the decades. This prevents to achieve a satisfying toxicological assessment and hampers an effective prediction and prevention of the adverse effect of mycotoxins. Such a complex gap is hard to get filled by-hand and requires structured and high-throughput approaches. In this respect, computational methods may deal with all this allowing the deep decryption of chemical aspects underpinning mycotoxin biology. Bioinformatics and computational chemistry have provided the base of knowledge to efficiently tackle mycotoxin research at many levels. As an example, in the last decades we showed how molecular modelling and the analysis of the chemistry beneath mycotoxin action can extend the toxicological understanding refining the early phases of risk assessment, as described for

instance for zearalenone and analogues. More recently, with a focus on ochratoxin A, we also demonstrated that bioinformatics succeeds to deal with toxicokinetic aspects providing a useful framework to investigate the inter-individual susceptibility to mycotoxin action. From an environmental perspective, *in silico* methods may allow for the systematic analysis of mycotoxin-dependent plant-pathogen interactions as well as the rational design of effective mitigation strategies via enzymatic or biological means to reduce the accumulation of mycotoxin in plants, food and related waste, and environment.

The modular nature of *in silico* tools and the use of high-performance computing facilities ensure setting high-throughput workflows to tackle complex system analysis, also using artificial intelligence. The complexity of the outcomes of such analysis can be then opportunely deconvoluted and applied at many levels of mycotoxin research under the One Health umbrella. This ultimately broadens the understanding of mycotoxin action and biology drawing mycotoxin research near the holistic perspective it would need starting from.

UTILIZING COMPUTATIONAL TOOLS TO IMPROVE THE BIOLOGICAL DETOXIFICATION OF MYCOTOXINS.

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Microbes possess a rich potential for removing toxins and pollutants from the environment. Despite the fairly wide availability of this potential, identifying suitable candidates and improving them remain challenging. Here, we explore the use of computational tools to discover strains and enzymes that detoxify harmful toxins. As a focus, we explore mycotoxins (fungus-produced toxins) that contaminate food and feed and cause serious health and economic consequences, and biological enzymes that are capable of detoxifying them to less harmful compounds. Biological detoxification is a promising solution to mycotoxin contamination because of its low cost, few undesired environmental side-effects, and potentially high efficiency and reliability. Identifying new detoxifying species is highly beneficial in that it can offer alternatives to overcome the limitations of existing biodegraders, such as narrow working conditions and low degradation rates. Additionally, exploring the mechanisms of detoxification by these organisms enables us to improve their detoxification capacity. Established and novel computational tools can be implemented to complement existing empirical approaches toward three main goals: (i) discovering detoxification potential in underexplored species; (ii) finding important cellular processes that contribute to detoxification; and (iii) improving the detoxification performance of discovered enzymes. We hope to create a synergistic conversation between researchers in computational biology and those in the bioremediation field. We showcase open bioremediation questions where computational researchers can contribute and highlight relevant existing and emerging computational tools that could benefit bioremediation researchers.

THE DESIGN OF EFFECTIVE MONITORING FOR MYCOTOXINS USING MACHINE LEARNING AND MULTIPLE CRITERIA DECISION MAKING

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The presence of mycotoxins in agricultural commodities for feed and food production around the world poses a threat to animal and human health, and economics. Governmental agencies and companies have monitoring programs in place to check for the presence of mycotoxins in raw materials and derived feeds and foods. Given limited resources and following a risk-based approach for food safety monitoring,

batches with the highest probability to be contaminated are checked. Nowadays, several methods are being explored for assisting in the design of risk-based monitoring programs for mycotoxins, such as machine learning and economic methods. In this presentation several case studies applying these methods will be presented.

The first case deals with the use of machine learning (ML) algorithms for the design of a risk-based monitoring program for aflatoxin B1 (AFB1) in feed materials. Historical monitoring data for AFB1 in feed products (2005–2016; 5003 records in total) were used to apply ML models aimed to predict the high-risk feed batches to be considered for further AFB1 sampling and analysis. Both monitoring cost and model performance were used as ML model selection criteria. Total monitoring cost included cost for sampling and analysis, disease burden, storage, and recalling and destroying contaminated feed batches. Results showed that ML algorithms were able to predict the high-risk batches. The Extreme gradient boosting (XGB) algorithm outperformed the other investigated ML and had an accuracy higher than 0.9. Using this ML algorithm to select the high-risk feed batches for further AFB1 sampling and analyses in practice would highly reduce the monitoring costs.

When other criteria come into play in the design of monitoring programs, Multiple Criteria Decision Analysis (MCDA) can be used. In a second case study, MCDA was applied for selecting an AFB1 monitoring schemes along the Dutch dairy chain. In this case, the optimal monitoring scheme was considered to best meet the views of stakeholders from the dairy cows' feed industry and dairy industry, including their possible conflicting interests. Three different intensity levels of the AFB1 monitoring program were considered at each of the feed mill and the dairy farm. The intensity levels were low (50%), medium (80%) and high (90%) probability of detecting AFB1 contamination (in case AFB1 was present). Five criteria were used, which were scored using quantitative models, literature, and input from experts. Weights of the criteria were obtained from stakeholders from the feed and dairy industry, using interviews. Stakeholders from the feed and dairy industry had similar view on the relative importance of the criteria, and the criterion 'public health' was considered most important. The preferred AFB1 monitoring program for the feed industry consisted of *high intensity monitoring* at both feed mills and dairy farms. The dairy industry preferred *medium intensity monitoring* at feed mills followed by *high intensity monitoring* at dairy farms.

The presented methods showed to be useful in the design in AFB1 monitoring programmes and can thus support authorities and industries in their monitoring tasks.

MONITORING MYCOTOXINS ACROSS SCALES: DIGITAL TOOLS FOR SMALLHOLDER FARMING SYSTEMS

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Mycotoxin-contaminated crops pose a significant challenge to the health of humans and livestock, especially in resource-limited settings. In sub-Saharan Africa, smallholder farming systems that rely on maize and groundnut as staple crops have often been hotspots of extreme mycotoxin exposure [1]. In such areas, conventional mycotoxin testing and regulation methods are not broadly feasible, and alternative strategies for monitoring must be explored. At the landscape scale, satellite remote sensing metrics of soil quality, plant health, and weather conditions are widely available and have been used to model spatiotemporal mycotoxin dynamics [2]. On the scale of a cup of ground maize, near-infrared reflectance (NIR) offers a rich source of information on a sample's quality and composition that may associate with mycotoxin contamination. This study aimed to understand how these digital tools might complement conventional wet lab analytical methods and expand the toolkit for modelling and predicting mycotoxins.

We surveyed local grain mills within a maize-based smallholder farming system in semi-arid Kongwa District, Tanzania. Farmers were interviewed at the end of the 2019 growing season, and milled samples of their locally grown maize (n=306) were acquired for analysis. Each ground maize sample was scanned with a SCiO NIR Micro Spectrometer (700-1100 nm), and then each sample was halved and

extracted for separate aflatoxin and fumonisin assays. Enzyme-linked immunosorbent assays (ELISA) were conducted for total aflatoxins and total fumonisins. For remote sensing data, georeferenced grain mill locations were used to define local grainsheds from which high-resolution (250 m^2) soil characteristics (soilGrids250m) and growing season plant health (normalized difference vegetation index (NDVI)) data were acquired for each sample [3-4]. ELISA results showed that maize mycotoxin levels exceeded the Codex Alimentarius Commission's standards for total fumonisins (2 ppm) in 19% of samples and total aflatoxins (10 ppb) in 78% of samples [5]. Mixed effect linear models were used to model and predict fumonisin or aflatoxin concentrations using different combinations of covariates: (i) only remote sensing data; (ii) only NIR data; and (iii) both remote sensing and NIR data. For both fumonisins and aflatoxins, model performance was modest (fumonisin R^2 range: 28.7-33.4%, aflatoxin R^2 range: 34.1-39.3%), but the combination of remote sensing and NIR data always produced the best performing model. Further, analysis of covariate effects confirmed previous remote sensing studies (e.g., reduced NDVI was associated with increased fumonisins, and lower soil bulk density was associated with higher aflatoxins). Also, NIR covariates implicated regions of the near-infrared spectrum associated with both aflatoxin and fumonisin accumulation.

This study demonstrates how inexpensive digital technologies that operate at different scales offer complementary information that can improve mycotoxin monitoring in low-resource areas.

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A UNIQUE MYCOTOXIN DATABASE: HOW IT CAN HELP IN THE PRIORITIZATION OF MYCOTOXIN TOXICITY ASSESSMENT

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Food production throughout the world is concerned by contamination with natural substances such as mycotoxins. These toxins are produced by fungi and responsible for diseases in livestock but also in humans with 50 000 hospitalizations estimated per year in the European Union. The concern due to the presence of mycotoxins in food and feed is increasing as the development of fungi is favoured by global warming. The data available on these substances are sometimes scarce but they are mostly present in a large source of information supports. In order to centralize and combine all kind of information that are available about mycotoxins, we are developing a mycotoxin database within the Interreg project Agritox. Although still under construction, it currently contains more than 2 000 mycotoxins, the synthesis pathways, the associated fungi as well as physico-chemical information. Mainly based on text mining approaches, the detection and quantification methods in food and feed will be also added to the database. Moreover, toxicological data for each mycotoxin will be implemented to this database: they are either collected from different databases or predicted by several QSAR and ADMET methods and are crossed with literature.

Two examples will be presented on the application of this database for predictive toxicology. The first example will show the selection of a small cluster of enniatin-like compounds and their study *in vitro* to outline a link with lung cancer. The second example will deal with the mutagenic and carcinogenic evaluation of 904 mycotoxins using 17 different QSAR models, 9 for mutagenicity and 6 for carcinogenicity. A scoring method is applied to take into account the prediction output of each model,

ending by a comparison with the alert prediction from DEREK Nexus. The predictions are further compared with experimental data to select the best method combination that will be further applied to the whole mycotoxin list. In conclusion, this database aims to prioritize the compounds to be tested and to identify the data gaps. We expect that it will be completed and released in one year. Any comment or suggestion will be welcome to improve it.

Acknowledgements
This research was supported by the Interreg Agritox project EAPA-998-2018.

CAN WE PREDICT AFLATOXINS OCCURRENCE IN AFRICA? THE APHLIS MYCOTOXINS RISK MODELLING, APPROACH, LESSONS LEARNED AND RE-ORIENTATION

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Mycotoxins in staple cereals and nuts are a major food safety concern and projections suggest risks will increase with the expected climatic changes. In the tropics, and in Africa in particular, mycotoxin contamination of staple foods is a serious problem, with known negative effects on human health and nutrition, animal productivity and on commodity trade and therefore national economies. For several crops, including maize and groundnuts, the development of *Aspergillus flavus* and other mycotoxin-producing fungi during the pre- and postharvest crop stages is to a large extent influenced by weather conditions. For example, drought stress during certain stages of plant growth can increase the risk of pre-harvest fungal contamination of the crop, and can stimulate mycotoxin production, which in turn leads to contamination of the food crop. Unusually heavy rainfall just before harvest or during the drying phase is also an important factor in postharvest losses, again favouring the infection and growth of various fungi.

The African Postharvest Losses Information Systems project (APHLIS), www.aphlis.net, has been studying postharvest cereal losses estimation in Africa for many years and started complementing its quantitative loss estimation with research on the main drivers of aflatoxins occurrence. The possibility of monitoring agro-climatic conditions favourable to mycotoxin growth in near-real time has also been explored to identify periods and areas of increased risk of contamination. This led to the production of experimental agro-climatic mycotoxin risk maps with the aim of providing early warning information of a possible high risk of mycotoxin contamination to guide targeted ground-level contamination surveying and risk mitigation. These maps, based on low resolution weather data and a generic agriculture phenology at the continental level have proven useful in signalling potential higher-risk locations based on some of the main known growth factors of mycotoxins (aflatoxins in particular) but cannot be quantitatively validated against mycotoxins occurrence. Further efforts need to be made to refine the model of these simple agro-climatic risk maps and avoid possible false positive warnings. Additionally, the model is insufficiently detailed to rule out causing possible negative consequences of inaccurate mycotoxin risk communications on food safety and product trade decisions. While these maps will continue to be used at the experimental level, the project initiated new scientific collaborations with experts from a selected number of countries to develop predictive models dedicated to specific areas and crops in an attempt to reach higher accuracy. The main aim of this collaboration is to explore the potential for ground-truthing of models for specific toxins and for specific geographic areas with high quality mycotoxins incidence data sets. The current APHLIS mycotoxins agro-climatic risks maps were presented at a workshop on predictive modelling of mycotoxins in Africa, organised collaboratively by the main mycotoxin research networks in October 2020, and the possible decision support role of the risk maps is mentioned in a review paper on predictive modelling of aflatoxins in Africa published in 2021 (https://knowledge4policy.ec.europa.eu/publication/predictive-modelling-aflatoxin-contamination-support-maize-chain-management_en).

FINAL PLENARY SESSION
LOOKING FURTHER AHEAD

Take a step back, take a deep breath, and look forward. What can be expected?

INCLUSION OF ANTIFUNGAL RESISTANCE IN ONE HEALTH POLICY AND DIALOGUE

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The rise of antimicrobial resistance (AMR), including both antibacterial and antifungal resistance, is a serious threat to public health and the economy, and the US federal government has pledged to work both domestically and internationally to combat AMR. To this end, the Presidential Advisory Council on Combating Antibiotic-Resistant Bacteria (PACCARB) was established in 2015. PACCARB is a U.S. federal advisory committee that provides advice, information, and recommendations to the U.S. Secretary of Health and Human Services regarding programs and policies intended to support and evaluate the implementation of U.S. Government activities related to combating antimicrobial resistance. The PACCARB is a One Health body that includes experts in human, animal, environmental health, and addresses AMR across all domains, including agricultural and crop. While the name indicates a focus on antibiotics, the discussion around growing antifungal resistance is of equal importance. Mycotoxins are a risk to the health of both the crops and animals in the agricultural sector, as well as a risk to the agricultural economy. Antifungals are an important tool used to minimize that risk. However, the fight against mycotoxins can have some unintended implications. In the USA and other countries, a growing rate in fungal infections in humans, including antifungal-resistant infections, has been noted. Additionally, the agricultural sector is reporting growing rates of triazole and multi-fungicide resistant agricultural pathogens. These experiences highlight the need to balance the fight against mycotoxins with antifungal stewardship, as well as the interconnectedness of all One Health domains regarding AMR. To help establish this balance, the Council has explored and made recommendations on the additional research gaps needed to combat resistant fungi and better understand the impact of antifungal use for both humans and crops.

EXPECT THE UNEXPECTED – FOOD TRENDS INFLUENCING MYCOTOXIN TRENDS

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The past few years we have seen some significant changes in food trends. One of the major trends is of course ‘plant-based everything’ – from plant-based alternatives for dairy products, meat products or any other product of animal origin. Some of these products are based mainly on pulses, such as soy, or cereals or nuts. ‘Superfoods’ is another trend, e.g., fermented products, green tea, berries, seeds, nuts, ingredients, such as hibiscus, cannabis, matcha or ancient grains, such as teff, spelt, sorghum. On top of that ‘sustainability’ or ‘organic’ continue to be trends as well as ‘ocean garden’, e.g., seaweed. The COVID pandemic put further emphasis on ‘health’ and ‘immunity’.

So, food consumption patterns are definitely changing. As a result, we also see a change in the exposure to mycotoxins. Of course, a higher consumption of cereal products might lead to a higher exposure of mycotoxins. Less well known is, e.g., the occurrence of ochratoxin in soy or other beans, an oat milk latte might contain ochratoxin contributed by the coffee but also by the oat milk, topped probably with some T-2/HT-2 as well from oat. Also, the ancient grains, such as spelt, teff, and quinoa, might be grown, harvested, and stored under conditions more favourable to mycotoxin formation and are maybe not

always on the radar for mycotoxin testing. The same might be true for dried products, such as green tea, cannabis, or other botanicals. Or who would have thought of mycotoxins in seaweed, such as kelp? Therefore, expect the unexpected. Food trends might introduce unexpected mycotoxins from unusual sources as well as change the total mycotoxin exposure due to the changes in consumption. In this presentation some examples of unexpected toxins will be presented.

MYCOTOXINS IN THE EUROPEAN HUMAN BIOMONITORING INITIATIVE (HBM4EU): LESSONS LEARNED, LOOKING FORWARD

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The European Human Biomonitoring Initiative (HBM4EU, https://www.hbm4eu.eu) is a project gathering 30 countries, funded under Horizon 2020, and running from 2017 to 2022. The goal of HBM4EU is to generate evidence on the current exposure of European citizens to chemicals and on their possible health effects to assess the associated risks. Following a systematic prioritization exercise, the mycotoxins deoxynivalenol (DON) and fumonisin B1 (FB1) were considered as priority substances around which the HBM4EU research programme was developed and followed by the Chemical Group Leaders (CGL) team.

Within the three pillars of the HBM4EU, i.e., 'Science to Policy', 'European HBM Platform', and 'Exposure and Health', several policy questions were addressed for these mycotoxins. Those questions concerned exposure biomarkers and associated analytical methods; exposure models and toxicokinetic data; exposure assessment in the EU population, including vulnerable population groups (e.g., workers); health outcomes from DON or FB1 exposure; related adverse outcome pathways development and effect biomarkers identification; and risk characterization. Data gaps and research needs were also identified. Key outputs for DON and FB1 under HBM4EU include: (i) a biomarker selected to assess human exposure to DON (total urinary DON) that will be used in the aligned studies; (ii) several European laboratories selected to perform DON analysis after passing an interlaboratory quality control study; (iii) a research protocol on human exposure and geographic variations in Europe; (iv) a risk assessment report dedicated to DON based on exposure in Europe; (v) a review of available toxicokinetics models; (vi) a draft on the possible mechanisms of FB1-induced adverse health effects; and (vii) a specific effect biomarker identified for FB1.

An overview by CGL on main project achievements, as well as lessons learned and research needs and gaps will also be discussed as a contribute for the next European partnership for the assessment of risks from chemicals (PARC).

Acknowledgements
Work co-funded by the HBM4EU project, Grant Agreement No. 733032 and by Portuguese national funds: FCT/MCTES through CESAM (UIDP/50017/2020 + UIDB/50017/2020) and ToxOmics (UIDB/00009/2020 & UIDP/00009/2020).

ANALYSING THE CHEMICAL EXPOSOME: DELUSION OR NEXT FRONTIER?

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Throughout our lifetime we are exposed to a multitude of food and environment-related molecules including mycotoxins. These exposures may impact the aetiology and course of a large share of human disease. Analytical technology remains a major limitation to enable exposome-wide assessment of chemical exposure. This contribution will present innovative workflows for the omic-scale investigation of natural and synthetic toxicants that are highly diverse in terms of physico-chemical properties, concentrations found *in vivo*, and toxicological impact/mode of actions. Newly established workflows based on liquid chromatography coupled to mass spectrometry (LC-MS) will be presented. This includes targeted, non-targeted and stable isotope-assisted approaches that have been thoroughly tested in first pilot applications. Abundant exposure data derived from proof-of-principal experiments will be presented to showcase the dynamics and complexity of different exposure scenarios. A particular focus will be on early-life chemical exposure as exemplified by the analysis of plasma samples from extremely premature infants. Moreover, the capacity of the newly established, broad methods will be demonstrated by reporting xenobiotics in breast milk and urine samples from diverse populations.

THE POWER OF GLOBAL NETWORKS FOR CAPACITY BUILDING IN MYCOTOXIN RESEARCH

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One of the most persistent food safety challenges worldwide are caused by mycotoxin producing fungi. Despite decades of research and technological progress, this problem has not been fully resolved. Many factors, including climate change, globalization, poverty, and conflicts, worsen the impact of unsafe food on human and animal health and are an obstacle in reaching the Sustainable Development Goal 2. Capacity building is defined as the process of developing and strengthening the skills, instincts, abilities, processes, and resources that organizations and communities need to survive, adapt, and thrive in a fast-changing world. As scientists, it is very important to join global efforts and networks to work in a most efficient way with the limited available financial resources. As an example, the still missing links between mycotoxin exposure and human disease outcomes – due to a lack of large-scale and well-designed epidemiological studies – need to be bridged through joint efforts. Only then, we will be able to provide the different stakeholders with strong evidence to support stringent policy measures. Global networks, such as the International Society for Mycotoxicology (ISM), MYTOX-SOUTH®, and other international societies play a key role in our mission towards a mycotoxin-safe world.

In this presentation, key challenges for sustainable mycotoxin research will be proposed that can only be successful through concerted actions:

- Building of equitable partnerships with focus on sustainable bottom-up human capacity building though training and education of young and gender-inclusive innovators in the food safety area, including the low- and middle-income countries (LMICs), as we are doing through the MYTOX-SOUTH® network.
- Investing in a harmonized global collection of accessible mycotoxin contamination data according to the FAIR principle, which means that data should be findable, accessible, interoperable and reusable. Open-access data for all stakeholders, from farmers to policymakers to consumers is necessary. Visualization of the mycotoxin contamination data for more efficient risk communication and decision-making should become a priority.
- Prioritization of high-quality food safety research, linking food safety-related exposure to disease outcomes, more specifically through combining large-scale epidemiological designs

along with mechanistic insights. Breakthrough innovation is expected from the exposome concept in which we measure all environmental influences and associated biological responses throughout the entire lifespan of humans, which consists of the analysis and integration of big data sets.
- Linking natural sciences with social sciences. Despite our efforts for technological innovations, implementation of mycotoxin mitigation measures and legislation is still running behind in LMICs. It is important to map the science-policy-society system to better understand how different actors influence the food safety systems in LMICs.

Examples on how global networks, such as ISM and MYTOX-SOUTH®, are contributing to these key challenges will be presented.

AN OUTLINE OF THE FOOD SAFETY COALITION PROJECT TO ADDRESS THE CHALLENGES OF AFLATOXIN CONTAMINATION IN RAW MATERIALS

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The Food Safety Coalition was formed ahead of the CEO Consultation in the lead up to UN Food Systems Summit in 2021. The coalition is a group of like-minded organizations committed to progressing insights and solutions at pace in a critical area of food safety, specifically data and knowledge sharing to address the challenge of aflatoxin contamination in raw materials. Aflatoxins are a key global challenge, posing a serious health threat to developing and developed worlds alike. As well as being linked to stunting, damage to the immune system, and maternal anaemia, they are estimated to play a part in around 28% of liver cancers globally. Evidence suggests aflatoxins are a challenge in mature economies and could become more so due to climate change. Furthermore, when aflatoxin levels are found to be above legal limits, food is disposed of, leading to food waste and an environmental and health risk as decontamination is not easy. Current solutions to tackle aflatoxins are imperfect and must be improved. Aflatoxin control can be targeted to both pre-and post-harvest interventions. Pre-harvest primarily focuses on managing the extent of toxigenic spore contamination, while post-harvest is focused on minimizing toxin formation and cross contamination; both are largely dependent on the application of good agricultural practices. Mitigation of aflatoxin is typically conducted through physical, chemical, and biological means. Lack of effective decontamination methods for aflatoxin tainted crops remains a major capability gap and has led to significant food waste and food fraud. There is an additional significant challenge of a lack of globally harmonized regulatory limits for aflatoxin contamination.

The Food Safety Coalition project has four interlinked work streams:
- Work stream 1: Understanding current methods for sampling and testing of aflatoxin in maize and to generate a best practice framework for each.
- Work stream 2: Providing a model framework for responsible, ethical, and fact-based risk assessment and communication of scientific findings.
- Work stream 3: Evaluating the accuracy of prediction aflatoxin levels compared to actual measurements; understanding the requirements for accurate and reliable aflatoxin predictions.
- Work stream 4: Developing a stakeholder informed conceptual framework to guide aflatoxin risk management outreach.

At the World Mycotoxin Forum, an overview of the coalition work will be presented, together with an update on the progress of each work stream and a timeline for programme completion.

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POSTER ABSTRACTS

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Iwona Ałtyn and M. Twarużek
Department of Physiology and Toxicology, Faculty of Biological Sciences, Kazimierz Wielki University, Poland

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Terenzio Bertuzzi[1], P. Giorni[2], G. Leni[1], P. Vaccino[3], C. Lanzanova[3] and S. Locatelli[3]
[1]Department of Animal, Food and Nutrition Science, Università Cattolica del Sacro Cuore, Italy; [2]Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Italy; [3]Research Center for Cereal and Industrial Crops, Council for Agricultural Reearch and Economics, Italy

P3 *The occurrence of mycotoxins in various spices purchased in retail stores in the USA*
Julie L. Brunkhorst, J. Bierbaum and R. Niemeijer
Trilogy Analytical Laboratory, USA

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S. Bonan[1], M. Peloso[1], P. Roncada[2], A. Guerrini[2] and **Elisabetta Caprai**[1]
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Carla Cervini[1], C. Verheecke-Vaessen[1], A. Mohammed[2], N. Magan[1] and A. Medina[1]
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Theodora I. Ekwomadu[1,2], R.E. Gopane[1] and M. Mwanza[2]
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Istituto Zooprofilattico Sperimentale della Sicilia, Italy

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Trouw Nutrition, the Netherlands

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Department of Food and Drug, University of Parma, Italy

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Department of Food Safety and Chemical Analysis, Institute of Agricultural and Food Biotechnology – State Research Institute, Poland

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Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Brazil

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Arnau Vidal[1], M. Devreese[2], S. De Baere[2], S. Croubels[2] and C. Gougoulias[1]
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M. Gajęcka[1], M.S. Majewski[2], **Łukasz Zielonka**[1], W. Grzegorzewski[3,4], E. Onyszek[5], S. Lisieska-Zołnierczyk[6], J. Juśkiewicz[7], A. Babuchowski[5] and M.T. Gajęcki[1]
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D. Greco[1], V. D'Ascanio[1], V. Marquis[2], D. Tricarico[3], M. Antonacci[3], A.F. Logrieco[1] and **Giuseppina Avantaggiato**[1]
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V. D'Ascanio[1], D. Greco[1], M. Abbasciano[1], A.F. Logrieco[1], D. Wilde[2] and **Giuseppina Avantaggiato**[1]
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Paul Bruinenberg[1] and M. Castex[2]
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G. Lo Dico1,2,3, S. Croubels[4], **Verónica Carcelén**[3] and M. Haranczyk[1]
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Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia, Spain

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Antonio Gallo[1], A. Catellani[1], M. Marotta[1], M. Mosconi[1], A. Mulazzi[1], S. van Kuijk[2] and Y. Han[2]
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Martha Cebile Jobe and M. Mwanza
Department of Animal Health, North-West University, South Africa

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Servei de Nutrició i Benestar Animal, Facultat de Veterinària, Universitat Autònoma de Barcelona, Spain

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Abdelhacib Kihal, M. Rodríguez-Prado and S. Calsamiglia
Servei de Nutrició i Benestar Animal, Facultat de Veterinària, Universitat Autònoma de Barcelona, Spain

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Jenna Lemmetty, T. Laitila, Y. Lee and N.H. Maina
Department of Food and Nutrition, Faculty of Agriculture and Forestry, University of Helsinki, Finland

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P52 *Zearalenone hydrolase ZenA (ZENzyme®) and its ability to degrade ZEN in the rumen of dairy cows*
Barbara Novak[1], T. Hartinger[2], J. Faas[1], M. Killinger[1], A. Höbartner-Gußl[1], B. Doupovec[1], D. Schatzmayr[1], Q. Zebeli[2] and G. Vogtentanz[1]
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P53 *Mycotoxin deactivator improves performance, antioxidant status, and reduces oxidative stress in gestating and lactating gilts fed diets containing mycotoxins*
S.P. Siqueira[1], H.F. De Brito[1], W.A.G. Araújo[1], M.S. Benfato[2], D.M. Canata[2], E.M. Gloria[3], **Damien P. Prévéraud**[4], D.V. Jacob[5] and B.A.N. Silva[6]
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P54 *Efficacy of mycotoxin deactivator on health and growth of broiler chickens under chronic dietary challenge of aflatoxins*
P.S. Ingewar[1], V. Patil[2], N. Kurkure[2], J. Dvorska[3] and **Damien P. Prévéraud**[3]
[1]A2 Livestock Farms and Research, India; [2]Department of Pathology, Nagpur Veterinary College, Maharashtra Animal and Fishery Science University, India; [3]Adisseo France SAS, France

P55 *The remediation of toxins in the food chain using a novel multicomponent mycotoxin detoxifying agent (MMDA)*
Jog Raj, H. Farkaš, Z. Jakovčević, S. Ćujić, J. Bošnjak-Neumüller and M. Vasiljević
PATENT CO, DOO., Serbia

P56 *Degradation of deoxynivalenol using Devosia lucknowensis NCIMB 30593*
Z. Jakovčević, **Jog Raj**, H. Farkaš, S. Ćujić, J. Bošnjak-Neumüller and M. Vasiljević
PATENT CO, DOO., Serbia

P57 *The effect of mycotoxin adsorbents on alleviating the negative effects of zearalenone in gilts: a field case*
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[1]Orffa Additives, the Netherlands; [2]First Ten Consulting Asia Pacific, Philippines; [3]Orffa Additives, Philippines

P58 *Evaluation of the efficacy of an anti-biotoxin solution supplemented in feed in dairy cows*
Clarisse Techer, A.-L. Tournay and L. Drouet
MiXscience, France

P59 *Carboxypeptidase immobilization on nylon nanofibrous membranes for ochratoxin A detoxification*
T. Calado[1,2], R. Ferreira[3], J.A. Lopes da Silva[3] and **Armando Venâncio**[1,2]
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P60 *Efficacy of TOXO®-XL on emerging mycotoxin adsorption at different pH levels*
Guan-Lin Wang and H.V.L.N. Swamy
Trouw Nutrition, the Netherlands

P61 *Efficacy of TOXO®-MX on ergot alkaloids adsorption at different pH levels*
Guan-Lin Wang and H.V.L.N. Swamy
Trouw Nutrition, the Netherlands

P62 *In vitro assessment of the potential interaction between vitamin B-complex and a commercial mycotoxin-mitigating product*
O. Daud, H.V.L.N. Swamy and **Guan-Lin Wang**
Trouw Nutrition, the Netherlands

P63 *Effect of Mycosorb A+ on the uptake and deposition of deoxynivalenol and zearalenone in growing pigs*
Alexandros Yiannikouris[1], S. Vartiainen[2], E. Koivunen[2], K. Raatikainen[2], J. Apajalahti[2] and C.A. Moran[3].
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P64 *Broiler performance and environmental impact of production in response to mycotoxins and yeast cell wall extract supplementation: a meta-analysis*
A. Weaver [1], D.M. Weaver [2], **Alexandros Yiannikouris**[1] and N. Adams[3]
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P65 *Partner-assisted artificial selection of a secondary function for efficient aflatoxin bioremediation*
M. Zaccaria[1], **Natalie Sandlin**[1], Y. Soen[2], M. Reverberi[3] and B. Momeni[1]
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CONTROLLING PLANT DISEASE AND MYCOTOXIN FORMATION

P66 *Optimizing fumonisin B1 production on liquid synthetic media*
G. Wiesenberger, M.M. Sopel, M. Lemmens, G. Adam and **Franz Berthiller**
University of Natural Resources and Life Sciences (BOKU Vienna), Austria

P67 *Enhancement of agri-food by-products: green extractions to obtain bioactive molecules against maize mycotoxigenic fungi*
Giulia Bulla[1], T. Bertuzzi[2], A. Mulazzi[2], G. Leni[2], M. Soldano[3], M. Tacchini[4], A. Guerrini[4], G. Sacchetti[4] and P. Giorni[1]
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P68 *Deoxynivalenol and pigmented grains: from organisms to cells*
Maria Cavallero[1], L. Righetti[2], M. Blandino[3], C. Dall'Asta[2] and E. Rolli[1]
[1]Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Italy; [2]Department of Food and Drug, University of Parma, Italy; [3]Department of Agricultural, Forest and Food Sciences, University of Turin, Italy

P69 *Bioformulate to reduce the accumulation of aflatoxins in maize based on a biopolymer as a carrier and support for growth of the biocontrol agent*
M.S. Alaniz Zanon[1], C. Oddino[1], D. Giovanini[1], C. Barbero[2], M.L. Chiotta[1] and **Sofia N. Chulze**[1]
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P70 *Influence of temperature and water activity on growth and aflatoxin production of Aspergillus flavus strains isolated from chickpeas*
C.J. Romero[1], J.F. Humaran[1], M.J. Nichea[1], V. Zachetti[1], E. Cendoya[1], L. Demonte[2,3], M.R. Repetti[2], **Sofia N. Chulze**[1] and M.L. Ramirez[1]
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P71 *From the treasure chest of plant bioactives to the future of new crop protectants for a sustainable agriculture: the possible exploitation of Citrullus colocynthis L. (SCHRAD.) extracts against Aspergillus flavus and aflatoxins and other stories*
Francesca Degola[1], M. Refifà[1], B. Marzouk[2], M. Commisso[3], S. Montalbano[4] and A. Buschini[1,4]
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P72 *Biocontrol activity of antagonistic yeasts against Penicillium expansum – major patulin producer in apples*
Tatjana Dudaš, D. Budakov, M. Loc, M. Grahovac and V. Stojšin
University of Novi Sad, Faculty of Agriculture, Department of Plant and Environmental Protection, Faculty of Agriculture, University of Novi Sad, Serbia

P73 *Pydiflumetofen co-formulated with prothioconazole: a novel fungicide for Fusarium head blight and deoxynivalenol control*
Simon G. Edwards
Harper Adams University, UK

P74 *Microbial modulation in wheat disease: mitigating the climate change impact of water scarcity using artificial intelligence*
C. Polano[1], I. Sanseverino[2], L. Gomez Cortes[2], A. Navarro Cuenca[2], S. Sarrocco[3], R. Baroncelli[4], P. Ermacora[1], **Monica Ermolli**[2], G. Firrao[1] and T. Lettieri[2]
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P75 A *rapid multiwell test to assay the effect of natural metabolites on growth and mycotoxin production of Aspergillus flavus*
Rosita Silvana Fratini[1], M. Beccaccioli[1], V. Cecchetti[1], R. Ragno[2] and M. Reverberi[1]
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P76 *Culture conditions for the enrichment of Fusarium spp. inoculum in food matrices*
Jéssica Gil-Serna, C. Melguizo, C. Vázquez and B. Patiño
Department of Genetics, Physiology and Microbiology, Complutense University of Madrid, Spain

P77 *The effect of fungi co-occurrence on plant and fungal gene expression profiles and mycotoxin production in maize kernels and in vitro*
Paola Giorni[1], A. Lanubile[1], T. Bertuzzi[2], A. Marocco[1] and P. Battilani[1]
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P78 *Gene editing of Aspergillus niger CBS 513.88 using a CRISPR-Cas9 based system*
Carolina Gómez-Albarrán, B. Patiño, C. Vázquez and J. Gil-Serna
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P79 *In-depth study of mycotoxin accumulation in relation to anthocyanin composition in pigmented wheat*
Marco Gozzi, M. Blandino, L. Calani, L. Righetti, C. Dall'Asta and R. Bruni
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P80 *Fungal growth and mycotoxin production in a new formulated meat product with structured emulsions as substitutes for pork rigid fat*
Ana Guimarães[1,2], A.J. Martins[3], M.A. Cerqueira[3], L. M. Pastrana[3], P. Sousa[4] and A. Venâncio[1,2]
[1]Centre of Biological Engineering, University of Minho, Portugal; [2]LABBELS – Associate Laboratory, Portugal; [3]International Iberian Nanotechnology Laboratory, Portugal; [4]Porminho Alimentação S.A., Portugal

P81 *The ability of cyclopiazonic acid production expressed by surface moulds isolated from dry cured meat products*
Tina Lešić[1], M. Zadravec[2], A. Vulić[1], N. Vahčić[3], N. Kudumija[1], I. Perković[4] and J. Pleadin[1]
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P82 *Antifungal activity of lemon peel medium fermented by lactic acid bacteria isolated from citrus fruits and metabolomic profile of citrus contaminating mycotoxigenic fungi*
Carlos Luz, L. Escrivá, C. Lafuente, M. Vitali, J. Quiles, J. Calpe, V. Dopazo, F. Illueca, B. Merenciano and G. Meca
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P83 *Ecology and control of Aspergillus flavus and aflatoxin B1 in chilli powder and whole red chillies*
D. Al-Jaza, A. Medina and **Naresh Magan**
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P84 *Climate change and acclimatization of Apergillus flavus strains influenced colonisation, biosynthetic gene expression and aflatoxin B1 production by A. flavus in raw pistachio nuts*
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P85 *Hanseniaspora uvarum as biocontrol agent against Aspergillus flavus*
Clara Melguizo, J. Gil-Serna, C. Vázquez and B. Patiño
Department of Genetics, Physiology and Microbiology, Faculty of Biology, Complutense University of Madrid, Spain

P86 *Antagonistic properties of selected Trichoderma fungi against Fusarium species biosynthesizing fumonisins and beauvericin*
Marta Modrzewska and M. Bryła
Institute of Agricultural and Food Biotechnology – State Research Institute, Poland

