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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.

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COMMITTEES

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

Local conference chairs
Prof. Sarah De Saeger  
Ghent University, Belgium  
Prof. Marthe De Boevre  
Ghent University, Belgium

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Dr Roger Pero-Gascon – Dr Esther De Rycke  
Ghent University, Belgium

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Cargill R&D Centre Europe, Belgium  
Prof. Hans-Ulrich Humpf  
University of Münster, Germany  
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Foshan University, China  
Dr Fernando B. Luciano  
Pontifícia Universidade Católica do Paraná, Brazil  
Prof. Angel Medina-Vaya  
Cranfield University, UK  
Dr Monique de Nijs  
Wageningen Food Safety Research, the Netherlands  
Dr Isabelle Oswald  
INRAE, France  
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Barilla, Italy  
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Canadian Grain Commission, Canada  
Dr Neriman Yilmaz  
University of Pretoria, South Africa  
Dr Guangtao Zhang  
Mars, China

About The World Mycotoxin Forum®
The main objectives of The World Mycotoxin Forum® are…..
- to provide a unique platform for the food and feed industry, regulatory authorities and science
- to exchange information and experiences on the various aspects of mycotoxins
- to review current knowledge related to mycotoxins in food and feed
- to discuss strategies for prevention and control of mycotoxin contamination ensuring the safety and security of the food and feed supply, and protecting human and animal health to promote solutions for the control of mycotoxin contamination along conventional and organic supply chains.
- for a sustainable, safe, and inclusive food future!
WELCOME TO ANTWERP

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 14th conference of the World Mycotoxin Forum® – WMFmeetsBelgium – 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        Local conference chairs
Rudolf Krska                     Sarah De Saeger
Chris Elliott                    Marthe De Boevre

About the venue

WMFmeetsBelgium will be held in ‘A Room with a ZOO’, a state-of-the-art conference centre located in the heart of Antwerp. ‘A Room with a ZOO’ is part of the Antwerp ZOO Society. Profits of congresses and events immediately support efforts on cherishing nature through animal welfare, education and awareness building. Your participation in WMFmeetsBelgium in ‘A Room with a ZOO’ contributes directly to nature conservation. In ‘A Room with a ZOO’, your engagement cherishes nature.

About Antwerp

Antwerp is an important cultural and trading centre, and home to more than 170 different nationalities. With more than 500,000 residents, Antwerp is Belgium’s second most populous city. As the city with one of the most exciting restaurant scenes, train station and ZOO in the world, Antwerp holds many more trump cards, such as Rubens, Plantin, diamonds, chocolate, fashion, museums, and Belgian beers.
SOCIAL EVENTS

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

Sunday 8 October 2023
18:30 – 20:00

The Welcome Reception – provided by R-Biopharm – will be held at ‘Chocolate Nation’, World’s largest Belgian chocolate museum in the centre of Antwerp. This free event includes drinks, snacks, and a tour of the museum. Take a magical chocolate trip, get to taste velvety Belgian chocolate and feel like ‘Charlie and the Chocolate Factory’ (Roald Dahl).

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

Chocolate Nation
Koningin Astridplein 7
2018 Antwerpen
Hands-on quality control for mycotoxins

Test systems for the detection of mycotoxins in food & feed

**RIDASCREEN®**
Sensitive ELISA for quantitative screening

**RIDASCREEN®FAST**
Quantitative ELISA for quick analysis

**RIDA®QUICK**
Lateral-flow tests for accurate, quantitative results

**Immunoaffinity columns and solid phase columns**
Sample purification for HPLC/GG/LC-MS and ELISA

**RIDA®QUICK**
Lateral flow tests for semi-quantitative screening

**Trilogy®**
Reference materials and mycotoxin standards for your quality control
Trilogy set out on a mission to revolutionize the conventional blueprint of analytical mycotoxin testing, which had long been burdened by extended result turnaround times, excessively high costs per analysis, and a lack of effective communication with clients. Recognizing the need for an alternative approach, the founders of Trilogy set out to build sample management process that placed the customer at the forefront of every step. Quick and efficient sample processing lies at the core of Trilogy’s overarching mission.

Trilogy firmly believes that everyone deserves to have access to the most accurate data available, accompanied by the ability to easily interpret the information provided to them. Analytical laboratories face a significant challenge in delivering analytical results that don’t require deciphering. By providing easy-to-understand results, customers are able to make informed decisions with ease based on the information presented to them.

There shouldn’t be anything complicated about it. Through relevant resources, education, a consultative environment partnered with a collaborative outlook, our team offers realistic solutions to evolving challenges for our customers.

“We are committed to helping you meet your quality goals.”

Explore Realistic Mycotoxin Solutions

The founders of Trilogy recognized a significant need in the mycotoxin industry: the necessity for fast result delivery while adhering to reference methods within a quality-driven analytical setting.

Shop analytical services, our line of QualiT™ products or sign up for a Custom E-Learning Session.
MYCOPEDIA

THE ULTIMATE GUIDEBOOK OF MYCOTOXINS IN FEED

Expand your knowledge of mycotoxins

Improve your understanding of mycotoxin impact on animal performance and health

Discover more about mycotoxin mitigation strategies

Use our knowledge to stay ahead of future trends and fight climate change

A RECORD-BREAKING BOOK

+30 contributing experts

+50 years of research summarized in a single book

+ 1,000 scientific publications referenced

388 pages to learn all about mycotoxins in feed and animal production

The book is available from Adisseo representatives
ZOO TOUR & CONFERENCE DINNER
(reservations only)

Tuesday 10 October 2023
19:00 – 23:00

Join the walking evening tour through Antwerp ZOO followed by a very special dinner with an unforgettable experience in the Marble Hall of ‘A Room with a ZOO’. Dinner Dress code: business casual.

Antwerp ZOO is one of the oldest and most famous zoos in Europe. Step inside and enjoy an oasis of calm in the heart of the city. The beautiful gardens, the characterful listed buildings and, of course, the animals being centre stage in the ZOO!

The ZOO 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 ZOO tour shall gather at 18:50 sharp in front of the entrance gate of Anwerp ZOO, located next to the conference venue.

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

- The tour ends in the Marble Hall, where the conference dinner takes place.

- Participants who do not want to join the ZOO tour but have registered for the conference dinner, shall gather at 19:55 sharp in front of the entrance gate of Anwerp ZOO, located next to the conference venue.
### Programme at a Glance

#### Sunday 8 October 2023

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
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<tbody>
<tr>
<td>18:30 – 20:00</td>
<td>Welcome reception – Provided by R-Biopharm</td>
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#### Monday 9 October 2023

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>11:00 – 12:30</td>
<td>Registration and light lunch</td>
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<tr>
<td>12:30 – 13:15</td>
<td><strong>Queen Elisabeth Hall</strong> OPENING SESSION</td>
</tr>
<tr>
<td></td>
<td>Welcome to WMFmeetsBelgium</td>
</tr>
<tr>
<td>13:15 – 14:15</td>
<td><strong>Queen Elisabeth Hall</strong> PLENARY SESSION</td>
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<tr>
<td></td>
<td>Grand challenges for a sustainable, safe, and inclusive food future</td>
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<tr>
<td>14:15 – 16:00</td>
<td><strong>Queen Elisabeth Hall</strong> Interactive debate – A world tour on climate change and the battle against mycotoxins</td>
</tr>
<tr>
<td>16:00 – 16:30</td>
<td>Networking break &amp; poster viewing</td>
</tr>
<tr>
<td>16:30 – 17:45</td>
<td><strong>Queen Elisabeth Hall</strong> Company pitches and Speed presentations</td>
</tr>
<tr>
<td></td>
<td>Short presentations by sponsors and by selected poster presenters</td>
</tr>
<tr>
<td>17:45 – 19:00</td>
<td>Wine &amp; Cheese tasting – Sponsored by dsm-firmenich/Romer Labs</td>
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#### Tuesday 10 October 2023

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
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<tbody>
<tr>
<td>08:30 – 10:30</td>
<td><strong>Marble Hall</strong> SESSION 1</td>
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<tr>
<td></td>
<td>Focus on mycotoxigenic fungi, plants, and soil</td>
</tr>
<tr>
<td>10:30 – 11:00</td>
<td>Networking break – sponsored by Charm Sciences – and poster viewing</td>
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<tr>
<td>11:00 – 12:30</td>
<td><strong>Marble Hall</strong> SESSION 3</td>
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<td>Mycotoxin exposure assessment and human health</td>
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<tr>
<td>12:30 – 14:00</td>
<td>Lunch break and poster viewing</td>
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<td></td>
<td><strong>Okapi Rooms 1-2-3</strong> Workshops</td>
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<tr>
<td>14:00 – 15:30</td>
<td><strong>Marble Hall</strong> SESSION 5</td>
</tr>
<tr>
<td></td>
<td>Managing and mitigating mycotoxin risks</td>
</tr>
<tr>
<td>15:30 – 16:00</td>
<td>Networking break and poster viewing</td>
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<tr>
<td>16:00 – 17:00</td>
<td><strong>Marble Hall</strong> SESSION 7</td>
</tr>
<tr>
<td></td>
<td>Managing mycotoxins in a sustainable future</td>
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<tr>
<td>17:00 – 18:00</td>
<td><strong>Okapi Room 1</strong></td>
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<tr>
<td></td>
<td>WMF Young Scientists Forum – Sponsored by Selko</td>
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<tr>
<td>19:00 – 23:00</td>
<td>ZOO tour and Conference dinner</td>
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<td>(reservations only)</td>
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<td>Time</td>
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<tr>
<td>08:45 – 10:15</td>
<td>Darwin Hall</td>
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<td>10:45 – 12:25</td>
<td>Darwin Hall</td>
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<td>12:25 – 12:35</td>
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<td>12:35 – 13:00</td>
<td>WMFmeetsBelgium</td>
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<tr>
<td>13:30 – 14:30</td>
<td>Darwin Hall</td>
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**WEDNESDAY 11 OCTOBER 2023**
CONFERENCE PROGRAMME

MONDAY 9 OCTOBER 2023

OPENING SESSION
WELCOME TO WMFmeetsBELGIUM

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

Local Conference Chairs:
Prof. Sarah De Saeger and Prof. Marthe De Boevre
Centre of Excellence in Mycotoxicology and Public Health, Ghent University, Belgium

12:30 Welcome
Prof. Anne De Paepe, chair Ghent University Association and Honorary Rector Ghent University, Belgium

12:45 Introduction to WMFmeetsBelgium
Prof. Sarah De Saeger and Prof. Marthe De Boevre

13:00 Tribute to Prof. Naresh Magan
Prof. Angel Medina Vaya, Cranfield University, UK

PLENARY SESSION
GRAND CHALLENGES FOR A SUSTAINABLE, SAFE, AND INCLUSIVE FOOD FUTURE

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

13:15 Food Systems Resilience: A major part of global food security
Prof. Chris Elliott, Institute for Global Food Security, Queen’s University Belfast, UK

13:35 Toxin-free food (against the backdrop of climate change)?
Prof. Rudolf Krska, Department IFA-Tulln, BOKU Vienna, Austria

13:55 Exploring the impacts of climate change on mycotoxin contamination in food systems:
Statements, gaps and perspectives
Dr Marco Camardo Leggieri, Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Italy
MONDAY 9 OCTOBER 2023

PLENARY SESSION
INTERACTIVE DEBATE – A WORLD TOUR ON CLIMATE CHANGE AND THE BATTLE AGAINST MYCOTOXINS

Hosted by the International Society for Mycotoxicology (ISM) and sponsored by dsm-firmenich, R-Biopharm, Romer Labs, and Trilogy, in collaboration with Affidia Journal.

Moderator:
Prof. Sarah De Saeger, Ghent University, Belgium and ISM

14:15 An esteemed panel of international experts from various mycotoxicology societies across the globe as well as from companies has been gathered. Each expert will provide a concise overview of thoughts and proposed strategies regarding the topic. Subsequently, the floor will be open for a live discussion involving all participants.

The panel consists of Prof. Amnart Poapolathep (Thai Society of Mycotoxicology), Prof. Kiminori Shimizu (Japanese Society for Mycotoxicology), Prof. Yemisi Jeff-Agboola (Mycotoxicology Society of Nigeria), Prof. Hans-Ulrich Humpf (Society for Mycotoxin Research), Dr. Lindy Rose (African Society of Mycotoxicology), and Dr. Juan Manuel Palazzini (Latin American Society of Mycotoxicology).

16:00 Networking break
Poster viewing

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)

Chair: Dr Esther De Rycke, Ghent University, Belgium

16:30 Company pitches
For details, see pages 24-30.

17:00 Speed presentations
For details, see page 35.
Suqin Shao (Canada) – Eva M. Biehl (Germany) – Jinquan Wang (China) – Łukasz Zielonka (Poland)

17:20 Company pitches
Alltech – EnviroLogix – Biönte – Bioeasy

WINE AND CHEESE TASTING – SPONSORED BY DSM-FIRMENICH/ROMER LABS
17:45 – 19:00
In the good tradition of the World Mycotoxin Forum®, a Wine and Cheese tasting party will be organised. A great way to meet all colleagues from the mycotoxin community.
TUESDAY 10 OCTOBER 2023

SESSION 1
FOCUS ON MYCOTOXIGENIC FUNGI, PLANTS, AND SOIL

Chairs: Dr Neriman Yilmaz, University of Pretoria, South Africa  
Dr Maria Agustina Pavicich, Ghent University, Belgium

08:30 Mycotoxicogenic fungus or not? Let’s have a look at the genome!  
Dr Jérôme Collemare, Westerdijk Fungal Biodiversity Institute, the Netherlands

08:45 Inter- and intraspecific genetic variability of mycotoxigenic fungi leading to differences in mycotoxin profiles  
Dr Antonio Moretti, Institute of Sciences of Food Production, National Research Council of Italy, Italy

09:00 Root uptake and metabolization of Alternaria toxins into wheat plants using a hydroponic cultivation system  
Dr Ahmed H. El-Khatib, Department Safety in the Food Chain, German Federal Institute for Risk Assessment, Germany

09:15 Resistance to Aspergillus flavus infection and aflatoxin contamination in maize  
Dr Kanniah Rajasekaran, Food and Feed Safety Research, Agricultural Research Service, US Department of Agriculture, USA

09:30 Mechanisms governing the regulation of mycotoxin biosynthesis and virulence in the plant pathogen Penicillium expansum  
Dr Edward Sionov, Agricultural Research Organization – Volcani Institute, Israel

09:45 Fusarium avenaceum mycotoxin production and pathogenicity in cereal and pulse crops  
Dr Nora Foroud, Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada, Canada

10:00 Silent saboteurs: Exploring the counterintuitive interplay between weak pathogens and the virulent Fusarium graminearum in Fusarium Head Blight disease suppression in wheat  
Prof. Kris Audenaert, Department of Plants and Crops, Ghent University, Belgium

10:15 Mycotoxin-soil interactions: The role of soil in the biosynthesis, fate and biological effects of mycotoxins  
Dr Katherine Muñoz, Institute for Environmental Sciences, RPTU Kaiserslautern-Landau, Germany

10:30 Networking break sponsored by Charm Sciences  
Poster viewing
TUESDAY 10 OCTOBER 2023

SESSION 2
SAMPLING AND MASS SPECTROMETRY-BASED APPROACHES FOR MYCOTOXIN ANALYSIS: AN UPDATE

Chairs: Dr Monique de Nijs, Wageningen Food Safety Research, the Netherlands
Prof. Michele Suman, Barilla, Italy

08:30 A quality control scheme designed to assess sample preparation performance
Dr Sheryl Tittlemier, Grain Research Laboratory, Canadian Grain Commission, Canada

08:45 Challenges towards sampling and analysis of ergot alkaloids in wheat gluten
Dr Johan De Meester, Cargill, Belgium

09:00 Recent advances in mycotoxin analysis at FDA
Dr Kai Zhang, Center for Food Safety and Applied Nutrition, Food and Drug Administration, USA

09:15 Interlaboratory study to normalize LC-MS mycotoxin determination using the N-alkyl-pyridinium-3-sulfonates (NAPS) retention index system
Dr Mark Sumarah, London Research and Development Centre, Agriculture and Agri-Food Canada, Canada

09:30 Comparison of UHPLC-MS/MS methodologies for human biomonitoring of multiple mycotoxins in serum
Dr. Roger Peró Gascón, Centre of Excellence in Mycotoxicology and Public Health, Ghent University, Belgium

09:45 Ergot alkaloids in cereals, results, and trends from a 6-year study of industry monitoring
Dr Susan MacDonald, Fera Science Limited, UK

10:00 Company pitches
For details, see pages 24-30.