P87 *Industrial manufacturing of aflatoxin biocontrol products allows farmers across Sub-Saharan Africa to produce safe crops*
L. Kaptoge[1], **Alejandro Ortega-Beltran**[1], J. Atehnkeng[1,2], P.J. Cotty[3,4] and R. Bandyopadhyay[1]
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P88 *Biocontrol of Aspergillus flavus and aflatoxin production with ecological vineyard isolated bacteria*
P. de la Huerta Bengoechea, J. Gil-Serna, C. Vázquez Estévez and **Belén Patiño Álvarez**
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P89 *Looking inside the maize/aflatoxin interplay: the effect of afaltoxin B1 interaction with maize plants in vitro unravelled*
F. Degola[1], **Enrico Rolli**[1], L. Righetti[2] and C. Dall'Asta[2]
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P90 *Combination of propidium monoazide (PMA) with real-time PCR and recombinase polymerase amplification coupled with SYBR Green I for the detection of patulin-producing fungi in apples and by-products*
Foteini Roumani[1, 2], J. Barros-Velázquez[2], A. Garrido-Maestu[1] and M. Prado[1]
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P91 *The role of maize kernels lipophilic antioxidants in resistance against Fusarium graminearum*
Jean-Marie Savignac[1], F. Richard-Forget[2], V. Ortega[1], V. Atanasova[2] and M.N. Verdal-Bonnin[2]
[1]Syngenta Seeds, France; [2]INRAE, UR1264, Mycology and Food Safety (MycSA), France

P92 *Effect of water activity and temperature on growth and trichothecene production by Fusarium cerealis isolated from durum wheat grains*
J. Erazo, S. Palacios, A. Del Canto, S. Plem, M.L. Ramírez and **Adriana M. Torres**
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P93 *Sensitive and accurate quantitative determination of aflatoxin M1 in milk from different species*
Francesca Bravin[1], A. Revello Chion[2], D. Giordano[2], F. Cavarero[2], R. Baudino[2] and F. Diana[1]
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P94 *Validation of aflatoxin M1 fluorescence quantitative rapid test for milk (15-150 ppt) for determination of aflatoxin M1 in milk*
W. Reybroeck, S. Ooghe and **K. Broekaert**
Research institute for Agriculture, Fisheries and Food, Belgium

P95 *The effects of grind and extraction size on result variability for aflatoxin, deoxynivalenol, fumonisin and zearalenone*
J. Bierbaum, **Julie Brunkhorst** and R. Niemeijer
Trilogy Analytical Laboratory, USA

P96 *How green are the sample preparations commonly used in multi-mycotoxin analysis?*
Laura Carbonell-Rozas[1], L. Van der Cruyssen[2], L. Righetti[1] and C. Dall'Asta[1]
[1]Department of Food and Drug, University of Parma, Italy; [2]Department of Bioanalysis, University of Ghent, Belgium

P97 *Multi-mycotoxin determination in apple puree samples by hplc-ms/ms*
Laura Carbonell-Rozas[1], L. Van der Cruyssen[2], L. Calani[1], L. Righetti[1] and C. Dall'Asta[1]
[1]Department of Food and Drug, University of Parma, Italy; [2]Department of Bioanalysis, University of Ghent, Belgium

P98 *Beer analysis: mycotoxins in brewing process by LC-MS/MS*
D. Rodrigo, A. Cantalapiedra and **Luís Gallego**
Chromatography Department, Analiza Calidad Madrid S.L., Analiza Calidad Group, Spain

P99 *The design, development and implementation of an LC-MS method as a 'toxicity alert system' in the animal feed sector*
Brett Greer, O. Kolawole, S. Haughey and C. Elliott
Institute for Global Food Security, Queen's University Belfast, UK

P100 *A new immunoaffinity column with high recovery and organic solvent tolerance for aflatoxins*
Jianmin Liu, J. Yu and L. Chen
VICAM Waters Corporation, USA

P101 *Exploiting hyperspectral imaging and chemometrics for the rapid on-site measurement of aflatoxin in chillies*
Natasha Logan, O. Kolawole, B. Greer, S.A. Haughey, J. Meneely and C.T. Elliott
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P102 *Analysis of aflatoxin and ochratoxin in vegan food products*
C. Mair, M. Norris, E. Marley, B. Houston, C. Milligan and **Elizabeth Manning**
R-Biopharm Rhône, UK

P103 *Acetonitrile extraction for the analysis of multi-toxins in animal feeds*
C. Mair, N. Mackay, J. Wilcox, E. Marley, C. Milligan and **Elizabeth Manning**
R-Biopharm Rhône, UK

P104 *Assessment of citrinin in spices and infant cereals using immunoaffinity column clean-up prior to HPLC-FLD*
C. Mair, M. Norris, C. Milligan, C. Donnelly, D. Leeman, P. Brown, E. Marley and **Elizabeth Manning** R-Biopharm Rhône, UK

P105 *Improving efficiency and analytical outcomes for the busy laboratory using the chronect symbiosis rida®crest robotic system for automated analysis of mycotoxins*
J. McGeehan, N. MacKay, E. Marley, C. Donnelly and **Elizabeth Manning**
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P106 *Analysis of aflatoxin and ochratoxin in cheese using immunoaffinity clean-up prior to FLD-HPLC detection*
G. Millar, P. Brown, M. Norris, J. Wilcox, E. Marley, B. Houston, C. Milligan and **Elizabeth Manning**
R-Biopharm Rhône, UK

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P108 *Molecularly imprinted polymers applied to the simultaneous coextraction of zearalenone and alternariol mycotoxins from oil samples*
Tamara Moya-Cavas[1], F. Navarro-Villoslada[1], J. Urraca[1], L.A. Serrano-González[2] and M.C. Moreno-Bondi[1]
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P109 *Comparative analysis of aflatoxins in dairy feeds by high-performance liquid chromatography with a fluorescence detector and liquid chromatography/tandem mass spectrometry*
Nancy Nleya[1,2], T.I. Ekwomadu[1], M. Sulyok[3], T.A. Dada[1], N. Lubanza[1] and M. Mwanza[1]
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P110 *OTA via 2D-LC-MS/MS: an elegant alternative for high-throughput ochratoxin A analysis*
Christina Pille[1], M. Reichel[1], N. Meyer[1], A. Dagane[1], F. Nack[1], K.Krampe[1,2] and J.S. Mänz[1]
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P111 *Z-scores arrow range (ZSAR) method – assessment of truncated mycotoxins values in proficiency testing*
Kees van Putten
Trilogy Europe B.V., the Netherlands

P112 *Prevalence of patulin in fruit juices and baby foods as determined by a novel ELISA*
Guilar Rosar, T. Glaze, F. Rubio and G. Yearwood
Eurofins Tecna Srl., Italy

P113 *Macroeconomic evaluation of mycotoxins and nutritional composition in stored maize through NIRS*
Denize Tyska[1,2], A.O. Mallmann[2], C.T. Simões[1], D.F. Soares[1], G. Prado da Rosa[1], J.K. Vidal[1], L.T. Gressler[3], E. da Silva Gubiani[1] and C.A. Mallmann[1]
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P114 *Extraction of small molecule analytes from a dried blood spot card matrix for stable and amenable toxicological analysis*
P.A. Kirkland, U. Fox and **Alexandros Yiannikouris**
Alltech Inc., USA

P115 *Evidence biochip technology – a multi-analyte semi-quantitative analysis for 6 main groups of mycotoxins in maize*
M. Plotan, C.A. Acaru, J. McNaughten, **Esin Yildiz**, J. Porter, S. Brockbank, R.I. McConnell and S.P. FitzGerald
Randox Food Diagnostics, UK

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P116 *FoodSafety4EU: a step towards improved engagement, cooperation, and transparency in the European Food Safety System*
Frederic Bayer[1], V.M.T. Lattanzio[2], N. Cito[2], B. Ciasca[2] and A.F. Logrieco[2]
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P117 *MycoTWIN – enhancing research and innovation capacity of tubitak mrc food institute on management*
Martina Loi[1], A.F. Logrieco[1], J. Calpe[2], G. Meca[2], C.P. Kodolbas[3] and H. Ozer[3]
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P118 *Implementation of the net-map analysis tool to unravel the science-policy-society collaboration system in mycotoxin risk analysis*
Celine Meerpoel[1], N. van der Linden[2], V.M.T Lattanzio[3], N.M. Cito[3], M. Tomaniova[4], S. De Saeger[1] and P.A. Luning[2]
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P119 *Five years of MYTOX-SOUTH®: achievements of a global partnership*
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P120 *How to get a gender-equal approach to aflatoxin training in Africa? An Ethiopian case study*
C. Cervini[1], B. Abegaz[2], A. Mohammed[3], A. Medina[1], R. Elias[3] and **Carol Verheecke-Vaessen**[1]
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OCCURRENCE – MYCOTOXIGENIC FUNGI AND MYCOTOXINS
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P1
THE RISK OF CONTAMINATION WITH MOULDS AND MYCOTOXINS, DIETARY SUPPLEMENTS IN THE FORM OF DRIED PLANTS

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According to the Act of 25 August 2006 on food and nutrition safety [Journal of Laws of 2015, Item 594], a dietary supplement is a foodstuff; therefore, legal regulations of these products are less restrictive than pharmaceutical products. The current legal status allows anyone to place a supplement on the market, provided they declare its composition to the sanitary authorities; in Poland, GIS, on the so-called notification. The NIK report from 2017 states that many supplements do not show features declared by producers. The research aimed to determine the level of moulds and mycotoxins in dietary supplements commercially available in Poland. The material consisted of 6 groups of dietary supplements based on *Plantago psylliumis* (n=6), *Crataegus oxyacantha* L. (n=16), *Lepidium meyenii* Walpers (n=16), *Silybum marianum* (L.) Gaertn (n=13), *Stevia rebaudiana* Bertoni (n=15), and *Epilobium parviflorum* L. (n=23), which were in the form of dried plants. The mycological examination was performed on YGC medium and the results were expressed as the number of colony forming units (cfu) per gram of sample. The obtained mould cultures were identified by genus. Mycotoxins were determined using HPLC-FLD (aflatoxin, ochratoxin A) and HPLC-MS/MS (deoxynivalenol, nivalenol, zearalenone, T-2 and HT-2 toxin, and patulin). Mycological analysis of dietary supplements in the form of dried plants showed a significant level of infection with moulds, the average number of which was 3.3×10^4 cfu/g. The most frequently identified mould types were *Eurotium* spp. (39%), *Aspergillus* spp. (15%) and *Penicillium* spp. (10%). The most frequently detected mycotoxins were patulin (50%), zearalenone (46%), and T-2 toxin (36%). Of all the samples, the most contaminated were dietary supplements based on milk thistle (100%) and hoary willowherb (87%). The conducted research confirms the presence of moulds and mycotoxins in dietary supplements in the form of dried plants. Producers should consider and monitor their level of contamination in these types of products. **Acknowledgements**. This study was supported by the Polish Minister of Education and Science, under the programme 'Regional Initiative of Excellence' in 2019–2022 (Grant No. 008/RID/2018/19).

P2
CO-OCCURRENCE OF MONILIFORMIN, FUMONISINS AND DEOXYNIVALENOL IN MAIZE AND WHEAT GROWN IN ITALY

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Fusarium species are able to produce several mycotoxins in cereals, including regulated ones, such as fumonisins (FBs), trichothecenes and zearalenone. In Italy, the most widespread mycotoxins for maize and wheat are generally FBs and deoxynivalenol (DON), respectively. Besides these toxins, moniliformin (MON) is an emerging *Fusarium* mycotoxin occurring in cereals; high levels have generally been found in maize. MON is a highly polar and acidic molecule and occurs as a water-soluble sodium or potassium salt. This toxin can be produced by different *Fusarium* species, in particular *F. subglutinans*, *F. temperatum*, *F. verticilloides* and *F. proliferatum*; the last two fungal species are also able to produce FBs. In this study, the co-occurrence of MON, FBs, and DON was evaluated in maize, durum and common wheat grown in different experimental fields located in several Italian regions. MON was determined by LC-MS/MS, adding lanthanum ions in the mobile phase. In maize, MON contamination was widespread and not negligible; the toxin was often detected (95.1% of positive samples) and exceeded 500 and 1000 µg/kg in 42.0% and in 18.5% of the samples, respectively. A significant positive correlation was found between MON and FBs concentrations. Moreover, a positive significant correlation was found between growing degree days (GDD) and MON values, when not droughty climate conditions occurred. MON occurrence in wheat was not widespread like in maize, and it was higher in durum wheat than in common wheat. In durum wheat, MON was detected in 45.0% of the samples with only 6 samples (7.5%) exceeding 500 µg/kg. In common wheat, the toxin was detected above the LOD in 18.7% of samples and exceeded 100 µg/kg in only two samples (2.5%). No correlation was found with DON contamination. Climate conditions influenced both MON and DON occurrence; for

durum wheat, MON production seemed to increase in cases of high GDD levels and moderate rainfall, while DON levels increased with low GDDs and high rainfall.

P3

THE OCCURRENCE OF MYCOTOXINS IN VARIOUS SPICES PURCHASED IN RETAIL STORES IN THE USA

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Mycotoxins are secondary metabolites produced by mould that can cause disease and death in humans and animals. They are proven potent carcinogens and are regulated in many countries. A limited survey was conducted of various spices, including garlic blends, cumin, paprika, chili powder and many others that were purchased at retail stores in the USA. These samples were analysed to determine the occurrence of aflatoxins, deoxynivalenol, fumonisins, zearalenone, ochratoxin A, T-2 and HT-2 toxin which are the most found mycotoxins. Spices tend to only be analysed for aflatoxin and ochratoxin, however, this study will show that more than these two mycotoxins can be present. Whether the contamination is due to weather conditions or storage conditions mycotoxins are present and may want to be considered.

P4

OCCURRENCE OF OCHRATOXIN A IN CHEESE FROM THE ITALIAN MARKET AND EFSA RACE TOOL RISK ASSESSMENT

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Ochratoxin A (OTA) is the most prevalent and toxic of the ochratoxins, a group of secondary metabolites produced by *Aspergillus* and *Penicillum* fungi. OTA has been classified by the IARC in Group 2B (possible carcinogen to humans) because of its genotoxic potential. In the European Union, guidance limits for OTA are set for a number of foods, but not for milk or dairy products. This study investigates the presence of OTA in 84 cheese samples collected in Emilia-Romagna region (Italy). The chemical analysis has been performed by LC-MS/MS after immunoaffinity columns sample clean-up; LOQ was 1 µg/kg. Fifty one out of 84 samples were ready-to-use hard grated cheeses that have been shown to be the products with the higher risk of OTA contamination. OTA was detected in seven samples of grated hard cheese (13.7%). OTA occurrence ranges from 1.3 to 22.4 µg/kg. This finding shows that a fairly high contamination by OTA is possible in this food category. As the detection of OTA in milk occurs infrequently and at low levels, the presence of OTA in cheese may origin from the growth of environmental ochratoxigenic moulds on cheese surfaces during ripening and it may be associated with the cheese derived from the grating of the rind. To understand if the consumption of OTA contaminated grated hard cheese could pose a risk for human health, it has been performed a risk assessment using the EFSA 'Rapid Assessment of Contaminant Exposure' (RACE tool) focused on the Italian population. The assessment considered the highest, the median and the lowest concentration of detected OTA, Foodex2 Item L5 'Extra hard cheese (parmesan, grana type)', whole population and consumers only group. The margin of exposure (MOE) was the assessed output. OTA exposure deriving from the consumption of grated hard cheese contaminated with 22.4 µg/kg (highest value) suggests a possible health concern for all population groups, while OTA contamination of 5 µg/kg (median) shows possible health risks for infants, toddlers, children and for all other population groups high consumers. OTA concentration of 1.3 µg/kg (lowest) results in MOEs higher than 10000 for all population groups except for cheese high consumption in children.

P5

HIGH OCCURRENCE OF AFLATOXINS AND CYCLOPIAZONIC ACID PRODUCING *ASPERGILLUS* SECTION *FLAVI* SPECIES ISOLATED FROM ETHIOPIAN PEANUT-GROWING AREAS

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In Ethiopia, peanuts can be used to produce ready-to-use therapeutic foods (RUTF) for the treatment of nutritionally affected children. The Ethiopian climate, characterised by episodes of drought stress or intermittent rainfall, can predispose the ripening groundnuts to infection by toxigenic xerophilic fungi

from the *Aspergillus* section *Flavi* group and results in contamination of local peanuts with mycotoxins such as the carcinogenic aflatoxins (AFs) or the toxigenic cyclopiazonic acid (CPA). In the present study, the mycotoxin-production profiles and chemotype diversity of forty-two *Aspergillus* section *Flavi* isolates from different districts of the Oromia region in Ethiopia were investigated. AFs (AFB_1, AFB_2, AFG_1, AFG_2), CPA, and kojic acid were detected using LC-MS/MS qTRAP. Using a combination of colony morphology, microscopic criteria, and mycotoxin production the isolates were identified as *A. flavus* (n=35), *A. parasiticus* (n=5), *A. minisclerotigenes* (n=1) and *A. tamarii* (n=1). All of them were toxigenic, producing at least one mycotoxin. Overall, total AFs and CPA were predominantly produced *in vitro* in the range of 1-10 µg/g and 10-100 µg/g, respectively. All the isolates produced kojic acid, a secondary metabolite found in all *Aspergillus* section *Flavi* species. Based on the different patterns of mycotoxins production, five chemotypes were differentiated. Amongst the *A. flavus* isolates, thirty-one (73.80%) produced both B type of AFs and CPA (chemotype I) and four (9.52%) were considered atypical producing AFB_1 only and CPA (chemotype II). Regarding *A. parasiticus*, all five isolates (100%) produced both B and G type of AFs (chemotype III). The single isolates of *A. minisclerotigenes* and *A. tamarii* produced B, G type AFs and CPA (chemotype IV) and CPA only (chemotype V), respectively. These results showed the biodiversity of mycotoxigenic *A.* section *Flavi* isolates contaminating peanut soils in Ethiopia. The high prevalence of AFs-producing species and the co-occurrence of other mycotoxins is of extreme concern and highlights the high risk of exposure of the Ethiopian populations to these toxic compounds. **Acknowledgments**. This research was part of the NutriNuts (105663) project funded by Innovate UK.

P6

OCCURRENCE AND VARIATION OF *FUSARIUM* REGULATED, MASKED AND EMERGING MYCOTOXINS IN MAIZE FROM AGRICULTURE REGIONS OF SOUTH AFRICA

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The presence of mycotoxins in cereal grain is a very important food safety issue with the occurrence of masked mycotoxins extensively investigated in recent years. This study investigated the variation of different *Fusarium* metabolites (including the related regulated, masked, and emerging mycotoxin) in maize from various agriculture regions of South Africa. The relationship between the maize producing regions, the maize type, as well as the mycotoxins was established. A total of 123 maize samples was analysed by a LC-MS/MS multi-mycotoxin method. The results revealed that all maize types exhibited a mixture of free, masked, and emerging mycotoxins contamination across the regions with an average of 5 and up to 24 out of 42 investigated *Fusarium* mycotoxins, including 1 to 3 masked forms at the same time. Data obtained show that fumonisin B1, B2, B3, B4 and A1 were the most prevalent mycotoxins and had maximum contamination levels of 8,908, 3,383, 990, 1,014, and 51.5 µg/kg, respectively. Deoxynivalenol occurred in 50% of the samples with a mean concentration of 152 µg/kg (max 1,380 µg/kg). Thirty-three percent of the samples were contaminated with zearalenone at a mean concentration of 13.6 µg/kg (max 146 µg/kg). Of the masked mycotoxins, DON-3-glucoside occurred at a high incidence level of 53%. Among emerging toxins, moniliformin, fusarinolic acid, and beauvericin showed high occurrences at 98%, 98%, and 83%, and had maximum contamination levels of 1,130, 3422, and 142 µg/kg, respectively. Significant differences in the contamination pattern were observed between the agricultural regions and maize types.

P7

PRESENCE OF OCHRATOXIN A IN RED AND WHITE WINES SOURCED FROM SOUTHERN ITALY (SICILY)

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Ochratoxin A (OTA) is a mycotoxin found in several matrices. It is produced mainly by fungi of the genera *Aspergillus* and *Penicillium*. The presence of OTA in wines is regulated by Regulation (EC) No 1886/2006 and Regulation (EC) No 401/2006. Samples of red wines (n=36) and white wine (n=46) were collected in Sicily during 2019-2020 and stored at -18°C until analysis. A validated and accredited LC-MS/MS internal method was used for the analyses. The extraction was conducted with solid-phase-extraction (SPE) OCHRAPREP® (R-Biopharm Italia. The chromatography run was resolved with an HPLC Column Hypersil Gold C18 50x2.1mm 1.9µm (Thermo Fisher Scientific, USA). Two samples of

white wines (4.35%) and two samples of red wines (5.56%) were over the screening target concentration (STC) of 2 ppb (µg/l) and non-compliant with Regulation (EC) 1886/2006. Wines were produced in the same year of the analyses 2020 in Catania and Palermo (red wines) and Ragusa and Palermo (white wines).

P8

SCREENING ON THE PRESENCE OF OCHRATOXIN A IN DRY CURED MEAT PRODUCTS COLLECTED IN SOUTHERN ITALY (SICILY) DURING 2019-2021

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The presence of ochratoxin A (OTA) was determined in traditional dry cured meat products derived from pork. A total of n=125 samples were analysed divided in dried pork meat (n=39), mortadella (n=40), salami (n=30), bacon (n=30). No European Regulation limits exist for OTA in dry cured meats; therefore, we used the Italian national limits of 1 µg/kg established by la Circolare del Ministero della Sanità n. 10 del 09/06/1999 [Gazzetta Ufficiale n.135 dell'11/06/1999]. An internal validated screening method was used for the analysis. The extraction and purification of samples were conducted with Ochraprep® immunoaffinity columns. High-pressure-liquid-chromatography coupled with tandem mass spectrometry was used as an instrument for analysis. A detectable level of OTA was found in dried meat (n=7) with a mean of 0.582±0.218, mortadella (n=6) 0.593±0.197, salami (n=6) 0.436±0.283, and bacon (n=6) 0.509±0.260. No samples were over the limit of 1 µg/kg.

P9

PRELIMINARY INVESTIGATION OF THE PRESENCE OF FUNGAL PATHOGENS AND MYCOTOXINS IN SEED HEMP VARIETIES

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In recent years, the cultivation of hemp (*Cannabis sativa* L.) in Italy has aroused interest among farmers for potential market opportunities. In fact, in the last 5 years the cultivation of hemp in Italy has increased from 400 ha in 2013 to almost 4,000 ha in 2018. The current legislation in Italy allows the production and trade of hemp-based products using varieties with a psychotropic cannabinoid content (THC) less than 0.2% of the dry weight of mature inflorescences. Thanks to the great versatility of this crop, there are opportunities for food (gluten free), cosmetics, energy and industrial fields. In the food supply chain, hemp is used mainly as whole and / or hulled seeds and their derivatives, flour, and oil. In the hemp plant, inflorescences and flower bracts produce glandular trichomes and accumulate cannabinoids. Residues of these terpenophenolic compounds could be present in hemp seeds affecting safety. Thanks to the great interest of hemp-based products, Italy is facing with the selection of hemp varieties for diversified uses and with the tuning of precise analytical tools capable of quantifying not only the amount of cannabinoids in plants and seed derivatives, but also to evaluate the health and safety of seeds. Hemp seeds may indeed be contaminated by fungal pathogens compromising their production and quality. During 2018 and 2019 on a panel of 6 hemp varieties more cultivated in Italy, with a low THC content, a preliminary investigation on fungal contamination was carried out. Moreover, the contamination level of fumonisins, aflatoxin B1, zearalenone, deoxynivalenol, T-2/HT-2 toxin, and ochratoxin A has been determined using ELISA assay and some confirmed with chromatographic methods. The presence of zearalenone was highlighted in some varieties with values beyond the legal limits considered for oil seeds. **Acknowledgements**. This work was not supported by any project. CREA carried out this work in the project 'MAIDET' (2014-2018) funded by the Italian Ministry of Agricultural, Food and Forestry Policies (MIPAAF, D.D. N. 88666 del 03/12/2014).

P10

FUNGAL SPECIES AND MYCOTOXINS IN WHEAT STRAW USED FOR DAIRY CATTLE FEEDING IN NORTH PORTUGAL

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Wheat straw is a crop residue consisting of the stem, occasionally with dried remnants of leaves, left over after the wheat grains are harvested. In Europe, it is the most important type of agricultural residue. In pig production, wheat straw used for bedding that is ingested by the animals has been shown to represent a significant source of deoxynivalenol and zearalenone, two mycotoxins known to be produced by *Fusarium* species. When stored under inappropriate conditions, wheat straw may become moist enough to promote fungal growth. In intensive dairy farms in North Portugal, wheat straw is chopped and included in total mixed rations of dry and pre-calving cows, reared heifers, and, at lower rates, lactating cows. Samples of wheat straw, some of them with visible signs of fungal development on some stems and/or leaf remnants, were taken from twelve dairy farms to isolate and identify the fungal species and determine the mycotoxins they contained. The straw had been stored in poorly insulated warehouses or sheds. Most fungal species isolated from wheat straw belonged to the genera *Alternaria*, *Aspergillus, Cladosporium*, *Fusarium* and *Penicillium*. There are few data about the presence of mycotoxins in wheat straw and analysis methods are scarce. Therefore, a new method based on a QuEChERS extraction followed by the ultra-high liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) detection has been developed for the analysis of mycotoxins this matrix. The method was in-house validated for the analysis of 32 mycotoxins in terms of limits of detection (LODs), limits of quantification (LOQs), linearity, recoveries, and precision; showing acceptable performance characteristics. The presence of ochratoxin A, sterigmatocystin, alternariol, alternariol monomethyl ether, beauvericin and enniatins was detected in some samples. Therefore, it would be necessary to monitor the presence of mycotoxins in this matrix when it will be used for animal feed.

P11

MYCOTOXIN PREVALENCE IN 2021: EUROPEAN FEEDS AND RAW MATERIALS

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Mycotoxins are proven to compromise animal health and performance when consumed via contaminated feeds or raw materials. A reliable and rapid understanding of mycotoxin prevalence in raw materials and feeds helps animal feed stakeholders to reduce, if not completely prevent, economic losses. Keeping this objective in mind, 12670 samples, collected from different countries in Europe in the year 2021, were analysed for 'Big 6' mycotoxins, aflatoxins (AF), ochratoxin A (OTA), T-2/HT-2 toxin (T2), DON, zearalenone (ZEA) and fumonisins (FB). Turkey, Ukraine, Czech Republic, Spain, France, Germany, Ireland, Italy, Hungary, Poland, Portugal, Romania, the Netherlands, and Russia participated in this analysis. As feed mills need to make a quick decision of accepting or rejecting raw materials, samples were analysed using Mycomaster, a rapid lateral flow device. When the analysis was conducted on the entire dataset, including all the raw materials and feeds, the highest contamination was observed for AF (61%), followed by ZEA (39%), DON (25%), OTA (14%), FB (13%), and T2 (12%). In the next step, average, median, minimum, and maximum concentrations, in ppb, were evaluated for the positive samples. The average concentrations revealed highest quantity for FB (1545ppb) followed by DON (1,324 ppb), ZEA (112 ppb), T2 (45 ppb), OTA (6.6 ppb) and AF (3.4 ppb). Median concentrations for all the above mycotoxins were lower than the average concentrations indicating higher variability in the data. It is, therefore, recommended to provide both average and median concentrations for various mycotoxins. All the mycotoxins tested in maize and maize by-products were at concentrations that can cause performance and health issues in animals. T2, ZEA and DON levels in wheat and its by-products were a concern while FB was an additional concern in wheat by-products. In other small grains, only ZEA and DON were at concern levels. In terms of protein sources, only ZEA was a concern in soybean meal while both ZEA and T2 were a concern in sunflower meal. Among various feeds tested, T2, ZEA, DON, and FB were found in pig feeds at levels that can compromise performance and health while only T2 and DON were a concern in poultry feeds. AF was a concern in

dairy cows from the milk quality perspective while ZEA and DON were a concern from the performance point of view.

P12
MYCOTOXIN PREVALENCE IN 2021: ASIAN FEEDS AND RAW MATERIALS
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The reliable and rapid understanding of mycotoxin prevalence in raw materials and feeds helps animal feed stakeholders to reduce, if not completely prevent, economic losses. Keeping this objective in mind, 3408 samples, collected from different countries in Asia in the year 2021, were analysed for “Big 6” mycotoxins, aflatoxins (AF), ochratoxin A (OTA), T-2/HT-2 toxin (T2), DON, zearalenone (ZEA) and fumonisins (FB). China, Japan, India, Indonesia, Myanmar, Philippines, Sri Lanka, Thailand, and Vietnam participated in this analysis. As feed mills need to make a quick decision of accepting or rejecting raw materials, samples were analysed using Mycomaster. When the analysis was conducted on the entire dataset, including all the raw materials and feeds, the highest contamination was observed for ZEA (86%), followed by OTA (76%), AF (70%), FB (70%), DON (43%), and T2 (16%). In the next step, average (mean), median, minimum, and maximum concentrations, in ppb, were evaluated for the positive samples. The average concentrations revealed highest quantity for FB (2456ppb) followed by DON (1,827 ppb), ZEA (100 ppb), T2 (36 ppb), AF (26.6 ppb) and OTA (10.4 ppb). Median concentrations for all the above mycotoxins were lower than the average concentrations indicating higher variability in the data. It is, therefore, recommended to provide both average and median concentrations in reports. AF, T2, DON, and FB in maize were at concentrations that can cause performance and health issues in animals. As expected, these mycotoxin concentrations were higher in maize by-products as compared unprocessed maize. AF, OTA, and DON were at significant levels in rice products and the same is true for DON in wheat products. AF and OTA levels in soybean meal were big enough to pose a concern. Among various feeds tested, AF, OTA, DON, and FB were found in poultry feeds at levels that can compromise performance and health while only AF, DON and FB were a concern in pig feeds. While AF, ZEA and DON were a concern in ruminant feeds, all mycotoxins except for T2 which was not analysed, were a concern in fish feeds. It can be concluded that rapid mycotoxin analyses in raw materials and feeds, at feed mills and farms, help Asian producers to make informed decisions on raw material selection and protect the performance of their animals, respectively.

P13
MYCOTOXIN PREVALENCE IN 2021: MIDDLE EAST AND AFRICAN FEEDS AND RAW MATERIALS
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The economic impact of mycotoxins on animal production in Middle East and Africa (MEA) is becoming clearer with increased monitoring. To further aid in the monitoring, 3408 samples collected from different MEA countries in the year 2021, were analysed for ‘Big 6’ mycotoxins, aflatoxins (AF), ochratoxin A (OTA), T-2/HT-2 toxin (T2), DON, zearalenone (ZEA) and fumonisins (FB). Cameroon, Cyprus, Egypt, Kenya, Nigeria, Rwanda, Saudi Arabia, South Africa, Sudan, Tanzania, and Uganda participated in this analysis. Samples were analysed using a rapid analytical tool, Mycomaster. When the analysis was conducted on the entire dataset, including all the raw materials and feeds, the highest contamination was observed for ZEA (89%), followed by OTA (84%), DON (59%), AF (57%), FB (39%), and T2 (25%). In the next step, average (mean), median, minimum, and maximum concentrations, in ppb, were evaluated for the positive samples. The average concentrations revealed highest quantity for FB (1,559 ppb) followed by DON (1,125 ppb), ZEA (95 ppb), T2 (25 ppb), AF (10 ppb) and OTA (6.1 ppb). Median concentrations for all the above mycotoxins were lower than the average concentrations indicating higher variability in the data. It is, therefore, recommended to provide both average and median concentrations in reports. AF, ZEA, DON, and FB in maize and maize by-products were at concentrations that can cause performance and health issues in animals. The concentrations of ZEA and DON in wheat and by-products can be a concern in some food producing animals while the same holds good for AF in soybean meal. The levels of mycotoxins seen in rice products does not seems to be of concern. Among various feeds tested, all the mycotoxin levels in poultry feeds, except ZEA, were a concern for poultry whereas the same holds good for only DON, ZEA and FB in pig feeds. While AF, ZEA and FB levels in feeds were a concern for fish species, AF, ZEA and DON levels were a concern for ruminant feeds. Pet food analysis indicated a significant challenge from AF, ZEA, DON and FB mycotoxins. It can be concluded that rapid mycotoxin analyses in raw materials and feeds, at feed mills

and farms, help MEA producers to make informed decisions on raw material selection and protect the performance of their animals, respectively.

P14

MYCOTOXIN PREVALENCE IN 2021: LATIN AMERICAN FEEDS AND RAW MATERIALS

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A reliable and rapid understanding of mycotoxin prevalence in raw materials and feeds helps animal producers to reduce, if not completely prevent, health, performance, and economic losses. Keeping this objective in mind, 5,892 samples, collected from different countries in Latin America (LATAM) in the year 2021, were analysed for 'Big 6' mycotoxins, aflatoxins (AF), ochratoxin A (OTA), T-2/HT-2 toxin (T2), DON, zearalenone (ZEA) and fumonisins (FB). Brazil, Mexico, Guatemala, Ecuador, Honduras, Dominical Republic, and Panama participated in this analysis. As feed mills need to make a quick decision of accepting or rejecting raw materials, samples were analysed using Mycomaster, a rapid lateral flow device. When the analysis was conducted on the entire dataset, including all the raw materials and feeds, the highest contamination was observed for OTA (72%), followed by FB (70%), ZEA (60%), DON (55%), AF (51%), and T2 (23%). In the next step, average, median, minimum, and maximum concentrations, in ppb, were evaluated for the positive samples. The average concentrations revealed highest quantity for FB (1,735 ppb) followed by DON (836 ppb), ZEA (272 ppb), AF (25 ppb), T2 (23 ppb) and OTA (3.9 ppb). Median concentrations for all the above mycotoxins were lower than the average concentrations indicating higher variability in the data. It is, therefore, recommended to provide both average and median concentrations for various mycotoxins. All the mycotoxins, except OTA, tested in maize, maize by-products, and wheat and its by-products were at concentrations that can cause performance and health issues in animals. On the other hand, only T2 was an exception for rice and its products. For small grains, including sorghum, barley, and oat, only DON and ZEA were present at concern levels. Among protein sources, only soybean meal samples were tested at enough numbers, and it can be concluded that AF, T2, ZEA and DON levels in this protein source can compromise animal performance. Among various feeds tested, pig feeds showed the maximum concern with five toxins (except OTA) out of six tested exceeding mycotoxin practical guidance values. Both AF and DON levels in poultry and ruminant feeds could compromise animal performance, while the same can be said for FB in poultry feed and ZEA in ruminant feed.

P15

ANALYSIS OF THE PRESENCE OF OCHRATOXIN A IN COFFEE AND COFFEE-BASED PRODUCTS COLLECTED FROM SOUTHERN ITALY (SICILY) DURING 2019-2021

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The presence of ochratoxin A (OTA) has been investigated in coffee and coffee-based products commercialized in Sicily during 2019-2021 (Southern Italy). Matrices analysed were roasted, decaffeinated coffee (n=3), ground coffee (n=70), roasted coffee (n=43), and coffee beans (n=6). A validated and accredited screening method was used for the analyses. Ochraprep® immunoaffinity columns were used to extract and purify samples. Analyses were conducted in high-performance liquid chromatography with tandem mass spectrometry. Only four samples of grounded coffee had a detectable level of OTA, and only one was over the EU Regulation limit of 5 (µg/kg). In particular, a sample collected in Palermo and produced in Guatemala showed a concentration of 18.29 (µg/kg). The results showed that a low percentage (0.82%) of coffee was contaminated with a relevant level of OTA.

P16

ENNIATINS AND BEAUVERICIN: EMERGING MYCOTOXINS OF CONCERN IN *FUSARIUM*

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Enniatins and beauvericin are *Fusarium* toxins for which no guidance levels have been laid down by the authorities up to now and are not subjected to regular monitoring. These mycotoxins contaminate several food and feed commodities, predominantly cereal-based products, and their presence co-occur with other *Fusarium* toxins. In the 2014 EFSA's scientific opinion [EFSA Journal 12 (2014) 3802], it was

concluded that “there might be a concern with respect to chronic exposure” to beauvericin and enniatins. Monitoring the dietary exposure to these toxins is, therefore, of primary importance. Recently, studies in terms of occurrence, have highlighted maximum values of enniatins and beauvericin of concern also in feed [Toxins 12 (2020) 686], in breast (human) milk as well as in farmed animals, such as some species of fish (including salmon) [Environment International 142 (2020) 105845; Food and Chemical Toxicology 101 (2017) 67]. EFSA, finally, included enniatins and beauvericin in 2021 calls of data to better understand their occurrence in food and feed. Mérieux NutriSciences LC-MS/MS validated method proved to be useful for the purpose since the majority of the analysed flour samples were found to be naturally contaminated with some of these *Fusarium* toxins. Numerous members of the fungal genus *Fusarium* can produce these non-ribosomal mycotoxins. Studies have found that beauvercin is toxic to human tissues and cells at concentrations lower than aflatoxin B1 [Frontiers in Pharmacology 9 (2018) 1]. Several members of the *Fusarium* genus have been utilized in the novel fermented microbial protein (FMP) production in the food industry for human consumption. Many of these products (burgers, cream cheese…) are already available in various markets. Extending the scope of this method to include matrices derived from FMP is important to verify the residues of these mycotoxins in the raw material and finished products. The above analytical strategy can be adopted to identify the distribution levels of contamination in foodstuffs and novel foods. Occurrence data can help to assess consumers exposure to potentially hazardous beauvericin and enniatins levels.

P17

NATURAL TOXINS IN PLANT COMMODITIES USED IN PLANT-BASED MEAT ALTERNATIVES: A SYSTEMATIC REVIEW

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Plant-based meat alternatives consumption is continuously growing mainly due to their perceived healthfulness by consumers. Adhering to plant-based diets is also considered to be pivotal in the transition towards sustainable food systems. However, the importance of natural toxins occurrence in plant commodities used for meat alternatives seems to be neglected. This systematic review was the first attempt at assessing the chemical risk of plant-based meat alternatives with natural toxins from European studies conducted between 2000-2021. While wheat/wheat-based food are monitored and regulated for mycotoxin contamination, seitan, soybean, chickpea, and pea, the most frequently used meat alternatives are understudied or not studied at all. Mycotoxins, either individual or mixtures were found in soy, pea, chickpea-based food, while tropane and β-carboline alkaloids were found in soy-based food. The findings indicate that there is a lack of data regarding the natural contamination of plant-based meat alternatives. To ensure proper risk assessment and assert the risk that consumers are exposing themselves to more data are required. The European Commission needs to set maximum limits for natural toxins in plant commodities used as meat alternatives to ensure consumer protection.

P18

OCCURRENCE OF MAJOR MYCOTOXINS, MASKED/MODIFIED FORMS AND EMERGING MYCOTOXINS IN EUROPE IN 2021 AS DETECTED BY SPECTRUM TOP® 50

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The innovative Multi-Mycotoxin Analysis Spectrum Top® 50 was introduced by Romer Labs and Biomin in 2018. It detects simultaneously over 50 different major mycotoxins, masked and modified forms, as well as the less studied ‘emerging mycotoxins’. In 2021, 3,470 samples were analysed worldwide, to provide insights on the occurrence of these metabolites. We describe the ten most common metabolites detected in the important crops maize, maize silage, wheat, and barley focusing on Europe. In maize (n=65), fumonisins B1, B2 and B3 (FUM) were the most prevalent mycotoxins (detected in 85% to 78% of samples). Average of positives was highest for FUM B1 (2,627 ppb). Also, highly prevalent were deoxynivalenol (DON) (77%, average of positives 368 ppb) and its masked form deoxynivalenol-3-glucoside (DON-3-G) (52%, 107 ppb), zearalenone (ZEN) (57%, 66 ppb) and the emerging mycotoxins moniliformin (MON) (72%, 636 ppb), enniatin (ENN) B and B1 (37% and 28%; average of positives 15 ppb and 8 ppb, respectively), as well as aflatoxin B1 (31%; 12 ppb). European maize silage (n=57) shows a different pattern with the emerging mycotoxins ENN B, B1, A1 and A among the top 5 (ranging in their abundance from 98% to 84%; ENN B with the highest average of positives 426 ppb). ZEN was very frequent in 91% of the samples (103 ppb). mycotoxins DON (81%, 1,277 ppb), FUM B1 (81%, 418 ppb), FUM B2 (56%, 163 ppb) and HT-2 (58%, 191 ppb) were also detected as well as the emerging mycotoxin MON (67%, 154 ppb). Emerging mycotoxins were commonly found in wheat (n=120): ENN

B1, A1 and A (ranging in abundance from 93% to 61%, highest average of positives B1 with 60 ppb), beauvericin (BEAU) (63%, 4 ppb) and MON (42%, 40 ppb) all among the 10 most frequently found metabolites. 71% of samples were positive for DON (191 ppb) and 55% for DON-3-G (30 ppb). ZEN occurred in 25% (30 ppb), nivalenol (NIV) in 23% of samples (48 ppb) followed by FUM B2 in 13% (16 ppb). Barley (n=70) showed a similar picture as wheat. The emerging mycotoxins ENN, BEAU and MON were highly abundant. DON, DON-3-G as well as NIV were detected. Contrary to wheat, ZEN and FUM were not among the top 10 metabolites but HT-2. High abundance and co-occurrence underline the need to monitor more than only the well-known major mycotoxins.

P19

FUNGAL DIVERSITY AND TARGETED METABOLOMICS IN STORED CEREALS UNDER DIFFERENT INTERACTING ABIOTIC FACTORS

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Environmental factors influence fungal growth and mycotoxin production in stored grains. However, the fungal diversity and multi-mycotoxin contamination (including bound mycotoxins) under different storage conditions has not been examined in detail. The objectives of this study were to examine (a) the fungal diversity of wheat, barley, maize, and oats (b) the effect of storage of natural grains and those inoculated with specific mycotoxigenic fungi (*Fusarium, Penicillium* and *Aspergillius* section *Flavi* species) under different water activity (0.85, 0.90 and 0.98 a_w) and temperature (20, 25 and 30^{o}C) conditions on the metabolomics profiles. This study investigated and compared the levels of free mycotoxins to masked mycotoxins in grains from the UK. 82 samples were analysed using LC-MS/MS. The fungal diversity and population of the four grains were examined and were commonly colonised by *Penicillium, Alternaria, Aspergillus* section *Nigri, Cladosporium, Aspergillus glaucus* group and *Mucor* species. The mycotoxin profiles analysis showed that grains were contaminated with regulated, masked, and emerging mycotoxins. Generally, the concentrations of free mycotoxins were higher than bound mycotoxins in most natural and inoculated grains and were influenced by storage aw levels and temperature except for DON-3-G concentrations in wheat grains inoculated with *Penicillium verrucosum.* Optimum production levels were recorded at 0.98 a_w at 25^{o}C (wheat) and 0.98 at 30^{o}C for maize. Gamma irradiated grains + inoculum of mycotoxigenic species had higher levels of mycotoxins than naturally contaminated grains. Thus, inoculated samples were mostly contaminated with deoxynivalenol, deoxynivalenol-3-glucoside and ochratoxin A in wheat. AFB1, AFM1 and fumonisin B1 in maize and T-2/HT-2, HT-2-Glu-NH_4 in oats. Emerging mycotoxins; moniliform and beauvericin were mostly present in the natural grains. Further studies will use CO_2 sensors to monitor onset of spoilage and mycotoxin production in these cereals stored at different water activities and temperatures. Findings will be used to develop a decision support tool for grain farmers/ companies to improve post-harvest management of grains thereby supporting Sustainable Development Goals 2 and 3.

P20

OCCURRENCE OF SELECTED *FUSARIUM* TOXINS IN SAMPLES OF POLISH MAIZE AND WHEAT CEREAL GRAIN FROM THE 2020 AND 2021 HARVEST

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Deoxynivalenol (DON), nivalenol (NIV), T-2 and HT-2 toxin are mycotoxins belonging to the group of trichothecenes. These compounds, like zearalenone (ZEN), are mainly secreted by fungi of the genus *Fusarium*, which infect crops. The aim of the research was to evaluate the content of DON, NIV, their glucosyl metabolites, T-2 and HT-2 and ZEN toxins maize and wheat collected in 2020 and 2021 in Poland. The dominant mycotoxins found in maize were ZEN, DON, and deoxynivalenol-3-glucoside (DON-3G) (70%, 63% and 58% of samples, respectively). The average content of these substances expressed in µg/kg was at 455.4 (10.5-2,699) for ZEN, 1,257.9 (173.2-3,801.8) for DON, and 291.6 (75.4- 707.5) for DON-3G. Up to 15% of maize samples exceeded the maximum allowable content (defined in EU law) of deoxynivalenol (1,750 µg/kg), while for ZEN it was the level of 30% (350 µg/kg). In case of 2% of maize samples, the sum of T-2 and HT-2 exceeded 200 µg/kg (Commission Recommendation 2013/165/EU). The average level of the sum of HT-2 and T-2 toxins in positive samples (21%) was 86.3 (7.6-652.8) µg/kg. DON, ZEN and the sum of HT-2 and T-2 toxins were dominant in wheat grain, which were identified in 34%, 25% and 12% of the samples, respectively.

Among the analysed mycotoxins in wheat samples, the permissible content was exceeded only for ZEN (3% of samples) (permissible content level 100 µg/kg). Both in the maize and wheat samples, the presence of NIV was found. Positive samples accounted respectively for 31% and 10% of the samples. The average content was 105.7 (41.3-506.8) µg/kg and 102.3 (51.4- 511.3) µg/kg, respectively. Different contamination levels with mycotoxin content were observed between years. In case of maize samples, the average mycotoxin content in 2020 expressed in µg/kg was: 1,543 (173.2-3,801.8) DON; 331 (75.4-707.5) DON-3G; 75 (41.1-197.6) NIV; <LOQ NIV-3G; 44 (10.2-91.7) HT-2 + T-2; and 451 (10.5-3,038) (ZEN); in 2021, 504 (139.8-1,678) DON; 196 (82.1-617.1) DON-3G; 230 (87.4-506.8) NIV; 200 (184-217.8) NIV-3G; 133 (7.6-652.8) HT-2 + T-2; and 464 (11.6-2,699) ZEN. Much lower levels of these toxins were recorded in the case of wheat samples. In the wheat samples collected in 2020, the average content in µg/kg was 303 (59.3-956.2) DON; 125 (76-201.4) DON-3G; 109 (108.7-108.7) NIV; <LOQ NIV-3G; 29 (19.1-39.8) HT-2 + T-2; and 14 (8.6-22.2) ZEN. In 2021, the average levels in µg/kg were as follows: 144 (58.1-465) DON; 102 (88.7-115.7) DON-3G; 102 (51.4-511.3) NIV; 87 (55.7-118.5) NIV-3G; 24 (5.4-60) HT-2 + T-2; and 74 (10.1-630.6) (ZEN).

P21

TOTAL MIXED RATION – MYCOLOGICAL AND MYCOTOXICOLOGICAL CONTAMINATION

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When feeding ruminants with TMR (total mixed ration), we feed the rumen microflora, for which the stability of the dose, with an appropriate balance, structure, and repeatability, is crucial. The components supplied to the mixer wagon cannot be of poor quality, and therefore it is advisable to control contamination with moulds and mycotoxins. The heating of TMR, especially in summer, changes the temperature and taste and modifies its microbiological balance as a result of the fermentation processes taking place. The study aimed to evaluate the moulds and mycotoxin level in TMR, as part of the standard quality control procedure all over Poland, delivered by farmers, feed producers, and veterinarians to the Mycotoxin Analytical Laboratory, Kazimierz Wielki University in Bydgoszcz in the years 2019-2022. Mycological examination of TMR was carried out on YGC agar medium, 5-7 days incubation in 25C±1˚C. The results are expressed as the number of colony forming units per gram of a sample. Identification of moulds was made to the genus. *Fusarium* toxins and fumonisins were detected via HPLC with MS/MS, whereas OTA and AF were detected via HPLC - FLD. A total of 97 TMR samples were analysed for the occurrence of *Fusarium* toxins. Additionally, 27 and 6 samples were randomly tested for OTA and AF contamination, respectively. DON, NIV, HT-2, and ZEN were found in all the tested samples (100%) (average concentration 251 µg/kg, 66.9 µg/kg, 31.4 µg/kg, and 28.0 µg/kg, respectively). OTA was detected in 26% of the samples with an average concentration of 0.76 µg/kg and a maximum content of 1.42 µg/kg. AF was not detected. Additionally, a mycological analysis was performed, which showed that the average number of fungi was 1.5 x 10^7 cfu/g, the average value of mould was 7.1 x 10^5 cfu/g, while the yeast level was 1.4 x10^7 cfu/g. The most frequently identified mould types were *Penicillium* spp., *Monascus* spp., *Aspergillus* spp., *Geotrichum* spp. Attention should be paid to the co-occurrence of mycotoxins. 100% of the TMR samples tested were contaminated with four mycotoxins. The presence of several different mycotoxins multiplies their toxicity (synergism) and the risk to animal health. **Acknowledgements**. This study was supported by the Polish Minister of Education and Science, under the programme ‘Regional Initiative of Excellence’ in 2019–2022 (Grant No. 008/RID/2018/19).

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OCCURRENCE OF MYCOTOXINS AND CROP AGRONOMIC CHARACTERISTICS IN MAIZE WITH DIFFERENT ENDOSPERM TEXTURES

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A study was conducted to evaluate the possible effects of different maize endosperm textures on the occurrence and concentration of mycotoxins as well as on crop agronomic characteristics. A total of 254 maize samples were provided from a field experiment conducted in the south region of Brazil. The maize hybrids most marketed in the region were cultivated in January 2020 and harvested in June 2020. The samples were classified according to the endosperm texture as dent (n=41), semi-dent (n=66), flint (n=64) and semi-flint (n=80). Crop yield (kg/ha), thousand grains weight (TGW; g) and the percentage

of damaged grains were measured and corrected for 13% of moisture. Aflatoxins (AFB1, AFB2, AFG1 and AFG2), fumonisins (FB1 and FB2), zearalenone, ochratoxin A, deoxynivalenol, diacetoxyscirpenol, 3-acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol (15-Ac-DON), nivalenol, fusarenon-X, T-2 toxin, HT-2 toxin, citrinin and cyclopiazonic acid were quantified by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Statistical analyses were conducted using the software Statgraphics Centurion XV. Data were previously submitted to descriptive statistics, transformed by $\log_{10}(x+1)$, and subjected to analysis of variance. Mean differences among maize types were compared by Tukey test ($P \leq 0.05$). The most prevalent metabolites were FB1 and FB2, being detected in 45.7% (mean 592 µg/kg) and 35.8% (mean 265 µg/kg) of the samples, respectively; AFB1 had the third highest positivity, with 12.2% (mean 1.34 µg/kg), followed by 15-Ac-DON, with 4% (mean 156 µg/kg). The other evaluated mycotoxins presented positive samples in less than 2% or were not detected in any sample. Comparison of means was only performed for FB1, FB2, FBT (FB1+FB2), AFB1 and AFB2 due to the positivity of these samples. There was no difference in aflatoxins concentration among maize types ($P > 0.05$), however, maize classified as dent presented higher means ($P < 0.01$) of fumonisins than the remaining types. Maize type had no effect on TGW ($P > 0.05$). Dent maize presented higher percentage of damaged grains ($P < 0.01$) than the other types whereas flint maize had the lowest crop yield ($P < 0.001$). These results indicate that the endosperm texture of grains may influence agronomic characteristics and mycotoxins contamination in different types of maize.

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POTENTIAL MYCOTOXIN-PRODUCING SPECIES IN ORGANIC CEREALS FROM SPAIN

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Production of organic cereals has become more and more popular, since society demands a chemical-free agriculture due to its concerns about human and animal health, as well as environmental problems that may arise from synthetic fungicides. However, there is no reports regarding the occurrence of mycotoxin-producing moulds in organic cereal crops to assess whether these agricultural procedures may affect the risk of mycotoxin presence. Therefore, the aim of this study was to analyse the presence of the main mycotoxigenic fungi in organic cereals from Spain using species-specific PCR protocols. A total of 83 cereal or pseudo-cereal samples (wheat, barley, spelt, oat, triticale, quinoa, teff, and buckwheat) were taken from the main Spanish cereal growing regions (Castilla y León, Castilla-La Mancha, and Madrid). The samples were milled, and 1 g was incubated in Sabouraud-Chloramphenicol broth for 24 h at 28ºC. Subsequently, the samples were filtered before the isolation of total genomic DNA. Species-specific PCR protocols were carried out to determine the presence of the main mycotoxin-producing species in cereals including aflatoxin-producing species (*Aspergillus flavus* and *A. parasiticus*), ochratoxin A producers (*A. carbonarius, A. westerdijkiae, A. steynii*, *A. niger*, and *A. welwitschiae*), fumonisin-producing species (*Fusarium verticillioides, F. proliferatum, F. subglutinans* and *F. temperatum*), trichothecene and zearalenone-producing species (*F. equiseti, F. langsethiae, F. sporotrichioides, F. poae, F. graminearum*, and *F. culmorum*) as well as the mycophenolic acid-producing *Penicillium brevicompactum*. All the samples analysed presented at least two mycotoxigenic species. The most frequently detected species was *A. flavus* (92% of contaminated samples) followed by *A. carbonarius* (75%), *A. niger* (60%)*, A. welwitschiae* (54%)*, and A. parasiticus* (53%). Regarding *Fusarium* species, *F. graminearum, F. sporotrichioides*, and *F. subglutinans* were found in a low percentage of samples (< 29%) and in no case *F. proliferatum, F. verticillioides,* and *F. culmorum* were detected in the organic cereals analysed. *P. brevicompactum* was detected in 35% of the samples. The presence of these fungi might be indicating a high risk of aflatoxin and ochratoxin A in the samples, as well as the co-occurrence of small levels of other mycotoxins. In this study, the presence of potentially mycotoxin-producing species in Spanish organic cereals was demonstrated, which could pose a health hazard if the environmental conditions are favourable for mycotoxin production. **Acknowledgements**. Work supported by Spanish Ministry of Science and Innovation (RTI 2018-097593-B-C21).

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STERIGMATOCYSTIN OCCURRENCE IN TRADITIONAL MEAT PRODUCTS OF HOUSEHOLDS SEATED IN DIFFERENT CROATIAN REGIONS

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Mycotoxins are toxic secondary metabolites produced by numerous types of moulds. They can contaminate both food and feed, so that they represent a serious public health threat. Sterigmatocystin (STC) is a mycotoxin mainly produced by different fungi of the *Aspergillus,* but also the *Emericella*, the *Chaetomium*, the *Humicola,* and *Botryotrichum* species. However, data on STC occurrence in different foodstuffs and feedstuffs are limited. Previous research has shown that STC can be found in bread, grains, nuts, coffee, spices, beer, and cheese, while data on the occurrence of STC in meat and meat products are not available. The production of Croatian traditional meat products (TMPs) includes the ripening period, during which moulds can overgrow the product surface, produce mycotoxins, and consequently contaminate the final product. TMPs under this study were produced using different production technologies and were sampled during a two-year period from households located in five climatically different Croatian regions. In total, 250 samples were taken and analysed using liquid chromatography tandem mass spectrometry (LC-MS/MS) preceded by the immunoaffinity sample preparation. STC was detected in 11 samples coming from Southern, Western, Northern and Central region. The mean STC concentration found in positives coming from the Southern, Western, Northern and Central region was 0.14±0.03 µg/kg, 0.29±0.18 µg/kg, 1.25±1.81 µg/kg, and 0.12±1.81 µg/kg, respectively. In the Eastern region, STC was not determined in any of the samples. Unlike other regions, in the Eastern region ripening took place during winter, i.e., at lower temperatures and higher precipitation amounts. Samples (of hams) taken from other regions were ripened during spring/summer, at higher temperatures and lower precipitation. As the members of the *Aspergillus* species, which are the main STC producers, grow in dry and warm environments, the absence of this mycotoxin in samples coming from the Eastern region can primary be linked to the weather during the ripening period. These findings emphasise the importance of temperature and humidity control during TMP production.

EXPOSURE ASSESSMENT AND HEALTH
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P25
EARLY-LIFE EXPOSURE TO MYCOTOXINS AND ITS IMPACT ON HEALTH – A CASE STUDY
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Considering the potential impact on health and the scarce data available regarding early-life exposure to mycotoxins, the earlyMYCO project (early-life exposure to MYCOtoxins and its impact on health) proposed to answer key questions: are pregnant women and infants until six months exposed to mycotoxins? Is this exposure a health threat? Does this early-life exposure influence the intestinal immune system development? Which is the burden derived from the exposure to mycotoxins? The earlyMYCO pilot study enrolled 19 pairs of mother and children, with a loss to follow-up ranging between 11% and 47% for different moments of observation. The mycotoxins' biomarkers detected were aflatoxin B1 (AFB1), ochratoxin A (OTA), deoxynivalenol (DON) and β-zearalenol (β-ZEL) in urine samples (mother and children), and AFB1, α-zearalenol (α-ZEL), fumonisin B1 (FB1), FB2 and FB3 in breast milk samples. Food consumption data revealed that foods consumed more frequently during the week were bread, dairy products, non-alcoholic drinks (tea and coffee), animal products (meat and fish) and pasta. Regarding infants, 22% were fed with infant formula and 78% were exclusively breastfed. Considering the exposure levels, a low risk of mothers' exposure to the main mycotoxins analysed is expected, since urine samples did not reveal detectable levels of these compounds; however, infants' urine samples presented a DON mean value of 14.8 ng/ml (corresponding to 148.0 µg/kg bw/day through reverse dosimetry), which could represent a risk for this population group. Notably, maternal exposure to AFB1 promoted an increase of overall T cell population, while it also resulted in a selective reduction of cytokine-producing innate lymphoid cells group 2 (ILC2) population in intestine of the progeny. These alterations were associated with decreased expression of Reg3b and Reg3g by the intestinal mucosa of progeny. Thus, these results indicate that maternal exposure to mycotoxins impacts the development of offspring intestinal immune system. An *in vitro* approach using intestinal cell lines Caco-2 and Caco-2/HT29-MTX models exposed to AFB1 during 24 h, confirmed the deleterious effects of AFB1 on intestinal membrane integrity and its effect on mucus layer. To assess the impact of AFB1 on early-life microbiota, faeces from litters of AFB1 treated female mice and controls were assessed by metagenomics. Although the overall diversity (Shannon diversity index) of the microbiome was not affected between groups, the microbiome composition varied between AFB1 and control faecal samples (Bray–Curtis dissimilarity index). In particular, some beneficial species were diminished in the litters from AFB1 treated females. Results emphasized the need for assessing the prenatal and lactation exposure to mycotoxins. **Acknowledgements**. This work was funded by FCT/MCTES through national funds, to earlyMYCO (PTDC/MED-TOX/28762/2017), and CESAM (UIDP/50017/2020+UIDB/50017/2020).

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INNOVATIVE 2D AND 3D *IN VITRO* MODELS TO EVALUATE TOXICOLOGICAL EFFECTS OF OCHRATOXIN A AND FUMONISIN B1 ON HUMAN CELLS
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The problem of mycotoxins is a matter of global importance in terms of feed and food safety, and therefore the assessment of human exposure to multiple contamination is of real interest [Toxicology Letters 280 (2017) 238]. Ochratoxin A (OTA) and fumonisin B1 (FB1), produced by various filamentous fungi belonging to the Aspergillus, Penicillium and Fusarium genera, are well-known to be toxic in cell cultures. To gain insight in such an important topic, *in vitro* toxicological approaches have recently been developed to evaluate the toxicity of mycotoxin mixtures produced by fungal species in diverse crops or in food products [Toxicology in Vitro 48 (2018) 188; Chemico-Biological Interactions 281 (2018) 51].

However, the information available concerning their toxicological relevance is still limited. The aim of this work was to evaluate different innovative *in vitro* models (2D and 3D) using mitochondrial activity and ATP assay, respectively, and to compare the toxicological effects with HepaRG and Caco-2 cells in differentiated and undifferentiated states. A transwell cell co-culture system was used to assess the potential bioavailability of OTA and FB1 in both cell types, by simulating mycotoxin transfer through the intestinal epithelial barrier in acute conditions. Cytotoxicity was carried out using the MTS assay with the range of 0.1 to 247.6 µM OTA and 0.07 to 138.5 µM FB1 concentrations at 48 h. For the bioavailability assay, HepaRG and Caco-2 cells were seeded in 96-well plates and co-culture in Transwell plates. The presence of mycotoxin concentrations and potential metabolization in extracellular and intracellular fractions was determined by LC-MS/MS-TOF. Cytotoxicity results showed that HepaRG cells were more toxic than Caco-2 cells, and an increase in HepaRG viability in co-culture with Caco-2 cells compared to HepaRG exposed alone. At all concentrations and models tested, differentiated cells were more sensitive than undifferentiated, except in OTA where undifferentiated HepaRG cells were more cytotoxic. For FB1, both cell lines presented similar values. The same effect in both cell types for each of the mycotoxins was obtained in the analytical studies. Overall, spheroid cells were observed to be more sensitive than 2D models. Our results suggest that, depending on the cell type, mycotoxins might act through different mechanisms of action which explains the importance of using more realistic models, and the impact of the intestinal barrier to protect against cytotoxic effects. Further studies will be necessary to confirm whether the metabolism may be more important than the elimination of the toxic in the apical compartment to provide new data for a better risk assessment. **Acknowledgements**. This research has been funded by the EU-FORA Fellowship Programme – The European Food Risk Assessment Fellowship, funded by EFSA, and the Laboratoire Universitaire de Biodiversité et Ecologie Microbienne, Université de Brest, France.

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PROLONGED VASCULAR CONTRACTILE RESPONSE INDUCED BY THE *R* AND *S*-EPIMERS OF THE ERGOT ALKALOID ERGOCRISTINE, AND ATTENUATION BY A NON-COMPETITIVE ANTAGONIST

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Vasoconstriction is a known pharmacological effect associated with ergot alkaloid consumption. The vascular contractile response is often sustained after exposure. Ergot alkaloids exist in two molecular configurations, the *R* and *S*-epimer. The sustained vascular contractile response to the *R*-epimers have been studied previously, unlike the *S*-epimers which are thought to be biologically inactive. Additionally, antagonists have been utilized to attenuate vascular contraction associated with the *R*-epimers of ergot alkaloids utilizing ex vivo techniques. This study utilized an arterial tissue bath to examine and compare the prolonged vascular contractile response attributed to ergocristine (*R*) and ergocristinine (*S*) using dissected bovine metatarsal arteries. The contractile blocking effect of a non-competitive alpha-adrenergic antagonist, phenoxybenzamine (POB), was also investigated in precontracted arteries. Arteries (n = 6/epimer) were exposed to a single dose of ergocristine or ergocristinine (1×10^{-6} M in buffer). Each of the epimer doses were followed by a POB (1×10^{-3} M) or methanol (control) treatment at 90 min and the response was observed for a further 90 min. Both epimers produced a prolonged contractile response over the 180 min total incubation period in the control groups. The *R*-epimer caused a greater prolonged contractile response from 60-180 min post epimer exposure, compared to the *S*-epimer ($P < 0.05$, Generalized Estimating Equations, Independent t-test). Phenoxybenzamine caused a decrease in the contractile response induced by ergocristine and ergocristinine from 105-180 min, compared to the control groups ($P < 0.05$, Generalized estimating equations, Paired t-test). Overall, these results demonstrate the presence of a sustained vascular contractile response attributed to the *R* and *S*-epimer of an ergot alkaloid with differences in contractile response between the epimers, suggesting differences in receptor binding mechanisms. Furthermore, this study demonstrated that a non-competitive antagonist could attenuate the sustained vasocontraction effects *ex vivo*. Additional investigation into *S*-epimers of ergot alkaloids is needed. This research contributes to the understanding of the ergot epimer-vascular receptor binding mechanisms, which may support the investigation of different approaches of minimizing ergot toxicity in livestock.

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COMBINATORY EFFECTS OF ENDOCRINE DISRUPTIVE MYCOTOXINS AND FOODBORNE XENOESTROGENS ON BREAST CANCER PROGRESSION

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The event of metastasis formation, including cell detachment from the primary tumour followed by unpredictable spread in the body, benchmarks tumour aggressiveness. In this light, potential influencing factors, including diet or lifestyle, can contribute to tip the balance between remission or disease progression. For adhesion and motility, cells rely on a complex membrane-cytoskeletal apparatus: these comprise, among others, proteins like integrins, which serve as anchoring structures to the extracellular matrix (ECM). In this study, we explored the potential of food constituents and contaminants to affect adhesion and motility of breast cancer cells exploiting the physiological binding versatility of integrins. On these molecular premises, we compared MCF-7 cells with MDA-MB 231 invasive breast cancer cells *in vitro*. As model substances we chose 4 known xenoestrogens, including two fungal secondary metabolites with endocrine disrupting potential like alternariol (AOH) and α-zearalenol (α-ZEL), the packaging plasticiser bisphenol-A (BPA) and the soy isoflavone genistein (GEN). In our experimental setup, exposure to single compounds increased the migration of MCF-7 (α-ZEL, BPA, GEN) and MDA-MB 231 (GEN). Intriguingly, combinatory treatments showed a marked antagonistic trend. Since xenoestrogens are known to act rather synergistically with the respect of the estrogenic potential [Molecular Nutrtion and Food Research 61 (2017) 1600526], other molecular pathways were necessarily activated to sustain these effects. Pursuing the hypothesis that the compounds could modify the binding capacity of integrins, cell adhesion and integrins expression/localization were also measured. Functional assays revealed that combined exposure to BPA + α-ZEL eased the detachment of MDA-MB 231. In turn, exposure to AOH and α-ZEL or binary mixtures including α-ZEL, increased the adherence of MCF-7 cells to the ECM. None of the abovementioned effects could be traced back to cytotoxicity, since metabolic activity and protein content were unaffected by the treatments. However, alterations in cell adhesion and motility were accompanied by morphological changes. Building on this, the compounds' potential to modulate the expression of activated integrin β1 or to tune actin cytoskeleton via the protease cathepsin D were also assessed. Preliminary data suggest an effect for the single compounds and the mixtures retracing the bi-directional readout on the cell migratory behaviour. In conclusion, exposure to AOH and α-ZEL modulated biophysical properties of breast cancer cells relevant for metastatic spread. Our studies highlighted novel pathways complementing the existing knowledge on the myco/xenoestrogen potentials and open a new perspective for the evaluation of the role of food constituent and contaminants in supporting breast cancer progression.

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CALCIUM DYSREGULATION IS INVOLVED IN ENNIATINS CYTOTOXICITY IN NEURONAL CELLS

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Enniatins (ENNs) are mycotoxins produced by *Fusarium* spp. whose mechanism of action remains elusive. The most frequent ENNs (A, A1, B and B1) present slight structural changes, so it has been traditionally believed that they have the same mechanism of action, acting as ionophores. The present study analyses the cytotoxicity, the type of death and the effects on intracellular calcium fluxes of ENNs A, A1, B and B1 in SH-SY5Y human neuroblastoma cells. Cells were treated with ENNs for 24 h at concentrations between 0.1 and 15 μM and cell viability was determined by MTT assay. Half inhibitory concentrations (IC_{50}) were calculated, finding that ENN B was the most toxic (IC_{50} =0.4 μM), followed by ENNs A, A1 and B1, with values among 2.0 and 2.7 μM. Co-staining with annexin V-FITC and PI revealed that the four ENNs caused apoptotic cell death of SH-SY5Y cells. Subsequently, as apoptosis could be associated with calcium signalling, the effects of ENNs on calcium fluxes were analysed. Dose-response treatments were performed with each mycotoxin at concentrations between 0.5 and 10 μM. ENN A1 produced the greater effect, inducing an acute Ca^{2+} depletion from intracellular compartments

and significantly increasing Ca^{2+} uptake when this ion was added to the bath solution. The effect of ENN A and ENN B1 on Ca^{2+} depletion from intracellular pools was smaller than the originated by ENN A1, but both toxins also induced calcium entry. In the case of ENN B, Ca^{2+} emptying from intracellular compartments was comparable to the produced by ENNs A and B1, however, the maximum Ca^{2+} influx generated by this toxin was found at the lowest concentration tested. The results obtained confirm that ENNs A, A1, B and B1 cytotoxicity is related to calcium dysregulation in neuronal cells, but each toxin acts differently, suggesting that ENNs have different mechanisms of action.

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POTENTIALLY HARMFUL EXPOSURE TO MYCOTOXINS DURING THE CRITICAL PRENATAL PERIOD: A HUMAN BIOMONITORING STUDY IN A PREGNANT COHORT IN RURAL BANGLADESH

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Aflatoxins (AFs), ochratoxin A (OTA), citrinin (CIT), fumonisin B1 (FB1), zearalenone (ZEN), and deoxynivalenol (DON) are mycotoxins that may contaminate diets, especially in low-income settings, with potentially severe health consequences. This study investigates the exposure of 439 pregnant women in rural Habiganj district, Bangladesh, to 35 mycotoxins and their corresponding health risks and links their exposure to certain foods and local stimulants. Overall, 447 first-morning urine samples were collected from pregnant women between July 2018 and November 2019. Mycotoxin biomarkers in first-morning urine samples were quantified by high-performance liquid chromatography-tandem mass spectrometry using a dilute and shoot approach (DaS-HPLC-MS/MS). Urinary concentration of frequently occurring mycotoxins was used to estimate dietary mycotoxin exposure. Crude median regression analyses were performed to investigate the direction of association between the consumption of certain foods and local stimulants, and urinary concentration of frequently occurring mycotoxins. Only in 17 of 447 urine samples (4%) were none of the investigated mycotoxins detected. Biomarkers for six major mycotoxins (AFs, CIT, DON, FB1, OTA, and ZEN) were detected in the urine samples. OTA (95%), CIT (61%), and DON (6%) were most frequently detected, with multiple mycotoxins co-occurring in 281/447 (63%) of urine samples. Under the lowest exposure scenario, dietary exposure to OTA, CIT, and DON was of public health concern in 95%, 16%, and 1% of the pregnant women, respectively. Consumption of specific foods and local stimulants – betel nut, betel leaf, and tobacco powder – were associated with OTA, CIT, and DON urine levels. In conclusion, exposure to multiple mycotoxins during early pregnancy is widespread in this rural community and represents a potential health risk for mothers and their offspring.

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DETERMINATION OF PHARMACOKINETIC PARAMETERS OF EFAVIRENZ AND AFLATOXIN B1: AN APPROACH TO UNRAVEL POSSIBLE INTERACTIONS

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Mycotoxins contamination is a global food safety issue leading to major public health concerns [Critical Reviews in Food Science and Nutrition 60 (2020) 2773. Repeated exposure to multiple mycotoxins not only has an impact on public health, but could also lead to interactions with other substances in the body - such as medicinal drugs - by altering pharmacokinetics and/or pharmacodynamics. In certain global regions, vulnerable populations are not only exposed to high levels of mycotoxins, but also to infectious agents such as the human immunodeficiency virus (HIV). Around 70 percent of the global infected HIV-population is located in Sub Saharan Africa [Open AIDS Journal 10 (2016) 34]. Efavirenz (EFV) is a frequently used HIV-blocker, in monotherapy or in combinations with other HIV-blockers [OMICS: A Journal of Integrative Biology 20 (2016) 575]. The purpose of this research is to determine pharmacokinetic parameters of aflatoxin B1 (AFB1) and EFV via *in vitro* research in human liver

microsomes (HLM) using liquid chromatography tandem mass spectrometry (LC-MS/MS). For the determination of the Michaelis-Menten constant (Km), i.e., the substrate concentration at half maximum velocity and maximum velocity (Vmax), a Michaelis-Menten curve was set up based on microsomal incubations at a specific microsomal protein concentration and ideal incubation time. The latter parameters are determined by performing linearity experiments, testing different protein concentrations and incubation times. Michaelis-Menten curves were set up by monitoring metabolite formation or parent compound depletion in function of the added substrate concentration [Bioscience Reports 37 (2017) BSR20171161]. For EFV the formation of 8-hydroxy-efavirenz (8-OH-EFV), formed by CYP2B6, is measured using an in-house validated method [Drug Metabolism & Disposition 38 (2010) 1218]. The depletion of AFB1 was measured, since not all AFB1-metabolites were available in analytical standards to develop a method for metabolite formation. Pharmacokinetic parameters, i.e., Km, Vmax and the intrinsic *in vitro* clearance, based on Km and Vmax, were determined for both EFV and AFB1 in order to gain more knowledge about these compounds in the framework of pharmacokinetics and/or pharmacodynamics. The currently determined parameters are used in specialized physiologically-based pharmacokinetic (PBPK) computer software to simulate potential *in vivo* human interactions between mycotoxins and medicinal drugs.