10:30 Networking break sponsored by Charm Sciences
Poster viewing
TUESDAY 10 OCTOBER 2023

SESSION 3
EXPOSURE ASSESSMENT AND HUMAN HEALTH

Chairs: Prof. Marthe De Boevre, Ghent University, Belgium
       Prof. Hans-Ulrich Humpf, University of Münster, Germany

11:00  New insights into aflatoxin B1 metabolism in vitro and in vivo by HPLC-MS/MS analysis in combination with intravital imaging
       Prof. Hans-Ulrich Humpf, Institute of Food Chemistry, University of Münster, Germany

11:15  The aflatoxin B1 misfortune never come alone: toxicity of the emerging mycotoxin versicolorin A
       Dr Laura Soler-Vasco, Toxalim Research Center in Food Toxicology, Université de Toulouse, INRAE, France

11:30  Unravelling the toxicokinetics of tenuazonic acid through a human toxicokinetic trial
       Lia Visintin, Centre of Excellence in Mycotoxicology and Public Health, Ghent University, Belgium

11:45  Accumulation of mycotoxins in human hair: novel approach for assessing chronic exposure
       Dr Alfonso Narváez, Laboratory of Food Chemistry and Toxicology, University of Valencia, Spain

12:00  Overall exposure of European adult population to mycotoxins by statistically modelled biomonitoring data
       Dr Barbara De Santis, Nutrition and Veterinary Public Health, Italian National Institute of Health, Italy

12:15  Exposomics study for investigating mycotoxins exposure and the association with biomolecular markers of aging and birth outcomes in rural Burkina Faso
       Yuri Bastos Moreira, Centre of Excellence in Mycotoxicology and Public Health, Ghent University, Belgium

12:30  Lunch break
       Poster viewing

WORKSHOPS (for details, see pages 21-22.
12:45 – 13:45

- Managing the matrix mayhem
  Sponsored by R-Biopharm, R-Biopharm Rhône, and Trilogy Analytical Laboratory

- Raw materials shortage and mycotoxins risk assessment: The role of screening and screening validation
  Sponsored by Gold Standard Diagnostics

- Unmasking mycotoxins: From detection to detoxification
  Sponsored by dsm-firmenich/Romer Labs
TUESDAY 10 OCTOBER 2023

SESSION 4  
MYCOTOXINS AND THEIR IMPACT ON ANIMAL HEALTH

Chairs: Dr Isabelle Oswald, INRAE, France  
Dr Fernando B. Luciano, Pontifícia Universidade Católica do Paraná, Brazil

11:00 Double Trouble: Mycotoxins and sub acute rumen acidosis’ impact on lactate-utilizing  
Megasphaera sp. in dairy cows – A rumen simulation system study  
Cameron Strachan, Unit of Food Microbiology, University of Veterinary Medicine, Austria

11:15 The hepatic metabolism of aflatoxin B1 explains the differences in susceptibility to the  
mycotoxin among major poultry species  
Prof. Gonzalo J. Diaz, Facultad de Medicina Veterinaria y de Zootecnia, Universidad Nacional  
de Colombia, Colombia

11:30 Dose-response effects of combined doses of fumonisins, deoxynivalenol, and zearalenone  
mycotoxins on major T-cell subsets and tight junction protein expressions in broiler chickens  
Dr Revathi Shanmugasundaram, Toxicology & Mycotoxin Research, Agricultural Research  
Service, US Department of Agriculture, USA

11:45 The mycotoxins T-2 and deoxynivalenol increase the translocation of Streptococcus suis  
across porcine ileal organoid monolayers  
Dr Regiane R. Santos, Department of Research and Development, Schothorst Feed  
Research, the Netherlands

12:00 Mycotoxin biomarkers for livestock species: How far have we come?  
Dr Veronika Nagl, dsm-firmenich, Austria

12:15 Company pitches  
For details, see pages 24-30.  
Impextraco – Cargill – Olmix – MiXscience

12:35 Lunch break  
Poster viewing

WORKSHOPS (for details, see pages 21-22).

12:45 – 13:45

- Managing the matrix mayhem  
  Sponsored by R-Biopharm, R-Biopharm Rhône, and Trilogy Analytical Laboratory

- Raw materials shortage and mycotoxins risk assessment: The role of screening and screening  
  validation  
  Sponsored by Gold Standard Diagnostics

- Unmasking mycotoxins: From detection to detoxification  
  Sponsored by dsm-firmenich and Romer Labs
TUESDAY 10 OCTOBER 2023

SESSION 5
MANAGING AND MITIGATING MYCOTOXIN RISKS

Chairs: Prof. Angel Medina-Vaya, Cranfield University, UK
       Prof. Chiara Dall’Asta, University of Parma, Italy

14:00 Potential mitigation strategies for free and modified *Fusarium* mycotoxins in oats
Dr Silvia Gratz, Rowett Institute, University of Aberdeen, UK

14:15 Nature vs. mycotoxins: affordable strategies to reduce mycotoxins in foods and animal feed
Dr Fernando Bittencourt Luciano, Laboratory of Agri-Food Research and Innovation, Pontifícia
       Universidade Católica do Paraná, Brazil

14:30 Pulsed light as a non-contact food decontamination technology for removing fungi and
       mycotoxins
Dr Yan Wang, College of Food Science and Technology, Zhejiang University of Technology,
       China

14:45 Reduction of deoxynivalenol in roller-milled fractions following washing and pearling of high
       and low-contaminated wheat
Dr Gunnar Sundstøl Eriksen, Toxicology Research Group, Norwegian Veterinary Institute,
       Norway

15:00 The need for HACCP-approach to manage mycotoxins in animal products
Dr Swamy Haladi, Selko, India

15:15 Developing a machine learning predictive model to strengthen the preventive measures for
       mycotoxins in food and feed
Prof. Chris Elliott, representing Agroknow, Greece

15:30 Networking break
Poster viewing
TUESDAY 10 OCTOBER 2023

SESSION 6
MYCOTOXINS AND ANIMAL PERFORMANCE

Chairs: Dr Johan De Meester, Cargill, Belgium
        Dr Tess Goessens, Ghent University, Belgium

14:00  Are mycotoxins in vegetable-based salmon feed a cause for concern? Effects of prevalent mycotoxins on welfare and growth of salmon in aquaculture
       Dr Kai Kristoffer Lie, Department of Marine Toxicology, Institute of Marine Research, Norway

14:15  Machine learning-aided design of composite mycotoxin detoxifier material for animal feed
       Dr Maciej Haranczyk, IMDEA Materials Institute, Spain

14:30  Complementary modes of action of a mycotoxin deactivator can support health and performance of animals
       Dr Damien Prévéraud, Adisseo, France

14:45  Untangling the complex web of aflatoxins and fumonisins using bentonite and fumonisin esterase as sustainable solutions for safer poultry production in Kenya
       Dr Phillis Ochieng, Nairobi University, Kenya and International Livestock Research Institute, Kenya

15:00  Mitigation of the combined toxicity of AFB1, DON and OTA in broiler breeder hens
       Dr Lv-Hui Sun, College of Animal Sciences and Technology, Huazhong Agricultural University, China

15:15  Cutting-edge strategy to mitigate the effect of deoxynivalenol on swine
       Dr Virginie Marquis, Phileo by Lesaffre, France

15:30  Networking break
       Poster viewing
TUESDAY 10 OCTOBER 2023

SESSION 7
MANAGING MYCOTOXINS IN A SUSTAINABLE FUTURE

Chair: Dr Sheryl Tittlemier, Canadian Grain Commission, Canada

16:00 Prioritization of mycotoxins for risk management action based on both public health risk and mitigation efficacy
Prof. Michele Suman, Barilla SpA and Università Cattolica del Sacro Cuore, Italy

16:15 Mouldy bread – a spoiled food waste or a future feedstock?
Dr Alessia Caprio, FFoQSI Austrian Competence Centre for Feed and Food Quality, Safety & Innovation, Austria

16:30 Ensuring the safety of plant-based meat alternatives: mycotoxin occurrence, risk-benefit assessment, and current research – Where we are and where we are supposed to be?
Dr Octavian Augustin Mihalache, Department of Food and Drug, University of Parma, Italy

16:45 The environmental impact of mycotoxin analysis: On the greenness of routine methods
Dr Stephan Freitag, Department IFA-Tulln, BOKU Vienna, Austria

WMF YOUNG SCIENTISTS FORUM
(for details, see page 23).

17:00 – 18:00

The challenges of conducting mycotoxin research – How to connect science to practical mycotoxin mitigation strategies?

Sponsored by Selko
TUESDAY 10 OCTOBER 2023

SESSION 8  
DATA-DRIVEN MYCOTOXIN MANAGEMENT

Chair: Dr Kanniah Rajasekaran, US Department of Agriculture, USA

16:00 An acronym soup for better risk assessment of DON: HBM, NAMs, TK-TD, MCMC, HDMI, and more!  
Prof. Weihsueh Chiu, Department of Veterinary Physiology and Pharmacology, Texas A&M University, USA

16:15 A new in silico tool for the prediction of mutagenicity, genotoxicity, and carcinogenicity of over 4,000 mycotoxins  
Dr Martina Palomino-Schätzlein, ProtoQSAR S.L., Spain

16:30 Predictive models to manage mycotoxin outbreaks in the USA  
Dr Lina Castano-Duque, Food and Feed Safety Research, Agricultural Research Service, US Department of Agriculture, USA

16:45 Resilience of the food supply chain to food safety shocks: case of mycotoxins  
Prof. Ine van de Fels-Klerx, Wageningen Food Safety Research, Wageningen University & Research, the Netherlands

WMF YOUNG SCIENTISTS FORUM  
(for details, see page 23).

17:00 – 18:00

The challenges of conducting mycotoxin research – How to connect science to practical mycotoxin mitigation strategies?

Sponsored by Selko
SESSION 9
UPDATE ON GLOBAL MYCOTOXIN RESEARCH AND NEW CONCEPTS

Chairs: Prof. Franz Berthiller, BOKU Vienna, Austria
Dr Lindy Rose, Stellenbosch University, South Africa

08:45  NutriNuts: Success story of industrial partnership toward sustainable mitigation of aflatoxin in Africa
Dr Carol Verheecke-Vaessen, Applied Mycology Group, Cranfield University, UK

09:00  Multi-actor collaboration: Everyone at the table for improved mycotoxin risk analysis – A perspective for the European and the African Union
Dr Celine Meerpoel, Centre of Excellence in Mycotoxicology and Public Health, Ghent University, Belgium

09:15  The Food Safety Coalition project to address the challenges of aflatoxin contamination in raw materials
Dr Yueju Zhao, Mars Global Food Safety Center, China

09:30  Mycotoxins in Asia: Impact on food safety and international trade
Dr Awansee Petchkongkaew, Thammasat University and International Joint Research Centre on Food Security, Thailand

09:45  Estimating the public health burden of aflatoxins – A global effort
Yuki Minato, Department of Nutrition and Food Safety, World Health Organization (WHO), Switzerland

10:00  Characterization of filamentous fungi and their metabolites in aquaponic production of herbs
Volha Akulava, Faculty of Science and Technology, Norwegian University of Life Sciences, Norway

10:15  Networking break
Poster viewing

FINAL PLENARY SESSION
FUTURE PERSPECTIVES
See page 20.
WEDNESDAY 11 OCTOBER 2023

SESSION 10
NOVEL AND ALTERNATIVE TECHNIQUES IN MYCOTOXIN ANALYSIS AND FUNGAL DETECTION

Chairs: Dr Mark Sumarah, Agriculture and Agri-Food, Canada
Dr Esther De Rycke, Ghent University, Belgium

08:45 Infrared spectroscopy in food safety: hype or hope
Prof. Boris Mizaikoff, Institute of Analytical and Bioanalytical Chemistry, Ulm University, Germany

09:00 Alternative approaches to the analysis of mycotoxins based on luminescent sensing coupled to biological or biomimetic molecular receptors
Dr Elena Benito Peña, Faculty of Chemistry, Complutense University of Madrid, Spain

09:15 Detection of T-2 toxin in wheat and maize with a portable mass spectrometer
Dr Chris Maragos, Mycotoxin Prevention and Applied Microbiology Research Unit, Agricultural Research Service, US Department of Agriculture, USA

09:30 Development of a portable microarray lateral flow immunosorbent assay for multiple mycotoxins detection
Dr Saowalak Adunphatcharaphon, National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Thailand

09:45 Application of electronic nose for feed safety and animal nutrition
Dr Matteo Ottoboni, Department of Veterinary Medicine and Animal Sciences, University of Milan, Italy

10:00 Accurate and non-destructive monitoring of mould contamination in foodstuffs based on whole-cell biosensor array coupling with machine-learning prediction models
Prof. Fuguo Xing, Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences, China

10:15 Networking break
Poster viewing

FINAL PLENARY SESSION
FUTURE PERSPECTIVES
See page 20.
WEDNESDAY 11 OCTOBER 2023

FINAL PLENARY SESSION
FUTURE PERSPECTIVES

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 Communicating with consumers: How to talk about food risk?
Dr Nina McGrath, European Food Information Council (EUFIC), Belgium

11:05 The chicken tikka masala and the importance of data quality: when the analytical performance may become a real hurdle
Prof. Chiara Dall'Asta, Department of Food and Drug, University of Parma, Italy

11:25 Shaping the future of mycotoxin management in Sub-Saharan Africa with pragmatism
Prof. Limbikani Matumba, Faculty of Life Sciences and Natural Resources, Lilongwe University of Agriculture & Natural Resources, Malawi

11:45 Think global, act local – on-site mycotoxin management challenges in the light of global climate change
Ronald Niemeijer, R-Biopharm, Germany

12:05 Mycotoxins and climate change: mission impossible for feed and food safety regulators?
Frans Verstraete, Directorate-General for Health and Food Safety, European Commission, Belgium

12:25 BEST POSTER AWARD – presented by Cargill

12:35 Top Five Answers learned at WMFmeetsBelgium
Prof. Rudolf Krska and Prof. Chris Elliott

13:00 Closing of WMFmeetsBelgium
Prof. Sarah De Saeger and Prof. Marthe De Boevre

Take your packed lunch to eat along the way!

GENERAL ASSEMBLY
INTERNATIONAL SOCIETY FOR MYCOTOXICOLOGY

13:30 – 14:30

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

ISM’s General Assembly is open to all participants of WMFmeetsBelgium.
WORKSHOP PROGRAMME

TUESDAY 10 OCTOBER 2023

12:45 – 13:45

MANAGING THE MATRIX MAYHEM – A COMPREHENSIVE TOOLBOX TO ADDRESS MATRIX EFFECT CHALLENGES IN MYCOTOXIN ANALYSIS WITH LC-MS/MS

Sponsored by R-Biopharm, Germany, R-Biopharm Rhône, UK and Trilogy Analytical Laboratory, USA

Mycotoxin contaminations of food and feed have a huge economic impact and solid analytical methods for mycotoxin analysis play an important role in protecting the consumers and minimizing the economic impact. Analytical methods based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) have proven to be very effective in mycotoxin analysis. As with all analytical methods in food testing components in the matrix to be tested may interfere with the analytical method.

This workshop will focus on the challenges associated with testing mycotoxins using liquid chromatography-tandem mass spectrometry (LC-MS/MS) in complex sample matrices. The presence of matrix effects can significantly affect the accuracy and precision of mycotoxin analysis, making it difficult to obtain reliable results. The workshop will cover the different types of matrix effects that can occur during LC-MS/MS analysis, including ion suppression and enhancement, and will discuss their underlying mechanisms. Various approaches to mitigate these matrix effects will be presented, such as solid phase extraction (SPE), immunoaffinity, matrix matched calibration, and the use of certified reference materials and quality control materials. To eliminate matrix effects, without compromising the sensitivity, a clean-up of the sample is necessary. For some samples a simple extraction is sufficient, but for other, more complex samples solid phase extraction / immunoaffinity extraction can make a big difference and can reduce or even eliminate matrix effects. Stable isotope labelled standards are often used to address matrix effects. They can help to correct for matrix effects. However, the use of stable isotope labelled standards can also be limited by factors as costs and availability. Matrix matched calibration can in some cases be a more cost-effective alternative method to obtain reliable results. Certified reference materials and quality control materials to monitor the accuracy and precision of their mycotoxin analyses, and to detect and correct any issues with their methods are important additional tools. These materials can help to ensure the quality of analytical results, and to meet regulatory requirements for method validation and quality control.