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IMPACT OF *FUSARIUM*-DERIVED MYCOTOXINS ENNIATIN B, B1 AND BEAUVERICIN FED TO WEANED PIGLETS

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Besides the regulated main mycotoxins, a wide range of less-investigated fungal metabolites such as the cyclic hexadepsipeptides enniatins and beauvericin are brought more and more into the scientific focus. As *in vivo* studies especially in mammals are scarce, we performed the first feeding trial with either enniatin B, B1 and beauvericin (EB) alone and together with deoxynivalenol (DON) in 28-29 days old piglets for a time period of 14 days. A control group fed a standard diet as well as a DON-contaminated group were included in the set-up. At the end of the trial, blood and faeces samples were taken as well as tissue from liver, jejunum, colon, and lymph nodes. The application of the combination diet with EB+DON resulted in a significant decrease in weight gain of piglets at day 14 ($P < 0.05$) of experiment and tended to be lower in the DON group ($P = 0.062$). Shotgun metagenomics revealed a substantial impact of EB contamination on gut microbiome resulted in a significantly lower faecal microbial richness and diversity. Furthermore, all mycotoxin-contaminated diets caused moderate to severe histological lesions in liver, intestine, and lymph nodes tissue. The intestinal-fatty acid binding protein (i-FABP), a biomarker for intestinal permeability, was significantly ($P<0.05$) lower in the groups fed the EB diets on day 14 compared to control group. No significant effects were seen on the expression of genes in liver or jejunum. Summarizing our results, we can assume that also these unregulated often neglected mycotoxins pose a certain risk especially to young pigs in the crucial phase after weaning. Therefore, scientific community and industry should deliver more data to the regulatory authorities that they can carry out a new risk assessment.

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ASSESSMENT OF MYCOTOXIN EXPOSURE OF RURAL WORKERS THROUGH DIET AND URINARY BIOMARKERS IN BRAZIL

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Exposure to mycotoxins occurs primarily through food consumption, although mycotoxins can also be found in airborne particles in environments where some susceptible products are handled, such as grain mills, bakeries, warehouses, factories, and laboratories. Thus, inhalation of contaminated air can generate an additional route of exposure to mycotoxins. Few studies have addressed the occupational exposure of rural workers to mycotoxins, especially in people involved in animal production. The objective of this work was to assess the occupational exposure through the simultaneous analysis of the main mycotoxins in food products consumed by volunteers from feed factories and rural properties in the State of São Paulo, Brazil, as well as to determine exposure biomarkers of mycotoxins in the urine of workers. Identification and quantification were performed by liquid chromatography coupled to mass spectrometry (LC-MS/MS). Twenty-eight feed handling workers from 3 feed factories and 4 rural

properties participated in the study, resulting in 27 airborne dust samples, 244 food samples and 97 urine samples analysed. Fumonisins (FBs) were detected in all airborne dust samples, at concentrations ranging from 7.8 to 16,839.0 ng/m^3. In all types of food, at least one type of mycotoxin was detected. Zearalenone (ZEN) was detected in samples of rice (52%), corn-based products (18%) and wheat flour-based products (42%), at median values of 3.58, 4.36 and 4.03 µg/kg, respectively. Only three wheat flour-based products and one popcorn samples had deoxynivalenol (DON) and fumonisins (FBs), respectively, at levels higher than the maximum limit allowed by Brazilian legislation. The analysis of urinary biomarkers revealed high frequency of DON (41%), but at low levels ranging from 0.61 to 14.57 ng/mg creatinine (median: 2.07 ng/mg creatinine), followed by FBs in 26% of samples at 0.49 to 63.98 ng/mg creatinine. The mean probable daily intake (PDI) based on estimated food intake (indirect approach) for aflatoxins (AFs), FBs, DON and ZEN were 0.005, 0.769, 0.673 and 0.012 µg/kg of body weight (bw)/day, respectively. Mean PDI values obtained through urinary biomarkers were 0.286, 0.101, 0.497, 9719 and 0.104 µg/kg body weight/day for AFs, DON, ochratoxin A (OTA), FBs and ZEN, respectively. The differences in the exposure data calculated via food intake and through the urinary biomarkers suggest an additional exposure due to occupational activities of the volunteers. This work is the first study in Brazil describing the possibility of occupational exposure to multiple mycotoxins in rural environments. **Acknowledgements**. Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Grant #2019/00990-7.

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A METAPROTEOMIC APPROACH TO ELUCIDATE THE EFFECTS OF DEOXYNIVALENOL AND ZEARALENONE ON THE PORCINE GUT MICROBIOME

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Deoxynivalenol (DON) and zearalenone (ZEN) cause a plethora of adverse health effects in pigs. After ingestion of contaminated feed, the gastrointestinal tract represents the first target of mycotoxins. While the impact of DON and ZEN on intestinal epithelial cells, gut barrier or local immune system have been described previously, their impact on the gut metaproteome is largely unknown. Therefore, the aim of our study was to employ metaproteomics to unravel the effects of DON and ZEN on the gut microbiome in the small intestine of piglets. To this end, female weaned piglets received either (i) uncontaminated feed (control), or feed contaminated with (ii) 870 µg/kg DON (DONlow), (iii) 2,500 µg/kg DON (DONhigh), (iv) 680 µg/kg ZEN (ZENlow) or (v) 16,00 µg/kg ZEN (ZENhigh) for 28 days. Thereafter, digesta and mucosal content of jejunum and ileum (three animals/group) were subjected to metaproteomics analysis. Following protein extraction, peptide mixtures were measured using a Q-Exactive HF-X mass spectrometer faced with an EasyLC 1000 nano-UHPLC. Identification and quantification of peptides/protein groups, their taxonomic assignment and functional annotation was done via MetaLab. Compared to the control, changes in the gut metaproteome composition were observed in the DONhigh and ZENhigh group, respectively. In the DONhigh group, peptides belonging to *Actinobacteria* were increased, whereas peptides belonging to *Firmicutes* were decreased. The ZENhigh group showed a similar trend, albeit lacking statistical significance. On functional level, DONhigh and ZENhigh affected the abundance of proteins associated with the ribosome and pentose-phosphate pathway as well as glycolysis and other carbohydrate pathways, and additionally increased the abundance of thioredoxin-dependent peroxiredoxin. Taken together, our study showed that DON and ZEN altered microbial metabolism, genetic processing, and oxidative stress response in piglets.

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THE FIRST EXTENSIVE AND GLOBAL MYCOTOXIN EXPOSURE SURVEY IN LIVESTOCK

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Mycotoxin exposure is a daily fact for farm animals and the development of more accurate methods for the determination of the actual animal exposure to mycotoxins is of high importance. As such, biomarker-driven research has been proven a successful method for the assessment of animal exposure to xenobiotics by determining concentrations of the parent compounds and/or metabolites in biological matrices like blood. However, mainly due to difficulties related to sampling collection, mycotoxin

biomarker analysis has not, yet, been adopted at farm level. Recently an UHPLC-MS/MS method targeting 23 mycotoxin biomarkers via dried blood spots was validated [Toxins 11 (2019) 541], which has now been extended to 36 biomarkers. Here the method enabled, for the first time, the analyses of mycotoxin biomarkers at an industrial scale through minimal blood volumes. Namely, a thousand animals were tested (511 poultry (broilers, breeders, layers, and turkeys) and 489 swine (piglets, sows, and fattening pigs)), from 20 different countries around the globe. Feed consumed at the moment of blood collection was also analysed for 16 mycotoxins with LC-MS/MS to establish a more complete mycotoxin risk assessment. To this, animal performance and health status were recorded with the intention to establish possible correlations with mycotoxin exposure. Results demonstrated that livestock is continually exposed to multi-mycotoxins as 97% of the studied animals were exposed to two or more mycotoxins simultaneously, and remarkably, more than 50% of which were exposed to six or more mycotoxins. Surprisingly, emerging mycotoxins produced by *Alternaria* and *Fusarium* spp. in combination with deoxynivalenol and ochratoxin A were the most predominant within the 31 mycotoxin biomarkers identified (ranging from trace level up to 2,354 ng/mL). Namely, tenuazonic acid was the most prevalent mycotoxin in blood (66 and 68% in poultry and swine farms, respectively) followed by ochratoxin A (44% and 57%, respectively) and enniatin B1 (37 and 57%, respectively). Additionally, biomarker analysis was able to uncover risk ignored by feed analysis as in 80% of the cases, blood analysis identified mycotoxin exposure missed in the latter. Interestingly, some tendencies between mycotoxin exposure and health status were identified. For example, sows presenting reproductive disorders were consistently exposed to zearalenone or *Alternaria* mycotoxins like alternariol and tenuazonic acid. In conclusion, the first of its kind extensive mycotoxin exposure survey elucidated that poultry and swine under real farming conditions are dominantly exposed to multi-mycotoxins and that biomarker analysis can be key in optimizing animal health and performance.

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CONCENTRATION OF ZEARALENONE, A-ZEARALENOL AND B-ZEARALENOL IN THE MYOCARDIUM AND THE RESULTS OF ISOMETRIC ANALYSES OF THE CORONARY ARTERY IN PREPUBERTAL GILTS

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The carry-over of zearalenone (ZEN) to the myocardium and its effects on coronary vascular reactivity in vivo have not been addressed in the literature to date. Therefore, the objective of this study was to verify the hypothesis that low ZEN doses (MABEL, NOAEL, and LOAEL) administered *per os* to prepubertal gilts for 21 days affect the accumulation of ZEN, α-ZEL and β-ZEL in the myocardium and the reactivity of the porcine coronary arteries to vasoconstrictors: acetylcholine, potassium chloride and vasodilator sodium nitroprusside. The contractile response to acetylcholine in the presence of a cyclooxygenase (COX) inhibitor, indomethacin and / or an endothelial nitric oxide synthase (e-NOS) inhibitor, L-NAME was also studied. The results of this study indicate that the carry-over of ZEN and its metabolites to the myocardium is a highly individualized process that occurs even at very low mycotoxin concentrations. The concentrations of the accumulated ZEN metabolites are inversely proportional to each other due to biotransformation processes. The levels of vasoconstrictors, acetylcholine, and potassium chloride were examined in the left anterior descending branch of the porcine coronary artery after oral administration of ZEN. The LOAEL dose clearly decreased vasoconstriction in response to both potassium chloride and acetylcholine ($P < 0.05$ for all values) and increased vasodilation in the presence of sodium nitroprusside ($P = 0.021$). The NOAEL dose significantly increased vasoconstriction caused by acetylcholine ($P < 0.04$), whereas the MABEL dose did not cause significant changes in the vascular response. Unlike higher doses of ZEN, 5 μg/kg had no negative influence on the vascular system.

MITIGATING THE NEGATIVE IMPACT OF MYCOTOXINS
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EFFICACY ASSESSMENT OF A YEAST CELL WALL-BASED PRODUCT IN REDUCING THE MYCOTOXINS ORAL BIOAVAILABILITY IN RATS
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In addition to the immunomodulatory and antimicrobial properties, yeast cell walls (YCWs) are widely used as feed additives for mycotoxin control. YCW are known to adsorb zearalenone (ZEA), while data on other toxins are conflicting. In this study, a new YCW product was developed to sequester a large spectrum of mycotoxins (aflatoxin B1 (AFB1), ZEA, ochratoxin A (OTA), and fumonisin B1 (FB1)), and its efficacy was assessed first *in vitro* by the isotherm approach, and then *in vivo* with rats. The product at 0.5% w/v dosage adsorbed simultaneously up to 80% ZEA, 65% OTA, 40% FB1 and 10% AFB1 from multi-mycotoxins buffers containing 1 µg/ml of each toxin. The adsorption isotherm approach allowed us to study the adsorption mechanism and to determine the parameters affecting mycotoxin uptake, i.e., type and toxin concentration, pH of the medium and adsorbent concentration. For the study with rats (0.3 kg initial bw), each mycotoxin was administered singularly by a single oral intragastric bolus containing the mycotoxin +/- the product. Two groups of rats were used for each mycotoxin: the control groups received the mycotoxins at the dose of 12, 1, 0.5 and 0.12 mg/kg bw for FB1, ZEA, OTA or AFB1, respectively, while the treated groups received the same dose of mycotoxins with the YCW (0.5% w/w of feed consumption). Feed intake was 100 g feed/kg bw. After toxin administration, rats were housed individually in metabolic cages to collect urine at different time points (4-72 h for AFB1, ZEA and FB1; 4-320 h for OTA). The mycotoxin biomarkers (AFM1, ZEA and its metabolites of phase I biotransformation, i.e., α-ZOL, β-ZOL and β-ZAL, OTA and FB1) were determined in urine by in-house validated HPLC/UHPLC methods. Mycotoxin content in urine was normalized to the respective creatinine concentration. Toxicokinetic parameters, i.e., area under the curve over 72/320h ($AUC0\rightarrow t$) and maximal urine concentration (C_{max}) were calculated for target mycotoxins/metabolites and were used to compare control and treatment groups. The YCW reduced the urinary excretion of ZEA, FB1 and OTA, but it was ineffective for AFB1. In particular, AUC was significantly reduced by -50% for OTA ($P = 0.014$) and -42% for FB1 ($P = 0.048$), while C_{max} value was reduced by -79% ($P < 0.029$) for ZEA and its metabolites. Taking into account overall findings, a good relationship was observed between *in vitro* and rat trials, and it can be concluded that the YCW may protect animals from the harmful effects of multi-mycotoxins contaminated feeds. **Acknowledgements**. This research was supported by the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No. 678781 (MycoKey) and by the European Union's Horizon 2020 research and innovation programme under Grant agreement No 952337 (MycoTWIN).

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FIRST *IN VITRO* EVIDENCES OF SPORIDESMINS ADSORPTION BY A CLAY-BASED MATERIAL
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Sporidesmin A is the most important mycotoxin produced by the fungus *Pithomyces chartarum* that grows on pasture litter during late summer and autumn. This fungus produces several variants of sporidesmin (from A to J), with sporidesmin A accounting for 80-90% of congeners. Sporidesmins affect the hepatobiliary system of ruminants and lead to the retention of phytoporphyrin which, reacting with light in peripheral tissues, causes the photosensitivity reaction known as "facial eczema". The disease produces losses in animal production and serious animal welfare problems in farmed ruminants in New Zealand and in warm temperate regions of the world. Zinc in several forms and with high dose rates is used as a control measure for facial eczema, but it poses safety issues. Other additives for sporidesmins control in feeds have not been evaluated. The use of adsorbing agents to reduce the exposure to mycotoxins by decreasing their bioavailability is a valuable strategy to cope with mycotoxins. To this scope, this study examines the ability of a clay-based agent in sequestering sporidesmins from liquid mediums at physiological pH values (2.5-6.5). The adsorption behaviour of the clay for sporidesmins was studied by equilibrium isotherms as a function of pH, clay dosage, initial toxin concentration, equilibrium time, and temperature. Physico-chemical, kinetic, and thermodynamic parameters related to the adsorption process were calculated, including the adsorbent dosage to achieve a 50 (C_{50}) and 100%

(C_{100}) of toxin adsorption, the maximum adsorption capacity (B_{max}) and the affinity (K_L), the rate constants, and the ΔG°, ΔH°, ΔS° parameters. The results showed that the clay exhibited high affinity for sporidesmins at ruminal pH (6.5), while its adsorption capacity slightly decreased with increasing pH from 2.5 to 6.5. In particular, the values of C_{50} and C_{100}, expressed as mg of clay/µg of sporidesmin A, were 10 and 64 mg/µg at pH 2.5 and 14 and 51 mg/µg at pH 6.5. B_{max} values (ng of sporidesmin A adsorbed/mg of clay) calculated at pH 2.5 and 6.5, were 54 and 26 ng/mg, respectively. K_L values (L/mg of sporidesmin A) were 16 and $7*10^2$ l/mg at pH 2.5 and 6.5. Kinetic studies suggested that sporidesmin A was adsorbed in less than 60 min following the pseudo second order model. Considering that ruminants are susceptible to sporidesmin A contaminated feed on a µg/kg basis, the results of this study suggest that the clay is promising for use as an effective adsorbent for sporidesmin decontamination.
Acknowledgements. The study was financially supported by the CNR-ISPA and ANPARIO plc Collaborative Research and Development Agreement, Grant No 2779/2020.

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EVALUATION OF A YEAST HYDROLYSATE FROM A NOVEL STRAIN OF *SACCHAROMYCES CEREVISIAE* FOR MYCOTOXIN MITIGATION USING *IN VITRO* AND *IN VIVO* MODELS

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Mycotoxicoses are diseases caused by exposure of animals to feeds contaminated with mycotoxins. One strategy to cope with mycotoxins is the use of adsorbing agents to reduce the exposure to mycotoxins by decreasing their bioavailability, which leads to a reduction of mycotoxin uptake as well as distribution to the blood and target organs. Yeasts and yeast cell wall extracts have shown *in vitro* adsorption efficacy for a number of mycotoxins, including zearalenone (ZEA), aflatoxin B1 (AFB1), T2-toxin, patulin and ochratoxin A (OTA). However, there are only limited and conflicting reports on deoxynivalenol (DON) adsorption by yeasts or by products derived therefrom. The objective of this work was to evaluate the efficiency of three selected yeast products as mycotoxin binder using *in vitro* and *in vivo* models. We have determined the *in vitro* binding capacity of three yeast hydrolysate products towards DON, OTA and ZEA by sequential incubation at pH 3.0, 5.0 and 8.5, mimicking the pH conditions during gastric passage in a monogastric animal. The results showed that only one product, an enzymatic yeast hydrolysate (YHY) of a novel strain *Saccharomyces cerevisiae* CNCM I-5405, effectively adsorbed about 45% of deoxynivalenol (DON) in solution, independent of the pH value. Next, we determined the effect of selected YHY on oral absorption of DON, ZEA and OTA using a toxicokinetic model in swine as developed by Devreese *et al.* [Toxins 6 (20214) 2998]. Pigs (± 20 kg) received an oral bolus of 3 mycotoxins (DON and OTA, 0.05 mg/kg bw; ZEA, 0.5 mg/kg bw) alone or in combination with YHY (100 mg/kg bw). Toxicokinetic modelling of the plasma concentration-time profiles of DON, OTA and ZEA-GlcA showed that YHY tended to reduce the maximal plasma concentration of OTA by 17%. YHY caused a numerical reduction in oral bioavailability of OTA, DON and ZEA-GlcA. The results of this work indicate that YHY may have the potential to reduce the absorption of multiple mycotoxins in animals after exposure. Furthermore, our toxicokinetic study indicate the need for a more thorough *in vivo* evaluation of the efficacy of YHY using natural mycotoxin contaminated feed to evaluate its capability in reducing mycotoxicoses in farm animals under field conditions.

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MACHINE LEARNING-AIDED DESIGN FOR ATOX NATURE SILVER DETOXIFIER FOR ANIMAL FEED

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The contamination of animal feed by mycotoxins fungi naturally occurs causing diseases and death in human and animals. The strict maximum mycotoxin levels regulations render crucial finding smart mycotoxin mitigation strategies. The most effective acknowledged approaches are forming bulky non-absorbable complexes with binding agents, reducing the bioavailability of mycotoxins. Among the detoxifiers, inorganic porous materials such as clays minerals are recognized effective especially for sequestering of aflatoxin B1. Similarly, organic compounds, such as activated charcoal, have been demonstrated powerful adsorbent for several mycotoxins, including deoxinivalenol (DON). However, the required doses for a significant detoxification leads to sequestrating of essential micronutrients. Our mycotoxin detoxifier Atox Nature Silver, being a biohybrid material establish a weighted compromise between diverse mode of action of the singular components. The latter was designed by exploiting

advanced machine-learning tools which provided costs and time saving especially in the contest of *in vivo* trials. The research involving living animals is guided by the principle of the '3Rs', i.e., replacement, reduction, and refinement when animals are used for scientific purposes to improve animal welfare and to minimize the environmental impact. Thus, the synergy of theory and experiment accelerates the screening of vast number of material formulations while incorporating the animal-derived models for detoxification testing. The computer-aided approach facilitates the design of effective materials and enable optimal design of *in vivo* experiments. In this contribution, we have built machine learning (ML) models that incorporate three distinctive factors underlying the detoxifier performance, i.e., the material formulation, the chemical structure of the targeted toxin and the process in which a mycotoxin-detoxifier (MDT) is applied. The models being trained using an extensive set of in vitro experiments are used as surrogates of real experiments for the exploration of promising MDTs. In this multidisciplinary study, we aimed to demonstrate three-fold applications of our approach (i) in the identification of the top performing formulation (named, Atox Nature Silver) for the regulated toxins, (ii) in predicting detoxification of a wide set of yet-unregulated mycotoxins, and (iii) in gaining insights into the *in vitro* detoxification mode of action through model feature importance analysis. Finally, biomarker detection-based *in vivo* validation of Atox Nature Silver is demonstrated in a challenging DON detoxification trial in broiler chickens.

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EFFECT OF LACTIC ACID BACTERIA IN OCHRATOXIN A AND AFLATOXIN B1 REDUCTION DURING BREAD FERMENTATION*

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* presented as an oral presentation in session 3

Aflatoxin B1 (AFB1) and ochratoxin A (OTA) are considered the most important mycotoxins with common presence in bread and bakery products. Biological detoxification of mould food spoilage and mycotoxin contamination by lactic acid bacteria (LAB) exhibits high potential on a cost-effective and large scale. The aim of the present study was to evaluate the potential effect of several LAB isolated from milk whey on reducing AFB1 and OTA concentration during bread making process. A screening of twelve LAB strains (B1, B2, B3, B4, B5, B6, B7, B9, B10, BS4, BS6, BS7) was performed by evaluating OTA and AFB1 reduction after 72 h incubation in MRS broth (37ºC). The most effective LAB were lyophilized and added as ingredient in bread formulation partially (50%) or totally (100%) substituting yeast, with five tested conditions: (i) control, (ii) yeast-B10, (iii) yeast-B3, (iv) B10, and (v) B3. Mycotoxins concentration was evaluated in both dough and bread after 4, 8 and 24 h of LAB fermentation by HPLC-qTOF/MS analysis. All conditions, as well as the control containing only yeast, were prepared in triplicate. Nine LAB reduced OTA (12-40%) in MRS broth with B3, B10, BS4 and BS7 as the most active ones. AFB1 was reduced by five LAB (11-35%), highlighting B3 activity. OTA was significative reduced in dough by yeast-B10 (22-29%), yeast-B3 (9-34%) and B3 (17-28%) fermentation compared to the control; while AFB1 was reduced up to 11% with yeast-B3. OTA showed significative reductions in bread after yeast-B10 (8-25%), yeast-B3 (14%), and B3 (19-26%) fermentation; while AFB1 reductions were observed in bread with yeast-B3 (35-52%) and B3 (32-55%) fermentation. For both mycotoxins, the highest reductions were obtained after 8 h fermentation with yeast-B3. The selected BAL showed OTA and AFB1 reduction during bread fermentation when added as ingredient alone or in combination with yeast, pointing to a potential biocontrol strategy for mycotoxins reduction in bakery products. **Acknowledgements**. Spanish Ministry of Science and Innovation (PID2019-108070RB-I00-ALI).

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A MYCOTOXIN-MITIGATING FEED ADDITIVE REDUCES THE CHRONIC ADVERSE EFFECTS OF MODERATE LEVELS OF *FUSARIUM* MYCOTOXINS IN DAIRY COWS

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Little is known about the adverse effects of commonly found levels of *Fusarium* mycotoxins on the performance of dairy cows, especially after a long period of exposure. To investigate the effects of moderate contamination levels of deoxynivalenol (DON), zearalenone (ZEA) and fumonisins B1 and B2 (FB) in total mixed ration (TMR) on dairy cows, 31 lactating Holstein cows were used in a completely randomized design. The animals were tested in two successive periods, spring (16 cows) and summer (15 cows). Both experimental periods included 7 days of adaptation, followed by 54 days of mycotoxin exposure. During exposure, the cows received one of three diets: (i) TMR with low DON, ZEA and FB

levels, at 284.5, 43.2 and 129.6 µg/kg dry matter (DM), respectively (CTR); (ii) TMR contaminated with mycotoxins at higher levels than CTR, but below U.S. and European Union guidelines (DON, ZEA and FB at 1021.0, 196.8 and 238.4 µg/kg dm, respectively; MTX); or (iii) MTX diet supplemented with a mycotoxin-mitigating product (TOXO® XXL, Trouw Nutrition, the Netherlands; 100 g/animal/day; with DON, ZEA and FB at 1009.6, 248.5 and 241.7 µg/kg, respectively; TOXO). Body weight, body condition score, dry matter intake (DMI), milk production and composition, or feed efficiency were measured both during adaptation and exposure periods. During adaptation, all animals were fed the same CTR diet and did not show differences in any parameters ($P > 0.05$). During exposure, the DMI was similar among groups with 26.0 kg/cow per day. Feeding MTX diet numerically reduced milk production from 38.23 kg/day (CTR group) to 37.55 kg/day (MTX group). Feeding the TOXO diet numerically increased the milk production up to 39.27 kg/day. Concerning milk quality, the milk fat concentration tended ($P = 0.07$) to be lower in CTR than MTX and TOXO groups (3.52% vs. 3.74% and 3.80%, respectively). No differences among groups and periods were measured for other parameters. In conclusion, a long-term exposure of dairy cows with moderate levels of *Fusarium* mycotoxins into diets numerically affected performance of dairy cows, and such negative effects were counteracted by supplementation of mycotoxin-mitigating product TOXO® XXL, the TOXO group producing +1.72 kg milk per day per cow than MTX group.

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IN OVO EFFECTS OF PHYTO-SYNTHESIZED SILVER/ZINC OXIDE NANOPARTICLES ON ATTENUATING AFLATOXIN B1 OXIDATIVE STRESS, LIVER INJURY AND GENE EXPRESSION IN INDIGENOUS CHICKENS

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Aflatoxin B1 (AFB1) is one of the predominant mycotoxin contaminants in food and feed, causing oxidative stress and hepatotoxicity, and it decreases antioxidant enzyme levels. Maternal and *in ovo* AFB1 exposure promotes immunological deficiency in developing embryos, making them more susceptible to infections and microorganisms during the growth and maturation of the compromised chicks. As a result, hens develop a low resistance to infectious illnesses and low immunity. The potential of silver/zinc oxide synthesized using *Urginea epigea* plant extract to alleviate AFB1-induced eggs was investigated in this study. Fertile eggs of indigenous chickens were divided into six treatment groups, including control groups: G1, no injection; G2, phosphate buffered saline; G3, 0.045 mg/kg AFB1; G4, 5 mg/ml AFB1; G5, 50 mg/ml Ag/ZnO nanoparticle; and G6, 5 mg/ml AFB1 + 50 mg/ml Ag/ZnO. After inoculation, eggs were incubated for 21 days, hatched, and reared in floor pens for 42 days in a complete randomized design with five replicates per treatment. After 42 days, liver and blood samples were collected for different analyses. AFB1 oxidative stress was evident in untreated AFB1-intoxicated chickens, as evidenced by a significant increase in hepatic transaminases, an increase in lipid peroxide biomarkers, a decrease in reduced glutathione concentration, and a decrease in antioxidant enzyme activities, specifically catalase, total superoxide dismutase, glutathione peroxidase and glutathione-S-transferase compared to control. Histopathological examination showed that after 28 days, the high concentration of AFB1 (5.0 mg/ml) induced liver injury in chickens, but Ag/ZnO supplementation partially ameliorated liver injury in a dose-dependent manner. RT-PCR data revealed that AFB1 significantly down-regulated Nrf2 and its downstream genes mRNA expression level. Moreover, Western blot analysis showed that Nrf2 protein expression level was markedly reduced in the AFB1-fed group. However, supplementation with Ag/ZnO ameliorated AFB1-induced liver injury via enhancing enzymes expressions and activity. Administration of Ag/ZnO resulted in amelioration of AFB1-induced effects compared to untreated AFB1-intoxicated chickens via an up-regulation of antioxidant enzyme gene expression, activation of the expressed genes, and increase in the availability of GSH. In conclusion, our study suggests that silver-zinc oxide encapsulated nanoparticle exerts a multi-active preventive role against AFB1 induced toxicity, oxidative stress by promoting antioxidative defense systems and limiting lipid peroxidation.

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REVIEW OF THE EFFICACY OF DIFFERENT MYCOTOXIN BINDERS TO ADSORB MYCOTOXINS *IN VITRO*

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The objective of this study was to determine the efficacy of different mycotoxin binders (MTB) to adsorb mycotoxins *in vitro*. A literature search was conducted to identify *in vitro* research papers from different databases. The search was based on 7 MTB, i.e., active carbon (AC), bentonite, clinoptilolite, hydrated sodium calcium aluminosilicate (HSCAS), sepiolite, yeast cell walls (YCW), and zeolite, and 6 mycotoxins, i.e., aflatoxin B1 (AF), deoxynivalenol (DON), fumonisin B1 (FUM), ochratoxin (OTA), T-2 toxin and zearalenone (ZEA). Inclusion criteria were: *in vitro* studies, incubation media and pH description, and percentage of mycotoxins adsorption. Sixty-eight papers with 1,842 data were selected and analysed with the PROC MIXED of SAS. The response variable was percentage mycotoxins adsorption by MTB, and the model included the fixed effects of pH, incubation media (water, methanol, HCl, citrate acetate phosphate (CAP) buffer, a simulation of the gastro-intestinal digestion and gastric juice (GI)) and their interactions, and the random effect of study. Incubation media was only different between CAP and GI ($P < 0.05$), and data from GI were excluded. The mycotoxins adsorption capacity was 62% ± 1.0 for bentonite (from 18 with DON to 93% with AF, $P < 0.05$), 52% ± 4.3 for clinoptilolite (from 0 with DON to 75% with AF, $P < 0.05$), 55% ± 1.9 for HSCAS (from 11 with DON to 83% with AF, $P < 0.05$), 76% ± 3.1 for MMT (from 9 with DON to 88% with AF, $P < 0.05$), 83% ± 1.0 for AC (from 53 with T-2 to 93% with AF), 44% ± 0.4 for YCW (from 19 with DON to 49% with AF) and 52% ± 9.1 for sepiolite (from 12 with DON to 95% with AF). The adsorption of AF was 76% ± 0.6 (from 49 with YCW to 95% with sepiolite, $P < 0.05$), for DON was 35% ± 1.6 (from 0 with clinoptilolite to 69% with AC, $P < 0.05$), for FUM was 50% ± 1.8 (from 25 with sepiolite to 86% with AC, $P < 0.05$), for OTA was 42% ± 1.0 (from 17 with sepiolite to 88% with AC, $P < 0.05$), for ZEA was 48% ± 1.1 (from 14 with clinoptilolite to 85% with AC, $P < 0.05$), and for T-2 was 27% ± 2.8 (from 5 with zeolite to 52% with AC). The absorption of OTA and ZEA was affected by pH ($P < 0.05$).

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META-ANALYSIS ON THE EFFICACY OF DIFFERENT MYCOTOXIN BINDERS TO REDUCE AFLATOXIN M1 IN MILK AFTER AFLATOXIN B1 CHALLENGE IN DAIRY COWS

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The objective of this meta-analysis was to determine the efficacy of different mycotoxin binders (MTB) to reduce aflatoxin M1 (AFM1) in milk. A literature search was conducted to identify *in vivo* research papers from different databases. Inclusion criteria where: *in vivo*, dairy cows, description of the MTB used, doses of MTB, aflatoxin inclusion in the diet and concentration of AFM1 in milk. Twenty-two papers with 108 data were selected. Binders used in the studies were: hydrated sodium calcium aluminosilicate (HSCAS), yeast cell wall (YCW), bentonite (BEN), and mixes of several MTB (MIX). The response variables were: AFM1 percentage reduction in milk, total AFM1 concentration excreted in milk per day and transfer percentage of aflatoxin from feed to AFM1 in milk; and AFM1 concentration in urine and faeces. Data were analysed with GLIMMIX procedure and WEIGHT Statement of SAS (SAS Institute, Inc., USA). The percentage reduction of AFM1 in milk was 54% ± 2.7 ($P < 0.05$) for BEN, 27% ± 2.9 ($P < 0.05$) for MIX, 18% ± 4.5 for YCW and 7% ± 3.5 for HSCAS. The excretion of AFM1 in milk (µg/d) was lower in HSCAS (14.4 ± 0.97), YCW (16.3 ± 1.21), and MIX (18.7 ± 1.27) ($P < 0.05$), and tended to be lower (13.9 ± 1.44) in BEN ($P < 0.08$) compared with control (22.6 ± 1.00). The transfer of AFM1 to milk was lower in HSCAS (1.5% ± 0.10), YCW (0.9% ± 0.03) and BEN (0.7% ± 0.10) ($P < 0.05$) compared with control (2.3% ± 0.11). Urine and faecal excretion were only identified in HSCAS and MIX treatments. Urine concentration of AFM1 (µg/l) tended to decrease with HSCAS (2.1 ± 0.70, $P < 0.08$) compared to control (7.6 ± 1.25). Faecal concentration of AFM1 (µg/kg) tended to decrease with MIX (4.9 ± 0.56, $P < 0.09$) compared to control (6.8 ± 1.05). The meta-analysis results showed that BEN has the highest capacity to reduce AFM1 from milk and YCW the lowest.

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EFFICACY OF A POSTBIOTIC YEAST CELL WALL IN ALLEVIATING EFFECTS OF NATURALLY CONTAMINATED FUSARIA TOXINS IN COMMERCIAL BROILER BIRDS.

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It has been reported in a large number of surveys that *Fusarium* toxins, especially deoxynivalenol (DON), are the most ubiquitous mycotoxins present in feedstuffs. The increased occurrence of extreme weather events and climate change could potentially result in widespread rise in contamination of livestock feed, a major concern expressed by EU and FDA regulators. The yeast cell wall (YCW) of *Saccharomyces cerevisiae*, more specifically the highly ramified and dynamic network of parietal β-glucans, has been exploited as key component for toxin mitigation strategies in feed to reduce bioavailability of toxins in the gastrointestinal tract and thus offset mycotoxicosis in livestock. YCW-based preparations have shown efficacy against *Aspergillus*-based toxins but could have a more diffuse impact against certain *Fusarium* type B trichothecenes found naturally in contaminated diet, and often occurring in larger amounts than other mycotoxins. In this study, we have investigated a combination of two *Fusarium* toxins, DON and T-2 toxin (T2) and the effect of two adsorbents, (i) a YCW extract (YCWE) and (ii) a postbiotic YCW-based product (PYCW) with the objectives of preventing the pathological effects in commercial broiler chicken. A total of 720-day-old non-vaccinated male Cobb broilers were randomly allocated into six treatments: (i) control diet, with background level of toxins (aflatoxins 6 µg/kg; cyclopiozanic acid 15 µg/kg; fusaric acid 25 µg/kg; fumonisin B1 310 µg/kg); (ii) diet 1 + YCWE at 0.2% inclusion rate to the diet; (iii) diet 1 + 0.2% PYCW; (iv) *Fusarium*-contaminated diet (3.0 mg/kg DON, 2.17 mg/kg acetyldeoxynivalenol, 0.104 mg/kg T-2 toxin, 0.079 mg/kg zearalenone); (v) diet 4 + 0.2% YCWE; and (vi) diet 4 + 0.2% PYCW. At the end of the 21-day and 42-day feeding periods, performance and immunological parameters, serum biochemistry, liver function, gut histomorphology and caecal SCFA were evaluated. Under the tested mycotoxin challenge, production performance, liver function (increased AST, ALP, ALT, LDH), blood metabolites (increased blood glucose and cholesterol levels) were significantly affected, and chicken were also found immunocompromised (NDV and IBV) ($P < 0.05$). Additionally, *Fusarium* toxins altered SCFA levels (reduced butyrate and iso-butyrate) in caecum, reduced goblet cell count in the gut epithelium and caused architectural aberrations in the epithelial integrity of intestinal villi. These ultrastructural changes were reversed by the inclusion of PYCW and in a lesser extend YCWE, for this latter confirming results found in a titration study, where villus height and crypt depth along with the number of goblet cells in villi were increased. Increased goblet cell count was similar to birds fed non-contaminated diet indicating normal mucin production and protection against bacterial assault and toxicants. The PYCW product provided nutritional benefits through sparing effects in energy metabolism, thus yielding better production performance and stimulation of the liver function (reduced liver enzymes in blood) and improved immunological function (Increased humoral immunity against NDV and IBV). Taken together, our research highlights the potential application of YCWE alone and in combination with nutritional bioactive (PYCW) that may act synergistically in alleviating the toxic effects induced by multiple mycotoxins present in naturally contaminated diet of broiler birds.

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ACCUMULATION OF FUMONISINS IN BROILERS TISSUE WITH OR WITHOUT ALGOCLAY TECHNOLOGY

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It was long considered that fumonisins (FB) do not accumulate in animals as they have a low oral absorption and a rapid plasma elimination. Nevertheless, recent studies demonstrated that the hepatic half-elimination of fumonisin B1 (FB1) was up to several days. Based on this latest research, the aim of this study was to evaluate fumonisins deposit in broiler tissues. 21-day old broilers were slaughtered after being fed a diet with 20 mg FB1+FB2/kg during 4 or 9 days prior to slaughtering. No sign of toxicity was observed on performances at this level of FB in feed but increased concentrations of sphinganine (Sa) and sphingosine (So) over time in liver were measured. An accumulation from 4 days to 9 days of exposure of FB1 was measured in liver with concentrations of respectively, 20.3 and 32.1 ng FB1/g observed at these two exposure periods. The same effect was observed with fumonisin B2 (FB2), from 0.79 ng/g at 4 days to 1.38 ng/g at 9 days. Even if FB1 levels were very low in breast muscles, an accumulation could also be observed from 4 days to 9 days exposure with concentrations of 0.036 and 0.072 ng FB1/g, respectively. When feeding algoclay technology (algae and clay combination) a significant reduction of FB1 accumulation was measured in liver and muscle by respectively, around

40% and 50% on day 9, whereas a non-significant effect was observed after 4 days exposure. Broilers fed algoclay also showed a decrease in Sa and So contents in the liver compared to the levels of Sa and So measured in broilers fed FB alone after 9 days exposure. Further studies are needed to confirm this fumonisins accumulation over the time in presence of fumonisins during longer time of exposure and different animal ages.

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THE ABILITY OF AN ALGOCLAY-BASED MYCOTOXIN DECONTAMINANT TO DECREASE THE SERUM LEVELS OF ZEARALENONE AND ITS METABOLITES IN LACTATING SOWS

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This study evaluated the effect of an algoclay-based mycotoxin decontaminant on the levels of zearalenone (ZEN), deoxynivalenol (DON), and their derivatives in the colostrum, milk, and serum of sows, as well as in the serum of weaned piglets after maternal mycotoxin exposure during the last week of gestation and during lactation of sows (26 days). For this, sows (n = 5) were fed diets artificially contaminated with 100 (LoZEN) or 300 (HiZEN) ppb ZEN, with or without an algoclay-based mycotoxin decontaminant in the HiZEN diet. All diets contained 250 ppb DON naturally occurring in the ingredients. Dietary treatments did not affect the performance of the sows and piglets. Only α-ZEL was significantly increased in the colostrum of sows fed the HiZEN diet, however, no differences in milk mycotoxin levels were observed at weaning. The highest levels of ZEN, α-ZEL, and β-ZEL were observed in the serum of sows fed the HiZEN diet. When the HiZEN diet was supplemented with the tested algoclay-based mycotoxin decontaminant the levels of ZEN and its metabolites were significantly decreased in the serum of sows. Although all sows were fed the same levels of DON, the serum level of de-epoxy-DON was increased in the serum of piglets from the sows fed the HiZEN diet but not in the serum of the piglets fed the HiZEN supplemented with the decontaminant. In conclusion, the tested algoclay-based mycotoxin decontaminant can decrease the levels of ZEN and its metabolites in the serum of sows and the level of de-DON in the serum of piglets.

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ADSORPTION OF AFLATOXIN B1 AND FUMONISIN B1 BY MAIZE, WHEAT, AND OAT BRAN

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Mycotoxins are secondary metabolites produced by fungi that contaminate food and agricultural products. In Africa, the most common mycotoxins are aflatoxin B1 (AFB1) and fumonisin B1 (FB1) [The Journal of Veterinary Medical Science 76 (2014) 789]. AFB1 is a well-known carcinogen to humans, while FB1 is considered a possible carcinogen. In several African countries, food insecurity, poverty, and poor regulation can cause continuous exposure to mycotoxin-contaminated foods. This leads to the prevalence of childhood stunting and an increased risk of morbidity, mortality, and impaired mental development. Studies are therefore needed to develop innovative solutions to reduce mycotoxin exposure through food. Recently, various food by-products have been investigated for their mycotoxin binding capacity, and plant fibres have shown promising results. The aim of this study was to determine the adsorption of AFB1 and FB1 by maize, wheat, and oat bran. The adsorption capacity of various brans for AFB1 and FB1 was evaluated at pH 7 and pH 3 conditions. Additionally, the effects of contact time, temperature, and bran amount were investigated. For AFB1 adsorption, maize bran (82.5% and 78.5%) showed the highest adsorption capacity compared to wheat (57.9% and 53.4%) and oat bran (24.0% and 29.8%) at pH 7 and pH 3, respectively. However, FB1 was not adsorbed by the brans, possibly due to its hydrophilicity. As the contact time and bran amount increased, the adsorbed AFB1 also increased. Lower temperatures (5°C and 25°C showed a higher adsorption efficacy for AFB1 in wheat and oat brans, while for maize bran, the highest absorption occurred at 37°C (94.1%). Nonetheless, the adsorption of AFB1 decreased above 37°C. This study showed that brans adsorbed AFB1 highly, indicating that bran consumption could reduce the risk of AFB1.

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DETOXIFICATION OF AFLATOXIN B1 BY MICROBIAL BINDING

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Aflatoxin B1 (AFB1) is a mycotoxin commonly produced by *Aspergillus flavus* and *A. parasiticus*. It is carcinogenic and mutagenic compound and possess both acute and chronic toxicities. In Africa, aflatoxins are one of the most common group of mycotoxins and usually contaminate maize. Although AFB1 levels in food are regulated, people facing food insecurity and poverty in Africa may consume crops that have AFB1 contamination above the maximum limits due to lack of choice. AFB1 contamination should always be avoided during pre- and post-harvest periods, nonetheless techniques for AFB1 decontamination are needed as mycotoxin production cannot always be fully avoided. Several binding agents, such as mineral and organic are used to detoxify contaminated feed. These adsorbents are only used in feed as they also reduce the bioavailability of nutrients. Biological adsorbents, however, such as lactic acid bacteria (LAB) are known to bind mycotoxins while maintaining the nutritional value of the decontaminated food [Food Additives & Contaminants Part A 28 (2011) 1590]. This study aimed to investigate the AFB1 binding ability of 13 different LAB strains. The binding capacity was evaluated with both viable and nonviable LAB cells. The binding studies were conducted at pH 7. In addition, strains showing the best binding capacity were selected and their binding stability was studied at pH 3, to mimic the pH conditions in the stomach. The viable LAB strains studied had the ability to bind 29.0-40.9% AFB1, indicating that the binding was strain dependent. Non-viable LAB cells had better binding capacity (44.9-65.7%) compared to viable cells, indicating that heat-induced lysis of the cells exposed more AFB1 binding sites. The binding capacity of both viable and non-viable cells was improved (up to 70% for the best strain) at lower pH, indicating that the binding is stable in the stomach pH condition. This study showed that LAB are a useful tool for AFB1 binding which may lower its bioaccessibility.

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EVALUATION OF WHEY FROM COW´S MILK FERMENTED BY LACTIC ACID BACTERIA AS A BREAD BIO-PRESERVATIVE AGENT

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Whey is a waste product from cheese industry. However, it contains proteins that have a high nutritional value and are an important source of antifungal peptides. Food deterioration caused by toxigenic fungi is one of the challenges of food safety. The aim of this study was to revaluate whey from cow´s milk by lactic acid bacteria (LAB) fermentation and the use of this ingredient as a bio-preservative in bread production. This work aimed to evaluate the antifungal activity and ability of selected lactic acid bacteria (LAB) to employed as novel application of bio-preservation of food and as reduction mycotoxins method in food. Whey was pasteurized and fermented by nine selected LAB with antifungal activity for 72 h at 37ºC. Subsequently, fermented whey (FW) was incorporated into the bread formulation, and the pH; antimicrobial metabolites, such as organic acids and volatile organic compounds (VOCs); total phenolic content; DPPH radical-scavenging activity of FW and breads were characterised. Also, the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC) of FW against 28 strains of toxigenic fungi belonging to the genus *Aspergillus, Penicillium* and *Fusarium* were determined. A study of shelf life of breads inoculated with a suspension of *Penicillium verrucosum* (ochratoxin A producer) and by natural contamination was carried out to study the reduction of fungal growth and mycotoxin production compared to bread without additive, bread with additive calcium propionate and bread with whey non-fermented. The highest lactic acid content was observed in the FW by *L. plantarum* TR7 (15.0 g/l) and *L. plantarum* TR2 (12.5 g/l). FW showed MIC and MFC values in range of 8-250 g/l. In addition, an increase in VOCs such as a hexanal, benzeneacetaldehyde, benzaldehyde and pyrazine tetramethyl was determined in bread with FW. FW evidenced an increase in radical scavengers, and this was reflected in a 33% rise in the DPPH- inhibitory activity of bread with FW compared with the control bread. Breads in which 100% of the water was replaced with FW by *L. plantarum* TR7 showed fungal growth at 10 days of storage and evidenced an improvement in the shelf-life of 4 and 7 days compared with a control containing calcium propionate at 0.3% and control bread, respectively. Bread with FW evidenced a reduction of fungal growth of 0.5-1 log spores/g and an ochratoxin A production in the range of 85-100%.

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ZEARALENONE HYDROLASE ZENA (ZENZYME®) AND ITS ABILITY TO DEGRADE ZEN IN THE RUMEN OF DAIRY COWS

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One of the most frequently detected mycotoxins, zearalenone (ZEN) can act both as an endocrine disruptor, causing fertility problems as well as having a negative impact on ruminal fermentation patters in dairy cattle. When ZEN enters the rumen, it gets largely metabolized into α-zearalenol (α-ZEL) and β-zearalenol (β-ZEL), which overall leads to a potentiation of its estrogenic effect. The feed enzyme Zearalenone hydrolase ZenA (ZENzyme®) has the ability to catalyse the hydrolysis of ZEN with the main metabolite of degradation being the non-estrogenic hydrolysed zearalenone (HZEN). Our objective was to test the ability of ZENzyme® to shift the ZEN metabolism in the rumen from the estrogenic potent metabolites ZEN, α-ZEL and β-ZEL to the formation of non-estrogenic HZEN. Therefore, a total of six dry, non-gestating Holstein-Friesian cows with rumen fistulas were used in longitudinal trial design with repeated measures. The first treatment consisted of a once per day fed portion of ZEN (5 mg ZEN mixed in 500 g wheat meal before morning feeding corresponding to a diet contamination of 500 ppb) for two days, followed by a two-day washout period. Following this washout period, the animals received the same amount of ZEN but supplemented with 100 U ZENzyme® (ZEN + ZENzyme®, corresponding to 10 U/kg DM feed). Rumen fluid samples were taken from the reticulum before, 15 min, 1 h, 3 h, 7 h and 10 h after administration of the toxin or toxin plus ZENzyme® and analysed for ZEN metabolites. Additionally, faeces samples were taken 24 h and 34 h after administration of treatments. If requirements were met, an independent t-test was performed, if not a non-parametric test was used (Mann-Whitney U test). Following the concentrations of both the parent compound and its metabolites, even 15 min after administration, a shift from the estrogenic ZEN and α-ZEL due to the addition of ZENzyme® was significant (ZEN content 15 min: ZEN 11.40 ng/ml vs. ZEN + ZENzyme® 0.84 ng/ml, $P < 0.001$; HZEN content 15 min: ZEN 0.50 ng/ml vs. ZEN + ZENzyme® 11.22 ng/ml, $P < 0.001$). This shift was visible at all sampling points as well as in the faeces samples, proving that ZENzyme® has a high efficacy in degrading the mycotoxin ZEN in the rumen of dairy cows.

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MYCOTOXIN DEACTIVATOR IMPROVES PERFORMANCE, ANTIOXIDANT STATUS, AND REDUCES OXIDATIVE STRESS IN GESTATING AND LACTATING GILTS FED DIETS CONTAINING MYCOTOXINS

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Ingestion of mycotoxins can result in many problems, including decreased growth rates, immune suppression, and high mortality. The present study aimed to evaluate the impact of a mycotoxin deactivator in diets containing added mycotoxins for gestating and lactating gilts on their performance and antioxidant status. A total of 60 commercial gilts (LD x LW) were used. On day 75 of gestation, the gilts were distributed among 3 dietary treatments according to a randomized block design: a negative control (CON), mycotoxin positive control (Myc), and a positive control added a mycotoxin deactivator (Myc+Deact at 2 kg/t; Unike® Plus, Adisseo). The total mycotoxins levels added to the diets 2 and 3 were: deoxynivalenol (DON) at 2.5 mg/kg, fumonisins (FBs) at 10 mg/kg, and zearalenone at 0.75 mg/kg. Sows received the experimental diets from day 75 of gestation until weaning (i.e., 21 days). At farrowing, total number of piglets born, born alive, stillborn, and mummies were measured. Piglets were individually weighed 24 h post-farrowing, and at weaning to determine litter performance during lactation. Sow mortality was registered at all moments. Antioxidant enzymes (glutathione peroxidase (GPx) and total superoxide dismutase (TSOD)), vitamins (A, E, and C), and malondialdehyde (MDA) were evaluated in erythrocyte and plasma samples. The effects of diet composition, blocks and initial weight were tested according to a general linear procedure analysis of variance (GLM procedure of SAS). During gestation, treatments influenced ($P = 0.022$) sow mortality rate: CON had no mortality and Myc 15.8% and Myc+deact 10.5%. The same observation was made for the lactation period ($P = 0.017$): CON (5.0%) showed a lower mortality rate compared to the mycotoxin fed sows (31.2 vs. 11.8%,

respectively for Myc and Myc+deact). Sow milk production yield was greater (P = 0.021) in CON (11.66 vs. 9.53 vs. 9.67 kg/d, respectively for CON, Myc and Myc+deact). As a consequence of that, treatments also influenced piglet (P = 0.047) and litter (P = 0.048) weight with a higher value for CON sows, followed by Myc+deact and Myc. Sows challenged with mycotoxins without deactivator presented lower performance traits, decrease in the efficiency of central antioxidant systems (↓GPx, ↓TSOD, ↓vit. A, ↓vit. E and ↓vit. C) and a higher oxidative damage to lipids (↑MDA) when compared to the control and deactivator associated treatment. Our findings showed that the use of the deactivator can mitigate the negative effects on performance when sows are subjected to diets contaminated by challenging levels of mycotoxins.

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EFFICACY OF MYCOTOXIN DEACTIVATOR ON HEALTH AND GROWTH OF BROILER CHICKENS UNDER CHRONIC DIETARY CHALLENGE OF AFLATOXINS

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Aflatoxins are hepatotoxic and carcinogenic, and display immunosuppression properties for both humans and animals. This is also why they are the most widely studied and regulated mycotoxins. Strategies to mitigate aflatoxins prevalence includes the use of clays in animal feed as sequestering agents. Innovative approach consists also in providing ingredients controlling inflammation and promoting immune. The objective of this study is to evaluate the impact of naturally contaminated diet with aflatoxins (AFB1) on health and performance of commercial broilers, and the efficacy of a mycotoxin deactivator based on clays, inactivated yeast and fermentation extracts. A total of 72,00 straight run Vencobb 430 chicks were assigned for 42 days to one of the 3 treatments in a randomized block design (8 pens per treatment): NC, negative control with residual mycotoxins; PC, positive mycotoxin control with 56 ppb AFB1; TN, PC + mycotoxin deactivator at 2 kg/t (ToxyNil®, Adisseo). Performance parameters were recorded on weekly basis whereas blood and liver samples were collected at day 21 and day 42. PC group shows significant lower bw than NC from 14 to 42 days, resulting in a decreased FCR (+2.6% for the overall period). TN can restore the performance compared to PC (-9.7% FCR) and to NC (-7.1% FCR). AFM1 in plasma, as a biomarker of chronic exposure, showed reduction of AFB1 bioavailability (from -42% to -75% for NC and PC groups, respectively; P<0.01) in the TN treated birds. AFB1 induces inflammation as shown by the increased secretion of pro-inflammatory cytokines like IL-1β, IL-6, IL-8, TNF-α between NC and PC. TN treatment aimed to decrease these parameters relative to PC and to promote the production of anti-inflammatory IL-10. Birds receiving TN treatment showed higher antioxidant activity such as superoxide dismutase, glutathione and glutathione peroxidase compared to PC and showed lower lipid peroxidation biomarker like malondialdehyde. Blood liver enzymes, such as AST, ALT and GGT, were significantly decrease by the use of the mycotoxin deactivator and were also highly correlated with pro-inflammatory cytokines, especially IL-6. Histomorphology analysis confirmed the necrotic state of hepatocyte in presence of AFB1 and the protective capability of mycotoxin deactivator to maintain liver health. This study highlights the complementary modes of action of this mycotoxin deactivator by inhibiting AFB1 in the intestine and by repairing damage caused at the gut and liver level on inflammation, immune response, and redox balance. This leads to sustain broiler resilience and performance.

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THE REMEDIATION OF TOXINS IN THE FOOD CHAIN USING A NOVEL MULTICOMPONENT MYCOTOXIN DETOXIFYING AGENT (MMDA)

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The main toxins contaminating the feed materials are mycotoxins, endotoxins, and algal toxins. The major mycotoxins affecting animal production are Aflatoxin B1 (AFB1), fumonisin B1 (FB1), T-2, ochratoxin A (OTA), zearalenone (ZEN), T-2 and deoxynivalenol (DON). Bacterial endotoxins are formed as a part of the outer membrane of the cell wall of Gram-negative bacteria that are released when cells are lysed (LPS-lipopolysaccharides). The algal toxins are produced by algae in the ocean and in fresh water. There is thus a critical need for a feed additive that can bind mycotoxins, endotoxins, and algal toxins in the animal gut. In this study, MycoRaid (MMDA), has been tested for adsorption and biotransformation of the main toxins using LC-MS/MS. For mycotoxins and algal toxins detoxification efficacy, 100 mg of adsorbent was put into a 15 ml Falcon tube. To this, 10 ml of 0.1 M phosphate buffer pH 3.0 and a solution containing 2 ppm of each toxin were added. Relevant controls with and without

toxins were prepared. The tubes were incubated on a rotary shaker for 60 min at 37°C and centrifuged at 4,500 rpm for 5 min; the supernatant was analysed by LC-MS/MS. Endotoxins from each bacterial source at 1ng/ml were incubated with the binder at 10 mg/ml in pH 6.5 buffer for 1 h at 37°C. The tubes were centrifuged and the endotoxin concentration in the supernatant was determined by the *Limulus* amoebocyte lysate (LAL) assay. The results were expressed as LPS binding capacity (%). The *in vitro* efficacy showed the net adsorption potential. The *in vitro* efficacy showed excellent removal of the toxins including 99.7% AFB1, 89.5% ZEN, 95.3 FB1, 98.7% OTA, 75.7% T-2, 93.5% citrinin, 98.8% ergot alkaloids, 98% *Pseudomonas* endotoxins, 92% *E. coli* endotoxins, 74% *Salmonella* endotoxins, and 81% saxitoxin (algal toxins). These results show that application of MMDA (MycoRaid) in feed can act as an effective solution for removal of these toxins in the feed and ultimately the food chain.

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DEGRADATION OF DEOXYNIVALENOL USING *DEVOSIA LUCKNOWENSIS* NCIMB 30593

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Aflatoxins (AF), zearalenone (ZEN), fumonisins (FB1 and FB2), T-2/HT-2, ochratoxin A (OTA) and deoxynivalenol (DON) are considered the most economically important mycotoxins in terms of their high prevalence and significant negative effects on animal performance. Biological degradation of mycotoxins has shown promise because it works under mild and environmentally friendly conditions. DON is a type B trichothecene mycotoxin produced by several *Fusarium* species that infest maize, wheat, barley, and oats. Food and animal feed contaminated with DON presents a significant health risk for both humans and livestock. For this research work, 5 soil samples were collected from different fields of alfalfa crop and pooled into one sample. Microbe, identified as *Devosia lucknowensis* by NCIMB (UK), was isolated by enrichment culture procedure using mineral salts medium and 1 ppm of DON as a sole carbon source. *D. lucknowensis* degraded 100% of DON in a 24 h period at 30°C under aerobic conditions. The microbe was able to reduce DON at pH levels ranging from 6-9 and temperatures ranging from 30-40°C. DON reduction level was quantified using LC-MS/MS.

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THE EFFECT OF MYCOTOXIN ADSORBENTS ON ALLEVIATING THE NEGATIVE EFFECTS OF ZEARALENONE IN GILTS: A FIELD CASE

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Zearalenone (ZEN) is one of the most prevalent mycotoxins, found in around 50% of the worldwide feed ingredients. The structure of ZEN resembles the structure of oestrogen, allowing ZEN to bind to oestrogen receptors which can result in reproductive disorders and infertility in animals. A very characteristic sign is the appearance of vulva hypertrophy (VH, swelling and redness) in gilts. The objective of this study was to evaluate the effects of three different mycotoxin adsorbents on the growth performance and the incidence of VH in gilts receiving feed with high levels of ZEN (30 – 40 ppm). The

Table 1. Performance results of gilts receiving three different mycotoxin adsorbents during trial 1 (73-180 days) and trial 2 (115-180 days).

	Adsorbent					
	A		B		C*	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
	73-98d	115-140d	73-98d	115-140d	73-98d	115-140d
Start BW, kg	25.7	46.64	18.8	54.38	17.3	49.02
Final BW, kg	41.2	69.86	37.4	73.00	33.4	68.06
ADG, g/d	644	842	775	776	669	793
ADFI, kg/d	1.02	1.77	1.07	1.65	0.91	1.66
FCR, kg/kg	1.58	2.10	1.38	2.13	1.36	2.09
Overall, 73-180d age	73-180d	115-180d	73-180d	115-180d	73-180d	115-180d
Final BW, kg	97.3	100.28	97.5	97.40	106.7	110.37
ADG, g/d	670	779	741	768	840	944
ADFI, kg/d	2.02	2.10	2.07	2.11	2.04	2.13
FCR, kg/kg	3.01	2.70	2.80	2.75	2.43	2.26
Reduction VH cases	0%	+7%	-18%	-35%	-54%	-34%

*Excential Toxin Plus. Body weight (BW), average daily gain (ADG), average feed intake (ADFI), feed conversion ratio (FCR).

study, performed on a commercial farm in the Philippines, consisted out of two trials: T1 performed in nursery gilts and T2 in growing gilts. Both trials started when the gilts showed signs of VH. Gilts from the same batch were allocated into three groups. The groups were fed three different types of multicomponent mycotoxin adsorbents A, B or C. For both trials, weight and feed intake were recorded and feed conversion ratio (FCR) was calculated. Vulva hypertrophy was noted per animal, initially and at the end of the trials. Both trials lasted until the gilts were 180 days old and were selected either as replacement gilts or sold as finishers with selection criteria being occurrence of oestrous during these first 180 days. In both trials, considering the overall period, gilts from group C had numerically higher final body weight and daily gain, and numerically lower FCR compared to groups A and B (Table 1). From all the gilts in T1, only two were selected as replacement gilts, both from group C. In T2, 12 gilts were selected as replacement (4 in group A, 3 in B and 5 in C). Group C resulted in the numerically highest reduction of VH cases (-54%) in T1. In T2, groups B and C reduced VH cases in more than 30%, with group A increasing appearance of VH. Overall, the inclusion of Excential Toxin Plus (group C) to the diets of replacement gilts resulted in improved growth performance and reduced signs of vulva hypertrophy. These results indicate that this mycotoxin adsorbent reduces the negative effects of ZEN on reproductive and growth performance compared to the two other adsorbents.

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EVALUATION OF THE EFFICACY OF AN ANTI-BIOTOXIN SOLUTION SUPPLEMENTED IN FEED IN DAIRY COWS

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Main mycotoxins effects on ruminants have been reported considering high dosage and/or single mycotoxins. However, little is known about the effects of naturally low levels of multiple mycotoxins on the performance, metabolism, and immunity of dairy cattle. The objective of the trial was to evaluate the efficacy of an anti-biotoxins product in feed containing naturally mycotoxins contamination in commercial farms. The trial was investigated in a French farm on 120 lactating Holstein dairy cows receiving diet supplemented by an anti-biotoxin (MPY) during 60 days at 50g/cow/day. Mycotoxins and metabolites analysis were performed in ration and urine. Milk production, immunological, blood serum chemistry, biomarkers of oxidative status and liver and kidney functions, serum immunoglobulins concentration were measured. Ration analysis have shown low but multiple *Fusarium* mycotoxins contamination. Deoxynivalenol (DON), fumonisins (FB1, FB2, FB3), zearalenone (ZEA) and H-T2 toxin were found during the whole studied period in the total mixed ration at levels above 0.36, 0.14, 0.02 and 0.02 mg/kg, respectively. A decrease of DON metabolite (DOM-1) in urine was observed during supplementation period. Milk production persistency was evaluated at 96.8% during the trial period, allowing a calculated gain of 0.9 kg milk/cow/day with supplemented diet (vs. milk persistency during pre- and post-trial period). Mean IgG concentration was 30 mg/ml at T0 and decrease in a range of 16-27 mg/ml after diet supplementation. Inflammation diagnostic, evaluated by total protein value and albumin/globumin ratio, indicated that animals were in chronic inflammation state and that supplementation allowed them to ameliorate their health status. Supplementation was associated with a decrease of liver/kidney functions biomarkers such as urea, total bilirubin, and creatinine. Our results indicated that feed contaminated with regular levels of *Fusarium* mycotoxins adversely affected the performance and immunity of dairy cows, and that supplementation with anti-biotoxin product (MPY) counteracted most of these negative effects.

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CARBOXYPEPTIDASE IMMOBILIZATION ON NYLON NANOFIBROUS MEMBRANES FOR OCHRATOXIN A DETOXIFICATION

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Ochratoxin A (OTA) is a mycotoxin that can be found in products as grape juice and wine. Biological detoxification methods are gaining interest as they constitute green processes and are less likely to cause loss in nutritional value and palatability of food. Carboxipeptidase A (CPA) was the first enzyme demonstrated to be effective in the degradation of OTA, being also used as a reference for other OTA-degrading enzymes. CPA acts on OTA by hydrolysing the amide bond, producing the less toxic ochratoxin alpha (OTα). So far, studies have assessed free enzymes on OTA degradation. However, free enzymes are very sensitive to pH, temperature, and the presence of inhibitors. Enzyme immobilization increases the rigidity of the attached molecule's structure, thereby enhancing its stability

and resistance, allowing its repeated application. The main aim of this work was to immobilize CPA into a nylon nanofibrous membrane and to verify if the OTA degradation capacity was maintained. In addition, an unusual spacer for enzyme immobilization was used, the bovine serum albumin (BSA), which has the advantage of showing low toxicity. Enzyme immobilization on nylon membranes was accomplish after activation with 12.5% aqueous solution of glutaraldehyde, washing with ultrapure water, immersion into BSA solution (0.1 mg/ml), and immersion into CPA solution in 10 mM sodium acetate, 5 mM calcium acetate buffer (pH 7.5). The OTA degradation assays were performed at 37°C and pH 8.5, during 168 h. The membranes loaded with immobilized enzyme were incubated in 200 µg/l OTA solution in tris buffer (pH 8.5). In the same way a subset of analyses was made with a solution of OTA and free CPA solution in tris buffer (pH 8.5). The OTA and OTα concentration was measured using UPLC-FL. The results showed that both the free and immobilized CPA caused OTA degradation, however, a reduction of CPA activity was observed for the immobilized enzyme when compared with assays using free enzyme. The optimization of the immobilization process is underway to improve the retention of enzyme activity and, at the same time, to achieve better stability and reuse. It is expected that the overall process will become cost-effective and that the developed material will contribute to make biocatalysis a feasible and attractive alternative for mycotoxin detoxification. **Acknowledgements**. Study supported by the Portuguese Foundation for Science and Technology (grants UIDB/04469/2020[1] and UIDB/QUI/50006/2020[3]) and by i-MultiSmart project (POCI-01-0145-FEDER-031924).

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EFFICACY OF TOXO®-XL ON EMERGING MYCOTOXIN ADSORPTION AT DIFFERENT pH LEVELS

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Emerging mycotoxins are those mycotoxins which are neither routinely analysed nor legislatively regulated, but the evidence of their incidence is rapidly increasing. Symptoms associated with emerging mycotoxins in animal production include reduced feed intake, increased intestinal permeability, hepatic necrosis, and increased morbidity. There is also potential additive or synergistic negative effect among emerging mycotoxins. In the present study, the adsorption efficacy of TOXO®-XL, an integrated mycotoxin-mitigating feed additive, on three emerging mycotoxins (roquefortine C, enniatin B and sterigmatocystin) was measured via an *in vitro* assay. 1000 ng/ml roquefortine C, enniatin B or sterigmatocystin each with 0.20% w/v TOXO-XL were performed at two pH levels (3.0 and 6.5; pH 3.0 to simulate stomach where main digestion occurs, while pH 6.5 to simulate small intestine where main absorption occurs) in two replicates per group. The adsorption efficacy of TOXO-XL was measured and expressed as a percentage relative to the control (emerging mycotoxins with no TOXO-XL). At pH 3.0, the binding efficacy of TOXO-XL on roquefortine C, enniatin B and sterigmatocystin was 96.4, > 85.0, and > 98.0%, respectively. At pH 6.5, the binding efficacy of TOXO-XL on roquefortine C, enniatin B and sterigmatocystin was 74.9, > 85.0, and 83.1%, respectively. In conclusion, TOXO-XL showed promising results in its adsorption efficacy on 1000 ng/ml roquefortine C, enniatin B or sterigmatocystin at both pH 3.0 and 6.5, which provides a solid basis from the solution development perspective. Further studies should be conducted to confirm the *in vivo* implications of the described *in vitro* results.

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EFFICACY OF TOXO®-MX ON ERGOT ALKALOIDS ADSORPTION AT DIFFERENT pH LEVELS

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Ergot alkaloids, a group of mycotoxins which are produced by fungi of the genus *Claviceps*, are well known to cause vasoconstriction of small arteries and consequent lameness or even gangrene, all of which lead to a suboptimal animal health and performance. The reported contamination of ergot alkaloids in rye, wheat, barley, and other cereal varieties in Europe has increased since 2021. In the present study, the adsorption efficacy of TOXO®-MX, a commercial bentonite-based mycotoxin adsorbent, on 213-221 ng/ml natural extract which consists of 12 ergot alkaloid varieties (ergometrin, ergometrinin, ergosin, ergosinin, ergotamin, ergotaminin, ergocornine, ergocorninin, ergocristin, ergocristinin, ergocryptin, and ergocryptinin) was measured at both pH 3.0 and 6.5. The adsorption efficacy was expressed as a percentage relative to the control (ergot alkaloids with no TOXO-MX). At pH 3.0, the adsorption efficacy on total natural extract of ergot alkaloids was 92.90%. At pH 6.5, the adsorption efficacy was 87.20%. In conclusion, TOXO-MX showed promising results in its adsorption efficacy on 213-221 ng/ml ergot alkaloids at both pH 3.0 and 6.5, which provides a solid basis from the

solution development perspective. Further *in vivo* studies should be conducted to confirm the described *in vitro* results.

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IN VITRO ASSESSMENT OF THE POTENTIAL INTERACTION BETWEEN VITAMIN B-COMPLEX AND A COMMERCIAL MYCOTOXIN-MITIGATING PRODUCT

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The availability of vitamin B-complex, a group of water-soluble essential nutrients in pig diet, is suspected to be influenced by the presence of commercial mycotoxin-mitigating products. In the present study, an *in vitro* assay was created, at which the recovery of vitamin B1(VB1), B2 (VB2) and B6 (VB6) out of 50 g pig complete feed were measured with or without the inclusion of 0.5 kg/t or 1.0 kg/t of TOXO®-XL (a commercial mycotoxin-mitigating product; tested samples were obtained from three random batches) at pH 3.0 and 6.5 in three replicates per group. The recovery of VB1, VB2 or VB6 was expressed as a percentage relative to the control group (pig feed without TOXO-XL). When the inclusion rate of TOXO-XL was 0.5 k/t, the recovery of VB1, VB2 and VB6 at pH 3.0 were 94.12, 96.11 and 95.27%, respectively. At pH 6.5, there recovery of VB1, VB2 and VB6 were 95.98, 96.42 and 98.12%, respectively. When the inclusion rate of TOXO-XL was 1.0 k/t, the recovery of VB1, VB2 and VB6 at pH 3.0 were 89.69, 94.68 and 93.00%, respectively. At pH 6.5, there recovery of VB1, VB2 and VB6 were 95.15, 94.68 and 97.35%, respectively. In conclusion, the results indicated that the availability of Vitamin B-complex at both pH 3.0 and 6.5 was still maintained at a high level, when pig complete feed had an inclusion of TOXO-XL at 0.5 or 1.0 kg/t. Further *in vivo* studies should be conducted to confirm the implications of the described *in vitro* results.

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EFFECT OF MYCOSORB A+ ON THE UPTAKE AND DEPOSITION OF DEOXYNIVALENOL AND ZEARALENONE IN GROWING PIGS

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Previous work indicated that uptake and metabolism of a single toxin – zearalenone (ZEA) – could be reduced and passage through the digestive tract increased in pigs fed a mycotoxin binder Mycosorb A+ (MSA+). The aim of this work was to investigate its efficiency in a multitoxin challenge comprising deoxynivalenol (DON) and ZEA and evaluate changes in zootechnical parameters, mycotoxins uptake and distribution, and relevant biomarkers. Experimental diets comprised: control (F0), base diet amended with 10% cracked wheat and Celite™ digestibility marker; challenge diet (F1), F0 diet + DON/ZEA (1.0 and 0.3 mg/kg, respectively); and mitigation diet (F2), F1 + MSA+ (4kg/t). Thirty pigs were adapted with F0 over a week. Ten pigs per group were then randomly allocated to F0, F1 or F2 for 4 weeks. At the end of the study, all pigs were sacrificed and blood, urine, bile samples, livers, kidneys, ovaries, intestinal tissues, digesta and faecal samples were collected. Samples were homogenized; glucuronide deconjugated; solvent extracted and purified via immunoaffinity chromatography before analysis by HPLC-FLD or GC-MS for ZEA and DON, respectively. Zootechnical parameters were not affected by treatments. Both DON, ZEA and metabolites could be detected in ovaries. MSA+ significantly reduced the concentration of total-DON (DON+DOM-1) in ovaries (by 18.2%). Both ZEA and alpha-zearalenol metabolite (α-ZOL) were present in equal quantities in ovaries (0.3 to 0.5 ng/g of tissue), with a significant decrease of α-ZOL and a numerical of ZEA with MSA+, for a total reduction of 35%. α-ZOL was decreased significantly in urine (39.9%) and ZEA in bile (34.7%) with MSA+. The main route of elimination of DON and DOM-1 was in urine with levels reaching 292 ng/mg of creatinine with F1, while MSA+ contributing to a numerical decrease to 205.5 ng/mg of creatinine. Total-DON recovered in faeces compared to amount administered accounted for 7%. Faecal analysis showed that unlike DON, < 40% of ZEA was found as α-ZOL, over 60% being as intact ZEA, for a total recovery of 20% of the initial ZEA fed in F1. The expression of six genes of interest in pig jejunal tissue highlighted trends in increased expression of genes associated to peptide transport, cytochrome P450 enzymes, xenobiotics enzyme transcription factors when pigs were fed F1 whereas pigs provided MSA+ (F2) saw a systematic numeric normalisation. At 35 days, calprotectin, a marker of intestinal stress and inflammation, found its highest level for F1, despite no statistical differences. Overall, MSA+ tended to decrease the recovery of DON, ZEA or their metabolites in the animal organism. These results indicate that MSA+ could reduce ZEA uptake and metabolism and partially decrease DON accumulation in specific tissues in pigs when toxins were fed concomitantly.

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BROILER PERFORMANCE AND ENVIRONMENTAL IMPACT OF PRODUCTION IN RESPONSE TO MYCOTOXINS AND YEAST CELL WALL EXTRACT SUPPLEMENTATION: A META-ANALYSIS

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A random-effects meta-analysis was conducted to evaluate the effect of mycotoxins (MT) on broiler performance without or with the inclusion of yeast cell wall extract (YCWE, Mycosorb®, Alltech, Inc.). Data on production performance including body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) and mortality rate was extracted from 25 research experiments comprising a total of 10,307 broilers. Using these performance parameters, the European Production Efficiency Factor (EPEF) was also assessed. Broilers fed MT had lower BWG (-217 g, $P < 0.001$), reduced feed intake (-264 g), increased feed conversion ratio (0.12), greater mortality by 2.01% and lowered ($P < 0.001$) EPEF. Inclusion of YCWE improved ($P < 0.001$) BWG (59 g) and FI (65 g), lowered FCR (-0.05), and reduced mortality by 1.74%. Feeding YCWE during the mycotoxin challenges also increased EPEF ($P < 0.001$). Finally, the carbon footprint of production was evaluated using results from the meta-analysis. In this scenario, broilers fed control diets without mycotoxins produced an estimated 1.93 kg CO_2-equivalent/kg liveweight (LW) while those fed MT produced 2.13 kg CO_2-equivalent/kg LW. In contrast to MT, the inclusion of YCWE during the mycotoxin challenge lowered the output to 2.03 kg CO_2-equivalent/kg LW which resulted in -25 tonnes less CO_2-equivalent output per 100,000 birds. In conclusion, this meta-analysis indicates that mycotoxins can play a role in reducing broiler performance and livability which overall lowers farm production output. Results from the emissions assessment scenario also indicate that mycotoxin consumption by broilers could increase the carbon footprint of production. Inclusion of YCWE in feed under a mycotoxin challenge was shown to improve broiler performance, livebility, and output, as well as lower carbon footprint of production. Taken together, the results of this meta-analysis suggest that inclusion of YCWE during mycotoxin challenges could play a role in improving farm efficiency, profitability, and environmental sustainability.