Case studies will be presented to illustrate how these approaches have been successfully applied in routine testing of a large mycotoxin testing laboratory.
TUESDAY 10 OCTOBER 2023

12:45 – 13:45

RAW MATERIALS SHORTAGE AND MYCOTOXINS RISK ASSESSMENT: THE ROLE OF SCREENING AND SCREENING VALIDATION

Sponsored by Gold Standard Diagnostics

Economic and political aspects combined to draught and climate change in general are impacting the raw materials availability, both worldwide or locally, and in several regions in the world a shortage of grains, vegetal commodities, feed ingredients have been reported. Changing the composition of dairy cattle diets, within the perimeters of procedural guidelines, might eventually impact the mycotoxins contamination risk, and, on the other hand, might affect the yield and the quality of milk. To mitigate the risk of using toxins-contaminated ingredients, screening test kits might be of support, only with adequate premises and an adequate validation approach.

With the relevant, valuable support of Prof. Antonio Gallo, Università Cattolica di Piacenza, the workshop will debate the challenges that farms, husbandries, and eventually laboratories might face when modifying the feed composition for livestock, with a special focus on lactating dairy cows.

Workshop led by Giulia Rosar, Senior Product Manager, Gold Standard Diagnostics Trieste.

TUESDAY 10 OCTOBER 2023

12:45 – 13:45

UNMASKING MYCOTOXINS: FROM DETECTION TO DETOXIFICATION

Gain new insights into mycotoxin management: exploring cutting-edge advancements in rapid detection and detoxification.

Sponsored by dsm-firmenich and Romer Labs

Dive into the world of mycotoxins from detection to quantification and mitigation. Gain insights into the DSM World Mycotoxin Survey and how it can help you. Quantification of mycotoxins in 4 minutes? Romer Labs will give you a hands-on experience and show you how that works. But what about mitigation? Watch dsm-firmenich as they show you FUMzyme-activity in real time. And for the first time ever at the World Mycotoxin Forum, you can have a 3D experience of mycotoxin deactivating enzymes working under practical conditions.
WMF YOUNG SCIENTISTS FORUM

TUESDAY 10 OCTOBER 2023

17:00 – 18:00

THE CHALLENGES OF CONDUCTING MYCOTOXIN RESEARCH
How to connect science to practical mycotoxin mitigation strategies?

During this year's Young Scientist Forum, Selko invites young scientists, researchers, and students to share experiences and learn from each other. In today's fast-changing and evolving industry, it can be a challenge to define research needs and connecting them to 'real' scenarios and creating cost-effective solutions for grain and animal producers around the world.

How to find the right model for your research? Which biomarkers to apply? In vitro or in vivo? Single or multi-mycotoxin, or mycotoxins combined with other environmental challenges? What are emerging technologies and how can they improve mycotoxin research in the future? These and other questions will be discussed during the informal brainstorm session, while enjoying some snacks and drinks. We hope to connect business needs with science and invite those young of age as well as young at heart to join in.
COMPANY PROFILES
Profiles of the companies presenting pitches

R-Biopharm
R-Biopharm is a leading developer of test solutions for clinical diagnostics and food & 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 (https://food.r-biopharm.com).

Trilogy Analytical Laboratory
Transforming mycotoxin testing
Trilogy Analytical Laboratory strives to be a pioneering force in the mycotoxin testing industry. The business was established with a visionary mission: to reimagine and simplify mycotoxin testing processes. We recognized the industry's pressing need for faster turnaround times, cost-effective solutions, and improved client communication, and we set out to redefine the status quo. Quality focused mycotoxin testing techniques. Our commitment to quality started with our founders and is the daily mission of our current team. It is the compass that keeps our team heading in the right direction. Quality leaders mentoring quality individuals using quality developed methodology. This is further demonstrated through the production of naturally contaminated quality control materials and analytical standards that are custom tailored to the laboratories methods giving the most robust and reliable methods offered. Putting customers first. At Trilogy, we have placed the customer at the forefront of every step in our sample management process. Quick and efficient sample processing is the cornerstone of our mission. We firmly believe that everyone deserves easy access to accurate data and results that don't require deciphering. Our goal is to empower our customers to make informed decisions with the reliability of our comprehensive solutions package for analytical testing and quality control products. Solutions for evolving challenges. Our team thrives on providing practical solutions to the ever-evolving challenges faced by our customers. We offer relevant resources, educational support, a consultative environment, and a collaborative outlook. Tailoring our solutions to be the perfect fit to our customer’s needs.

dsm-firmenich Animal Nutrition & Health
The world has a lot on its plate. But not enough of the good stuff. One in eight people struggles to get enough protein in their diet. That's why we, as dsm-firmenich Animal Nutrition & Health, are on a mission to help the animal farming industry feed our growing population more sustainable. Our farm-to-fork solutions help farmers reduce their environmental impact, improve animal performance and welfare, and secure sustainable livelihoods. All while putting high-quality animal protein on our plates: protein that's healthy, nutritious, safe, and affordable.

Romer Labs
Romer Labs is a global leader in innovative diagnostic solutions for food and feed safety. With a focus on mycotoxins, food allergens, GMOs, and microbial contaminants, we strive to meet the ever-changing demands of our customers while upholding our reputation for exceptional service. We have been providing exceptional service to our customers for over 40 years. Our dedicated technical support team is always available to provide assistance, and our technical sales team is committed to helping customers find the right solutions to meet their unique needs. In addition, we operate six analytical services labs across three continents, ensuring that our customers have access to reliable and accurate testing services whenever and wherever they may need them. Our core mission at Romer Labs has always been the same: Making the World's Food Safer®. Through our innovative products and exceptional service, we remain dedicated to providing our customers with the tools they need to ensure the safety and quality of their products.
Selko
At Selko, the feed additives brand of Nutreco, we are committed to developing specialty feed additives that optimise animal performance. Decades of experience, ongoing research, and a commitment to developing functional and more sustainable solutions help our customers’ animals achieve their full potential. Backed by sound science and manufactured to meet the highest quality standards, the Selko specialty feed additive portfolio helps support feed safety, antibiotic reduction, trace mineral optimisation, animal health and performance, mycotoxin risk management and much more. Our passionate and driven team is dedicated to solving challenges across the feed-to-food chain. With a presence in 105 countries and manufacturing plants on several continents, we serve feed producers, farmers and home-mixers, integrators, distributors, and food processors. At Selko, we understand that combining the power of nutrition, specialty feed additives and good farm management can transform our industry – and even our planet. Selko’s planet-to-plate approach is challenging how today’s feed-to-food chain works and helping our customers secure a brighter future. For more information, please go to www.selko.com.

Adisseo
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...
- Unike® Plus: maximum protection against challenges posed by broad-spectrum mycotoxin contamination
- Toxy-Nil® Plus: powerful protection against broad-spectrum mycotoxin contamination
- Toxy-Nil®: reliable protection against moderate-level mycotoxin contamination
... in order to propose the best-suited solution to a specific challenge!
Adisseo is one of the world’s leading experts in feed additives. The group relies on its 8 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 2,650 employees, it serves around 4,200 customers in over 110 different countries through its global distribution network. In 2022, Adisseo achieved a turnover of over 2.04 billion Euros. Adisseo is one of the main subsidiaries of China National BlueStar, leader in the Chinese chemical industry with nearly 12,300 employees and a turnover of 7.5 billion euros. Adisseo is listed on the Shanghai Stock Exchange. Corporate website: www.adisseo.com

Gold Standard Diagnostics
Global provider of diagnostic test kits and instruments
Gold Standard Diagnostics is a global provider of fast, reliable, and easy to use diagnostic test kits and instruments in the fields of bioanalytical testing for the food, feed, environmental, biopharma, and clinical industries. For mycotoxin analysis, our comprehensive portfolio of ELISA test kits suits all needs for effective mycotoxin detection in grains, feed and vegetal commodities. Additionally, I’screen AFLA M1 Milk, a quantitative immunoassay for the analysis of aflatoxin M1, has been granted AOAC Research Institute Performance Tested Methods℠ status (AOAC Certificate No. 072002) for use with raw bovine whole milk, skim milk and powdered milk. To complete mycotoxin screening, we provide solutions including matrix-based control materials and standard solutions. These products are relevant both in the evaluation and validation process and in routine Internal Quality Control and External Quality Assurance. Our new immunoaffinity column range SENSIPure offers multiple advantages: in addition to quantifying the capacity and the cross-reactivity, the extended quality control includes inspecting blank contamination and verifying recovery in matrix. Multiple mycotoxin assays can also be run by our automatic analysers and have been validated for The BOLT™ and ThunderBOLT®, two compact, user-friendly ELISA robots that can create considerable savings. The in-house R&D teams have expertise in developing a wide range of innovative methods and applications to meet the testing needs of both research and industry. Our experts at Gold Standard Diagnostics are able to customize programs to serve specific needs with competitive pricing and high-quality standards.
Phileo by Lesaffre
At Phileo by Lesaffre we are committed to push the boundaries of animal care to better nourish and protect the planet. We believe that yeast and bacteria with fermentation technologies and biotransformation are the future of animal health through nutrition. They are the answers to the challenges of a global demand for animal protein and greater sustainability. We believe that effective mycotoxin management is about seeing the whole challenge, from the farm to the feed mill and from risk assessment to feed management. Using a combination of modern management tools, the Alltech® Mycotoxin Management Program provides a complete holistic solution to help producers take control of mycotoxin contamination. The program is built around class-leading risk identification technology, data analysis and insights, and mycotoxin binder solutions designed to reduce the damaging effects of mycotoxins on animal health and production potential. A robust research and development program has helped maintain strong scientific stewardship and leadership through in-depth interaction with key experts in the field. This allowed us to develop a multi-faceted exploration of mycotoxins’ impact on animal systems and remediation, using *in silico*, *in vitro*, *ex vivo*, *in situ*, and *in vivo* methodologies, pushing the frontiers in search of successful mitigation strategies.

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

EnviroLogix
Founded in 1996, EnviroLogix is a global provider of rapid GMO, mycotoxin, and allergen testing solutions for the grain, milling, and pet food industries. Our portfolio of smart, simple diagnostic solutions – formulated to provide maximum accuracy, simplicity, and speed - are an ideal fit for inbound grain inspection and internal lab use. EnviroLogix also provides laboratory-based testing and data management solutions to deliver a complete quality management program for its global customers.

BIÖNTE
BIÖNTE is a spin-off of Andrés Pintaluba SA (APSA), head of the Pintaluba Group, a multinational conglomerate of 12 companies based in 6 countries. The company is backed by +20 years of experience in the mycotoxins adsorbent market thanks to QUIMITOX® product. In 2022, BIÖNTE takes over from APSA the experience in the mycotoxins adsorbent segment to face a more demanding environment with specialization and technical service as differentiating elements. Our commitment, in its broadest sense, is to remain a global benchmark in mitigating the effects of mycotoxins through innovation and teamwork with the scientific community, providing effective and sustainable solutions for a better animal nutrition. Not all clients pose the same challenges; raw materials, target species, and regions also raise different challenges. Therefore, the only way to mitigate the negative effects of mycotoxins is through specialization and personalized service as well as an effective, safe and profitable product.
At BIÔNTE we make all our knowledge available to our business partners and clients, as well as the necessary resources to undertake comprehensive plans as a key to success. Our knowledge of the market and of the global challenges of animal nutrition, the experience and international vocation of the technical and commercial team of BIÔNTE, together with the technological and operational support of the Pintaluba Group, are the axes of work that guide the beginning of our activity as an independent company. More information: www.bionte.com | bionte@bionte.com

Bioeasy
Shenzhen Bioeasy Biotechnology Co., Ltd., founded in 2007, is a high-tech enterprise engaged in food safety, animal health, public safety and other fields. With a focus on rapid detection, we are dedicated to provide our customers with high quality products, services and overall solutions to tackle current and emerging food safety problems, protecting our food from farm to table. Our products include rapid test kits and instruments for detection of antibiotics, aflatoxin, pesticides and other food additive residues, serving clients all over the world in fields like dairy, meat and seafood, feed, grain and oil, food processing, etc.

Charm Sciences
Charm Sciences is the global leader in providing rapid food safety diagnostic tests across many industries. Our reputation was achieved by our commitment to our customers’ needs and by developing reliable, simple, and innovative technologies that meet different regional guidance and regulations around the world. Vertical integration is a hallmark of our business, with reagent and equipment manufacturing, software design, and firmware design located in one of our three manufacturing facilities in Massachusetts, USA. As food safety risks evolve, Charm offers customized solutions to develop faster, user-friendly test solutions to proactively prevent contamination. Charm’s portfolio includes test kits and systems for pesticides, alkaline phosphatase, pathogens, end product microbial assessment, allergen control, water quality, antibiotics, ATP sanitation verification, and mycotoxins (ROSA® portfolio includes aflatoxin, DON, fumonisin, ochratoxin, T-2/HT-2 toxin, and earalenone tests with test option using water-based single extraction for multiple toxins). Cutting-edge data management allows customers to link test results with real-time corrective action. Charm products serve the food, beverage, water, pharmaceutical, medical, personal care, environmental, and industrial markets in more than 100 countries. Our goal – and source of pride – is to provide peace of mind using Charm’s technology meet our customers’ food safety needs and protect their brand!

FOSS
FOSS is the leading global provider of analytics for the food and agricultural industries. We help producers maximize the value of their production, ensure food safety, and make the best possible use of valuable natural resources. Value for the customer and value for the environment go hand in hand. For instance, our fast and dedicated analytical solutions will measure the level of mycotoxins in grain, scan meat to determine fat content and check for unwanted objects such as bone splinters, measure moisture in grain, and help decide whether the cow gets the right feed, is healthy, and produces good and nutritious milk. We use many different technologies, such as flow cytometry, FTIR, NIR, X-ray, Kjeldahl distillation, and falling number. Based on sophisticated algorithms, our solutions provide a wealth of data and are implemented and developed with the newest software technologies. This enables us to develop solutions that are faster than traditional methods. For instance, our MycoFoss™ solution enables testing of up to 6 mycotoxins in less than 8 min by combining flow cytometry and immuno-assay technology. With the addition of FOSS networking software, you can connect the MycoFoss™ solution in a network of analysis instruments and get full access to data from all your instruments and across sites. Link up your instruments to get consistent quality throughout your organization, better monitoring to ensure adherence to food safety regulations and standard operational procedures and store your data safely with back up and long-term data storage.

SCIEX
Advances in human wellness depend on the power of precise science. At SCIEX, our mission is to deliver solutions for the precision detection and quantification of molecules, empowering our customers to protect and advance the wellness and safety of all. SCIEX has led the field of mass spectrometry for 50 years. From the moment we launched the first ever commercially successful triple quad in 1981, we have developed groundbreaking technologies and solutions that influence life-changing research and outcomes. Today we continue to pioneer robust solutions in mass spectrometry and capillary electrophoresis. But we don’t just develop products. It is what we do together with our customers that sets us apart. That’s why thousands of life science experts around the world choose SCIEX to get the answers they can trust to better inform critical
decisions; decisions that positively impact lives. We proudly stand behind our tagline: The Power of Precision.

**Waters**
Waters Corporation is the world's leading specialty measurement company, and has pioneered chromatography, mass spectrometry, rapid testing and thermal analysis innovations for more than 60 years. Waters delivers solutions for food, environmental, pharmaceutical, biopharmaceutical and materials 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 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. The combination of clean-up using immunoaffinity columns (IAC), and analysis by liquid chromatography or fluorescence detection is a routine, cost-effective way to check compliance. The use of ACQUITY UPLC technology enables laboratories to shorten analytical run times and remove the need for post-column derivatization. If the desire is to add higher specificity with your methodology, Waters can offer fully supported workflow solutions with LC-MS/MS with high sensitivity, often targeting multiple analytes. 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.

**ProGnosis Biotech**
ProGnosis Biotech is an innovative biotechnology company, specialized in developing and manufacturing modern in vitro testing technologies for use in the food, beverage, agriculture, and clinical diagnostics markets. Based in Greece, ProGnosis Biotech is an export-oriented enterprise which through a large network of distributors, exports ELISA kits and lateral-flow tests to more than 62 countries worldwide. In a market where the competition is highly sophisticated and within a continuous challenging environment, Prognosis Biotech has achieved to possess an important role in the food diagnostics industry by providing innovation solutions. The company is always devoted in continuous improvement as it invests in R&D more than 15% of the earnings of each fiscal year. ProGnosis Biotech maintains high product quality by adhering to GMP and ISO standards and holds ISO 9001:2015 and ISO 13485:2016 certifications for development, manufacturing, and marketing of diagnostic and laboratory products.

**Fianovis**
*Act together for food integrity!*
Fianovis is a biotechnology laboratory based in France and specialized in R&D, production and commercialization of innovative and reliable solutions dedicated to food & feed safety, especially mycotoxin analysis. With more than 15 years of expertise in detection and quantification of mycotoxins and substantial investments in R&D and human talents, Fianovis laboratory has developed specific expertise and technology for uniform and complete labelling by isotopic enrichment with carbon 13 (stable isotope). This innovative technology, supported by high added-value know-how ensures accurate mycotoxin detection by utilizing reference calibration solutions and internal standards as molecular tracers. Fianovis supports the growth of your business, meeting your specific needs in terms of mixing, concentration, and conditioning. Additionally, as a trusted partner to laboratories, food industry stakeholders, and research centres, Fianovis guarantees high quality and reliability of its products. The company commitment to its ecosystem is based on quality and reliability, expertise and innovation, support, and responsiveness.