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PARTNER-ASSISTED ARTIFICIAL SELECTION OF A SECONDARY FUNCTION FOR EFFICIENT AFLATOXIN BIOREMEDIATION

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Microbial enzymes have a broad potential to detoxify aflatoxins, but their native performance often does not match specific applications of interest. In attempting to evolve strains to match expectations, one challenge is to establish aflatoxin degradation as a having positive fitness effect on the producer. As a result, a conventional selection scheme cannot be used to improve such secondary functions. We propose an alternative 'partner-assisted artificial selection (PAAS)', in which an assisting population acts as an intermediate to create a feedback from the function of interest to the fitness of the producer. We use a simplified model to examine how well and under what conditions such a scheme leads to improved aflatoxin degradation. We find that selection for improved growth in this scheme successfully leads to improved degradation performance, even in the presence of other sources of stochasticity. We find that standard selection considerations apply in PAAS: a more restrictive bottleneck leads to stronger selection but adds uncertainty. We also examine how much stochasticity in other traits can be tolerated in PAAS. Our findings offer a roadmap for successful implementation of PAAS to evolve improved aflatoxin degradation.

CONTROLLING PLANT DISEASE AND MYCOTOXIN FORMATION
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OPTIMIZING FUMONISIN B1 PRODUCTION ON LIQUID SYNTHETIC MEDIA

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Fumonisins are mainly produced on maize by several *Fusarium* species, such as *F. verticillioides* or *F. proliferatum*. Besides free fumonisins, also non-covalently bound 'hidden' and covalently 'bound' fumonisins of largely unknown structure occur naturally. In a project [Austrin Science Fund (FWF): P33011]] aiming to study the formation and bioavailability of modified fumonisins, we optimized fumonisin production to find suitable growth conditions for the later production of ^{13}C-FB1 and ^{14}C-FB1. These labelled forms of FB1 will subsequently be used for treatment of fast flowering mini maize and a maize suspension cell culture to investigate the formation of modified fumonisins *in planta*. Fifty-one *Fusarium* strains from the BOKU collections were screened on different complex and synthetic media under several growth conditions. Glucose in general lowered production. Interestingly, FB1 production on solid (cracked maize) media and synthetic liquid media showed no meaningful correlation. The strain performing best on synthetic liquid media containing 3% glucose as sole carbon source had been isolated in the Tulln area from maize and produces close to 1 g/l FB1. Recently it was reported that the proposed fumonisin transporter Fum19 acts as a repressor of FB1 production in a *F. verticillioides* strain [mBio 11 (2020) e00455-20]. Deletion of the gene encoding this ABC transporter led to a 25-fold increase in FB1 production, while overexpression causes reduction of FB1 formation. This prompted us to investigate if deletion of *FUM19* might further improve the FB1 yield of our isolates. We deleted the *FUM19* gene in seven of our best fumonisin producers and compared the FB1 production by the deletion strains to that in untransformed and ectopically transformed control strains. Surprisingly, six of our *fum19Δ* strains produced less FB1 than the control strains on various synthetic media. In one *F. verticillioides* strain deletion of *FUM19* caused no significant change in FB1 production. Further investigation on the role of Fum19 as possible repressor of FB1 production is warranted.

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ENHANCEMENT OF AGRI-FOOD BY-PRODUCTS: GREEN EXTRACTIONS TO OBTAIN BIOACTIVE MOLECULES AGAINST MAIZE MYCOTOXIGENIC FUNGI

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Maize (*Zea mays*) is the major agricultural crop grown worldwide and one of the most important cereals produced for human and animal consumption in the European Union (EU). Depending on weather conditions and location, maize can be exposed to the contamination by different fungal species able to produce mycotoxins, secondary metabolites with toxic effects for humans and animals, and causing important economic losses. Food waste extracts represent a potential source of natural compounds with biological, biostatic, and biocidal activity against fungi. Using extraction processes with high technological level and high sustainability, by-products of food production of fruit, wine and beer could be used as environmentally friendly alternatives to protect plants from fungal infection and mycotoxins contamination, limiting the use of chemical products. In this study, in *vitro* tests were carried out to verify the potential use of 19 extracts obtained from by-products of the agri-food chain in reducing the development of the mains mycotoxigenic fungi of maize *Aspergillus flavus, Fusarium verticillioides* and *Fusarium graminearum*, and in contain the production of their relative mycotoxins: aflatoxins (AFs), fumonisins (FBs) and trichothecenes (TCNs), respectively. Fungal strains were centrally transferred on Petri dishes containing potato dextrose agar (PDA) and 1 ml of the different extracts considering two concentrations (320 ppm and 1000 ppm). After 14 days of incubation at 25°C, fungal growth was determined measuring fungal colony along two orthogonal lines while the analysis of mycotoxins was performed using HPLC-FLD, LC-MS/MS and GC-MS systems. The reduction percentages for fungal growth and mycotoxins were calculated comparing results obtained with those obtained by the same fungi cultivated on PDA medium without the presence of the extracts. The results obtained were very interesting; in fact, while fungal growth was only limited affected by natural extracts used with maximum percentages of reduction going from 22% to 42% in comparison with untreated thesis and depending

on the fungus considered, more effective and promising results were obtained on mycotoxins with reduction higher than 70% for aflatoxins, fumonisins and trichothecenes. A different efficacy was obtained by each natural extract in relation with the target fungus-mycotoxin considered. **Acknowledgements**. Funding, Initiative under the 2014-2020 Emilia-Romagna Rural Development Programme – Operation Type 16.1.01 – Operational Groups of the European Innovation Partnership 'Agricultural Productivity and Sustainability' – Focus Area 3A – Innovation Plan 'MIlk_COntrollo'.

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DEOXYNIVALENOL AND PIGMENTED GRAINS: FROM ORGANISMS TO CELLS

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The study of plant-mycotoxin interaction is commonly carried out by collecting naturally contaminated samples from cultivated fields. To evaluate the physiological response of plants to mycotoxins, *in vitro* systems, such as plantlets, organs, or cells cultures, are often preferred to fields trials and greenhouse experiments, despite their obvious distance from natural conditions. *In vitro* techniques, besides being a consolidated approach to investigate the metabolic fate of mycotoxins, allow to speed up the analysis due to a germplasm collection, which ensures working regardless of the seasons. To aid data interpretation regarding deoxynivalenol (DON) biotransformation, we propose a metabolic comparison between plantlets (differentiated tissue) and cells (undifferentiated), i.e., a metabolic study at the cellular level taking advantage of suspensions of undifferentiated cells of wheat (*Triticum aestivum*) varieties. The cultures were grown in appropriated medium and, during the exponential phase, 100 µg DON was inoculated directly into the suspension. Periodically, the medium was sampled for an indirect test concerning toxin adsorption. At the end of the experiment (10 days), cells were collected and analysed. At the same time, similar experiments were conducted using whole plants with the same varieties in aseptic culture. DON solution (100 µg) was dissolved in the liquid medium in plantlets-containing jars. As described above, at various times, medium was collected and at the end of experiment roots and leaves were separated and frozen. All samples (medium, cells, roots, and leaves) were subsequently subjected to LC-MS/MS analysis. Particular attention, in both tests, was put on the absorption trend of DON, much faster for cells than in whole plants. Regarding the plantlets results, roots contained a high amount of untransformed DON, while leaves are able to effectively biotransform DON to DON-3-Glc. Deoxynivalenol and its gluco-conjugated derivatives were also found in the wheat suspension culture, indicating the capability of undifferentiated cell to biotransform DON as in differentiated tissue. None of these biotransformation metabolites were detected in the culture media at the end of the tests, indicating an evident cell retention. Different DON to DON-3-Glc ratios were found depending on the wheat varieties, suggesting different susceptibility/resistance towards the accumulation of DON present in undifferentiated cells of the varieties used. These preliminary data may suggest the use of undifferentiated cells as a faster but reliable model to investigate wheat response to DON accumulation.

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BIOFORMULATE TO REDUCE THE ACCUMULATION OF AFLATOXINS IN MAIZE BASED ON A BIOPOLYMER AS A CARRIER AND SUPPORT FOR GROWTH OF THE BIOCONTROL AGENT

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Maize (*Zea mays* L.) is the cereal with the highest volume of production worldwide, and the second most important in Argentina. The presence of aflatoxins in the different stages of the maize agri-food chain is a current problem in food safety and it is caused by the contamination with species of *Aspergillus* section *Flavi,* mainly *A. flavus*. During many years the research group has focused on the development of a biological control strategy based on the competitive exclusion mechanism. Several studies have demonstrated the effectiveness of the non-toxicogenic *A. flavus* AFCHG2 strain developed by solid state fermentation on long grain rice. However, considering the United Nations Sustainable Development Goal of zero hunger, it was proposed to replace this substrate and to develop a biopolymer that allows the growth and transport of the biological control agent to be applied to crops. such as maize and peanuts. In this sense, different natural, economic, and starch-rich substrates were analysed: cassava starch (10 and 15%), rice flour (10 and 15%), and maize starch (5, 10 and 15%). In addition, urea was added as a nitrogen source and citric acid as promoter of greater crosslinking of starch chains. Also, the

development of the biocontrol strain in polymers with the addition of glucose or sucrose was evaluated. The diameter of the pores of each polymer was determined and those with a pore diameter of 93-97 µm were selected assuming they allow a better use of the entire substrate by the biological control agent. In addition, the growth of the biological control strain in the different preparations was analysed. The synthesis of this biopolymer included stages of gelation, cooling, freezing, thawing, drying, sterilization and curing, hydration, pH regulation, inoculation, incubation, and final drying. The effectiveness of the bioformulate evaluated under field showed a reduction of 81% in aflatoxin accumulation in maize kernels in comparison with the non-inoculated controls. The development of this biotechnological tool allowed us to present a process and product patent that is currently pending. In addition, it offers to producers an eco-friendly, economical, and safe alternative that contributes to food quality and safety.

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INFLUENCE OF TEMPERATURE AND WATER ACTIVITY ON GROWTH AND AFLATOXIN PRODUCTION OF *ASPERGILLUS FLAVUS* STRAINS ISOLATED FROM CHICKPEAS

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Chickpea (*Cicer arietinum* L.) is one of the most cultivated pulses in terms of world production. There is a high demand for world production due to the crop's nutritional value. In Argentina, most of chickpea production is exported. Chickpea is susceptible to more than 25 well-documented fungal pathogens that cause seed deterioration and contamination with mycotoxins. The most worldwide prevalent fungi in chickpeas are species belonging to *Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria*, and *Rhizopus* genera. In a previous study, we observed that *A. flavus* was the prevalent fungi isolated from chickpea. Considering that *A. flavus* has the ability to produce aflatoxins (compounds classified in group 1 by IARC) and aflatoxin production and fungal growth of *A. flavus* can be influenced by abiotic conditions, the effect of water activity (a_w, 0.99, 0.98, 0.96, 0.94, 0.92, 0.90 and 0.87), temperature (15, 25, and 30°C), incubation time (5, 10, 14, and 21 days), and their interactions on mycelial growth and aflatoxin production in a chickpea-based medium by three *A. flavus* strains isolated from chickpea in Argentina was evaluated. Maximum growth rates were obtained at a_w 0.99 and 30°C, with growth decreasing as the a_w of the medium was reduced. Maximum amounts of aflatoxins were produced at 0.99 a_w and 25°C after 5 days of incubation for 2 strains, and at 25°C and 0.96 a_w after 21 days of incubation for the third strain. Aflatoxin concentrations varied depending on the a_W and temperature interactions assayed. Two-dimensional profiles of a_W by temperature interactions were developed from these data to identify areas where conditions indicate a significant risk from aflatoxin accumulation on chickpea. This study provides useful data about conditions representing a high and a low risk for aflatoxin contamination of chickpea which is of greater concern because chickpea is destined mainly for human consumption.

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FROM THE TREASURE CHEST OF PLANT BIOACTIVES TO THE FUTURE OF NEW CROP PROTECTANTS FOR A SUSTAINABLE AGRICULTURE: THE POSSIBLE EXPLOITATION OF *CITRULLUS COLOCYNTHIS* L. (SCHRAD.) EXTRACTS AGAINST *ASPERGILLUS FLAVUS* AND AFLATOXINS AND OTHER STORIES.

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The world of plant extracts and natural compounds have long been regarded as a promise land for the individuation of healthy alternatives to chemical preservatives, against microbial contamination, in food and feed commodities. A plethora of aromatic and medicinal plant species have been studied from decades to explore their antimicrobial and antioxidant properties, in order to both validate their ethnobotanical use for healing microbial illnesses and assess their suitability as food preservation agents. In fact, after terrestrialization and during the following evolutionary pathway, plants had to develop chemical compounds – constitutive and/or induced – for defense against specific pathogens, therefore becoming a potential source of new natural products usable with antimicrobial purposes. Aside from the most common contaminants that could occur in foodstuff, mycotoxigenic fungal species

represent a big concern, mainly in cereals and derived products: aflatoxins in particular are the most dreaded among such toxic and cancerogenic secondary metabolites, and the control of the main producer *Aspergillus flavus* is currently one of the most pursued goals in the field of food safety. As aromatic and medicinal plants have a long history of use in the Mediterranean basin for both food preservation and pest control in crops, the exploitation of native species for the control of mycotoxigenic phytopathogens is almost rationale. The present work discusses the suitability of some natural extracts/compounds as novel crop protectants, exploring the promises of plant species from harsh or still scarcely mined environments for this purpose; more specifically, we are aimed to provide novel insights into the possible use of *Citrullus colocynthis* extracts as antimycotoxigenic additives, demonstrating, for some of them, a feasible application as crop and food protectants with specific regard to aflatoxin contamination. Additionally, the evaluation of their cytotoxic potential and nitric oxide production on human cell lines has been reported for the first time.

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BIOCONTROL ACTIVITY OF ANTAGONISTIC YEASTS AGAINST *PENICILLIUM EXPANSUM* – MAJOR PATULIN PRODUCER IN APPLES

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Penicillium expansum causes apple blue mould and it poses a serious threat to human health due to patulin production. Traditionally, the pathogen is controlled by synthetic fungicides, but nowadays, sustainable production requires alternative control methods to avoid environmental and health hazards and the development of fungicide resistant populations of the pathogen. Using yeasts in biocontrol of *P. expansum* is a promising alternative to chemicals. Healthy apple fruits that were not treated with fungicides were collected from 15 localities in Serbia. Putative biocontrol yeast strains were isolated from the surface of apple fruits using the swab streaking technique onto the YPDmin medium and incubated for 48 h at 25°C. After incubation, individual yeast colonies were purified and 80 isolates were obtained. They were screened for the ability to inhibit *P. expansum* growth on PDA plates using modified dual culture assay [Postharvest Biology and Technology 24 (2002) 123]. In 90 mm Petri dishes with PDA medium, a total of 10 µl of *P. expansum* spore suspension (10^6 spores/ml, prepared from 7 days old colonies grown on PDA) was placed 10 mm from the centre of the plate. On the other half of the petri dish, 20 mm from the pathogen suspension, a loop of tested yeast (3-days old, grown on PDA) was streaked as a 40 mm vertical line. Plates inoculated only with pathogen suspension represented negative control. The experiment was conducted in four replicates. Plates were incubated at 25°C for 10 days and the colony diameter of the pathogen in dual culture was compared with the control. Three yeast strains inhibited *P. expansum* growth by more than 45%, most of the strains (38) caused 30-45% growth inhibition, 17 showed less than 30% inhibition, while the rest (22) did not affect the pathogen growth. *In vitro* inhibition of fungal growth is only one aspect in the assessment of biocontrol potential of yeast strains, so further studies such as inhibition of pathogen growth and patulin production and induced resistance in apple fruits will be conducted.

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PYDIFLUMETOFEN CO-FORMULATED WITH PROTHIOCONAZOLE: A NOVEL FUNGICIDE FOR *FUSARIUM* HEAD BLIGHT AND DEOXYNIVALENOL CONTROL

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Legal limits exist in many countries for the mycotoxin, deoxynivalenol (DON) which is produced as a result of the disease, *Fusarium* head blight (FHB). In the European Union (EU), the legislative limit for wheat is being reviewed with a lower limit of 1000 ppb under consideration. This will require farmers to take greater efforts to grow wheat within legal limits. There are numerous integrated pest management (IPM) strategies to control FHB resulting in reductions in the mycotoxin contamination of cereals. However, no one strategy is consistently effective at reducing mycotoxins to below legislative limits when disease pressure is high. Current fungicide control is largely restricted to the azole chemistry with prothioconazole being the most effective. Reliance on a single chemical group increases the selection pressure for resistance within the pathogen population and as endocrine disruptors, several of the azoles are being removed from the market, further reducing the choice of chemical control. Adepidyn™ is a trademark owned by Syngenta for pydiflumetofen currently undergoing EU registration. It is a succinate dehydrogenase inhibitor (SDHI) but unlike other SDHI fungicides, Adepidyn™ has good activity against *Fusarium* species. Field experiments were conducted over several years to identify the

activity of Adepidyn™ to control FHB compared to the standard triazole fungicide (prothioconazole) separately and when combined in a co-formulation at various rates and at four application timings (flag leaf fully emerged, mid-head emergence, early flowering, and late flowering). Plots were inoculated with *F. graminearum* infected oat grains in early spring, mist irrigated at flowering and assessed for FHB at milky ripe and grains were analysed for deoxynivalenol (DON) at harvest. In all experiments, Adepidyn™ outperformed prothioconazole with the co-formulation of both active ingredients giving the greatest reduction in FHB and DON. Optimum timing was at early flowering for all chemistry (greater than 90% DON reduction for the co-formulation applied at full rate). The activity reduced for earlier or later applications but the co-formulation still gave more than 50% reduction in DON when applied as a foliar spray to the fully emerged flag leaf. This gives an opportunity to target FHB at two key fungicide timings rather than a single application at flowering. As an alternative to current triazole chemistry, Adepidyn™ will provide an important additional tool within IPM to control FHB and resulting mycotoxin contamination delivering a greater reduction in toxins, greater flexibility in fungicide timing and improved resilience of fungicide programs against FHB.

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MICROBIAL MODULATION IN WHEAT DISEASE: MITIGATING THE CLIMATE CHANGE IMPACT OF WATER SCARCITY USING ARTIFICIAL INTELLIGENCE

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Wheat and durum wheat are among the most cultivated cereals in the world. Unfortunately, an important part of production is lost to diseases, such as *Fusarium* head blight (FHB), which is also cause of mycotoxin contaminations of great socio-economic importance. Risks to grain yields and quality have led to widespread and sometimes excessive use of fungicides. The identification of strategies to limit the impact of FHB is therefore of primary economical and environmental importance. In consideration of recent geo-political events, the production of cereals has assumed an unprecedented strategic importance in the European context. The spread of FHB is favoured by the alternation of periods of drought, that produce stress in plants and make them more susceptible, with periods of submersion due to intense rains, that favour the spread of fungal spores. Global warming is expected to exacerbate this alternation, so countermeasures will need to be designed and implemented. Current strategies that use biological means to control the disease are on the whole less effective than traditional compounds, due in part to difficulties in their application. This project aims to develop a strategy based on the upstream reduction of the infectious potential of pathogens, acting on the soil microbiota in the early phases of cultivation. The poster will show the overall project which aims to evaluate the composition of the microbiota and its temporal evolution during the most sensitive phases of cultivation. DNA has been extracted from the rhizosphere, under experimental conditions of variable water stress, and it will be characterised with next generation sequencing (NGS) technologies in terms of microbial, bacterial, and fungal species present. The most innovative aspect consists in the adoption of neural network (NN) technology to determine the most favourable composition of the microbiota over time, influenced by the experimental parameters. NNs have shown their effectiveness in numerous situations, such as the reconstruction of fragmented data, a case close in principle to the problem this project intends to tackle. The analysis will consider not only metagenomic data, but also the presence or scarcity of water, and will make use of sequencing data analysis techniques, but also of statistical tools typically part of NN development. Agronomic and climatic knowledge will also be taken into account to evaluate the results of the modelling.

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A RAPID MULTIWELL TEST TO ASSAY THE EFFECT OF NATURAL METABOLITES ON GROWTH AND MYCOTOXIN PRODUCTION OF *ASPERGILLUS FLAVUS*

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Plants and microorganisms can be natural sources of bioactive metabolites with antioxidant, anti-microbial, or anti-inflammatory effects. The use of plant or microbial bioactive molecules in the integrated management of crop diseases represent an innovative sustainable choice to counteract the

contamination of mycotoxins produced by phytopathogenic fungi, such as *Aspergillus flavus*. Here we report a rapid and effective methodology, multiwell test, employed to understand the effect of natural metabolites on the *A. flavus* growth and aflatoxins (AF) production. Multiwell test allows monitoring simultaneously the effect on *A. flavus* of several natural metabolites at numerous concentrations. Plate spectrophotometer/fluorimeter was used to monitor the fungal growth (from 1 to 3 days post-inoculation) and AF biosynthesis (by fluorescence); at 7 days post-inoculation, AF were extracted directly from the wells and analysed by LC-MS. Natural metabolites tested derive from fungi and plants. Fungal compounds were obtained from *Trametes versicolor*, *Pleurotus eryngii*, and *Schyzophyllum commune* (Basidiomycota). Notably, *T. versicolor* produces a bioactive exo-polysaccharide, Tramesan, efficient in blocking completely AF synthesis. Plant-based extracts and compounds were obtained from officinal plants, such as *Heracleum persicum*, *Crocus sativus*, *Peganum harmala*, *Trachyspermum ammi, Rosmarinus officinalis*, *Anethum graveolens*, *Berberis vulgaris*, and *Berberis thunbergii* via an hydroalcoholic extraction and from *Linaria purpurea* via aqueous extraction and subsequent purification. The multiwell tests indicate that the bioactive molecules derived from fungal cultures and plants extracts do not significatively affect the growth of *A. flavus* but have a role on the aflatoxin production. In particular, aflatoxins levels showed a significant decrease in the presence of *T. versicolor* compounds and their production was completely inhibited in the presence of *H. persicum*, *P. harmala, T. ammi*, and *L. purpurea* plant extracts. AF inhibition occur at the transcriptional level for most compounds. Further investigations with machine learning algorithms will be undertaken in a quantitative composition-activity relationship (QCAR) fashion to shed light on the role of the extract components and pure compounds in modulating the aflatoxin levels. Our findings encourage the use of natural bioactive compounds to manage mycotoxin contamination in pre- and post-harvest diseases of agricultural crops.

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CULTURE CONDITIONS FOR THE ENRICHMENT OF *FUSARIUM* SPP. INOCULUM IN FOOD MATRICES

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Direct detection of mycotoxigenic fungi in foodstuffs is a good tool for predicting the risk of mycotoxin contamination. Our group has designed several specific PCR-based protocols in order to detect the main mycotoxin producers directly in food matrices without fungal isolation in culture. However, food products are often co-contaminated by several fungal species, some of them occurring at very low levels; therefore, it is necessary to perform a short pre-incubation to increase fungal biomass to effectively detect them by PCR. However, some fungi such as *Aspergillus* spp. or *Rhizopus* spp. present an extremely high development rate, and they often overgrow other slow-growing species such as mycotoxigenic *Fusarium* spp. Taking all these aspects into account, the aim of this work was to select the best culture conditions for maximizing *Fusarium* spp. growth and reduce the development of fast-growing fungi. Four media were selected on the basis of the information on the available literature: Dichloran Glycerol agar (DG18), Dichloran Rose Bengal Chloramphenicol agar (DRBC), Malaquite Green agar (MGA25), and Czapek Dox Iprodione Dichloran agar (CZID). Moreover, fungal growth was also evaluated in Sabouraud-Chloramphenicol agar since it is the most common medium for sample preincubation. Seven *Fusarium* species were studied including trichothecene, zearalenone and fumonisin producers (*F. poae, F. acuminatum, F. tricinctum, F. avenaceum, F. brachygibbosum, F. langsethiae*, and *F. verticillioides*). Moreover, four fast-growing fungi were selected including *Aspergillus flavus*, *A. niger*, *Rhizopus* sp., and *Penicillium* sp. which frequently co-occurred in food matrices with toxigenic *Fusarium* spp. The plates were incubated for 7 days at 20 and 25°C to establish the most suitable temperature for favouring *Fusarium* spp. growth. In general, non-*Fusarium* species were favoured in Sabouraud-Chloramphenicol at both temperatures tested compared to *Fusarium* spp. which confirms that this medium might not be adequate for the preincubation step. Regarding the other media, all *Fusarium* species reached their maximum growth in CZID plates, whereas non-*Fusarium* fungi were considerably inhibited. The results were similar at both temperatures tested except for *F. langsethiae* which was more favoured at 25°C. Taking into account this is one of the main type A trichothecene producers, and one of the most difficult fungi to be detected by direct PCR, CZID medium and this temperature will be selected in future experiments to perform sample preincubation in order to detect mycotoxigenic *Fusarium* species directly by PCR in food products. **Acknowledgements**. Work supported by CAM/UCM, project PR65/19-22428.

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THE EFFECT OF FUNGI CO-OCCURRENCE ON PLANT AND FUNGAL GENE EXPRESSION PROFILES AND MYCOTOXIN PRODUCTION IN MAIZE KERNELS AND *IN VITRO*

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Compelled by increasing temperature and elevated CO_2 during summer, a rising incidence of *Fusarium verticillioides* and *Aspergillus flavus*, as well as of their mycotoxins, in European maize can be predicted. In the current study, we aimed to: (i) gain deeper insight into the cross-talk between maize and its main associated mycotoxigenic fungi (*F. verticillioides* and *A. flavus*) utilizing a maize kernel inoculation assay; (ii) enrich the knowledge about fungal co-culture performance in *in vitro* experiments; and (iii) quantify the influence of fungal co-occurrence on maize and fungal transcriptional profiles and mycotoxin production in different temperature regimes. The expression profiles of two pathogenesis-related (PR) genes and four mycotoxin biosynthetic genes, *FUM1* and *FUM13*, fumonisin pathway, and *AFLR* and *AFLD*, aflatoxin pathway, as well as mycotoxin production, were examined in kernels and in artificial medium after a single inoculation with *F. verticillioides* or *A. flavus* or with the two fungi in combination, under different temperature regimes (20, 25 and 30°C) over a time-course of 21 days. In maize kernels, *PR* genes showed the strongest induction at 25°C in the earlier days post inoculation (dpi) with single fungi. A similar behaviour was maintained with fungi co-occurrence, but with enhanced defence response at 9 dpi under 20°C. Regarding *FUM* genes, in the kernels inoculated with *F. verticillioides* the maximal transcript levels occurred at 6 dpi at 25°C, decreased with the co-occurrence of *A. flavus* while the highest gene induction was detected at 20°C. Similar results were observed in fungi grown *in vitro*, whilst *A. flavus* presence determined lower levels of expression along the entire time-course. For *AFL* genes, considering both *A. flavus* alone and in combination, strengthened up-regulation levels were reached at 30°C during all time-course both in infected kernels and in fungi grown *in vitro*. Regarding mycotoxin production, no significant differences were found among temperatures for kernel contamination, whereas *in vitro* the highest production was registered at 25°C for aflatoxin B_1 and at 20°C for fumonisins FB_1+FB_2 in case of single inoculation. In fungal co-occurrence, both mycotoxins resulted reduced at all the temperatures considered compared to the amount produced with single inoculation. All together these findings represent pivotal steps for elucidating complex fungal competition and better managing and controlling pathogen co-occurrence under climate change scenario. **Acknowledgements**. This research was funded by the MycoKey project 'Integrated and Innovative Key Actions for Mycotoxin Management in the Food and Feed Chain' (EU Project H2020-GA 678781.U.3.2-678781).

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GENE EDITING OF *ASPERGILLUS NIGER CBS 513.88* USING A CRISPR-CAS9 BASED SYSTEM

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Conventional genetic disruption in fungi has been found to be limited in many species due to their low capacity for homologous recombination. CRISPR-Cas9 has emerged as a good alternative to solve this problem and offers some other advantages such as time-saving and improved efficiency. Under the direction of a single guide RNA (sgRNA), the endonuclease Cas9 recognizes and cleaves specific and targeted DNA sequences, producing double-strand breaks (DSB) in the genome. DSB are primarily repaired by non-homologous end-joining (NHEJ), resulting in small mutations such as base-pair deletions, insertions, or substitutions. Gene disruption using CRISPR-Cas9 is usually performed using plasmids for heterologous expression of Cas9; however, this would limit the application of mutants as biocontrol agents since they would be considered transgenic. For this reason, the aim of this work was to develop a gene editing protocol using directly an exogenous Cas9 protein with the sgRNA generated *in vitro* in the mycotoxin-producing fungi *Aspergillus niger.* The widely used CBS 513.88 was selected since it is used in biotechnological processes and its complete genome is available on databases. Although the main objective is the disruption of genes involved in ochratoxin A (OTA) synthesis, the protocol was optimized with the pigmentation gene AYG1 to facilitate the selection of mutants. A final concentration of $5x10^6$ spores/ml of *A. niger* was inoculated into YPD medium and incubated during 16 h. Protoplasts were generated using lysing enzymes, and transformation was performed using either PEG-Ca or different electroporation conditions. Prior to the transformation into fungal protoplasts, RNP complexes were assembled. Mutant selection was based on conidial pigmentation and the mutations were tested by PCR and sequencing. A single pigmentation mutant was found using electroporation

transformation (600 Ω, 10 μF, 2 kW). The positive transformant has a 108 bp deletion in the pigmentation gene *AYG1* but located off-target. These preliminary results show that gene editing of *Aspergillus niger* is possible directly transforming with the Cas9 enzyme and sgRNA. However, further research is needed to understand why off-target mutations occur and how to minimize off-target effects. **Acknowledgements**. Work supported by Spanish Ministry of Science and Innovation (RTI 2018-097593-B-C21). C. Gómez-Albarrán is supported by a FPI fellowship PRE 2019-087768.

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IN-DEPTH STUDY OF MYCOTOXIN ACCUMULATION IN RELATION TO ANTHOCYANIN COMPOSITION IN PIGMENTED WHEAT

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Secondary metabolites, such as flavonoids and phenolic acids, are known to be involved in defence mechanisms against fungal infection, strengthening the cell wall or acting as antimicrobial and antioxidant compounds or signalling molecules [European Journal of Plant Pathology 152 (2018) 1]. Pigmented wheat (*Triticum aestivum* L.) varieties are rich of different phytochemicals among which anthocyanins, antioxidant molecules that are differently located within the grain and responsible for its colour (blue, black, purple or red) [Trends in Food Science and Technology 110 (2021) 240]. The presence of these metabolites makes pigmented grain useful as functional food and represents a selectable trait for breeding programme. A better understanding of the metabolic pathways involved in defence mechanisms in the frame of the current intraspecific diversity may offer a new tool in fighting *Fusarium* and its related mycotoxins. As already reported [Frontiers in Microbiology 7 (2016) 566], secondary metabolites with antioxidant activity, including flavonoids may counteract toxigenic *Fusaria* and mycotoxin accumulation in wheat. However, little is known about the possible contribution of anthocyanins to resistance to FHB and mycotoxin accumulation except for some studies carried out on barley [Plant Pathology 61 (2012) 509], maize [Scientific Reports 10 (2020) 1417], and non-pigmented wheat [Plant Physiology and Biochemistry 83 (2014) 40]. The aim of this work is to evaluate a potential correlation between anthocyanins composition and multiple *Fusarium*-related mycotoxins accumulation in twelve varieties of differently pigmented wheat collected over two years. By quantifying 14 different anthocyanins we have found noticeable differences, with a clear clustering among varieties based on grain colour and phytochemical profile. By normalizing data according to hectolitre weight, DON and DON-3Glc content clustered according to the colour group with a superimposable pattern in both harvesting years. The contribution of anthocyanins and their precise tissue accumulation in defence mechanisms needs further evaluation.

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FUNGAL GROWTH AND MYCOTOXIN PRODUCTION IN A NEW FORMULATED MEAT PRODUCT WITH STRUCTURED EMULSIONS AS SUBSTITUTES FOR PORK RIGID FAT

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One of the major changes in dietary habits of the last half-century has been the increase of meat-based products consumption. This increase has contributed to a diet rich in saturated fats, which can result in adverse health effects. A recent trend in food industry is the development of new meat products based on traditional ones, though having less saturated fats and adding healthy ingredients. In this work, the growth and mycotoxin production of three mycotoxigenic species that colonize meat products was evaluated in a new formulation of cured salami, where rigid pork fat was replaced by O/W structured emulsions based on sunflower oil. Together with the traditional formulation (TS), two degrees of rigid pork fat substitution were analysed, 50% (SF1) and total substitution (SF2). To evaluate fungal and mycotoxins contamination, salami produced in industrial environment were grinded and mixed with agar. This medium was inoculated with the toxigenic species *Aspergillus nomius*, *Aspergillus westerdijkiae* and *Penicillium nordicum.* At the end of the incubation period, fungal growth did not show significant differences between the three analysed samples for *A. nomius*; for *P. nordicum*, results show a variation of approximately ±10% of SF1 and SF2 in relation to TS; as for *A. westerdijkiae*, SF1 and SF2 showed growth inhibitions up to 20% and 40%, respectively, when compared with TS samples. Despite fungal growth not being affected in the different, mycotoxin analysis revealed an almost complete inhibition of aflatoxin production by *A. nomius* in the SF2 medium and up to 90% for SF1, when compared with

traditional salami. OTA production by *P. nordicum* was hindered by 15 to 30% for SF2 and up to 20% for SF1; and *A. westerdijkiae* OTA production was reduced by approximately 15%, in SF2 medium and up to 10% for SF1 medium, when comparing with traditional salami. Fatty acid profile shows a reduction of total fat of approximately 30% for SF1 and 60% for SF2, when compared with the traditional salami. More expressive differences are observed in palmitic, stearic, and oleic acid, where concentrations drop to half in SF2 samples. It is hypothesized that reduction of these fatty acids may be related to the reduced levels of mycotoxins, specially, in SF2 samples. Further studies are being developed to determine the factors responsible for mycotoxin inhibition in these new formulations. **Acknowledgements**. Study supported by the Portuguese Foundation for Science and Technology (grant UIDB/04469/2020) and by project BetterFat4Meat (POCI-01-0247-FEDER-039718).

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THE ABILITY OF CYCLOPIAZONIC ACID PRODUCTION EXPRESSED BY SURFACE MOULDS ISOLATED FROM DRY CURED MEAT PRODUCTS

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Cyclopiazonic acid (CPA) is an under-investigated mycotoxin of toxicological significance, produced by several *Penicillium* spp. (*P. griseofulvum, P. camemberti, P. dipodomyicola* and *P. commune)* and *Aspergillus* spp. (*A. flavus, A. oryzae* and *A. tamarii)* species. Moulds of the *Penicillium* and the *Aspergillus* genus overgrow the surface of dry cured meat products during ripening. Although *P. commune* is one of the predominant mould species isolated from dry cured meat products, CPA concentrations in products of this type are generally unexplored. The ability of moulds to produce mycotoxins is affected by various environmental and biological factors, such as the presence and expression of biosynthetic genes. In this study, 200 samples of Croatian traditional dry cured meat products were analysed for the presence of CPA-producing moulds. Surface moulds were identified using both the traditional and the molecular method, the latter employing β-tubulin and calmodulin loci sequencing. The isolates of mould species that can produce CPA were tested for the presence of *dmaT* gene encoding dimethylallyl tryptophan synthase involved in CPA production using real-time PCR. CPA concentrations in dry cured meat products were analysed using LC-MS/MS (liquid chromatography-tandem mass spectrometry). Species identified from the surface of TMP samples, potentially able to produce CPA, were *P. commune* (70 isolates), *A. flavus* (12 isolates), and *P. polonicum* (13 isolates). Several studies have indicated the need for testing the CPA production potential of *P. polonicum* as well. The results revealed the presence of *dmaT* gene in 17% of *A. flavus,* 64% of *P. commune,* and none of *P. polonicum* isolates. In 24 samples, the CPA concentrations were above the limit of detection (LOD = 2.17 µg/kg) and increased to 66.35 µg/kg. *P. commune* comprising *dmaT* gene was identified in roughly half of the CPA positives (11 out of 24), while *A. flavus* was not identified in any of CPA-contaminated samples. It can be concluded that *P. commune* commonly possesses the CPA biosynthetic gene and therefore represents a potential public health hazard. CPA contamination of samples in which *P. commune* or other CPA producers were not identified can either be explained by the previous contamination of meat product ingredients, or by the possibility that *P. commune* (or other CPA producers) were overgrown by other mould species at the end of the ripening stage and therefore skipped detection.

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ANTIFUNGAL ACTIVITY OF LEMON PEEL MEDIUM FERMENTED BY LACTIC ACID BACTERIA ISOLATED FROM CITRUS FRUITS AND METABOLOMIC PROFILE OF CITRUS CONTAMINATING MYCOTOXIGENIC FUNGI

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The important productive activity of the juice sector generates huge amounts of waste materials every year, such as peels, membranes, and seeds, representing citrus peel waste alone almost 50% of the wet fruit mass. Fungal rots are the leading cause of postharvest losses (about 30%) of citrus and can greatly reduce its shelf life. Some postharvest fungal pathogens of citrus fruits produce mycotoxins that can diffuse through the peel and be found in juices. The aims of this study were to re-evaluate lemon peel waste to develop a fermented product by isolated bacteria from citrus fruits with antifungal activity, explored their antagonistic properties against the main mycotoxigenic pathogens of citrus fruits,

belonging to *Aspergillus* spp., *Penicillium* spp., *Cladosporium* spp., *Phytophthora* spp. *Fusarium oxysporum*, and *Plenodomus tracheiphilus*. Isolated bacteria were tested against the pathogens with overlay assay that showed inhibition by direct contact. Antifungal bacteria were identified by MALDI-TOF/MS and fermented in lemon peel broth formulation to produce secondary metabolites with antifungal activity. Preliminarily, the 2 cell-free supernatants (CFS) were evaluated by agar diffusion assay in which the activity was established by the presence of the inhibition halo. Subsequently, the minimum inhibition concentration and the minimum fungicidal concentration (MIC and MFC) were determined. In addition, antifungal compounds such as organic acids, phenolic acids, and volatile organic compounds (VOCs) present in CFS were identified by ESI-LC-MS-TOF and GC-MS. Also, metabolomic profile of citrus contaminating fungi was determined by ESI-LC-MS-TOF. A total of 30 strains were isolated of which 13 isolates, identified belonging to the genera *Pediococcus*, *Lactobacillus* and *Leuconostoc*, showed clear zones of inhibition on the tested pathogens. The cell-free supernatants (CFS) of 13 isolates inhibited the growth of most tested pathogens the CFS of isolates *L. plantarum* H1 and L1 showed good activity with MIC values between 15.6-125 mg/ml and MFC values between 15.6-250 mg/ml.

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ECOLOGY AND CONTROL OF *ASPERGILLUS FLAVUS* AND AFLATOXIN B1 IN CHILLI POWDER AND WHOLE RED CHILLIES

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Chillies and chilli-based products are important spices on a global basis. The production, processing, transport, and storage phases of chillies are prone to infection by *Aspergillus* Section *Flavi* and contamination with aflatoxins (AFs), especially aflatoxin B1 (AFB1) for which legislative limits exist in many countries. We have examined the effect of the interacting abiotic factors of water availability (water activity, a_w; 0.995-0.850 a_w) and temperature (15-37°C) on growth and AFB1 production to identify the optimum ad boundary conditions for colonisation and toxin production by three *A. flavus* strains on a 10% chilli-based medium. Studies with whole red chillies + *A. flavus* conidial inoculum on` AFB1 contamination during storage for 10-20 days at 30°C were also carried out. This was complimented with studies on the use of different food-grade preservatives for the control of growth and AFB1 contamination of chilli powder and whole red chillies. Ecologically, there was no statistical difference in growth between the three strains. Optimal growth was at 37°C and 0.982 a_w with no growth at 0.85 a_w. Optimal temperature x a_w conditions for AFB1 production were at 30°C and 0.982 a_w with no statistical difference in production between strains. No AFB1 was produced at 15-20ºC at 0.901 and 0.928 a_w levels, respectively. *In situ* studies with *A. flavus* inoculated whole red chillies at 0.90 and 0.95 a_w found that this species became the major component of the total fungal populations at 30°C after 10-20 days storage. AFB1 contamination was above the European legislative limits 5 µg/kg) for spices at 0.90 a_w after 20 days storage and at 0.95 a_w after 10 and 20 days. This suggests that storage conditions of ≥0.90 a_w, especially at ≥25-30°C represents a significant risk of contamination with AFB1 at levels where rejection might occur, even after only 10-20 days storage. Subsequently seven different food grade preservatives were examined for control of aflatoxins in chillies. Of these compounds, sodium metabisulphite (NaMBS) was the most effective in controlling growth and AFB_1 production by *A. flavus* strains at 0.93, 0.98 and 0.95 aw. No growth or production of AFB1 occurred with 500-2,500 mg/l NaMBS. In situ studies with chilli powder or whole red chillies (naturally contaminated or + *A. flavus* inoculum) with NaMBS controlled total fungal populations and *A. flavus*. Studies with commercial laminated sheets containing slow-release layers of NaMBS (SO_2) in stored chillies showed significant reductions in fungal populations and AFB1 contamination.

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CLIMATE CHANGE AND ACCLIMATIZATION OF *ASPERGILLUS FLAVUS* STRAINS INFLUENCED COLONISATION, BIOSYNTHETIC GENE EXPRESSION AND AFLATOXIN B1 PRODUCTION BY *A. FLAVUS* IN RAW PISTACHIO NUTS

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Pistachio nuts are an important economic tree nut crop which is used directly or processed for many food-related activities. They are colonized by mycotoxigenic spoilage fungi, especially *Aspergillus flavus*, resulting in contamination with aflatoxins (AFs), especially aflatoxin B1 (AFB1). The prevailing climate in which these crops are grown changes as temperature and atmospheric CO_2 levels increase, and episodes of extreme wet/dry cycles occur due to human industrial activity. In addition, such fungal pathogens may evolve resilience when acclimatized for several generation in elevated CO_2. The objectives of this study were to evaluate the effect of interacting climate change-related abiotic factors of temperature (35 vs. 37°C), CO_2 (400 vs. 1000 ppm) and water stress (0.98-0.93 water activity, a_w) on (i) colonization, (ii) *aflD* and *aflR* biosynthetic gene expression, (iii) AFB1 production by strains *A. flavus* (AB3, AB10), and (iv) acclimatization for 5 generations in elevated CO_2 on colonization of raw pistachio nuts and AFB1 contamination. The *A. flavus* strains were very resilient in terms of colonization of pistachio nuts with no significant difference when exposed to the interacting three-way climate-related abiotic factors. The relative expression of the structural *aflD* gene involved in AFB1 biosynthesis was decreased or only slightly increased, relative to the control conditions at elevated CO, regardless of the a_w level examined. For the regulatory *aflR* gene expression, there was a significant ($P < 0.05$) increase in 1000 ppm CO_2 and 37°C for both strains, especially at 0.95 a_w. There was a significant ($P < 0.05$) stimulation of AFB1 production at 35°C and 1000 ppm CO_2 for both strains, especially at 0.98 a_w. At 37°C, AFB1 production was either decreased, or remained similar depending on the strain when exposed to 1000 ppm CO_2. Acclimatized strains of *A. flavus* (5 generations) showed changes in colonization patterns and some stimulation in AFB1 production in pistachio nuts. This suggests that *A. flavus* strains are very resilient to climate change factors, with differential effects on AFB1 production that may be strain dependent. This will impact on the relative toxin risks during processing of this tree nut under future climate-related abiotic factors and the development of appropriate control strategies.

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HANSENIASPORA UVARUM AS BIOCONTROL AGENT AGAINST *ASPERGILLUS FLAVUS*

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Mycotoxin contamination of crops is a critical problem that affects economy and global health. Among all mycotoxins, aflatoxin B1 (AFB1) is considered the most relevant since it is a potent natural carcinogen, being *Aspergillus flavus* the most common producer. Several authors have reported an increase in the occurrence of this fungus due to climate change. Biocontrol agents are one of the most promising methods to prevent the presence of mycotoxigenic species. In previous works carried out in our laboratory, the potential of *Hanseniaspora uvarum* U1 as a biocontrol agent was described. The aim of the present work was to study the effect of its co-application with a commercial bio-fungicide in order to control *A. flavus*. CYA plates were supplemented with a final concentration of 10^3 cells/ml of *H. uvarum* U1 and 10 ml of the commercial bio-fungicide or 6.4 x 10^2 cells/ml of its main bioactive compound (*Bacillus subtilis*). Then, 2 µl of a spore suspension (10^6 spores/ml) of *A. flavus* was inoculated in the centre of the plate. The assays were carried out in triplicate using three isolates of *A. flavus*. In all cases, treatment with *H. uvarum* U1 and *B. subtilis* alone as well as their combination produced a significant reduction on fungal growth. Treatment with the bio-fungicide combined with *H. uvarum* U1 reduced significantly fungal-growth although to a lesser extend than the treatment with *H. uvarum* U1 or *B. subtilis* alone. However, treatment with bio-fungicide alone did not affect fungal growth. The concentration of AFB1 in CYA plates was evaluated by competitive ELISA. AFB1 concentration was reduced 98% when the yeast was used alone or supplemented with *B. subtilis*. This decrease was 20% higher than that obtained in the treatment using *B. subtilis* alone. Neither the bio-fungicide nor the bio-fungicide combined with the yeast had any effect on AFB1 concentration, which suggests that there is some interference between the bio-fungicide and the yeast. These preliminary results confirm the potential of *H. uvarum U1* to reduce the growth of *A. flavus* and the concentration AFB1. However, the

actual formula of the bio-fungicide tested does not reduce the growth of *A. flavus* or its ability to produce AFB1. **Acknowledgements**. Research funded by RTI 2018-097593-B-C21R.

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ANTAGONISTIC PROPERTIES OF SELECTED *TRICHODERMA* FUNGI AGAINST *FUSARIUM* SPECIES BIOSYNTHESIZING FUMONISINS AND BEAUVERICIN

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The present study aimed to examine the abilities of eight isolates belonging to three different *Trichoderma* species (*T. atroviride*, *T. viride*, and *T. viridescens*) to inhibit the mycelial growth and mycotoxin production by two *Fusarium* strains (*F. proliferatum* and *F. poe*). Dual-culture bioassay on potato dextrose agar (PDA) medium clearly documented that all *Trichoderma* strains used in the study were capable of influencing the mycelial growth of *Fusarium*. The qualitative evaluation of the interaction between the colonies after 5 days of co-culturing on PDA medium showed that six *Trichoderma* strains were completely overgrown and sporulated on the colony of at least one of the tested *Fusarium* species. Of all tested *Trichoderma* strains, *T. atroviride* AN 215 and *T. atroviride* AN153 were also found to be the most efficient suppressors of mycotoxins (fumonisin B1, fumonisin B2, fumonisin B3, and beauvericin) production by both *Fusarium* species on PDA medium (91-100% toxins reduction) and rice samples (98-100% toxins reduction). This research suggests that *T. atroviride* AN215 and *T. atroviride* AN153 can be promising candidates for the biological control of toxigenic *Fusarium* species.

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INDUSTRIAL MANUFACTURING OF AFLATOXIN BIOCONTROL PRODUCTS ALLOWS FARMERS ACROSS SUB-SAHARAN AFRICA TO PRODUCE SAFE CROPS

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In sub-Saharan Africa (SSA), many crops are contaminated with aflatoxins by *Aspergillus* section Flavi fungi. This negatively impacts health, trade, income, and development sectors. Farmers, industries, and governments need sound management strategies to effectively limit aflatoxin throughout the value chain and reduce human and animal exposure to aflatoxins. Effective technologies exist but institutional, infrastructural, and policy inadequacies impede their use. Biocontrol products containing atoxigenic (i.e., non-aflatoxin producing) isolates of *A. flavus* as active ingredient effectively mitigate aflatoxin contamination. Biocontrol use allows farmers to produce crops with safe aflatoxin content—80% to 100% less aflatoxin than crops from neighbouring, untreated fields. Effectiveness of biocontrol depends largely on application at the right stage (2 to 3 weeks before flowering) and the right dose (10 kg/ha). Although biocontrol is highly effective by itself, its use in conjunction with other management tools further strengthens biocontrol effectiveness. Several country-specific products under the tradename Aflasafe have been developed, each containing as active ingredient four atoxigenic isolates of *A. flavus* native to major agricultural regions of the target country and containing natural defects across the aflatoxin biosynthesis gene cluster. Over 15 years ago, promising results were obtained in field tests in Nigeria with a manually manufactured product, but it was clear that tangible impact would be achieved only if the manufacturing process was scaled up. Industrializing biocontrol manufacturing coupled with incentivization, commercialization strategies, advocacy and effective partnerships has allowed producing more than 5 thousand tons of biocontrol to treat crops and produce over a million tons of aflatoxin-safe maize, groundnut, and sorghum. This has contributed to increased food safety, security, and income in various SSA countries. A long road has been traversed with modest beginnings in the laboratory to identify candidate atoxigenic fungi and producing few kilos of biocontrol, extensive interactions with regulators to achieve registration, novel approaches to design its industrial manufacturing and transfer the technology through a well-designed process. To date, private sector companies in Nigeria, Senegal, Tanzania, Mozambique, and a public sector institution in Kenya have been licensed to manufacture and distribute Aflasafe products. Product development, testing, registration, and tech transfer are at different stages in another 17 SSA countries. The industrial process was instrumental to allow thousands of farmers accessing the product. Food safety and security across Nigeria have increased and these experiences were capitalized to make biocontrol manufacturing a reality in other countries.

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BIOCONTROL OF *ASPERGILLUS FLAVUS* AND AFLATOXIN PRODUCTION WITH ECOLOGICAL VINEYARD ISOLATED BACTERIA

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The presence of mycotoxigenic fungi of the genus *Aspergillus* in vineyards, is a problem for food safety and economy. Although ochratoxin A has been considered the main mycotoxin in grapes, the rising of temperatures due to climatic change are modifying existing microbial communities, causing the replacement of some fungal communities and the raise of other types of mycotoxins such as aflatoxins. The use of microorganisms as biocontrol agents (BCA) is one of the most promising strategies to prevent fungal growth and toxin production on this type of crops. In a previous work carried out in the group, 542 microorganisms were isolated from ecological vineyard soils from different Spanish regions. Sixteen bacterial strains achieved all the requirements to be considered appropriate BCA. They were identified by sequencing and nine bacteria belonging to four different species of actinobacteria were selected to test their potential to control three toxigenic strains of *A. flavus*, one of the main mycotoxigenic fungi in grapes. The experiment was performed by supplementing CYA plates with a final concentration of 10^6 cells/mL of the selected bacteria and a spot of spores of mycotoxigenic fungi were deposited on the centre of the plates. All but one the potential BCA, significantly reduced fungal growth. Actinobacteria of to the genus *Arthrobacter* and *Rhodococcus* showed the highest ability to reduce fungal growth, reaching reduction percentages of up to 17.5% and 35.6%, respectively. Furthermore, aflatoxin B1 production were evaluated by ELISA, and in all cases the mycotoxin concentration was reduced, specially with the use of *Arthrobacter*, *Rhodococcus* and *Bacillus* strains. These results show that the selected microorganisms are good candidates for possible commercialization as BCA to reduce mycotoxin risk in vineyards as a sustainable option. **Acknowledgements**. Work supported by Spanish Ministry of Science and Innovation (RTI 2018-097593-B-C21).

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LOOKING INSIDE THE MAIZE/AFLATOXIN INTERPLAY: THE EFFECT OF AFLATOXIN B1 INTERACTION WITH MAIZE PLANTS *IN VITRO* UNRAVELLED

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Aflatoxins represent a severe health risk for human and animal health, generally occurring in maize crops and maize-based foodstuff. Unfortunately, crops contamination by mycotoxigenic fungal species depends on a broad spectrum of synergistic as well as antagonistic variables involving environmental and genetic factors (geographical location and local climate, and the cultivar varieties), agronomic practices and others. Due to these limitations, scarce is still the available information about the direct effect of aflatoxins on *Aspergillus flavus* infected plants; additionally, it is likely difficult to separate the general effects induced during the plant-pathogen interaction from the specific molecular response, at a cellular level, of the exposed tissues to the toxin. Thus, through metabolomic and proteomic approaches, we aimed to investigate the variations induced by the exposure of maize plantlets, *in vitro*, to AFB1, with the scope of uncover the possible mechanisms of response – or defense – of the plant. Samples from different organs of *Zea mays* L. plantlets (roots, stems and leaves) exposed to AFB1 were subjected to a metabolomic comprehensive analysis to evaluate several molecular components, such as polar primary metabolites next to lipids or proteins, while a two-dimensional electrophoresis technique has been employed to analyse changes in the proteome of treated plants.

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COMBINATION OF PROPIDIUM MONOAZIDE (PMA) WITH REAL-TIME PCR AND RECOMBINASE POLYMERASE AMPLIFICATION COUPLED WITH SYBR GREEN I FOR THE DETECTION OF PATULIN-PRODUCING FUNGI IN APPLES AND BY-PRODUCTS

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Nowadays, the application of rapid DNA-based methods, such as PCR/qPCR, and isothermal techniques, such as recombinase polymerase amplification (RPA), has emerged in order to overcome some of the drawbacks of classical culture-based methods due to their high sensitivity and specificity. One of the main disadvantages of these techniques is their inability to differentiate between live and dead microorganisms. Patulin is a mycotoxin typically produced by fungi belonging to *Penicillium* spp. that can cause acute and chronic toxic effects to humans and animals. In the present study, a real-time PCR assay and an RPA coupled with naked-eye SYBR Green I (SG) detection were developed for the detection of patulin-producing fungi. Primers and probe were designed based on the *idh* gene of the patulin metabolic pathway. Furthermore, the addition of the PMA dye was implemented prior to the DNA extraction for the selective detection of viable fungi. In the tested concentration the PMA was capable of inhibiting the amplification of DNA coming from spores with a concentration of up to 10^7 spores/ ml. The developed assays were able to detect down to 1.25 pg/ µl (qPCR) and 23.8 pg/ µl (RPA-SG) of pure *P. expansum* DNA. Finally, when artificially inoculated apples and by-products were analysed the LOD_{50} of the qPCR was found to be 8.1×10^3 spores/5 g of food sample. In the case of the RPA-SG, the determined LOD_{50} was 5.8×10^4 spores/5 g. The developed qPCR assay proved to be more sensitive compared to the RPA-SG; however, the proposed RPA assay has the advantage of naked-eye detection by the addition of SYBR Green in the amplified product. As a consequence, this assay can be easily implemented for on-site testing and can be a helpful tool for an early screening of the fruits.

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THE ROLE OF MAIZE KERNELS LIPOPHILIC ANTIOXIDANTS IN RESISTANCE AGAINST *FUSARIUM GRAMINEARUM*

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Fusarium graminearum is the main causal agent of Gibberella ear rot (GER) a devastating fungal disease affecting maize. GER leads to significant economic losses and serious health issues due to the ability of *F. graminearum* to produce mycotoxins such as type B trichothecenes. Many factors including environmental, agronomic or genetic ones affect the levels of mycotoxins accumulating in the grains and there is an urgent need to implement efficient and sustainable management strategies to reduce mycotoxin contamination. To durably solve the problem of mycotoxin accumulation, the breeding of tolerant genotypes is one of the most promising environment-friendly strategies. While numerous reports have addressed the role of phenylpropanoids in plant resistance to *Fusarium graminearum,* very few have focused on lipophilic antioxidants, including carotenoids and tocochromanols. And yet these last compounds are potent antioxidants, are likely to interfere with cellular signalling by altering plant hormone levels and some of them have been described as antifungal and anti-mycotoxins agents [Journal of Agricultural and Food Chemistry 61 (2013) 3389; Journal of Agricultural and Food Chemistry 64 (2016) 4545]. The present study aims to evidence and investigate the contribution of lipophilic antioxidants to the chemical defense employed by maize to cope with *F. graminearum*. In a first step, the carotenoid and tocochromanol compositions of a wide set of maize genotypes have been characterized and statistically related with the GER resistance level of the different genotype. In a second step, the antifungal and anti-mycotoxin activity of the major lipophilic antioxidants have been clarified. Overall, our data support the contribution of some carotenoid compounds that could be interesting biochemical markers to be used in phenotyping and breeding strategies.

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EFFECT OF WATER ACTIVITY AND TEMPERATURE ON GROWTH AND TRICHOTHECENE PRODUCTION BY *FUSARIUM CEREALIS* ISOLATED FROM DURUM WHEAT GRAINS

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The major pathogen associated to fusarium head blight (FHB) is included in the *Fusarium graminearum* species complex. However, recently there have been reports of *F. cerealis* causing the disease in wheat and barley. This pathogen is able to produce deoxynivalenol (DON) and nivalenol (NIV). Nevertheless, the effect of environmental factors on growth and mycotoxin production by this species have not been studied so far. The aim of this study was to determine the effect of water activity (a_w, 0.99-0.90) and temperature (15, 20, 25 and 30°C) on growth and DON and NIV production by three *F. cerealis* strains (RCFG6046, RCFG6029, RCFG6076) isolated from durum wheat grains. A wheat-based medium was used and adjusted to the different a_W with glycerol. Plates were inoculated centrally and incubated during 28 days (three replicates per treatment). Growth rate (mm/day) was determined and mycotoxin production was analysed after the incubation period by HPLC-UV. All strains were able to growth at all temperatures and all a_w except at 0.90. Maximum growth was observed at 0.99 a_w and 25°C and it decreased as water availability was reduced. Minimum growth was observed at 0.93 a_w and 15°C. Mycotoxin production was strain dependent. Strains RCFG6046 and RCFG6076 produced both DON and NIV, being NIV the most produced, while RCFG6029 just produced DON. Strains RCFG6029 and RCFG6046 just produced DON at the optimum growth condition unlike RCFG6076 that produced only NIV. For some conditions, RCFG6076 was able to produce both toxins simultaneously in contrast to RCFG6046 that cannot produce both toxins at the same time. Maximum NIV production (9,796.5 µg/kg) was observed for RCFG6076 at 0.97 a_w and 30°C and the minimum level (1,075.57 µg/kg) was detected at 0.95 a_w and 15°C for the same strain. No NIV was detected at 0.93 a_W at any temperature. DON production was observed for all conditions tested. The highest DON concentration (2,954.35 µg/kg) was produced at 0.93 a_w and 30°C for strain RCFG6076 while the minimum was detected at 0.99 a_w and 20°C for the same strain. In conclusion, *F. cerealis* was able to produce both toxins in a wide range of a_w and temperatures, however, this production was strain dependent. Maximum levels were produced during stress conditions and NIV was produced in high levels. Considering that NIV is more toxic than DON, this presents a risk for human consumption since these strains were isolated from durum wheat.

SAMPLING AND ANALYSIS
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SENSITIVE AND ACCURATE QUANTITATIVE DETERMINATION OF AFLATOXIN M1 IN MILK FROM DIFFERENT SPECIES

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Aflatoxins are a group of mycotoxins produced by filamentous fungi belonging to different genera of *Aspergillus*, which grow on agricultural commodities pre- and postharvest at relatively high moisture contents and temperatures. All aflatoxins are classified as Group 1 carcinogens by IARC, because they are highly toxic, mutagenic, teratogenic, and carcinogenic compounds. Aflatoxin M1 (AFM1), the hydroxylated derivative of Aflatoxin B1 (AFB1), is formed as a metabolite in the liver and it is excreted into the milk in the mammary glands of animals that have been fed with AFB1-contaminated feed. Due to high consumption of milk in the human diet, contamination with AFM1 is therefore a relevant health concern, and for this reason regulatory limits were set worldwide for AFM1 in milk, ranging from 0.05 µg/kg in the European Community to 0.5 µg/kg according to the US FDA and the Codex Alimentarius. Different methods of analysis can be used by dairy industries, and screening methods like enzyme-immunoassays are a valid tool for the rapid and sensitive detection, and for high-throughput quantitation of AFM1. The objective of this study was to demonstrate that I'screen AFLA M1 ELISA kit, previously fully validated for the quantitative analysis of AFM1 in raw and powdered bovine milk, can be a proper screening tool also for sheep, goat and buffalo milk. Assay performance was evaluated on raw blank and spiked milk samples from different species collected in Northwest Italy and tested as whole as well as skimmed. Blank samples (n = 60 for sheep; n = 35 for goat; n = 20 for buffalo) turned to be < 5 ng/l, showing that no matrix effect is evident, neither in the presence of fat in the sample. Milk samples spiked at 5 ng/l were always detected, indicating high assay sensitivity. Further spiking tests carried out in the range 20-100 ng/ml, demonstrated good accuracy and precision, being the recovery 83 ± 5 % for sheep milk (n = 45), 118 ± 12 % for goat (n = 36), 116 ± 17 % for buffalo (n = 48), and the mean RSDr for all milk species ≤ 10%. Again, no significant difference in performance was observed due to fat presence in the sample. The collected data indicate therefore I'screen AFLA M1 as a sensitive, accurate and precise tool for the screening of AFM_1 in raw milk samples from different species.

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VALIDATION OF AFLATOXIN M1 FLUORESCENCE QUANTITATIVE RAPID TEST FOR MILK (15-150 PPT) FOR DETERMINATION OF AFLATOXIN M1 IN MILK

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The Aflatoxin M1 Fluorescence Quantitative Rapid Test for Milk (15-150 ppt) (Shenzhen Bioeasy Biotechnology Co., Ltd., China) was validated at ILVO under the AOAC Research Institute Performance Tested Method℠ program. The validation concerned evaluation of selectivity, recovery, bias, false-positive results, robustness, and suitability for various milk types and milks from various species. The test is selective for aflatoxins: high cross-reactivity was noted for aflatoxin B1, B2, G1 and G2 and limited for M2. The cut-off, discriminating samples with aflatoxin M1 concentrations possibly exceeding the maximum level (ML), is 36.9 ng AFM1/l (95% detection). The method demonstrated a 15% false violative rate at 25 ng AFM1/l, and a false positive rate of 3% based on 18 false positive samples upon a total of 300 farm and 300 tanker milk samples. It is worth noting that retesting of the presumptive positive milk samples resulted in 16 out of 18 cases in a negative result. Quantitative readings had a mean bias of -3.2 ng AFM1/l at 50 ng AFM1/l with a standard deviation of 5.9 ng AFM1/l. The test provides a quantitative value except for blank milk indicated as '<15 ng AFM1/l'. Hence the limit of detection (LOD) and limit of quantification (LOQ) were calculated following two different statistical approaches resulting in a LOD of 5.7 or 14.8 ng AFM1/l and a LOQ of 17.2 or 36.0 ng AFM1/l, respectively. Regarding test repeatability a standard deviation of 4.2 ng AFM1/l and a coefficient of variation of 11.2% was noted. These values are acceptable for a quantitative dipstick test. Regarding reader repeatability for three different readers low (≤1.7%) and acceptable coefficients of variation were obtained. The impact of milk quality (somatic cell count, aerobic plate count), milk composition (fat and protein content) and milk pH were tested. Out of the results could be concluded that the method is applicable to normal raw cows' milk with increased loss of detection capability for milk with a high protein content. Lowering the cut-off to 30.0 ng AFM1/l could be a solution. The Aflatoxin M1 Fluorescence Quantitative Rapid Test for Milk could be used for screening of milk for aflatoxin M1 at EU-ML level.

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THE EFFECTS OF GRIND AND EXTRACTION SIZE ON RESULT VARIABILITY FOR AFLATOXIN, DEOXYNIVALENOL, FUMONISIN AND ZEARALENONE

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Mycotoxins are found throughout the world in various concentrations. Several questions arise when discussing sampling, analytical sample size and sample grind size. Over the past years grind size and analytical sample size were evaluated for total aflatoxin, total fumonisin, deoxynivalenol and zearalenone. Sample preparation for products being tested for mycotoxins is a critical part of the analytical process. The difference in sample grind size, as well as the amount of sample extracted can also contribute to the overall result variability. Naturally contaminated materials were ground to various mesh sizes, homogenized and various sample sizes were extracted. The extractions performed were acetonitrile/water (84/16) for aflatoxin, deoxynivalenol and zearalenone, and methanol/water (3/1) for fumonisin. All samples were extracted on an Eberbach shaker. The extracts were then analysed by LC-MS/MS for deoxynivalenol and zearalenone, and HPLC for aflatoxin (AOAC 994.08) and fumonisin (AOAC 995.15). Data presented shows the effect grind size and sample extraction size has on each mycotoxin result variability.

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HOW GREEN ARE THE SAMPLE PREPARATIONS COMMONLY USED IN MULTI-MYCOTOXIN ANALYSIS?

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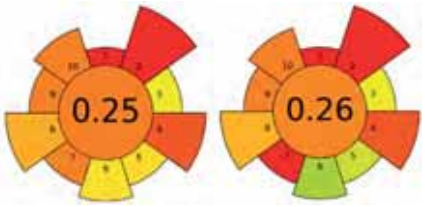
Sample preparation is a key step in the analytical procedure essential for the enrichment of mycotoxins to accomplish the legislation, the minimization of matrix interferences and/or to ensure compatibility with the measurement technique. However, sample preparations usually employed in multi-mycotoxins analysis present some disadvantages such as involving high volume of organic solvents and being time-consuming. This in disagreement with the new trends in Green Analytical Chemistry (GAC) that encourages to follow the 10 principles of Green Sample Preparation (GSP) [Trends in Analytical Chemistry 148 (2022) 116530]. In order to assess the greenness of different sample preparations used in multi-mycotoxin analysis, a new metric tool recently developed known as 'AGREEprep' was used. AGREEprep is an analytical greenness metric for the sample preparation step, which is a user friendly, intuitive, and free to download software. It is based on the 10 principles or criteria of GSP which are calculated from 0 to 1 scale sub-scores, and then, used to calculate the final assessment score, being 1 the greenest score possible [Trends in Analytical Chemistry 149 (2022) 116553]. In this work, we have selected and evaluated two sample preparation previously reported both based on a QuEChERS procedure [Journal of Chromatography A 1362 (2014) 145; Talanta 121 (2014) 263]. We have carefully checked the variables involved in each procedure and taking into consideration the criteria of the AGREEprep software, it has been calculated a score for each one. As can be seen in the pictograms, similar scores were obtained in both cases; 0.25 and 0.26. These scores are low due to the fact that reagents were neither from sustainable nor renewable sources, waste generation was high, many steps were involved during the process and the hazards of chemical were considerable. Slight differences were observed in terms of size of sample and sample throughput, as it is reflected in the color of criteria 5 and 6 of the pictograms, being favorable for the second proposed QuEChERS procedure [Talanta 121 (2014) 263]. To conclude, regarding sample preparation, these methodologies are not environmentally friendly and they could be improved reducing or replacing organic solvents with other green alternatives and also reducing waste in order to be in compliance with GAC.



P97

MULTI-MYCOTOXIN DETERMINATION IN APPLE PUREE SAMPLES BY HPLC-MS/MS

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In this work, we propose a simple and fast Solid Liquid Extraction (SLE) followed by liquid-chromatography coupled to tandem mass spectrometry detection (LC-MS/MS) for the simultaneous determination of mycotoxins in apple puree samples. Among them, patulin (PAT) has been the most commonly investigated mycotoxin in these samples. However, it is also important to consider the *Alternaria alternata* mycotoxins such as alternariol (AOH), alternariol monomethyl ether (AME) and tentoxin (TEN), and aflatoxins (AFB1, AFB2, AFG1, AFG2) which could be potential contaminants in fruits and fruit-based products. The European Union has set the maximum content of PAT in 25 µg/kg for apple puree and 10 µg/kg for infant products. The variables affecting the SLE were carefully optimized in order to reduce the organic solvent consumption and avoiding sample dilution. Thus, different organic solvents such as methanol (MeOH), acetonitrile (MeCN) and methyl tert-butyl ether (MTBE) were investigated. Afterwards, 4 ml of MeOH were selected as optimum using an extraction time of 10 min. The chromatographic separation was performed using a C18 SunShell column (2.6 µm, 2.1 i.d. x 100 mm) and a mobile phase consisted of MeOH with 0.2% acetic acid as solvent B and ultrapure water with with 0.2% acetic acid and 5 mM ammonium acetate as solvent A. Matrix-matched calibration curves were established in apple puree samples with limits of quantification (LOQs) below 0.1 µg/kg for AME, AFB1, AFB2 and TEN, 1 µg/kg for AOH, AG1, AG2 and 5 µg/kg for PAT. Precision, expressed as relative standard deviation (% RSD), lower than 15% and recoveries higher than 80% were obtained in all cases. Finally, the proposed method was applied for the analysis of 10 apple puree samples. PAT was found at a high concentration ranging from 1.5 to 2 mg/kg and being above the maximum content allowed. AOH and AME were also frequently found with the highest amount of 214 µg/kg and 340 µg/kg, respectively in the same sample. Moreover, TEN was found in the same samples but at low concentration levels. AFB1 and AFB2 were also found in one sample with a total content of 6 µg/kg. To sum up, the proposed SLE-HPLC-MS/MS method is an efficient and sensitive method for the simultaneous determination of mycotoxins in apple puree samples. In addition, this an environmentally friendly alternative if compared with those proposed sample preparations for multi-mycotoxins analysis.

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BEER ANALYSIS: MYCOTOXINS IN BREWING PROCESS BY LC-MS/MS

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Beer is one of the oldest and most consumed alcoholic beverage worldwide. It is a cereal-based product elaborated from malted barley, hops, yeast, and water, while wheat or other cereals also may be added. Various mycotoxins have been identified in the cereals used in beer preparation but, aflatoxins, ochratoxin A, trichothecenes of type A and B, fumonisins and zearalanone are commonly founded in these cereals. Other mycotoxins that may be present in these cereals are fusaproliferin, moniliformin, beauvericin, and enniatins produced by *Fusarium* spp. Fumonisin B1 and deoxynivalenol were most commonly contaminant found in beer and also significant concentrations of deoxynivalenol-3-glucoside, a metabolite produced by glycosylation of DON, was detected. Emerging mycotoxins are not routinely determined as they are not regulated by legislation. Due to their water solubility, mycotoxins can be present in every stage of the brewing process. For that reason, new, precise, and sensitive detection procedures are necessary to overcome this problem. Detection by liquid chromatography coupled with mass spectrometry (LC-MS/MS) is the most common, sensitive, and efficient method for mycotoxin analysis. Therefore, we have applied a very efficient chromatographic separation; significantly reducing the run time improved the detection sensitivity a lot and largely avoided signals due to interfering compounds. The analysis of different mycotoxins provides a good basis for the evaluation of these compound during the storage of cereals, the brewing process, bottling, distribution, sale, and consumption. Thus, with the analysis of mycotoxins by LC-MS/MS, we can ensure an acceptable level of quality for the starting materials, the intermediate products, and the the beer consumer.

P99

THE DESIGN, DEVELOPMENT AND IMPLEMENTATION OF AN LC-MS METHOD AS A 'TOXICITY ALERT SYSTEM' IN THE ANIMAL FEED SECTOR

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An LC-MS method utilising dilute-and-shoot (DnS) was developed and implemented for the analysis of the most important mycotoxins from a food safety perspective, with the final method consisting of 30 mycotoxins. The suite of mycotoxins includes those with regulated or guideline values as set by the European Food Safety Authority (EFSA) and the European Commission (EC), and some masked/modified versions of deoxynivalenol (DON) due to their potential reconversion to DON. In addition, the method contains the most important emerging mycotoxins as selected by an expert mycotoxin working group and which are under consideration from regulatory bodies such as EFSA due to their potential toxicological effects and presence in feed. A few natural toxins belonging to the ergot class have also been included due to legislation for these scheduled for implementation in 2024. After conducting a literature review on the use of DnS and its evolution to include the analysis of solid matrices such as feed/foodstuffs for mycotoxins, it was decided to implement this extraction and clean-up methodology alongside LC-MS/MS. The chromatography developed resolved the suite of mycotoxins with a run time of 13 minutes, incorporating both DON) and zearalenone (ZEN) radiolabelled internal standards to correct for matrix effects (SSE). The final methodology was applied to the analysis of complete animal feed for ruminants (dairy and beef) and poultry as part of a 'toxicity alert system' in collaboration with an industrial stakeholder in Northern Ireland, UK. Samples (ruminant and poultry feed) were tested on a monthly basis, with the results of the main mycotoxins of importance from an animal feed perspective, DON, ZEN and fumonisins B1 and B2, fed back to the stakeholder, with the industrial contact alerted if any of the aforementioned mycotoxins were above the guidance values for that commodity and species. Furthermore, levels of emerging mycotoxins detected were reported in order that trends could be monitored and the data used to help shape future guideline values. The results of our analysis correlated favourably against that of the Food Fortress programme currently being used by the industry at present, with both analyses showing a similar trend over time, indicating a positive result for our developed method. Furthermore, some of the emerging *Fusarium* mycotoxins, such as enniatins, specifically B and B1, as well as moniliformin, appear to indicate the highest levels over the period analysed and, therefore, should continue to be monitored.

P100

A NEW IMMUNOAFFINITY COLUMN WITH HIGH RECOVERY AND ORGANIC SOLVENT TOLERANCE FOR AFLATOXINS

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Aflatoxins are carcinogens and mutagens produced by certain moulds, such as *Aspergillus* species. Aflatoxin testing is routinely performed worldwide in many labs for food safety. Immunoaffinity column has been widely used during aflatoxin analysis. Most aflatoxin antibodies are developed against aflatoxin B1, it has less reactivity with aflatoxin G types, therefore aflatoxin G recovery is low in some assay condition particularly when high stringency washing and high contend of organic solvent is used. To achieve a high recovery for all aflatoxins B and G under high organic solvent conditions, VICAM has developed a new aflatoxin affinity column, Aflatest WB SR^+. Samples are extracted with 80% acetonitrile or 80% methanol, and extracts are diluted with phosphorate buffer (PBS), run through column. Aflatoxins are eluted and analysed by UPLC with florescent detector. Studies by serially increasing solvent in PBS have demonstrated that the highest content of acetonitrile and methanol can be used for Aflatest WB SR+ column is 30% and 40%, respectively, in PBS without significant affecting capacity of 500 ng total aflatoxins. The LOD and LOQ of aflatoxins for the testing procedure in corn sample are 0.007-0.037 ppb and 0.012-0.083 ppb, respectively. Results show a very high degree of linearity (r^2 = 0.9999) for aflatoxins with a test range of 2 to 500 ng. High recoveries (> 90% for B and G types) were also achieved in naturally contaminated samples. The VICAM new Aflatest WB SR+ column provides a new and reliable clean-up method for analysing aflatoxins and potential other complex matrix such as animal feed.