**Neogen**
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MiXscience develops and markets a product offer composed of premixes, minerals, innovative specialties, biocontrol solutions and liquid feed adapted to different livestock species. Expert services enrich this offer. Alongside its customers (feed manufacturers, integrators...) and in partnership with its network of distributors, MiXscience contributes to the development of sustainable farming: economically
efficient, respecting health and animal welfare, preserving the environment, and producing feed of high sanitary and nutritional quality. As a major player in animal nutrition in France and abroad, the company has a total turnover of 150 million euros and operates in more than 50 countries. 10 million tons of feed equivalent are produced each year using MiXscience techniques. Based in Bruz (France), the company is also located in Brazil and Vietnam and develop its products and services in more than 50 countries. MiXscience is part of Avril group. Founded in 1983 as an initiative of the agricultural world to ensure sustainable outlets for French production, Avril is the main industrial and financial player in the vegetable oil and protein sector. Present in sectors as diversified as consumer food, animal nutrition, and expertise, renewable energies, and green chemistry, Avril relies on a portfolio of well-known brands that are leaders in their fields both in France and abroad: Bunica (Romania), Costa d'Oro (Italy), Lesieur, Oleo100, Oleon, Puget, Sanders, and Taous (Morocco)... For nearly 40 years, the group has remained true to its original mission: feeding people and animals, and preserving the planet. In view of the current challenges posed by the climate emergency and the demographic growth that is putting a strain on resources, Avril has chosen to reaffirm its power to act, expressed through its purpose: Serving the Earth. In 2022, Avril generated a revenue of €6.9 billion employing nearly 7,350 people working in 73 industrial sites around the world. As part of its sustainable approach to animal health and welfare, MiXscience has developed the Multiprotect range of products and services to counter the harmful effects of biotoxins (mycotoxins and bacterial toxins). By combining different modes of actions (deactivation, degradation, and bioprotection), Multiprotect in-feed solutions are intended to reduce the consequences of biotoxins risk exposure and to help animals to handle stress that their induce. A service offer focused on analysis and decision tools completes this range of products.
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FOOD SYSTEMS RESILIENCE: A MAJOR PART OF GLOBAL FOOD SECURITY

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The ability or indeed inability to feed the world’s growing population has come into sharp focus over recent years. Discussions around food security, food insecurity and sustainability have intensified at national and international levels and have become topics discussed and debated at major economic and political fora. More recently the term ‘food system resilience’ (FSR) has entered into these discussions and one of the best definitions for this concept is ‘Food system resilience: capacity over time of a food system and its units at multiple levels, to provide sufficient, appropriate and accessible food to all, in the face of various and even unforeseen disturbances’. The focus of FSR is clearly around the having the ability to cope with major shocks to a national food system. A range of examples includes climate change, rapid urbanisation, population changes, natural disasters, financial and political crises, and how food systems themselves respond and adapt to these processes and events. Some of these are ongoing processes that have already started to impact the global food system while others can be very much country or even region specific. On the global scale, our changing climate is without doubt the biggest issue we are facing. It is no longer a case of looking at problems that may arise in the future, but a combination of how these are currently impacting different countries in different ways and how these impacts will continue to escalate unless urgent actions are taken.

The presentation will review some of the consequences of supply chains disruptions and discuss some of the strategies to increase their resilience. A number of case studies will be used to illustrate these points.

TOXIN-FREE FOOD (AGAINST THE BACKDROP OF CLIMATE CHANGE)?

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Toxin-free food is wishful thinking, yet a view shared by many consumers. However, even in highly developed Europe, with its comprehensive measures to ensure food quality and safety from field to fork, consumers are confronted with food-related health risks resulting from dietary exposure to harmful environmental chemicals. Thus, food without contaminants including mycotoxins seems to be more of an illusion than a reality [1]. It is important to be able to better assess and classify the health risks to which consumers are exposed through the long-term intake of harmful substances via food. To support this, we conducted a study to determine how safe our food really was. In this study [2], we scrutinised a total of more than 100 risk assessments conducted at the European level, mainly by the European Food Safety Authority (EFSA). As a result, we evaluated, in detail, those potentially harmful chemical substances in food to which consumers were chronically exposed to. Finally, as part of our studies, we attempted to rank the chronic risks of the identified contaminants. This was done by considering both the type of critical effect they induced and their eventual daily intake via food. In this ranking, the contaminants from food processing are in first place, followed by aromatic petroleum hydrocarbons. This is due to their genotoxicity and carcinogenicity and their widespread distribution in many foods consumed daily. In third place are aflatoxins due to their high carcinogenic potency, causing liver cancer in humans, coupled with the high consumption of cereal-based foods in Europe. Although aflatoxins
appear to play only a minor role in the development of liver cancer in Europe, their chronic dietary exposure still poses a potential risk to Europeans, even if the legal limits for maximum concentrations of aflatoxins in food are respected. Exposures to other regulated mycotoxins in Europe, such as deoxynivalenol, T-2 and HT-2 toxin, zearalenone and fumonisins do not pose a health risk for average consumers in Europe.

Climate change will continue to affect fungal growth and thus the formation of aflatoxins and other mycotoxins. It is also projected that changes in climate will shift the geographic distributions of mycotoxin-producing fungi and hence the patterns of mycotoxin occurrence in the world. The amounts of mycotoxin-contaminated crops are generally anticipated to increase with global warming, but at the same time major variations in (emerging) mycotoxin contamination are also expected [3]. Due to a lack of data, the available risk assessments for many emerging mycotoxins, such as enniatins, are, however, not highly robust. However, their frequent occurrence and the resulting exposure from foods suggest a potential health risk for average European consumers. A balanced diet is the best way to avoid exceeding the harmful daily dose of food that may be contaminated with harmful (carcinogenic) substances!

References

EXPLORING THE IMPACTS OF CLIMATE CHANGE ON MYCOTOXIN CONTAMINATION IN FOOD SYSTEMS: STATEMENTS, GAPS AND PERSPECTIVES

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Climate change is a complex phenomenon with the potential to significantly alter marine, terrestrial and freshwater ecosystems all around the world. It is projected that global warming of 2°C will be exceeded during the 21st century and some extreme events such as severe drought, wildfires and heavy precipitation have already emerged in all their detrimental nature in different areas of the world. It is therefore evident how climate change can impact on multiple food safety hazards, of which mycotoxin contamination is of increasing significance. Research focusing on the effects of climate change on mycotoxin contamination in crops and the associated risks to human and animal health has developed considerably throughout the years. Therefore, a comprehensive literature search was carried out to collect available studies in scientific literature on the topic ‘Mycotoxins and climate change’ published between 2000 and 2023. The selected papers highlighted the major findings on the topic and exposed the existing gaps and future research needs. In particular, it is acknowledged how warmer temperatures are enabling the migration, introduction and increased abundance of thermophilic and thermotolerant fungal species, including those producing mycotoxins. Certain mycotoxigenic fungal species, such as Aspergillus flavus and Fusarium graminearum, are expected to acclimatize to new conditions and potentially become more aggressive pathogens. Furthermore, abiotic stress factors resulting from climate change are expected to weaken the resistance of host crops and make them more vulnerable to fungal infections, increasing the risk of mycotoxin contamination. Changes in the interaction of mycotoxigenic fungi are likewise expected, resulting in different community structures and dominances of different species, with the effect of influencing the prevalence and the co-occurrence of mycotoxins in the future. The impact of climate change on mycotoxin production is complex and varies depending on fungal species and environmental conditions. Improving predictive modelling, expanding research on different pathosystems and facilitating the application of effective mitigation strategies are all matters of utter importance. Future research efforts should concentrate on investigating how the co-occurrence of mycotoxigenic fungi could change in a climate change scenario, as well as the production of modified mycotoxins. Farmers will need to adapt their farming practices to new conditions, and new strategies for mycotoxin contamination control in crops will need to be developed in response to climate change. Understanding these complexities is crucial to ensuring food safety and security and preventing harmful effects on human and animal health.
PLENARY SESSION
INTERACTIVE DEBATE – A WORLD TOUR ON CLIMATE CHANGE
AND THE BATTLE AGAINST MYCOTOXINS

The International Society for Mycotoxicology (ISM) proudly presents its first interactive debate on climate change and the battle against mycotoxins.

Representatives of different mycotoxicology societies together with the sponsoring companies will share their views on the topic based on data from the respective countries/continents. Expert panel members are Amnart Poapolathep, president of the Thai Society of Mycotoxicology, Hans-Ulrich Humpf, representing the Society for Mycotoxin Research, Juan Manuel Palazzine, representing the Latin-American Society for Mycotoxicology, Kiminori Shimizu, representing the Japanese Society of Mycotoxicology, Lindy Joy Rose, representing the African Society for Mycotoxicology, and Yemisi Jeff-Agboola, president of the Mycotoxicology Society of Nigeria.

The debate will start with a short introduction on ISM by Antonio Moretti, general secretary of ISM, followed by presentations by Gerd Schatzmayr (dsf-firmenich) with data from the global mycotoxin survey related to climate/weather data and Julie Brunkhorst (Trilogy Analytical Laboratory) presenting data on mycotoxin contamination from North America.

This first part will be followed by the discussion amongst the different panel member moderated by Sarah De Saeger, president of ISM and Esther De Rycke, Ghent University. Different topics will be targeted among which specific climate-change related research results and forecasting models from the different countries/continents. Questions on how the countries/continents are preparing for climate-change related mycotoxin management will be discussed. Moreover, the importance of data sharing will be elaborated and what role the societies could play. Questions from the audience are welcomed as this debate will be interactive.

Finally, the debate will come up with a top-10 future needs for climate-change readiness.

Acknowledgements
The debate is sponsored by dsm-firmenich, R-Biopharm, Romer Labs and Trilogy Analytical Laboratory; in collaboration with Affidia Journal.
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 81-168).

P25
The interaction of deoxynivalenol and natural microbial flora
Suqin Shao
Guelph Research and Development Centre, Agriculture and Agri-Food Canada, Canada

P84
Targeted inoculation with Fusarium culmorum: Tracing Fusarium toxins from barley to beer
Eva M. Biehl
Chair of Analytical Food Chemistry, Technical University of Munich, Germany

P93
Machine learning for predicting zearalenone occurrence in pet food
Jinquan Wang
Institute of Feed Research, Chinese Academy of Agricultural Sciences, China

P96
Production of mealworm larvae biomass with the use of feed materials with a high concentration of deoxynivalenol
Łukasz Zielonka
Department of Veterinary Prevention and Feed Hygiene, University of Warmia and Mazury in Olsztyn, Poland
MYCOTOXIGENIC FUNGUS OR NOT? LET'S HAVE A LOOK AT THE GENOME!

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The production of mycotoxins is major food safety threat which requires to carefully monitor their presence in food and feed. This issue is becoming even more important in the biotechnology era as more and more fungal-derived products are being produced, from biocontrol agents to mycoproteins and fungal leather. Like any other fungal secondary metabolites, mycotoxin production is tightly regulated and induced under specific conditions that are often not known. Thus, using analytical chemistry methods to detect the production of mycotoxins is only providing information about the tested condition. For applications like biocontrol and feed, this traditional approach does not fully guarantee that the fungus of interest will not produce mycotoxins. A more reliable approach to guarantee the safety of a fungal product relies in genome analyses. Indeed, the biosynthetic pathways of monitored mycotoxins, such as aflatoxins and fumonisins, and of even emerging mycotoxins, such as sporidesmins, are known. Combining genome sequencing and phylogenetic dereplication is a strategy that provides a clear answer about the capacity of a fungus to produce known mycotoxins or not. This approach is also useful to monitor the production potential in field isolates as mycotoxigenic ones may not produce mycotoxins under laboratory conditions. I will provide examples of both applications. Although this approach does not give information about uncharacterized biosynthetic pathways, it accurately certifies on the safety of a fungal strain regarding known mycotoxins.

INTER- AND INTRA-SPECIFIC GENETIC VARIABILITY OF MYCOTOXIGENIC FUNGI LEADING TO DIFFERENCES IN MYCOTOXIN PROFILES

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Toxigenic fungi that colonize crops of agro-food interest are reason of particular concern since they can produce mycotoxins, and accumulate them in the final products, being a serious risk for human and animal health. Among the species that produce mycotoxins, those belonging to Aspergillus and Fusarium genera are the most common, showing a great variability of their mycotoxin profiles, even in species phylogenetically closely related. In addition, such variability can be detected at intraspecific level. The results of ISPA team studies, carried out in cooperation with other research institutions, and conducted through metabolomics analyses and whole genome sequencing approach, are here reported. In particular, we will report: (i) the variability of ochratoxin A (OTA) and fumonisin B2 (FB2) production related to the occurrence of gene clusters (ota and fum), in Aspergillus niger clade, where both the intact and deleted gene clusters co-exist; (ii) variability of beauvericin (BEA) production and BEA gene cluster in Fusarium subglutinans and Fusarium temperatum, two phylogenetic sister species where toxigenic potential is related to single nucleotide polymorphism occurrence in bea1 gene; (iii) variability of fumonisins (FBs) production and FUM genes cluster in Fusarium proliferatum isolated from fig and maize, two populations with apparently same toxigenic potential, but different FBs production capacity; and (iv) variability of trichothecenes (TRI) production in the Fusarium equiseti incarnatum species complex and related variability in TRI genes cluster. Taken together, these data show that mycotoxin gene clusters can dramatically differ within a single species or among very closely related species and the lack of a given mycotoxin production, at least in vitro conditions, is frequently, but not always, related to the absence of related gene clusters. Due to the examples of genetic variants in several toxigenic fungi with respect to mycotoxin gene clusters, we need continuous epidemic studies from different crops and geographic areas by using a consistent and statistically significant approach to generate data that better would reflect the current dynamics of species profile and their genetic variability, also in the
perspective of the current climate change scenario. In addition, a further current challenge should be to use genomic approach to better understand the relationships between genotyping and phenotyping for pathogenicity, mycotoxin profile and stress related genes.

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ROOT UPTAKE AND METABOLIZATION OF ALTERNARIA TOXINS INTO WHEAT PLANTS USING A HYDROPONIC CULTIVATION SYSTEM

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Mycotoxins can leach out of contaminated plants or crop debris into the soil entering the plant via the roots. The presence of aflatoxin B1, ochratoxin A, T-2 toxin, deoxynivalenol (DON), nivalenol and zearalenone (ZEN) in the soil has been reported. However, data on the natural occurrence of Alternaria toxins (ATs) in soil are lacking so far. The transfer from soil into the plant has already been shown for several xenobiotics but only limited data are available for mycotoxins. Few studies on the uptake of mycotoxins by plants and crops in in vitro systems, greenhouse and field experiments were performed. The incubation of seedlings of durum wheat and barley as well as tissue cultures with ZEN demonstrated its absorption by plant organs and the formation of several metabolites that were extracted from roots and leaves [1,2]. Similar studies have been performed on biotransformation of T-2 and HT-2 toxin [3] and DON [4]. Even though fungi of the genus Alternaria are ubiquitous in the environment, no studies on the transfer of ATs from soil into the plant have been carried out so far.

The aim of this work was to evaluate the importance of this entry pathway and its contribution to the overall content of ATs in wheat plants to better understand the soil-plant-phytopathogen system. A hydroponic cultivation system was established and wheat plants were cultivated for up to two weeks under optimal climate conditions. One half of the plants was treated with a nutrient solution spiked with alternariol (AOH), alternariol monomethyl ether (AME) and tenuazonic acid (TeA), whereas the other half was cultivated without mycotoxins. Plants were harvested after one and two weeks and analysed using a QuEChERS-based extraction and an in-house validated LC-MS/MS method for quantification of the ATs in roots, crowns and leaves separately. ATs were taken up by the roots and transported throughout the plant up to the leaves after one as well as two weeks of cultivation with the roots showing the highest ATs levels followed by the crowns and the leaves. In addition, numerous AOH and AME conjugates like glucosides, malonyl glucosides, sulfates and di/trihexosides were detected in different plant compartments and identified by high-resolution mass spectrometry.

This is the first study demonstrating the uptake of ATs in vivo using a hydroponic system and whole wheat plants examining both the distribution of ATs within the plant compartments and the modification of ATs by wheat plants [5].

References
RESISTANCE TO ASPERGILLUS FLAVUS INFECTION AND AFLATOXIN CONTAMINATION IN MAIZE

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Aflatoxin contamination in maize, a major food and feed crop, caused by Aspergillus flavus, is a global concern that compromises food safety and marketability. Aflatoxins are potent carcinogens that adversely impact human and animal health worldwide. The most efficient and practical approach to reduce pre-harvest contamination in maize is the development of resistant lines. Our laboratory has been working to achieve this objective through conventional and molecular breeding. Our molecular breeding approaches include: (i) expression of antifungal synthetic peptides, for effectiveness against toxin-producing A. flavus strains in addition to their broad-spectrum control of other pathogens. Transformation of maize lines with genes encoding synthetic peptides has yielded up to 95% control of aflatoxin contamination. (ii) Host-induced RNAi-mediated gene silencing of key A. flavus genes responsible for growth, infection and toxin production which enables down-regulation of specific genes. So far, we have successfully demonstrated this approach so that transgenic RNAi maize lines can shut down key fungal genes needed for growth (alpha-amylase, p2c or polygalacturonase, alkaline protease) and/or aflatoxin production (aflatoxin biosynthesis pathway genes and global regulators such as aflC, aflS, aflM, veA, nsdC) resulting in significant aflatoxin reduction in transgenic kernels. Some of these lines have been evaluated under field conditions to demonstrate significant resistance to aflatoxin contamination and c) identification and overexpression of key secondary metabolites in maize, such as flavonoids, carotenoids and polyamines, that can contribute to resistance to aflatoxin contamination.

A summary of our research efforts along with other complementary approaches and their effectiveness in reducing the aflatoxin contamination to safe levels in food and feed crops, such as maize, will be elaborated.

MECHANISMS GOVERNING THE REGULATION OF MYCOTOXIN BIOSYNTHESIS AND VIRULENCE IN THE PLANT PATHOGEN PENICILLIUM EXPANSUM

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Penicillium expansum is one of the most harmful post-harvest pathogens and the causal agent of blue rot disease in apples and other agricultural products. This fungus poses a major concern for global food security because of its widespread occurrence and ability to produce the toxic secondary metabolites patulin and citrinin that can affect virulence and, in addition, make the fruit inedible. Our recent studies have been focused on the regulation of these two mycotoxins, which pose a serious threat to human and animal health. In this regard, the widely studied global regulator of secondary metabolism in fungi, LaeA, was shown to positively regulate patulin production. Deletion of laeA in P. expansum strains led to a significant decrease in patulin biosynthesis due to down-regulation of the expression of the patulin biosynthesis genes. Patulin was also found to be positively regulated by PacC, the pH regulatory transcription factor. Another transcription factor, CreA, the carbon catabolite repressor, was reported to function as a positive regulator of both patulin and citrinin production. In addition to their key role in regulating of secondary metabolism, the loss of all these global transcription factors results in attenuated virulence of P. expansum on apple fruits. Moreover, our recent study has shown that epigenetic mechanisms are involved in the production of secondary metabolites in P. expansum. We found that the epigenetic reader SntB regulates P. expansum development, patulin and citrinin biosynthesis and virulence on apples. SntB is a positive regulator of laeA, creA, and pacC expression, which may explain some of its impact on fungal biology.

In order to identify common signaling pathways affecting mycotoxin accumulation in fruits, we performed RNA-seq analysis of the P. expansum wild-type, ΔsntB, ΔcreA, ΔlaeA and ΔpacC strains isolated from
apples after inoculation. Using genome-wide transcriptome profiling of *P. expansum* strains, we identified three uncharacterized transporters co-regulated by SntB, LaeA, CreA and PacC, which are involved in mycotoxin production. Elucidation of mechanisms controlling mycotoxin production in *Penicillium* and other pathogens will stimulate new approaches to postharvest disease control, as decreased mycotoxin synthesis may result in reduced fruit rot during storage and present improved health outcomes for consumers.

**DISSECTING THE ROLE OF *FUSARIUM AVENACEUM* MYCOTOXINS IN CEREAL AND PULSE CROP DISEASES**

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*Fusarium avenaceum* is a generalist pathogen capable of infecting a variety of important crops, among other plant species. The pathogen has caused problems for two important groups of crops in regular rotation in the Canadian Prairies: cereals and pulses. In cereals, *F. avenaceum* is involved in *Fusarium* head blight (FHB), an important disease of the inflorescence that results in mycotoxin contamination of cereal grains. In pulses, such as peas and lentils, *F. avenaceum* is one of the major pathogens in the root rot complex and causes significant yield losses. *F. avenaceum* isolates originating from pulses can infect cereals, and *vice versa*, which is an important consideration in cereal/pulse crop rotations. The main FHB pathogen of cereals, *F. graminearum*, produces trichothecene mycotoxins, such as deoxynivalenol (DON), that have been shown to be involved in virulence in wheat. *F. avenaceum* does not produce trichothecenes, but has the genetic machinery to produce a wide variety of secondary metabolites and mycotoxins, with the genome encoding in the neighborhood of 80 key enzymes involved in their production. Our team has been investigating the role of secondary metabolites and mycotoxins produced by *F. avenaceum* in wheat and pulse crop diseases, as their role in disease has not been previously characterized. One of the most abundant mycotoxins produced by *F. avenaceum* are the enniatins (ENNs), a group of cyclohexadepsipeptides that can cause necrosis in at least some plant tissues, including wheat leaves. ENNs are known to accumulate in *F. avenaceum* infected cereal grains and act as virulence factors in potato tuber necrosis caused by a group of *Fusarium* species. More recently they were also found to influence virulence on barley seedlings and inflorescence. We disrupted the ENN synthase (ESYN) gene, which encodes the main enzyme involved in ENN production, in two Canadian *F. avenaceum* isolates. We did not observe any effect on virulence in durum wheat inflorescence and pea roots; however, a more detailed study in hexaploid wheat suggests that there may be a small effect on FHB virulence.
SILENT SABOTEURS: EXPLORING THE COUNTERINTUITIVE INTERPLAY BETWEEN WEAK PATHOGENS AND THE VIRULENT *FUSARIUM GRAMINEARUM* IN FUSARIUM HEAD BLIGHT DISEASE SUPPRESSION IN WHEAT

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In plant pathology, researchers often study the response of plants to pathogenic infections through a bilateral interaction between one pathogenic strain and a host. However, in agro-ecosystems, disease development is always the result of complex interactions involving multiple species. This intricate web of interactions presents an intriguing ‘crime scene’ for investigating how plants cope with simultaneous invasions by various pathogens. Fusarium head blight (FHB) represents a significant fungal disease affecting wheat, with *Fusarium graminearum* and *Fusarium poae* being the predominant species associated with the disease in Europe. Interestingly, while both species can be found in diseased kernels, only *F. graminearum* is considered pathogenic, while *F. poae* lacks virulence. The role of such weakly pathogenic species in disease development remains a mystery. To shed light on this, our study utilized a time series of (co)inoculations, along with multispectral imaging, transcriptional profiling, and mycotoxin analyses, to investigate the temporal interaction between both species and wheat. Our findings revealed that co-inoculation of *F. graminearum* and *F. poae* inhibited symptom development, but mycotoxin accumulation remained unaffected compared to single *F. graminearum* infection. In contrast, pre-inoculation of *F. poae* reduced both FHB symptoms and mycotoxin levels when compared to a single *F. graminearum* infection. Intriguingly, *F. poae* demonstrated increased growth in dual infections, indicating that this weak pathogen thrives in the presence of *F. graminearum* infection. RT-qPCR analyses further revealed that *F. poae* induces jasmonic acid and salicylic acid responses, suggesting that these pathways might hinder a subsequent *F. graminearum* infection. Then, we investigated the potential impact of several biocontrol bacteria against *F. graminearum* and *F. poae*. *Streptomyces* sp. and *Rhodococcus* sp. were tested for their efficacy in reducing FHB symptoms and mycotoxin production by *F. graminearum*, both in the presence and absence of *F. poae*. Our results indicated that both actinobacterial strains successfully reduced FHB symptoms and DON levels in wheat ears inoculated with *F. graminearum*. While *Streptomyces* exhibited direct antagonistic effects, *Rhodococcus*-mediated suppression of *F. graminearum* was linked to the archetypal salicylic acid and jasmonic acid defense pathways. Surprisingly, the biocontrol efficacy was significantly reduced when *F. poae* was co-inoculated with *F. graminearum*.

In conclusion, our study highlights how control strategies to reduce FHB are hindered by the presence of the weakly pathogenic *F. poae* in the disease complex. This research provides valuable insights into the complexity of controlling plant diseases caused by multiple pathogens, offering potential directions for future control strategies.

MYCOTOXIN-SOIL INTERACTIONS: THE ROLE OF SOIL IN THE BIOSYNTHESIS, FATE AND BIOLOGICAL EFFECTS OF MYCOTOXINS

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Mycotoxins are secondary metabolites also occurring in soils, as part of a chemical-ecological response. Consequently, mycotoxins have an ecological role in terrestrial ecosystems. Levels of mycotoxins in soils are in the range of ng/g, which is very low in comparison with levels in food and feed matrices. The occurrence of mycotoxins in soils obey to different processes: the indirect contamination of soils from...
adjacent systems, i.e., mobility with rainwater from contaminated fields or incorporation via animal manure. But mycotoxins in soils are also produced in situ. In this context, we observed that in agricultural soils mycotoxin concentrations increased after pesticide application and in nutrient depletion scenarios, in example of deoxynivalenol, indicative of a stress-mediated response. In soil monitoring studies, we observed that deoxynivalenol is the most frequently detected mycotoxin, however its distribution is heterogenous, which point out the need of suitable sampling and analytical strategies. In the case of aflatoxin B1 and deoxynivalenol, the residence time in soils is highly dependent on soil properties rather than concentrations. Biological degradation has the major contribution to the degradation of mycotoxins in comparison to abiotic factors such as light, or the presence of organic acids, resulting in a degradation of almost 80% after few days. This point out that the status of the soils, in particular the soil microbiome, is key for the persistence of mycotoxins. Regarding the effects of mycotoxins in soils, we observed in preliminary studies that deoxynivalenol takes part in soil biogeochemical processes related to N-status in the soils. Furthermore, we observed that mycotoxins exert temporal effects on the structure and functions of the soil microbiome, in example of the fungi:bacteria ratio and the catabolic response of the soil in the substrate utilization patterns. The results of these studies indicate that mycotoxins do occur in soils and that they are produced to follow an ecological role in this ecosystem.

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A QUALITY CONTROL SCHEME DESIGNED TO ASSESS SAMPLE PREPARATION PERFORMANCE

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Many mycotoxins are not evenly distributed in agricultural commodities and foods. It is well known that sampling and sample processing, as opposed to the analytical testing method, are the major contributors to the variability of mycotoxin measurements. Proficiency tests evaluate the performance of the analytical testing method by providing comminuted material for participants to (in some instances) sub-sample, and then extract and analyse as per the participant’s analytical method. Therefore, the format of these proficiency tests does not assess performance of the crucial steps of sampling and sample processing.

In this work, we have developed a scheme to assess ‘performance’ of preparing a laboratory sample of whole grain wheat for analysis of deoxynivalenol (DON). The scheme involves preparation and analysis of duplicate laboratory samples using a participant’s own method for sub-sampling, comminuting, and other handling, up to preparation of the test portion for extraction. Material mimicking Fusarium damaged kernels was prepared with the aim of minimizing the variability of DON content. Various approaches were taken to produce this material; the most acceptable approach produced ‘kernels’ with an average DON concentration of 2,500 mg/kg and mass of 0.034 g. The test material was used to fortify portions of blank whole grain durum wheat. The durum had been visually inspected and determined to be free of fusarium damage as well as sub-sampled and analysed for DON, which was not detected above the LOQ of 0.05 mg/kg. Concentrations of DON in the DON-containing test material and fortification levels of the whole grain laboratory samples were chosen to approximate a relevant scenario of whole grain DON concentration of 1 mg/kg and low Fusarium damage. Trial analyses of the fortified whole grain durum, representing good and poor sample preparation practises, were conducted. The variance in DON data attributed to sample preparation was 0.001 mg²/kg² for the good practise. In comparison, the bad practise had a variance of 2.5 mg²/kg². The variance due to sample preparation was comparable to the variance due to the analytical test for the good sample preparation practise. However, for the poor practise the variance due to sample preparation was approximately 600x greater than the variance due to the analytical test. The ability to distinguish the good and poor sample preparation practises based on variance demonstrates the potential of this scheme as a tool to assess the ‘performance’ of sample preparation.

CHALLENGES TOWARDS SAMPLING AND ANALYSIS OF ERGOT ALKALOIDS IN WHEAT GLUTEN

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Ergot, caused by the fungal pathogen Claviceps purpurea, infects the female flowers of a range of cereal crops. At harvest time, the dried purple to black coloured fruit bodies can be present in the commercial crop. Due to climate change more frequent infestations are established in different cereals. The sclerotia contain different classes of alkaloids. At higher levels and over a broad range, statistically significant linear relationship between the content of sclerotia and the levels of ergot alkaloids are observed. However, this relationship could not always be demonstrated at lower levels which indicates that the absence of sclerotia does not exclude the presence of ergot alkaloids. One reason is the highly varying content of alkaloids in sclerotia. Handling of cereals breaks the sclerotia, resulting in ergot dust, which is then adsorbed to the cereal grains and are difficult to mitigate in processing plants. Despite the
mentioned discrepancy, the EU Food Contaminant Regulation 1881/2006 was amended in October 2015 to introduce a maximum level for ergot sclerotia of 0.5 g/kg for unprocessed cereals (excluding maize and rice) and establishing categories for ergot alkaloids without setting limits. The amendment included a call for the monitoring of ergot (alkaloids). In January 2022, maximum levels entered into force for the sum of 12 ergot alkaloids: ergocornine/ergocorninine; ergocristine/ergocristinine; ergocryptine/ergocryptinine (alpha- and beta-form); ergometrine/ergometrinine; ergosine/ergosinine; ergotamine/ergotaminine in cereal processed products and wheat gluten.

An EURL report of 2019 compared three rapid test methods from which the results did not seem to correspond with the best available method: LC-MS/MS. An update will be presented on comparing recent obtained results. Analytical data on the content of ergot alkaloids in a set of sclerotia collected in at least two different countries will be provided and discussed, as well as the distribution of ergot alkaloids in a truck loaded with wheat gluten. Although two studies have been published on the distribution of ergot alkaloids in dry different milling fractions of barley and durum wheat, no recent study is available on the distribution of ergot alkaloids in soft wheat. Results will be discussed on the distribution of ergot alkaloids during dry milling of soft wheat used in the European starch industry.

References

RECENT ADVANCES IN MYCOTOXIN ANALYSIS AT FDA

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To protect and promote food safety, the FDA has established regulatory levels of mycotoxins in a wide range of food and feed products and has incorporated mycotoxin analysis into regulatory monitoring and surveillance programs. Due to the variation in the physicochemical properties of mycotoxins and the complex process of mycotoxin analysis, the development of robust analytical protocols for mycotoxin detection must address challenges related to sampling, sample homogenization, subsampling, mycotoxin co-occurrence, method validation, and the availability of certified matrix reference materials. Furthermore, much of the sample preparation for mycotoxin analysis still needs to be handled manually, leading to laborious operations with low throughput for routine sample analysis. Therefore, in recent years, we have developed practical tools for mycotoxin analysis to address these challenges.

This presentation will review some of the existing technical issues and difficulties encountered in the analysis of mycotoxins in food samples and will highlight tools for automated sample preparation, the validation and extension of LC-MS based methods for mycotoxin analysis, the characterization of sample homogeneity using laser diffraction particle size measurements and real-time imaging analysis, and the application of mycotoxin certified matrix reference materials to evaluate method uncertainty and establish metrological traceability of measurements. Our results demonstrate that the incorporation of these newly developed tools not only expands the FDA’s capabilities to monitor mycotoxins in foods but also sheds the light on future directions for mycotoxin research at the FDA.
INTERLABORATORY STUDY TO NORMALIZE LC-MS MYCOTOXIN DETERMINATION USING THE N-ALKYLPYRIDINIUM-3-SULFONATES (NAPS) RETENTION INDEX SYSTEM

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A major challenge for analytical chemistry is the ability to compare LC-MS data between laboratories and across instrument platforms. Currently, mycotoxin determination relies on dereplication strategies based on physicochemical properties such as the \( m/z \) of the precursor and product ions as compared to standards. Unlike these intrinsic properties, retention time (tR) is an extrinsic property impacted by the chromatography conditions, making exchange of data between groups difficult. To address this, we are promoting the incorporating an electrospray compatible, retention index (RI) system based on a series of N-alkylpyridinium-3-sulfonates (NAPS). Our group at Agriculture and Agri-Food Canada along with the Nation Research Council of Canada are conducting an interlaboratory retention index study for mycotoxins. Each participating laboratory will be asked to run both the mycotoxin mixes and NAPS standards by LC-MS using their normal pre-validated multi-mycotoxin methods, report the retention time for each analyte, and upload the raw data files. The goal of this study is to help get the mycotoxin community to move away from reporting retention times from individual instruments using specific columns and elution gradients to normalized retention indices. This will reduce the number of misidentified mycotoxins in the literature and will increase the ease of data and method sharing between laboratories.