P101

EXPLOITING HYPERSPECTRAL IMAGING AND CHEMOMETRICS FOR THE RAPID ON-SITE MEASUREMENT OF AFLATOXIN IN CHILLIES

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Naturally occurring toxins (e.g., mycotoxins) remain one of the greatest food safety concerns globally. Chilli (*Capsicum* spp.) is one of the most important and largest produced spice in India, however, they are continuously susceptible to aflatoxin (AF) contamination at every stage of production due to the climatic conditions. High temperatures, high rainfall and humidity in these areas are the perfect conditions for fungal proliferation and toxin production. The main technological challenge is that AF testing commonly relies on analytical techniques, such as liquid chromatography with tandem mass spectrometry (LC-MS/MS) or high-performance liquid chromatography combined with fluorescence (HPLC-fluor), which are extremely accurate and sensitive. However, they are not applicable outside of the laboratory and require highly skilled and trained personnel to conduct. Immunosensor based tests such as, ELISAs and lateral flow tests (LFT) are also available. However, these tests require lengthy procedures and incubations, multiple steps, and the incorporation of recognition elements, which can be costly and ultimately lead to cross reactivity and/or matrix effects. Therefore, there is a demand to improve on-site analysis using rapid, portable, and cost-effective techniques. Hyperspectral Imaging (HSI) is rapid, portable and can provide enhanced information about the chemical and physical properties of samples through spatial imaging combined with spectroscopy. During this preliminary study, chilli samples from India (n = 300) were analysed using two analytical techniques. HSI was conducted alongside confirmatory analysis, LC-MS/MS to quantify levels of AF and kojic acid. The correlation between the two analytes was addressed and the LC-MS/MS results were fused with the spectral data obtained using HSI for statistical modelling. Several statistical models were developed using chemometrics to determine low, medium, and high-quality chillies and AF contamination with detection limits down to 20 ppb. A leave-30%-out cross validation was conducted to assess model predictability and accuracy. Overall, the results from HSI (combined with LC-MS/MS) and chemometric modelling revealed much promise for the future on-site determination of quality parameters (e.g., discoloration and degradation) and AF contamination in chillies. Future work will focus on improving datasets and model robustness.

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ANALYSIS OF AFLATOXIN AND OCHRATOXIN IN VEGAN FOOD PRODUCTS

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Pseudo cereals are seeds of non-grasses and are a staple in vegan diets. Examples of pseudo cereals include quinoa, millet, bulgur wheat and spelt grain. They are gluten-free and can therefore serve as a substitute for true cereals that cannot be consumed by people with gluten intolerances. Pseudo cereals have a high nutritional value, acting as a good source of protein, vitamins, and starch. Dairy-free alternative milks are also popular item for vegans and lactose-intolerant individuals alike. Examples of popular dairy-free alternative milks include hazelnut, almond, soya, pea, coconut, and oat milk. Both pseudo cereals and dairy-free alternative milks are susceptible to contamination with aflatoxin and ochratoxin and so methods are required for these commodities to ensure EU regulations pertaining to maximum mycotoxin concentrations are adhered to. This poster looks at methods using immunoaffinity clean-up with either single or multi-toxin columns; EASI-EXTRACT® AFLATOXIN, OCHRAPREP® and AFLAOCHRA PREP®, to analyse the concentration of aflatoxin and ochratoxin in pseudo cereals and dairy-free alternative milks. Results demonstrate that the developed methods meet the relevant acceptance criteria for each product with all %RSDs being below 20%. These methods are therefore suitable for the analysis of the relevant food products.

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ACETONITRILE EXTRACTION FOR THE ANALYSIS OF MULTI-TOXINS IN ANIMAL FEEDS

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EU regulations for mycotoxins are complex with varying limits applied to specific commodities. This has resulted in an increasing trend in multi-mycotoxin analysis within the food and feed industry. As a result, there has been greater demand for multi-toxin immunoaffinity columns to effectively remove sample

matrix from complex commodities such as animal feeds to ensure compliance with EU method performance criteria. This study summaries the validation of a new acetonitrile extraction prior to clean-up using a multi-toxin immunoaffinity column; 11^{+}Myco MS-PREP® for animal feeds such as silage and forage. A multi-toxin dried distillers grains (DDGS) reference material (Trilogy Analytical Lab, USA) was also included to assess the accuracy and reliability of this clean-up method. Samples were extracted and passed through n = 3 replicate IAC and calculated contamination, % recovery and % RSD were reported. For the reference material, the calculated contamination was corrected for recovery prior to reporting. Solvent-based calibration standards were used for quantification throughout and were compared against matrix-matched calibration standards to assess matrix effects. Excellent recoveries were obtained for all spiked animal feed samples and ranged from 75 to 103% with %RSD generally < 10 % demonstrating an accurate and reliable method that complies with EU method performance criteria (Commission Regulation (EC) No. 401/2006). Matrix effects were < 10% for all analytes, demonstrating excellent clean-up with 11^{+}Myco MS-PREP®.

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ASSESSMENT OF CITRININ IN SPICES AND INFANT CEREALS USING IMMUNOAFFINITY COLUMN CLEAN-UP PRIOR TO HPLC-FLD

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Citrinin (CIT) is a secondary fungal metabolite produced by several species of the genera *Aspergillus*, *Penicillium*, and *Monascus*. It commonly effects cereals and red yeast rice but more complex matrices like spices and infant foods are becoming more widely assessed. Methods were therefore developed for the analysis of spices and infant food deploying immunoaffinity clean-up with HPLC-FLD. Extraction of CIT from samples was conducted by blending with 75% methanol, filtration, and dilution prior to IAC application. The methods were validated by assessing recovery from samples spiked at 0.2, 0.5, 1 and 2x legislative level of a similar mycotoxin (ochratoxin A, OTA). The method for spices provided a recovery between 81.8-85.4% with a maximum %RSD of 2.56. Similarly, the method for infant food gave a recovery between 102.2-108.4% with a maximum %RSD of 8.31. The methods were then applied to a variety of samples: cinnamon, nutmeg, ginger, paprika, turmeric and chilli for spices and semolina, baby rice, oatmeal, spelt cereal, and multigrain porridge for infant foods. Recoveries ranged from 77.2-99.5% for spices and 77.5-126.5 for infant foods. It was noted during analysis that the nutmeg and multigrain porridge contained significant concentrations of CIT naturally, above legislative level for OTA. This highlights the importance of monitoring these matrices for CIT.

P105

IMPROVING EFFICIENCY AND ANALYTICAL OUTCOMES FOR THE BUSY LABORATORY USING THE CHRONECT SYMBIOSIS RIDA®CREST ROBOTIC SYSTEM FOR AUTOMATED ANALYSIS OF MYCOTOXINS

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Busy laboratories normally have high numbers of analysis to perform combined with a lack of time and resources. This can lead to mistakes at the bench resulting in reduced quality and performance of analysis. Laboratories are often tasked to improve efficiency without increasing headcount or reducing the quality of results. This poster demonstrates the benefits of the new CHRONECT Symbiosis RIDA®CREST system, a fully automated clean up system which uses IMMUNOPREP® ONLINE affinity cartridges for testing a range of mycotoxins from sample extraction to final detection. The system offers a front-end solution and combines with most detectors including UV fluorescence or mass spectrometry with different modules to meet individual laboratory needs. Each affinity cartridge is suitable for up to 15 analyses, saving space and offering the possibility to include a QC check or sample blank to meet the strictest accreditation standards. The system with IMMUNOPREP® ONLINE affinity cartridges is shown to remove sample interference leading to improved chromatography and better sensitivity. Manual steps are precisely controlled, decreasing analytical error, and significantly reducing data variability leading to improved performance and greater laboratory efficiency.

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ANALYSIS OF AFLATOXIN AND OCHRATOXIN IN CHEESE USING IMMUNOAFFINITY CLEAN-UP PRIOR TO FLD-HPLC DETECTION

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Cheese is a very popular food item around the world and is available in several varieties, such as cheddar, cottage cheese, blue cheese, gouda, and parmesan. Like cereals, spices and fruits, cheese is also susceptible to mycotoxins. Although it is rather obvious that aflatoxin M1 contaminated milk used in cheese-making will lead to the presence of aflatoxin M1 and is therefore common to analyse for this mycotoxin in various cheese samples the presence of ochratoxin A in the milk of ruminants occurs less frequently and therefore may not always be analysed. Recently, a risk assessment has identified low levels of ochratoxin A in blue cheese. Considering this discovery, there has been discussion regarding the introduction of new legislation for the detection of ochratoxin in cheese products to ensure that levels remain low. Suitable methods are well established for the analysis of aflatoxin M1 in a range of cheeses however it is now worth considering such methods for the analysis of ochratoxin A. A new method for the analysis of ochratoxin A has been developed by R-Biopharm which utilises an immunoaffinity column clean-up which has the benefit of isolating and concentrating the toxin from the complex cheese matrix enabling detection at low levels. The recoveries for both mycotoxins were found to be consistently above 80% and RSD were less than 7%. In conclusion, both methods provided precise and reliable results and should be considered suitable for the analysis of either aflatoxin or ochratoxin in various cheese samples at low levels.

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POSTER WITHDRAWN BY AUTHOR

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MOLECULARLY IMPRINTED POLYMERS APPLIED TO THE SIMULTANEOUS COEXTRACTION OF ZEARALENONE AND ALTERNARIOL MYCOTOXINS FROM OIL SAMPLES

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Molecularly imprinted polymers are tailor made materials containing specific recognition cavities with a predetermined selectivity for a specific analyte or group of similar analytes. In order to broaden up the application of these materials, in this work, we report the optimization of MIPs for the simultaneous pre-concentration of zearalenone (ZEN) and alternariol (AOH) from oil samples using molecularly imprinted solid phase extraction (MISPE). The polymers were prepared with *N*-(2-aminoethyl) methacrylamide as functional monomer, methacrylamide as co-monomer and ethylene glycol dimethacrylate as cross-linker. Two template molecules, namely, 3,8,9-trihydroxy-6H-dibenzo[b,d]pyran-6-one and cyclododecyl 2,4-dihydroxybenzoate, were used as surrogates of AOH and ZEN, respectively, for MIP synthesis. The MISPE method was optimized using a chemometric approach that maximized the selective retention of both mycotoxins in the MIP vs. the NIP. In the optimized conditions the cartridges, filled with 150 mg of a mixture (50:50, w/w) of the AOH/ZEN selective MIPs, were loaded with 30 ml of sample, followed by a washing step with 2 mL of ACN/water (20/80, v/v) and elution with 2.5 ml of trifluoroacetic acid/MeOH (3/97, v/v). The extracts were analysed by HPLC coupled to a fluorescence detector (FLD). No cross-reactivity was observed in the presence of mycotoxins from other families. The optimized MISPE-HPLC-FLD method, has been applied to the analysis of the mycotoxins in oil samples (2 g) with a limit of detection of 2 and 5 µg/kg, respectively. Recoveries were in the range of 94% to 113% (RSD < 5%, n = 6) for AOH and 92% to 108% (RSD < 6%, n = 6) for ZEN in the analysis of spiked maize oil samples. The method has been validated based on Commission Decision 2002/657/EC. The results have been confirmed by HPLC-MS/MS. **Acknowledgements**. Work funded by the Spanish MCIN (grant RTI2018-096410-B-C21). TMC thanks the MCIN por a predoctoral grant.

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COMPARATIVE ANALYSIS OF AFLATOXINS IN DAIRY FEEDS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH A FLUORESCENCE DETECTOR AND LIQUID CHROMATO-GRAPHY/TANDEM MASS SPECTROMETRY

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Aflatoxins, naturally occurring secondary metabolites of some *Aspergillus* members belonging to section *Flavi,* have detrimental effects on both humans and animals even at very low concentrations. As a result of this detrimental effect, robust methods have been developed for precise detection and quantification of aflatoxins in feed and food matrices. In this study, the closeness of two methods namely high-performance liquid chromatography with a fluorescence detector (HPLC-FLD) and liquid chromatography/tandem mass spectrometry (LC-MS/MS) detection and quantification of aflatoxins B1, B2, G1, and G2 in dairy feed samples were compared. Aflatoxins were detected in all the feed samples analysed, in the range of 0.177-176.279 µg/kg (average = 25.912 ± 41.522 µg/kg) for total aflatoxin by HPLC-FLD whereas for LC-MS/MS total aflatoxin range was 0.000-111.88 µg/kg (average = 16.453 ± 27.221 µg/kg) and the detection was in only 60% of the samples. One sample t-test gave $P = 0.00$ ($P < 0.05$) showing significant differences at 95% confidence level in the total aflatoxin means obtained using the two methods. Recovery rates for LC-MS/MS (30.8-36.6%) were much lower than those for HPLC-FLD (85%). The low recovery in LC-MS/MS extraction could have resulted from the inability to achieve high extraction efficiency of all individual mycotoxins in a single extraction as they have different physicochemical properties, such as polarity and solubility, as well as the absence of an ultraviolet detector, a major parameter required for aflatoxin detection. Correlation analyses results showed a significant positive linear relationship between the two analytical methods for individual and total aflatoxin analysis with $P < 0.01$. Regression analysis showed the highest level of agreement between the methods for aflatoxin G1 samples (r^2=0.823), followed by B2, AF_{total}, B1 and G2 (0.409, 0.397, 0.182, and 0.173, respectively). These results shows that HPLC-FLD remains the gold standard for aflatoxin detection and quantification. However, LC-MS/MS can be used for aflatoxin screening followed by verification and quantification by HPLC-FLD.

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OTA VIA 2D-LC-MS/MS: AN ELEGANT ALTERNATIVE FOR HIGH-THROUGHPUT OCHRATOXIN A ANALYSIS

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Extraction and detection of ochratoxin A (OTA) in food matrices remains a difficult task. Sugary dried fruits, polyphenol-rich coffee and highly pigmented spices are just few examples that require good purification methods. To find an efficient and cost-effective overall procedure, protocols based on immunoaffinity column (IAC) clean-up followed by liquid chromatography and fluorescence detection (LC-FD) were compared with simple QuEChERS (quick, easy, cheap, efficient, rugged, safe) extraction and separation by two-dimensional LC and mass spectrometric detection (2D-LC-MS/MS).
Purification by IAC enables good matrix separation, concentration of the analyte, and is extremely specific. Thus, even with inexpensive LC-FD detection, the demanded method requirements of Commission Regulation (EC) No 401/2006 are fulfilled. Otherwise, antibody-based columns can be subject to batch variations. Furthermore, the IAC accounts for ~84% of the total consumable costs, which are crucial for the total test costs. Antibodies used for immunoaffinity columns tolerate only certain pH values and are not compatible with organic solvents, especially in higher concentrations. Therefore, extracts must be diluted with buffer. However, the column feed volume limits the dilution factor particularly for high-throughput analysis with liquid handlers. A QuEChERS-based extraction with 2D-LC-MS/MS measurement was tested as alternative. Since tandem mass spectrometry is highly selective as well as specific, rapid, and cheap sample preparation can be applied. Without antibody-based steps, pure organic extracts do not pose a problem. QuEChERS extraction and phase separation were optimized with respect to the recovery rate of OTA. As mass spectrometry is susceptible to matrix effects, good chromatographic separation of analyte and matrix is required before ionization to allow sensitive measurements. Different chromatography columns and eluents were tested to optimize both 1D- and 2D-separation. Due to the high price of a 2D-LC-MS/MS system, the chromatographic run time has the greatest impact on test cost. By establishing a timesaving measurement method (~ 8 min), the

instrument costs per sample remain within a manageable range when the instrument is highly utilized. Due to approx. 40% lower consumable costs and shorter sample preparation times, the advantages of the new method outweigh those of the IAC method in a high-throughput lab. The validation for different coffee matrices, e.g., instant, roasted and raw coffee, as well as grain is currently performed in our laboratory and shows promising results. In summary, 2D-LC-MS/MS is a powerful analytical tool for OTA quantification as matrix effects are reduced to a level comparable with the more costly immunoaffinity column clean-up step.

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Z-SCORES ARROW RANGE (ZSAR) METHOD – ASSESSMENT OF TRUNCATED MYCOTOXINS VALUES IN PROFICIENCY TESTING

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Trilogy Europe B.V. organizes proficiency tests (ISO 17043 accredited) and provides reference materials (ISO 17034 accredited) in the food and feed area. The focus of Trilogy Europe B.V. is on analytical, physical, microbiological, and microscopic quality assurance for laboratories and companies to monitor, guarantee and improve their quality. Proficiency tests are an essential part of any laboratory quality assurance system. Proficiency tests are mandatory to obtain or maintain your laboratory accreditation and recognition. Each proficiency test provides your result with a Z-score allowing you to decide if any corrective actions are needed. But how to access your score if your proficiency test results are truncated values (< LOD or cut off values)? The ISO standard 13528:2015 describes three options how to deal with truncated results in statistical calculations. All these three approaches have their (dis)advantages but could also lead to misinterpretation by the participating laboratories. Furthermore, the chosen PT providers approaches could lead to contradictories Z-scores. Trilogy Europe B.V. uses the Z-Scores Arrow Range (ZSAR) method [Accreditation and Quality Assurance 20 (2015) 355] for truncated values. This ZSAR method is based on a *z*-score range between the lowest possible *z*-score and the highest possible *z*-score. This information gives the participant a good, reliable, and independent indication if the values of the truncated values are in the range of $|z| = 2$ and $|z| = 3$. The ZSAR method is an excellent additional quality tool in proficiency tests. Displayed graphically and tabularly, it gives you the opportunity to see easily if your truncated values are comparable with other participants, or that the used preparation method and/or technique (adjustment) is not sufficient and needs to be improved. In this poster, we will present and discuss the ZSAR method in proficiency testing for mycotoxins quality management purposes.

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PREVALENCE OF PATULIN IN FRUIT JUICES AND BABY FOODS AS DETERMINED BY A NOVEL ELISA

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Patulin (4-hydroxy-4H-furo[3.2-C]pyran-2(6H)-one) is a toxic polyketide metabolite produced mainly by *Penicillium expansum*. Although it can occur in infected fruits, grains, and other foods, the main route of exposure to patulin is through the ingestion of infected apples and some of its derivatives, such as juices and compotes. The Joint FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization) Committee of Experts on Food Additives (JECFA) established in 1995 a provisional maximum tolerable daily intake (PMTDI) for patulin of 0.4 µg/kg of body weight/day. Based on this recommendation, the European Union has established maximum allowed levels of patulin of 50 µg/kg for juices, 25 µg/kg for apples, and 10 µg/kg for foodstuffs intended for children consumption. This study evaluates the Eurofins Abraxis Patulin ELISA test kit for the rapid quantitative analysis of patulin, as a means of obtaining reliable results (up to 41 samples) in 3 h without the need of expensive analytical instrumentation and with minimal operator training. We show that this ELISA has an LOD of 7 µg/kg and an LOQ of 10 µg/kg and characterize commercially available fruit juices and baby foods to assess the potential health risk from this mycotoxin.

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MACROECONOMIC EVALUATION OF MYCOTOXINS AND NUTRITIONAL COMPOSITION IN STORED MAIZE THROUGH NIRS

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High-efficiency production requires previous economic evaluation of ingredients for suitable destination, therefore, fast technologies are highly needed in storage units. The storage process is dynamic and may trigger mycotoxins production and changes in nutritional characteristics; knowing the asset before its use allows the correct destination and economic evaluation. This work conducted the analytical monitoring of maize stored in 71 silos and nine warehouses in a cooperative located in Paraná State, Brazil, totalling 455 thousand tons. Data related to mycotoxicological contamination (aflatoxin B1, fumonisins B1 and B2, deoxynivalenol, zearalenone and ochratoxin A) and determination of a_w and nutritional assessment (crude protein, moisture, starch, ether extract, mineral matter, crude fibre, and apparent metabolic energy in poultry) were analysed through near-infrared (NIRS) technology; physical classification (moisture, impurities, broken grains, damaged grains, and rotten grains) was also performed. Due to heterogeneous distribution of mycotoxins in the grain mass, sample collection followed a protocol that varied with the unit size. A pneumatic probe was introduced into the grain mass, at several points, collecting samples from all depths, top to bottom, thus making them as representative as possible. The sample generated at each point was physically classified and then fully ground in a crusher (3 mm sieve). The generated volume was reduced in a Jones-type splitter to ~ 500 g. Afterwards, a final grinding was done (1 mm sieve) and reading was performed on NIRS. All silos and warehouses were registered in the data management system and the concentrations and criteria referring to the analysed parameters were established by the Company as low, medium, and high. The results were mathematically equalized according to their importance in terms of animal species and production stage, resulting in the cost of formulating the diets. Thus, each sampling point was represented by colours that varied with the contents found in the samples, as well as the visualization of the degree of risk for each mycotoxin in the given species and production phase. The average cost ton/feed (broilers, starter stage) considering the variability of the evaluated parameters and the inclusion of antimycotoxin additive, was US$ 370.56 (range US$ 367.47 – 374.26). This new reality makes it possible to select the storage units offering the ideal raw material for the specific animal category, thus allowing the decision to be based on proper and more economical information, with speed and low investment cost, promoting health and the rational use of maize.

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EXTRACTION OF SMALL MOLECULE ANALYTES FROM A DRIED BLOOD SPOT CARD MATRIX FOR STABLE AND AMENABLE TOXICOLOGICAL ANALYSIS

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Chronically occurring mycotoxicity have significant economic repercussions on feed and animal production systems, more often involving lost in productivity and attrition of feed operations. Dramatic advancements have been made in programmatic prevention of mycotoxin exposure, but the lynchpin of these measures' effectiveness lies in the ability of accounting for exposure in the animal and detection and quantitation of selected mycotoxins. Often, logistical roadblocks exist that make toxicological sampling difficult or even impossible by traditional means, brought about by sub-optimal sample storage conditions, cost, and slow sample transit times to extra-regional or international test facilities. Here, we describe a detailed adaptation of a robust methodology for the efficient extraction of mycotoxins from a dried blood spot sample preserved on an absorbent paper card matrix (DBS) and subsequent validation using UPLC-ESI(+)-IMS-qTOF-MSE (Waters Corp.) following the ISO 17025 normative reference guidelines. Fruition of this benchtop protocol will allow for simple, inexpensive, and convenient sample collection, shipment of toxicological samples, and stable, long-term storage of whole blood or blood fraction samples, uniform sample extraction and analysis of biological material after weeks in transit or storage at ambient temperature and low moisture with very few other requisites. Blood samples (50 μl) were collected from different animal sources and immediately deposited on DBS cards using a small gauge needle and syringe or haematocrit capillary tubes, and immediately sealed in foil pouch envelope. At reception, cards were punched with 3 mm excision punch and extracted with a water:acetone: acetonitrile (30:35:35, v/v/v) overnight at room temperature. Samples were then concentrated to dryness

and reconstituted with methanol:water (60:40, v/v), followed by centrifugation. Samples were placed in LC vials and UPLC-eluted using a reverse phase CORTECS® C18+ column over a gradient of water/methanol, 0.1% formic acid. Under blind test conditions, blood samples spiked with different levels of deoxynivalenol (1 to 20 μg/ml) were tested. Matrix match evaluation determined a +36.3% signal suppression for deoxynivalenol. Calibration's linear regression correlation coefficient was >0.9914 with a dynamic range of 7.0 to 2830 μg/ml. Coefficient of variations for intra/inter-day precisions were 9.5% and 10.9%, respectively. Extraction efficiency evaluated in triplicate for low to high concentrations averaged 84.5%, and accuracy 91.1%. If the primary challenge associated with this method development arose from physical and chemical complexity of blood specimens, we were able to develop and validate a method using a DBS application for the correct determination of deoxynivalenol, which could find direct application in controlled animal trial experimentation.

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EVIDENCE BIOCHIP TECHNOLOGY – A MULTI-ANALYTE SEMI-QUANTITATIVE ANALYSIS FOR 6 MAIN GROUPS OF MYCOTOXINS IN MAIZE

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The continuous development of knowledge and availability of the most up-to-date risk assessment reports on co-exposure, effect of low-level cocktails, metabolites and conjugated forms of predominant mycotoxins is of pivotal importance for both human and animal food chains. The need for testing is consequently important. Maximum limits and/or guidance levels are well established for aflatoxins, fumonisin, deoxynivalenol, zearalenone, ochratoxin and T-2/HT-2 toxins across various global jurisdictions. Generally, most commercial technologies available to the industry lack both multiplexity and validation to required standards, which limits their screening capacity and reliability. Therefore, the availability of multi-analytical screening methods is beneficial to maximize mycotoxin detection in testing settings. This study summarizes the performance data following Commission Regulation (EU) 519/2014 of Myco 6 Array, utilizing biochip array technology, thus allowing multi-mycotoxin screening of 22 toxins from a single maize sample. Maize is one of the first matrices validated on Myco 6 due to its highest global production of all the cereals. Myco 6 Array employs six simultaneous chemiluminescent immunoassays, defining discrete test regions on the biochip surface. The Evidence Investigator biochip analyser was used and enables the analysis of up to 54 biochips at a time. Mycotoxins were extracted from corn by a single generic solid/liquid extraction. Screening results were semi-quantitative. Myco 6 Array presented broad specificity profiling allowing detection of aflatoxins, fumonisins, ochratoxin A, deoxynivalenol, zearalenone, T-2 toxin and HT-2 toxin. The limits of detection ranged from 1 ppb for aflatoxin B1 to 200 ppb for fumonisin B1. Nineteen maize quality control materials (65 measuring points) provided by Trilogy, FAPAS and BIPEA evaluated on Myco 6, were all within Z-score concentration ranges and showed perfect recovery with 91% samples passing recovery criteria for confirmatory methods specified under Commission Regulation (EU) 519/2014. Out of the 19 samples tested, 11 presented multi-mycotoxin contamination. In Conclusion, Myco 6 Array allows multi-mycotoxin screening of both well-established and emerging mycotoxins from a single corn sample. Myco 6 array provides a unique commercial market analytical solution for the detection of either single or multi-mycotoxin contamination. The methodology is rigorously validated according to EU regulations applying both naturally contaminated samples and CRMs. Innovative multiplex technology is the future of mycotoxin control, which gives an opportunity to adjust to future legislations, including decreasing maximum limits or increasing spectrum of mycotoxins being under control.

CAPACITY BUILDING
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P116
FOODSAFETY4EU: A STEP TOWARDS IMPROVED ENGAGEMENT, COOPERATION, AND TRANSPARENCY IN THE EUROPEAN FOOD SAFETY SYSTEM
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Food safety is a top priority for the European Commission, which policies aim at sustaining a high level of protection of human health and consumers' interests, while ensuring an effective functioning of the internal EU market. Under the new transparency regulation (Regulation (EU) 2019/1381), the EU-funded FoodSafety4EU project (www.foodsafety4.eu) kicked off in January 2021, represents a significant step for the European Food Safety System (FSS) towards more transparency, better engagement and closer cooperation. FoodSafety4EU aims at designing, developing, and releasing a multi-stakeholder platform for the future European FSS. This platform is gathering a network of FSS actors at national, European, and international levels, with the goal of providing scientific advice and technical support for EU food safety policies, by enabling actors to access, share and exchange scientific knowledge, resources and data more efficiently, better synchronise food safety research strategies, and contribute to a more transparent communication regarding the FSS. Through a structured, digitally supported, participatory process, the platform hosts the co-design of future research and innovation strategies, as well as risk communication models tailored to the specificities of various target groups. For example, through so-called Food Safety Operational Labs, multi-actors workgroups find new ways to tackle selected food safety challenges, e.g., food contact materials, mycotoxins, consumer practices education, perception of the role of industries in food sustainability, etc., following a continuous process, from co-designing to implementing and evaluating relevant pilot actions. By engaging EU citizens in these structured dialogues, FoodSafety4EU supports a clearer understanding of the scientific evidence at the base of any risk management decision, thus enhancing public confidence in the European FSS. The FoodSafety4EU Network currently consists of 23 consortium partners and around 50 stakeholders – food safety authorities, consumer associations, academia, research centres and networks, food industries and sector associations, thinktanks, etc. This community is expected to grow into a collaborative European Food Safety Forum by the end of the project, late 2023. **Acknowledgements**. This project has received funding form the European Union's Horizon 2020 research and innovation programme under grant agreement No. 101000613.

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MYCOTWIN – ENHANCING RESEARCH AND INNOVATION CAPACITY OF TUBITAK MRC FOOD INSTITUTE ON MANAGEMENT
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MycoTWIN is an EU H2020 funded project, which aims to significantly strengthen the toxigenic fungi and mycotoxins field of research in TUBITAK (an institute from a widening country) by linking it with the National Research Council of Italy and University of Valencia, two internationally leading research institutes in Member States Countries. MycoTWIN will support the entire value chain from cropping system to cultivation, harvest, storage, transport, processing of crops and their consume, since all these can be affected by mycotoxins contamination. This multi-actor partnership approach will integrate the EU Commission food safety policy as well as relevant supranational guidelines (e.g., CODEX). MycoTWIN will provide knowledge and experience transfers on mycotoxigenic fungi and mycotoxins achieved from other FP7 and H2020 projects (e.g., MycoRed and MycoKey), and clustered in three operational areas: (i) integrated information system; (ii) toxigenic fungi and mycotoxin monitoring; and (iii) prevention, intervention and remediation. MycoTWIN will enhance the scientific and technological capacity of all three institutions in this specific field with a principal focus on TUBITAK. Five specific objectives have been identified: (i) to increase knowledge, experience, and skills of research staff with a specific focus on involvement of early stage researchers through exchange of knowledge among project partners with scientific activities; (ii) to achieve rapid and effective communication with relevant parties/stakeholders in the field of mycotoxigenic fungi and mycotoxins; (iii) to provide effective dissemination and exploitation of MycoTWIN outcomes by organizing scientific events open to all parties/stakeholders including industrial/commercial user groups; (iv) to raise awareness on MycoTWIN

through external scientific events and scientific papers; and (v) to strengthen cooperation and formulate scientific strategy among the project partners for further researches in the field of mycotoxigenic fungi and mycotoxins. Collaborations within MycoTWIN consortium, with external academia partners and stakeholders are crucial for the creation of future collaborative projects in the Mediterranean area and team works among Mediterranean research institutions and industries. **Acknowledgements**. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 952337.

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IMPLEMENTATION OF THE NET-MAP ANALYSIS TOOL TO UNRAVEL THE SCIENCE-POLICY-SOCIETY COLLABORATION SYSTEM IN MYCOTOXIN RISK ANALYSIS

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The current legal framework of European risk analysis is undergoing significant change; risk assessment and risk communication have been specifically targeted in Regulation 2019/1381 on the transparency and sustainability of the EU risk assessment in the food chain. Therefore, developments in risk assessment should be supported and a legitimate platform is needed to establish a dialogue between risk assessment and risk management by developing tools for evaluating procedures and enforcement practices and analysis of Science-Policy-Society collaboration systems. This is one of the multiple goals of FoodSafety4EU, a Horizon 2020 collaborative action focused on the design, development, and release of a multi-stakeholder platform for the future European Food Safety System. Currently, many challenges are affecting European food safety regulations. Through interviews with European food safety authorities, issues regarding (emerging) mycotoxins were often highlighted as one of these challenges. Occurrence data for emerging mycotoxins are lacking, and the uncertainty on the tolerable daily intake calculation is large. Moreover, many gaps regarding their metabolites and modified forms remain, for instance, data gaps on chemical structure and toxicokinetics. Therefore, several steps in risk analysis are incomplete, and this case was used to identify stakeholders involved in this risk analysis and how they interact through a social network analysis tool called Net-Map. Net-Map is an interview-based mapping tool that can be used to visualise implicit knowledge and understand the interplay of formal and informal networks, power relations, and stakeholders' goals; uncover sources of conflicts as well as potentials for cooperation; facilitate knowledge exchange and develop visions and strategies to achieve common goals. The original Net-Map tool from Schiffer and Hauck [Field Methods 22 (2010) 231] was adjusted and digitalised to map the stakeholders and their relations in the Science-Policy-Society collaboration system in risk analysis of emerging mycotoxins and to identify constraints in relations, resources, and capabilities. Two Net-Map workshops were organised online in Italy and Czech Republic. First, stakeholders and their main goals in mycotoxin risk analysis were identified. The next step was to characterise how the stakeholders were linked in the system, followed by an assessment of the stakeholders' influence. Finally, a discussion took place on constraints compromising the risk analysis. The workshops resulted in complex Net-Maps, visualizing how the SPS collaboration system regarding mycotoxin risk analysis is set up. The obtained Net-Maps will be presented on the poster, including identified constraints in the SPS collaboration system. **Acknowledgements**. This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement No. 101000613.

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FIVE YEARS OF MYTOX-SOUTH®: ACHIEVEMENTS OF A GLOBAL PARTNERSHIP

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The majority of the world's food crops are contaminated with mycotoxins. These mycotoxins have many dangerous effects on human health and are an important source of food loss. The most affected crops are those being produced in low- and middle-income countries, which already face food safety related challenges due to poverty, population growth and climate change. Furthermore, mycotoxins have a substantial economic impact, depriving low- and middle-income countries from trading with the rest of

the world. While researchers have taken enormous steps forward in mycotoxin analysis, prevention, and mitigation strategies, low- and middle-income countries still lack awareness and have limited capacity to implement legislation, prevention, and monitoring systems. To tackle this mycotoxin problem, an international approach is needed, which is offered by MYTOX-SOUTH®. MYTOX-SOUTH®, founded in 2017, harnesses the expertise and infrastructure available at Ghent University and partner institutions to strengthen the worldwide capacity to tackle the mycotoxin problem and the associated food safety and food security issues. Co-creation between partners in the 'North' and 'South' has become priority. Aiming to build human capacity, bridge the gap between different actors and create a strong international network, many activities were organized, of which a selection is presented. Traineeships to several students from different low- and middle-income countries were granted, allowing them to learn about mycotoxin analysis, operate analytical machinery, work in a lab environment and network with many other students from different cultures. Moreover, training sessions were organized in partner countries, for instance the Farmer's Lab in Soweto, South-Africa. Efforts have been made over the 5 years to expand the network, and strategic and sustainable partnerships were established (2017 = 30 partners; 2022 = 47 partners), including 5 Ghent University faculties, 2 USA partners, 3 China partners, and 6 EU partners. Moreover, MYTOX-SOUTH® consists of 18 Sub-Sahara African and 4 Latin-American partners. Our network will be further broadened and extended to South-East Asian countries as well. MYTOX-SOUTH® launched 2 surveys and 2 international webinars in order to map the existing mycotoxin legislation and constraints in Latin-America and Africa, respectively. This research will enable the network to take action on the identified gaps, in order to improve the food safety system of the future. During the five years of its existence, MYTOX-SOUTH® managed to meet the initial goals and is ready to further increase its societal impact in the future. **Acknowledgements**. MYTOX-SOUTH® receives funding from VLIR-UOS and Ghent University Global Minds.

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HOW TO GET A GENDER-EQUAL APPROACH TO AFLATOXIN TRAINING IN AFRICA? AN ETHIOPIAN CASE STUDY

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Ethiopia is one of the countries with the lowest gender-equality performance in sub-Saharan Africa being ranked 121/134 in terms of the magnitude and scope of gender disparities by the United Nations Women Organisation. Within the farming communities, women are suspected to represent 70% of the labour force. However, historically mycotoxin training events run by Ethiopian Universities (e.g., Haramaya University) has been attended by men only. The objective of this project was to interview Ethiopian women to understand how we could develop mycotoxin-training systems that can reach Ethiopian women. Three hundred and fifty-two women were surveyed from two Ethiopian region producers of maize and peanuts. The survey included questions on socio-economic background, farming practices, mycotoxin knowledge, training availability, and the impact of the COVID-19 pandemic. The first survey results are showing that 48.15% of women answered positively about knowing what mycotoxins are. Among these, 80.85% reported that mycotoxins are an organism (living or dead) and 71% stated that aflatoxins have no impact on health. Moreover, only 44.97% acknowledged that they could do something to prevent exposure. Further analysis revealed that only 0.24% of women have been trained on mycotoxins mitigation techniques. Further studies are in progress to develop and propose training routes for women in Ethiopia with potential application throughout Africa. **Acknowledgements.** This research was supported by a Global Challenges Research Fund Quality-Research to Cranfield University.

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