COMPARISON OF UHPLC-MS/MS METHODOLOGIES FOR HUMAN BIOMONITORING OF MULTIPLE MYCOTOXINS IN SERUM

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Human biomonitoring, which is the direct measurement of biomarkers of exposure and effect in biological fluids, is increasingly being accepted as an efficient way to assess human exposure to mycotoxins and to investigate the impact of mycotoxins on human health [1]. Ultra high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) has become a powerful tool for both quantitative and qualitative analysis of multiple mycotoxins in various matrices. In particular for human biomonitoring, there is a need for high-throughput, selective and sensitive methods in limited sample volumes. We have developed a methodology for the screening of 176 mycotoxins and metabolites in human serum by UHPLC-high resolution MS/MS using a Synapt G2-Si HDMS (Waters®, Manchester, UK) followed by data analysis using UNIFI software (Waters®). UNIFI was used to perform automatic peak processing and subsequent compound identity confirmation employing an in-house library containing MS/MS information found in the literature as well as the retention time, experimentally determined when commercial standards were available or predicted with retention time prediction Retip R package in absence of standards [2]. The method was validated by spiking standards of 40 mycotoxins and metabolites in human serum and the limit of detection (LOD), and lower limit of quantification (LLOQ) ranged from 1.1 to 30 µg/l and 2.0 to 55 µg/l, respectively. Compared to targeted analysis by UHPLC-multiple reaction monitoring MS using a triple quadrupole XEVO TQ-XS (Waters®), the screening method had a LOD and LLOQ about 10 times higher for most mycotoxins. However, targeted analysis was restricted by the availability of standards of the mycotoxins and their metabolites. Conversely, the screening method did not require standards for qualitative analysis, allowing detection of the mycotoxins and metabolites included in the library and retrospective data analysis if more compounds or information are added to the library in the future. The developed screening and targeted methods were applied to human serum samples of oesophageal cancer patients and healthy controls from Ethiopia (Ethical dossier number 024/21/DMIP), in which citrinin, ochratoxin A and tenuazonic acid were detected and quantified.
ERGOT ALKALOIDS IN CEREALS, RESULTS AND TRENDS FROM A 6-YEAR STUDY OF INDUSTRY MONITORING – A UK PERSPECTIVE

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A project to monitor the occurrence of mycotoxins and other contaminants started in 2016. The project, funded by AHDB, has four industry trade body partners, AIC, BOBMA, MAGB and UK Flour Millers, that each submit samples. Since the project started, 2,800 samples have been analysed for a range of mycotoxins and other contaminants and agrochemicals. Sampling takes place at two time points each year delivering freshly harvested and stored products, that include wheat, barley, oats, wheatfeed, oat feed and malt. The focus of this paper will be to present and review the results of the ergot alkaloid analyses. Each year the freshly harvested products are analysed for twelve ergot alkaloids using an LC-MS/MS method accredited to ISO17025. During the lifetime of the project this method has also been adopted as the European CEN standard method. The ergot alkaloid results for seven years of monitoring will be presented alongside industry data on ergot sclerotia monitoring to review how the analytical results for ergot alkaloids tally with the industry sclerotia testing results. Combining alkaloid data with the sclerotia results will allow an overall assessment of ergot occurrence in UK cereals over an extended time period. This will provide an impression of the overall picture on ergot alkaloids in UK cereals to be developed. Trends will be reviewed to assess how ergot alkaloid levels have changed over the life of the project, and what might be contributing factors, including changes in farming practices, weather conditions and how recent research knowledge on the movement of ergot alkaloids within grains may provide some insight into ergot alkaloid occurrence in the UK.
NEW INSIGHTS INTO AFLATOXIN B1 METABOLISM IN VITRO AND IN VIVO BY HPLC-MS/MS ANALYSIS IN COMBINATION WITH INTRAVITAL IMAGING

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Aflatoxin B1 (AFB1) is known to exert species-dependent toxicity and carcinogenicity that may be related to its metabolism and formation of DNA adducts in the respective species [1]. The first part of the study focused on the kinetics of AFB1 metabolism in primary hepatocytes of mouse, rat and human origin by HPLC-MS/MS analysis. Selective and sensitive quantification of AFB1 and its metabolites, such as aflatoxin P1 (AFP1), aflatoxin M1 (AFM1), aflatoxin Q1 (AFQ1), aflatoxicol (AFL), AFB1-N-acetyl-cysteine, AFB1-glutathione (AFB1-GSH), AFB1-guanine and AFB1-lysine in cells and cell medium. Upon treatment of cells with AFB1, samples were collected up to 24 h. In addition, the cellular DNA was analyzed for adducts (AFB1-guanine) which are linked to the carcinogenicity and mutagenicity of AFB1. Fast metabolization and comparably high levels of the far less toxic AFP1 as main metabolite were observed for mice hepatocytes. Furthermore, AFB1-GSH and AFM1 were formed quickly, and only low amounts of AFB1-DNA adducts were detected, which disappeared almost completely from the DNA up to 24 h. In contrast, rat hepatocytes metabolized AFB1 significantly slower and formed mainly AFM1 and AFB1-GSH. Compared to mouse hepatocytes higher amounts of AFB1-DNA adducts were detected, explaining the higher carcinogenic potency of AFB1 in rats in comparison to mice. The main human metabolites were AFM1 as well as AFQ1 and AFL, which had only a minor role in the other species.

In the second part, AFB1 was applied to mice and rats, and samples from plasma, urine and bile were collected for up to 24 h. The metabolite pattern of this in vivo time-course showed comparable results to the in vitro experiments with primary rodent hepatocytes. In order to analyse also the tissue distribution a two-photon microscopy-based technique for intravital imaging of AFB1 based on its blue fluorescence was applied [2]. The results show a very rapid uptake of AFB1 in hepatocytes of mice and enrichment in the nuclei with kinetic differences between mice and rats.

In summary, the results demonstrate a strong correlation between the sensitivity of certain species towards AFB1 and their respective metabolism and distribution.

References
THE AFLATOXIN B1 MISFORTUNE NEVER COME ALONE: TOXICITY OF THE EMERGING MYCOTOXIN VERSICOLORIN A

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During the last steps of the biosynthesis of the dangerous carcinogen Aflatoxin B1 (AFB1), a distinctive structure (a double bond in the 8-9-position of the terminal furan ring) appears. The mutagenicity of AFB1 stems in this structural feature, so precursors sharing this characteristic are potentially dangerous. While this was confirmed for sterigmatocystin, much less is known on versicolorin A (VerA). Our data have confirmed that VerA, like AFB1, is dependent on its biotransformation to become mutagenic according to results of the SOS/umu and Ames test. Metabolomic analysis indicated that VerA can be transformed in epoxide and hydroxylated metabolites that might be responsible for this effect. We have observed that the genotoxicity of VerA, characterized by the induction of DNA strand breaks, replication stress and genomic instability appears after a short exposure and at low concentrations, inducing permanent DNA damage without affecting cell viability. Moreover, our results suggest that the toxicity of VerA is quite complex, inducing profound changes at the transcriptome and proteome level. The functional analysis of our results indicate that VerA toxicity involves important mechanisms including transcription inhibition, mitochondrial damage and the induction of phenotypic changes in cells related with cell shape and adhesion capacity. In conclusion, VerA is a highly dangerous molecule even at low concentrations, and the co-contamination of foodstuff with VerA and AFB1 together represent an uncharacterized hazard warranting further investigation.

UNRAVELLING THE TOXICOKINETICS OF TENUAZONIC ACID THROUGH A HUMAN TOXICOKINETIC TRIAL

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Mycotoxins are toxic fungal secondary metabolites, produced by genera such as Aspergillus, Fusarium, Alternaria, and Penicillium. It was estimated that up to 80% of the world’s food crops are contaminated by mycotoxins. Tenuazonic acid (TeA) is the major secreted mycotoxin of Alternaria alternata, which is a dominant pathogen of numerous plants, fruits, and vegetables. The European Food Safety Authority declared that the European population’s dietary exposure to TeA is by far the highest among the different Alternaria toxins. Apart from its acute toxicity toward rodents (LD₅₀=81-186 mg/kg bw) and chicken embryos (LD₅₀=0.55 mg/egg), TeA showed adverse effects in several animal-feeding trials, such as vomiting and haemorrhages in lungs and gastrointestinal tract. To date, toxicokinetic parameters, biomarkers of exposure, and/or metabolites of TeA are unknown, and the assessment of dietary TeA exposure is only achieved by biomonitoring the mycotoxin itself, whilst ignoring potential metabolization pathways. In this study, 11 different subjects received a single oral dose of TeA at the Threshold of Toxicological Concern to investigate the toxicokinetics of TeA in blood and urine. A total of 113 urinary samples and 128 blood samples (collected via Mitra® devices with VAMS®) were extracted with and without enzymatic hydrolysis and analysed using ultrahigh performance liquid chromatography-Xevo TQ-XS for the quantification of TeA and its phase II-metabolized fraction. Non-compartmental analysis
of preliminary blood data resulted in toxicokinetic parameter estimates for free TeA of $t_{1/2}=1.06\pm0.54$ h, $T_{max}=0.5\pm0.38$ h, $C_{max}=0.85\pm0.59$ ng/ml, $\text{AUC}_{0-\text{inf}}=1.36\pm0.99$ ng $\cdot$ h/ml, $\text{Cl/F}=1.77\pm1.43$ l/(h $\cdot$ kg bw), and $V_d/F=2.12\pm1.26$ l/kg bw. TeA was detected unmodified in urine for 13 h after ingestion, with maximum concentrations ranging between 19.31 ng/ml and 104.05 ng/ml. The cumulative urinary excretion of TeA in 48 h accounted for 40±17% of the dose administered. A subset of the urinary samples was analysed along with samples collected from a control group using UPLC-Orbitrap Exploris for the acquisition of the polar metabolome. The compounds of the metabolome that were effective in the discrimination of the two groups were filtered using orthogonal partial least squares-discriminant analysis and subsequently annotated. Finally, the urinary metabolome was compared with possible metabolites of TeA predicted \textit{in silico} by the use of GLORY, GLORYx, and Compound Discoverer software, leading to the identification of 9 novel metabolites.

**ACCUMULATION OF MYCOTOXINS IN HUMAN HAIR: NOVEL APPROACH FOR ASSESSING CHRONIC EXPOSURE**

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Human exposure to mycotoxins represents a concern for public health worldwide due to their increasingly known toxicological potential. To assess this exposure, human biomonitoring (HBM) studies stand as the most useful tool, based on the measurement of mycotoxins or their metabolites in biological samples. Several biomarkers of exposure have been validated in urine and blood/plasma/serum, providing information about short-to-medium term exposure, but the scientific community is still dealing with some limitations when assessing chronic exposure. In this context, hair emerges as a promising matrix that could provide a wider window of surveillance, from months to years, that is already used for assessing exposure to other dietary contaminants such as heavy metals. Therefore, the objective of this study was to develop and validate an analytical procedure to quantify multiple mycotoxins in human hair for performing a pilot study with volunteers from Spain, using liquid chromatography coupled to Q-TOF high resolution mass spectrometry (LC-Q-ToF-HRMS$^2$) as analytical tool.

Hair samples were subjected to a thorough washing with a non-ionic detergent followed by an enzymatic digestion using dithiothreitol and pronase E. Then, samples (n=100) underwent an acetonitrile-based salt-assisted liquid-liquid extraction (SALLE) prior to LC-HRMS$^2$ analysis. Finally, a retrospective non-targeted screening was also conducted using the spectral library Agilent Mycotoxins and Related Metabolites PCDL. This procedure was validated for beauvericin (BEA), enniatins (ENN) A, A1, B1, B2, aflatoxins (AF) B1, B2, G1, G2 and T-2 toxin (T-2). Limits of quantification (LOQs) were 2.2 ng/g for BEA, 2.2 ng/g for AFs, 8.7 ng/g for T-2, and ranged from 0.6 to 2.2 ng/g for ENNs. After the analysis of the collected samples, results showed contamination in 43 out of 100 samples. ENNs (up to 18%, range: 3.4-106 ng/g) and AFB1 (7%, range: 5-24 ng/g) resulted to be the most relevant mycotoxins. These values corresponded to a cumulative exposure over five months according to the length of the samples. The prevalence of AFs was significantly higher in hair samples belonging to women (p-value=0.04). Retrospective non-targeted screening showed the presence of 128 mycotoxins or related metabolites, including relevant toxins such as patulin or zearalenone. These results confirmed the accumulation of mycotoxins in human hair and aims to be the starting point for a novel approach in exposure assessment studies. Nevertheless, further studies are required in order to establish a quantitative relation between the concentration ingested and the dietary intake of specifics mycotoxins.

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OVERALL EXPOSURE OF EUROPEAN ADULT POPULATION TO MYCOTOXINS BY STATISTICALLY MODELLED BIOMONITORING DATA

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Human biomonitoring (HBM) measures the internal dose of toxins or their metabolites in human biological samples such as urine or blood. The HBM approach considers exposure from different sources providing a more accurate estimate of the body burden. The urinary biomarkers have gained great importance to assess individual exposure to mycotoxins, since to date a huge number of BM data are available in the literature, this work aims to present the attempt to assess the exposure to mycotoxins by using already published BM data. For targeting the objective, a probabilistic methodology was adopted using the goodness-of-fit method (KS-gof tests) to overcome the initial biomarkers data scarcity, to assess the uncertainty and provide a reliable estimation of the BM concentration. Biomarker data from two research projects, ‘Mycotoxin mixtures in food and feed: holistic, innovative, flexible risk assessment modelling approach: MYCHIF” and ‘Experimental study on deoxynivalenol biomarkers in urine – DONEXPO’ was used. The modelled biomarker concentrations were used as input for the exposure assessment to single mycotoxins, calculated by adopting the approach of the probable daily intake (PDI). Several exposure scenarios have been selected depending on body weight and mycotoxin biomarker concentration class, namely mean, lower bound and upper bound; the exposure has been assessed also clustering scenarios by geographical area (North vs South Europe).

The main output obtained refers to a concern for public health about AFM1, FBs, T-2/HT-2 and NIV, and a low concern for OTA, DON and CIT. The margin of exposure for AFM1 did not respect the reference value of 10,000 considered of low priority for risk; for Fusarium toxins, FBs and T-2 and HT-2 PDI values resulted about ten times higher than their tolerable daily intake (TDI) and NIV presented the most critical situation with a calculated PDI 30 times higher than the reference TDI value. North and South Europe scenarios were also depicted by clustering biomonitoring data for geographical location, OTA and DON showed to be prevalent in Northern countries and the opposite was noticed for ZEN, higher in Southern countries. Notwithstanding the caution in the conclusive outputs, considering the numerosness of data, the limited number of countries feeding the dataset for some biomarkers and the uncertainty in the ER values available in the literature, the output of European scenarios presented is considered a valuable initial exercise evocative of adults’ exposure obtained by a statistical model which gave remarkable results.

EXPOSOMICS STUDY FOR INVESTIGATING MYCOTOXINS EXPOSURE AND THE ASSOCIATION WITH BIOMOLECULAR MARKERS OF AGING AND BIRTH OUTCOMES IN RURAL BURKINA FASO

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Maternal undernutrition is a public health challenge in many African countries, and often results in poor birth outcomes such as small-for-gestational age (SGA), low birthweight (LBW), or preterm. The 2016 World Health Organization antenatal care guidelines suggests that pregnant women in undernourished nations should receive balanced energy-protein (BEP) food supplementation. A food supplement was then developed by the Bill and Melinda Gates Foundation in 2016. In the present Biospecimen Study, pregnant women (n=300) in rural Burkina Faso were individually and randomly allocated to an
intervention or a control group, during pregnancy and again in the post-natal period. The intervention group received a BEP supplementation and the standard iron folic acid (IFA) tablet, whereas the control group only received the tablet. Blood, urine, stool and breastmilk samples were collected from mothers-new-borns pairs according to a well-defined sampling scheme. Biospecimen analyses provide the opportunity to identify biomarkers, of exposure and effect, that can address questions in many areas of interest, including the impact of environmental exposure to toxic compounds, such as mycotoxins and black carbon, and the supplementation effect on biomolecular markers of aging like the telomere length (TL) and mitochondrial DNA content (mtDNAc). Results suggested that combined daily BEP supplement and IFA tablet did not affect newborn TL or mtDNAc as compared to an IFA tablet alone. Exploratory analyses indicated higher, but non-significantly different mtDNAc among children born either SGA, LBW, or preterm. This presentation will highlight the level of exposure to mycotoxins, to investigate the association with TL and mtDNAc as well as birth outcomes.

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DOUBLE TROUBLE: MYCOTOXINS AND SUB ACUTE RUMEN ACIDOSIS’ IMPACT ON LACTATE-UTILIZING MEGASPHERA SP. IN DAIRY COWS – A RUMEN SIMULATION SYSTEM STUDY

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As ruminant livestock currently consume a third of the crops grown on earth, optimizing rumen function can significantly impact global food production and agricultural sustainability. Yet, efforts to manipulate the rumen microbiome have the potential to be undermined by omnipresent mycotoxins in animal feeds. Here, we carried out bioreactor experiments inoculated with rumen content to screen for groups of microorganisms whose growth was hindered by a fungal extract containing deoxynivalenol, zearalenone and aurofusarin. Then, because of its direct susceptibility to these mycotoxins, we characterize the diversity and ecological role of a bacterial lineage that clusters tightly with Megasphaera hexanoica. To do so, we leverage environmental genomic datasets and representative isolates to resolve two divergent sister lineages of Megasphaera and link differential metabolic traits with distinct habitat distributions. This showed that the M. hexanoica lineage harbours low population-level diversity and is preferentially adapted to the adult rumen. Furthermore, M. hexanoica is proposed to glean lactic acid before it accumulates with decreasing pH, unlike its well-characterized sister species, M. elsdenii, which rapidly consumes lactate at high concentrations. This results in fatty acids that are the product of lactate-based chain elongation and considered beneficial to the host. Thus, by studying the differentiation of key lactate utilizers, we describe an overlooked metabolic niche that may be critical for rumen function during phases of pH depression while highlighting mycotoxins as a threat to the microbes inhabiting it.

THE HEPATIC METABOLISM OF AFLATOXIN B1 EXPLAINS THE DIFFERENCES IN SUSCEPTIBILITY TO THE MYCOTOXIN AMONG MAJOR POULTRY SPECIES (CHICKENS, DUCKS, TURKEY AND QUAIL)

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Aflatoxins are probably the most important mycotoxins, not only because they were the first group of fungal toxins to be discovered and characterized, but also because they are highly toxic for some animal species. For mammalian species, aflatoxins in general, and particularly aflatoxin B1 (AFB1) are extremely toxic for pigs, horses, rabbits, dogs, and cats; however, rodents like mice and rats and lagomorphs like chinchillas, are highly resistant. Among poultry species it has been known for decades that ducks are extremely sensitive to the adverse effects of AFB1, while chickens are extremely resistant. The hepatic metabolism of AFB1 of ducks, turkeys, quail and chickens has been studied by
our laboratory for several years. In our studies, we have found important differences in metabolism that correlate with the known in vivo sensitivity to AFB1 and that can explain the large differences in the toxicological response to the toxin among these species.

In general, all poultry species studied produced the same metabolites from AFB1; however, the amount and speed at which the metabolites are produced are very different. The major metabolite of AFB1 is the aflatoxin B1-8,9-exo-epoxide (AFBO), which is very unstable and within seconds transform into a toxic electrophilic metabolite known as AFB1-dihydrodriodiol (AFB1-dhd). Duck hepatocytes produce this metabolite in large amounts while chickens produce it in very small amounts. Further, the detoxication of AFBO through conjugation with the nucleophilic compound glutathione (GSH) is very efficient in the chicken and least efficient in the duck. AFB1-dhd can be detoxified by aflatoxin B1 aldehyde reductase (AFAR) and again, its activity is much higher in chickens that in ducks. Finally, the cytosolic reduction of AFB1 into aflatoxicol, a non-toxic metabolite of AFB1, is highest in chickens and lowest in ducks. Taken together, the results of these series of studies indicate that the hepatic biotransformation of AFB1 explain the extreme sensitivity of ducks and the chickens’ high resistance to the toxin. We postulate that these differences are the result of the very different evolutionary biology scenarios of these two species.

DOSE-RESPONSE EFFECTS OF COMBINED DOSES OF FUMONISINS, DEOXYNIVALENOL, AND ZEARALENONE MYCOTOXINS ON MAJOR T-CELL SUBSETS AND TIGHT JUNCTION PROTEIN EXPRESSIONS IN BROILER CHICKENS

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Fumonisins (FUM), deoxynivalenol (DON), and zearalenone (ZEN) mycotoxins impair gut health and integrity in broilers by decreasing enterocyte proliferation, tight junction protein synthesis, and trans-epithelial electrical resistance. The presence of multiple mycotoxins, even at subclinical doses below the US-FDA tolerance limits (50 mg/kg for FUM and 5 mg/kg for DON), can have a negative impact on poultry health. This study evaluated the compounded effects of graded subclinical doses of FUM, DON, and ZEN on the T cell percentages and jejunal tight junction proteins in broilers. A total of 960 one-day-old chicks were assigned to either one of the following eight treatments with six replicates each: (i) control diet (0.8 mg FUM and 0.4 mg DON); (ii) 33 mg FUM + 3 mg DON + 1 mg ZEN; (iii) 26 mg FUM + 1 mg DON + 0.2 mg ZEN; (iv) 14 mg FUM + 3.5 mg DON + 0.7 mg ZEN; (v) 7.7 mg FUM + 0.4 mg DON + 0.1 mg ZEN; (vi) 3.6 mg FUM + 2.5 mg DON + 0.9 mg ZEN; (vii) 0.8 mg FUM + 1.0 mg DON + 0.3 mg ZEN; and (viii) 1 mg FUM + 0.5 mg DON + 0.1 mg ZEN per kg diet. Data was analysed using ANOVA, and Tukey’s posthoc test was applied. On d14, 3.6 mg FUM + 2.5 mg DON + 0.9 mg ZEN significantly (p<0.05) decreased the CD4+ cells, CD8+ cells, and CD4+CD25+ cell percentages in the caecal tonsils and spleen, and this trend continued until day 35 (p=0.07), compared to the control group. At day 14, 33 mg FUM + 3 mg DON + 1.0 mg ZEN significantly decreased the total IgA (p<0.05) by 20% in bile, and this trend continued until day 35 (p=0.08). At day 21 and 28, 0.8 mg FUM + 1.0 mg DON + 0.3 mg ZEN significantly decreased the total IgA concentration by 20 and 17%, respectively, compared to the control group. At day 35, 3.6 mg FUM + 2.5 mg DON + 0.9 mg ZEN significantly decreased the jejunal Claudin-1 mRNA by 4.8-fold and Claudin-4 mRNA by 6.9-fold, compared to the control group. Claudin-2 expression did not differ significantly between the treatment groups. Broilers fed diets contaminated with at least 3.6 mg FUM + 2.5 mg DON + 0.9 mg ZEN had decreased T-cell percentages, total IgA, and Claudin-1 mRNA expression. Thus, broilers are sensitive to the ingestion of multiple mycotoxins, which, even at subclinical doses, leads to immunosuppression and gut inflammation, decreasing the overall health and welfare of broilers.
THE MYCOTOXINS T-2 AND DEOXYNIVALENOL INCREASE THE TRANSLOCATION OF STREPTOCOCCUS SUIs ACROSS PORCINE ILEAL ORGANOID MONOLAYERS

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Chronic exposure to the Fusarium mycotoxins deoxynivalenol (DON) and T-2 toxin may impair intestinal morphology and function. It is known that both DON and T-2 disrupt the intestinal barrier, increasing the risks of infections. The bacterium Streptococcus suis is often found in pigs and sometimes outbreaks are observed. When the pigs are exposed to stressful conditions, including dietary stress, signs of S. suis infection can be observed, e.g., arthritis, meningitis, and sepsis. In the present study, we hypothesized that co-exposure of the intestinal cells to S. suis and Fusarium mycotoxins can be deleterious even when the toxins are present at levels that will not cause acute hazard. To investigate the interaction between S. suis and mycotoxins ileal porcine organoids were used because of their ability to mimic the complexity of the intestinal mucosa. Therefore, ileal organoids were exposed to 0.1 μM DON, 0.01 μM T-2, S. suis or in different combinations and we measured effects on intestinal viability, permeability, and translocation of S. suis. The tested mycotoxins did not affect S. suis growth, but the ileal organoids affected the bacterial dynamics, possibly due to the antimicrobial peptides produced by this minigut. The mycotoxins alone did not affect cell permeability, but with S. suis a negative impact on the gut barrier was observed. Furthermore, DON and T-2 together decreased the transepithelial electrical resistance and favoured bacterial translocation.

MYCOTOXIN BIOMARKERS FOR LIVESTOCK SPECIES: HOW FAR HAVE WE COME?

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According to Baldwin et al. [1], mycotoxin biomarkers can be classified into two categories: mechanisms-based biomarkers and exposure-based biomarkers. Mechanism-based biomarkers refer to a biological response caused by mycotoxins, such as alterations in protein, enzyme or gene expression levels. This is exemplified by the effects of fumonisins on the sphinganine-to-sphingosine ratio, currently recognized as the best-known mechanism-based mycotoxin biomarker in livestock species. On the other hand, exposure-based biomarkers involve the direct measurement of the mycotoxin itself and/or its metabolites within biological samples. Driven by the inherent specificity of exposure-based biomarkers and significant advances in the field of mass-spectrometry, research on these biomarkers has intensified. As a result, important progress has been made in recent years to elucidate the toxicokinetics of various (non-regulated) mycotoxins across different livestock species and production stages. A widely anticipated step is to transfer the knowledge gained under experimental settings to farm level. Although it is well-known that mycotoxins impair animal health, diagnosis of mycotoxin-induced disorders on farms remains a complex task [2]. While feed analysis represents a crucial tool in this regard, it might be accompanied by challenges, such as the unavailability of relevant feed lots. Consequently, mycotoxin biomarkers are often seen as promising avenue for the on-farm diagnosis of mycotoxicosis. Unfortunately, essential prerequisites for this application, such as the establishment of reference values, are not yet fulfilled. Based on selected examples, this presentation will highlight advancements in mycotoxin biomarker research in livestock species as well as address existing knowledge gaps that still need to be resolved.

References
POTENTIAL MITIGATION STRATEGIES FOR FREE AND MODIFIED FUSARIUM MYCOTOXINS IN OATS

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Oats are frequently contaminated with *Fusarium* mycotoxins including type A and B trichothecenes and zearalenone, and their glucoside conjugates have also been reported. Agronomy practices, cereal variety and climate conditions have been suggested to play a role in driving *Fusarium* infection in oats. Removal of outer husks during processing has been suggested to remove large portions of mycotoxin contamination. The current study investigates levels of free and conjugated *Fusarium* mycotoxins in Scottish oats collected over 4 years and assess the mitigation potential of organic production, cereal rotation and de-husking. Cereal samples were collected from Scottish farms and analysed for 12 *Fusarium* mycotoxins (type A trichothecenes T-2-toxin, HT-2-toxin, diacetoxyscirpenol; type B trichothecenes deoxynivalenol, nivalenol; zearalenone and their respective glucosides) using LC-MS/MS. The prevalence of type A trichothecenes T-2/HT-2 was highest (95-100% of conventional oats, 50-83% of organic oats) whereas type B trichothecenes were less prevalent and zearalenone was rarely found. T-2-glucoside and HT-2-glucoside were the most prevalent conjugated mycotoxins in oats and co-occurrence between type A and B trichothecenes was frequently observed. Organic oats were contaminated at significantly lower average concentrations than conventional oats, and husks contained higher levels of mycotoxins than de-husked kernels. Cereal intensity within the crop rotation was identified as a significant factor driving mycotoxin contamination. Our results clearly indicate that free and conjugated T-2- and HT-2-toxins pose a major risk to Scottish oat production and that organic production, low cereal intensity rotation and de-husking offer potential mitigation strategies.

NATURE VS. MYCOTOXINS: STRATEGIES TO REDUCE MYCOTOXINS IN FOODS AND ANIMAL FEED


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Mycotoxins, toxic compounds produced by fungi, pose a grave threat to human and animal health through contamination of grains and animal feed. Mycotoxin occurrence in grains can reach up to 60-80%, with about 25% exceeding safety standards. Despite preventive efforts, mycotoxins remain a global concern, particularly in tropical regions where mould impacts crops. Radiation, ozone gas, acid/alkaline treatment, low-pressure cold plasma and processing have been used to reduce mycotoxins in grains and their derivates with limited success. In addition, clays and glucomannans are adsorbents used as ingredients in animal feed, but their ability to scavenge mycotoxins is limited to a few molecules.

The fungi that produce mycotoxins thrive in imbalanced environments, affecting soil and plant microbiomes. Research in the past 25 years has focused on finding biological agents to prevent mycotoxin growth or detoxify them. These biocontrol agents can be found in the market. Filamentous fungi such as non-pathogenic *Aspergillus*, non-pathogenic *Fusarium* spp., *Rhizopus* spp., *Trichoderma* spp., yeast, such as *Trichosporon* and *Saccharomyces*, and bacteria, such as *Bacillus*, *Pseudomonas* and lactic acid bacteria, can outgrow pathogenic fungi in the field and significantly minimize mycotoxin production. These are usually microorganisms isolated from healthy soils that can be affordably produced and inoculated in seeds or in the field to produce better environments for plant growth. Our research group has isolated different bacteria such as *Bacillus amylo liquefaciens plantarum* CICC
23985, \textit{Bacillus subtilis} CECT 499, \textit{Bacillus velezensis} CL197, and \textit{Streptomyces griseus} CECT 3276, which can outgrow pathogenic fungi and break down zearalenone in non-toxic compounds. These microbes hold potential for practical deployment in the field and as a probiotic in animal feed, as they present the ability to efficiently detoxify zearalenone even within the gastrointestinal environments of pigs and poultry. Several companies have introduced similar microorganisms to the market, representing a wise and cost-effective strategy for mitigating the detrimental economic and health impacts of mycotoxins.

Fungicides are used in fields and storage, but their handling requires care. Plant compounds, such as essential oils, inhibit mould growth. Allyl isothiocyanate from mustard and other plants inhibits mould growth (i.e., \textit{Aspergillus} and \textit{Penicillium} species) at low doses (as low as 100 ppb), protecting stored crops like corn and wheat. Air circulation in storage silos enhances its efficiency. Developing countries with essential oil production could benefit from this strategy.

Our research group has recently conducted a systematic review followed by metanalysis to evaluate the effect of current genetic modifications applied in commercial maize in the appearance and concentration of mycotoxins in these grains. Transgenic maize reduced overall mycotoxin content by 54\%, particularly fumonisins, aflatoxins, and zearalenone, which decreased by 56, 49, and 51\% respectively. Genetic modifications to resist insects, herbicides, antibiotics, and \textit{Aspergillus flavus} contamination played an important role. GM crops can offer safer harvests in less technological regions.

To combat mycotoxins, a comprehensive approach is crucial. Vigilant monitoring, best practices, and proper storage conditions are vital from field to storage. Natural biocontrol agents, including fungi and bacteria, can deter fungal growth and break down mycotoxins. Genetic modifications in maize show promise in reducing mycotoxin levels, uniting science and agriculture to secure food and feed supplies while preserving health and industry.

\textbf{PULSED LIGHT AS A NON-CONTACT FOOD DECONTAMINATION TECHNOLOGY FOR REMOVING FUNGI AND MYCOTOXINS}

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Food commodities are highly susceptible to contamination by fungi and mycotoxins, which cause great economic losses and threaten public health. Compared to conventional food processing methods, pulsed light as an emerging non-contact food physical processing technology can effectively kill fungi and degrade mycotoxins while having relatively minor negative effects on the functional, sensory, and nutritional properties of the treated foods. Pulsed light was used for control of \textit{Aspergillus flavus} and \textit{Aspergillus carbonarius}, two common fungi in agricultural products, and the optimal conditions for pulsed light were determined. The mechanism of lethality of \textit{Aspergillus} sp. spores by pulsed light technology was investigated, and a bactericidal kinetic model was developed. Pulsed light inhibits hyphal growth, increases cell membrane permeability, destroys cell wall integrity, leaks cell contents and increases conductivity. The expression of mycotoxin biosynthetic genes and growth and development related genes was inhibited by pulsed light through transcriptome and qRT-PCR analysis. Meanwhile, the results showed that the Weibull+Tail model had a better fit and could better describe the inactivation characteristics of \textit{Aspergillus flavus} spores under the sterilization mode of pulsed light in peanut. Moreover, pulsed light can effectively reduce the content of AFB$_1$ and OTA, and the chemical structure (bond) of toxins is destroyed by photochemical reactions and photophysical effects. Pulsed light technology offers a novel way to prevent or control fungus and mycotoxin contamination and has broad application potential.
REDUCTION OF DEOXYNIVALENOL IN ROLLER-MILLED FRACTIONS FOLLOWING WASHING AND PEARLING OF HIGH AND LOW CONTAMINATED WHEAT

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Fusarium fungi and the mycotoxin deoxynivalenol (DON) is present in the vast majority of cereal samples. The toxin is generally present in highest concentrations in the outer parts of the cereal. In this study, we aimed to investigate if traditional pre-processing treatments, such as washing and pearling, can be relevant strategies to decontaminate wheat kernels for DON to obtain safer milled wheat fractions. Both high and low contaminated wheat were investigated, where the latter is more realistic to be used in real cereal food products.

The low contaminated batch of wheat was naturally contaminated (~600 μg DON/kg), while the high contaminated (~12,000 μg DON/kg) was from an artificially Fusarium-infected batch. Samples from the two batches were either neither treated, washed, pearled or washed and pearled prior to milling. Samples were subject to either hammer milling resulting in a wholemeal flour or roller milling resulting in an endosperm fraction and a fraction passed through a bran finisher to separate bran from endosperm residues still adhering to the bran (here termed endosperm rest). Overall, the processing resulted in four fractions from each of the two batches, wholemeal flour, endosperm, endosperm rest and bran. All samples were analysed for DON, DON-3-glucoside and acetylated DON using HR LC-MS. The highest DON content was as expected found in the bran fractions of both high and low contaminated wheat, followed by the wholemeal flour, and lowest in both endosperm fractions. DON did not differ between endosperm and endosperm rest. Pre-processing by both washing, pearling and their combination resulted in high significant reduction of DON, especially in the bran fractions, with highest relative reduction with the low contaminated wheat (>60% compared to control). Combined treatments had only minor additional reducing effects. Interestingly, none of the pre-processing treatments had significant effects on the DON-3G content in any of the fraction types obtained from conventional wheat, but with high contaminated wheat, pearling resulted in increased DON-3G content compared to unprocessed grain in all fractions except the bran. Pre-processing had also impact on the content of 3Ac-DON in all fraction types from high contaminated wheat, though with partly opposite effects as pearling, but 3 Ac-DON concentrations were low compared to DON concentrations. Overall, the results shows that the introduction of a pre-process step on whole wheat kernels could reduce the DON concentrations in the final milling products. This is especially relevant for conventional wheats (low contaminated), that regulatory are allowed to be used for food purposes.

THE NEED FOR HACCP-APPROACH TO MANAGE MYCOTOXINS IN ANIMAL PRODUCTS

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The economic impact of mycotoxins on animal industry is well documented. It is fortunate that only few of hundreds of mycotoxins that are known today leave significant residues in animal products such as milk, meat, eggs, and organs like liver and kidneys. Notable ones are aflatoxin M1 (AFM1) in milk and milk products, ochratoxin A (OTA) residues in pork meat and kidneys, T-2 toxin in eggs, aurofusarin in eggs etc. Among these, AFM1 residue in milk and milk products garnered greater attention as the ratio of conversion of aflatoxin B1 (AFB1) to AFM1 is smaller than other mycotoxins. Aflatoxin M1 can cause liver cancer in animals and humans and hence regulated in most countries. According to the European regulatory authorities, AFM1 levels in milk for human consumption cannot exceed 0.05 parts per billion (ppb) which means complete feedstuffs are limited to 5 ppb AFB1 when used in dairy diets. FDA (US) regulations are less restrictive with an AFM1 maximum level of 0.5 ppb in milk and 20 ppb AFB1 in dairy feed. Any milk that contains AFM1 above the regulatory limit is considered unfit for human consumption and should be discarded. The amount of AFM1 in dairy milk may represent at least 1-2% of the ingested AFB1; however, several factors can affect this percentage, and high-yielding dairy cows can have AFM1 levels in milk that are even above 6% of ingested AFB1 [1]. For example, model calculations showed...
that vulnerable high-yield cows given feed that was within the EU limits for AFB1 might produce milk with AFM1 levels above the EU limit. The presence of AFM1 in the milk may also lead to AFM1 contamination in yoghurt and cheese. Therefore, people who consume milk or dairy products from high-yielding cows might be exposed to harmful levels of AFM1.

An integrated HACCP approach is required to reduce mycotoxin residues in animal products. Five steps that can be taken to protect not only the health of animals, but also the safety of milk and human health.

- Work with your milk company, nutritionist, and feed supplier – Analyse and detect AFB1 in the cereals and feed as well as AFM1 in the milk.
- Understand the level of contamination in milk – As aflatoxin M1 in milk is quite stable, the level in milk will help you to back calculate the levels of total aflatoxin in the feed.
- Understand the contribution of feedstuffs – Monitoring of grains is very critical as this fraction of the diet contributes the most to the total AFB1 intake. Both silages and corn grain can harbour aflatoxin, but do not forget to include by-products and other purchased feedstuffs.
- Understand critical points of contamination – The identification of the critical risk points for mould growth and mycotoxin contamination in dairy operations, and the good management of silage and feed can prevent additional mycotoxin production.
- Take action – Reduce the use of contaminated raw materials, use alternative clean forages and raw materials, use a proven mycotoxin binder – Supportive strategies, such as the use of a mycotoxin binding product, should be added to the feed to alleviate the effects of mycotoxins to the animals and to reduce the transfer of aflatoxin M1 to the milk [2].

References

DEVELOPING A MACHINE LEARNING PREDICTIVE MODEL TO STRENGTHEN THE PREVENTIVE MEASURES FOR MYCOTOXINS IN FOOD AND FEED

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Between 60-80% of our food is contaminated with mycotoxins. During the last 10 years, there is an increasing trend of mycotoxin issues in food and feed according to recorded incidents data. In addition to that we have new emerging mycotoxins for which we do not (yet) have regulatory limits. The research community and the food industry are working towards limiting important mycotoxins issues in our supply chain by proposing new tools to mitigate the mycotoxins risk. Predictive models are one of the tools that we can use to mitigate the risk of mycotoxins. Different types of models such as mechanistic, empirical and hybrid models have been proposed during the last years to predict the occurrence of mycotoxins in food and feed. Recently, numerous studies have been published showing the potential of machine learning and big data in predicting mycotoxins in food and feed.

The proposed solution includes a machine learning predictive model and an interactive dashboard that can be used to design a risk-based monitoring program for mycotoxins. To train and test the model, the monitoring results of the EU monitoring programme for chemical contaminants occurrence in food and feed were used. To validate the predictive model both accuracy-based criteria and non-accuracy criteria were used. The machine learning model is able to predict the number of batches that are potentially non-compliant and should be analysed and those that should not be analysed. To transform the prediction results into the cost of monitoring programme, a cost model that was proposed recently in the literature, was used. The results of applying this predictive model for a specific real use case in order to set up a risk-based approach for a mycotoxins monitoring programme in food and feed will be presented. The goal of setting up a risk-based approach is to make the monitoring programme for mycotoxins more efficient in terms of cost but most importantly in terms of ensuring that we will identify the batches of high risk. The prediction results of the proposed approach can be used by the companies to become more proactive. The mycotoxins prediction results can be used by a food company to communicate the risk internally in the organization, to proactively adjust the testing plan, supplier practices and the audit plan.
ARE MYCOTOXINS IN VEGETABLE-BASED SALMON FEED A CAUSE FOR CONCERN? EFFECTS OF PREVALENT MYCOTOXINS ON WELFARE AND GROWTH OF SALMON IN AQUACULTURE

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The increased demand for salmon feed ingredients and the aspiration to make salmon farming more sustainable has rendered the feed composition to largely being plant based. The use of vegetable feed ingredients has also introduced new contaminants and anti-nutrients previously not associated with the farming of marine carnivorous fish species such as Atlantic salmon. Screening of currently used Norwegian salmon plant-based feeds as well disclosed a high prevalence of mycotoxins. Enniatins (ENNs) were present in over 80% of the examined feeds of which rape seed oil is likely the major source. Due to lack of knowledge on the long-term effects in food producing animals, no upper limits are presently set for these mycotoxins in animal feeds. Recent in vivo and in vitro studies investigating the effect of ENNB and beauvericin (BEA) on salmon, has demonstrated that the levels of mycotoxins occasionally observed in salmon feed could affect the health and growth of the fish. In vitro and in vivo transcriptomic assessment revealed novel mechanisms of toxicity related to iron and heme metabolism. The perturbation of iron and heme metabolisms might be one of the key mechanisms of toxicity following long term exposure to ENNB and BEA in salmon. Although the two mycotoxins show roughly the same toxicity in cell studies, they had different effects on growth in in vivo feeding trials. The group that had been fed BEA became thinner despite eating more than the control group. The poor feed utilization could be due to both increased energy turnover (need for more energy) and poor nutrient uptake linked to the observed increased intestinal leakage. Although no significant effects on the intestines were observed as a result of ENNB in the feed, ENNB had a marked effect on length growth and blood parameters. The present experiments have only examined the effect of single toxins, but a summary of levels of ENNB and BEA in salmon feed shows that they rarely occur alone. If there are high levels of ENNB, there may also be high levels of other mycotoxins and other forms of enniatins.

MACHINE LEARNING-AIDED DESIGN OF COMPOSITE MYCOTOXIN DETOXIFIER MATERIAL FOR ANIMAL FEED

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The development of additives that mitigate the presence of mycotoxins in animal feed involves the finding a material with specific properties that enable the desired function while minimizing the adverse effects related with their interference with the concurrent complex biochemistry of the living organisms. Traditionally, this process is heavily dependent on costly and time-consuming in vitro and in vivo experiments. In this presentation, we will present an alternative, accelerated approach to design clay-based composite materials for mycotoxin removal. The approach can accommodate various material compositions and different toxin molecules. With application of machine learning trained on in vitro results of mycotoxin adsorption-desorption in the gastrointestinal tract, we have searched the space of possible composite material compositions to identify formulations with high removal capacity and gaining insights into their mode of action. An in vivo toxicokinetic study, based on the detection of biomarkers for mycotoxin-exposure in broilers, validated our findings by observing a significant reduction in systemic exposure to the challenging to be removed mycotoxin, i.e., deoxynivalenol (DON), when the optimal detoxifier is administrated to the animals.
Each mycotoxin has specific modes of action, they also manifest different traits in each animal species. This means that each mycotoxin can pose a different kind of harm depending on the host. So, a simple one-act mitigation plan cannot successfully address all or at least the majority of the single effects of mycotoxins at micro (cell and microbiota functions) and macro (organs, animal and its performance) levels. Therefore, a holistic approach must include a number of actions designed to reduce mycotoxins as well as their impacts, and the ‘prevention is better than cure’ rule applies here as well. Mycotoxin-detoxifying agents, including adsorbing agents, reduce the exposure to mycotoxins by decreasing their bioavailability. According to the Commission Regulation (EC) No 386/2009, these nutritionally inert adsorbents are defined as ‘substances for reduction of the contamination of feed by mycotoxins: substances that can suppress or reduce the absorption, promote the excretion of mycotoxins or modify their mode of action.’ Among adsorbents, mineral clays are the most commonly used binders. Combinations of modified yeast cells and of inorganic minerals such as zeolite, bentonite or aluminum silicate are also widely used to deactivate mycotoxins present in feeds. The effectiveness of mycotoxin adsorbents seems to depend both on their chemical structure and the mycotoxin considered. These binders are effective with AFB1 but have limited activity against other types of mycotoxins.

Besides the adsorption process, the natural bio-inactivation of mycotoxins is also considered to be very efficient in the mitigation strategy. Only bio-inactivation leads to detoxification or reduction of the toxicity of potential metabolites, while processes of degradation and transformation can also result in the formation of substances that remain toxic for the host. An animal’s natural detoxifying capabilities are not only restricted to the protective function of the rumen or crop microbiota. A variety of microorganisms including bacteria, yeast and fungi from the small and large intestines of different animal species have been recognized for their ability to bio-inactivate mycotoxins into less toxic metabolites through routes such as binding, (de)acetylation, oxygenation, ring/side chain cleavage, de-epoxidation, isomerization or glucosylation. Detoxification of mycotoxins can also happen in the cells of the intestinal epithelium, liver, and kidneys. Cellular biotransformation of many compounds usually occurs in two steps: phase I consists of oxidative, reductive and hydrolytic reactions that make the molecule more reactive, and phase II involves conjugation reactions.

Mycotoxins have different toxic effects but most of them target the immune system. Depending on the toxin, the concentration and the parameter studied, they have immunostimulatory or immunosuppressive effects. As a consequence of that, it can increase the susceptibility to bacterial and viral diseases, and decrease the effectiveness of vaccines. Inflammation has also been observed with ingestion of mycotoxin contaminated diets. To reduce the negative effects of mycotoxins on mucosal immunity, the use of immunomodulators, including yeast derivates, can be a reliable option. Finally, it is well documented that among nutritional stresses, mycotoxins are major contributors to oxidative stress. Their detrimental effects (among others) are related to damaged mitochondria, which is a major source of free radicals and excessive RONS production in biological systems, and compromised expression and activities of antioxidant enzymes, including SOD, GPx and catalase, first line of the antioxidant defense network. Dietary supplementation with natural antioxidants could be considered a key tactic in managing mycotoxins in farm animal feeds and keeping their negative consequences at bay.
UNTANGLING THE COMPLEX WEB OF AFLATOXINS AND FUMONISINS USING BENTONITE AND FUMONISIN ESTERASE AS SUSTAINABLE SOLUTIONS FOR SAFER POULTRY PRODUCTION IN KENYA

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This study evaluated efficacy and safety of two mycotoxin detoxifiers (bentonite and fumonisin esterase) to protect broiler and layer chickens against negative effects of aflatoxins (AFs) and fumonisins (FBs), alone or in combination, on health and productivity as well as AFs carry-over to chicken products. The experiments were conducted under conditions representative of small-scale poultry farming in Kenya. Broiler and layer chickens were fed 20 diets consisting of either a control (with no added mycotoxins/mycotoxin detoxifier), AFs (range: 54.6 to 546 μg aflatoxin B1 (AFB1)/kg feed) and/or FBs (range, 7.9 to 7.43 mg/kg feed. Selected diets were supplemented with bentonite (AFB1 binder) and/or fumonisin esterase (FBs modifier). Each treatment had 20 chickens housed in 4 pens for trial periods of 5 weeks for broilers and 4 weeks for layers. Growth and productivity, liver gross pathological changes, blood biochemical changes, organ weights and mortality were used to evaluate animal productivity and health following the different treatments. The safety of chicken products (plasma, muscle, liver and eggs) was evaluated through analysis of AFs residues using validated UPLC-MS/MS methods. The different treatments did not affect growth and productivity of broilers, but egg production was reduced in layers fed high AFB1 diet. Contaminated diets caused alterations of serum total proteins, albumin and uric acid of the chickens. Increased relative weight of liver, spleen and gizzard were observed in layers fed AFs alone or with FBs. Interactions between AFs and FBs resulted in pronounced effects on weights of spleen, heart and gizzard of the chickens. Residues of AFs were detected in plasma, liver and eggs of layers and broilers fed diets with high AFs. Highest mean residues of AFB1 (0.66±0.76 μg/kg tissue) were observed in liver samples from layers fed 546 μg AFB1/kg and 7.9 mg FBs/kg feed. The maximum carry-over rates of AFB1 from feed to liver was 0.12% in layers and 0.06% in broilers. The detoxifiers improved egg production and egg weight and reduced the negative effects of AFs and FBs on the changes of weights of organs, blood biochemistry as well as transfer of AFs to tissues and eggs. The two mycotoxin detoxifiers were safe as they caused no effect on productivity or health of the chickens. Mycotoxin detoxifying agents thus provides a sustainable post-harvest intervention strategy to counteract negative effects of mycotoxins on animal health and productivity, and human exposure through animal-source foods.

MITIGATING OF THE COMBINED TOXICITY OF AFB1, DON AND OTA IN BROILER BREEDER HENS

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The objective of this study was to evaluate the efficacy of an integrated mycotoxin-mitigating agent in reducing the adverse effects of co-occurring dietary AFB1, DON and OTA on broiler breeder hens. Three hundred and sixty 30-week-old broiler breeder hens were randomly allocated into four experimental groups with 10 replicates of 9 birds each. The four groups received: (i) a basal diet (BD; Control); (ii) a
BD supplemented with 0.15 mg/kg AFB1 + 1.5 mg/kg DON + 0.12 mg/kg OTA (Toxins); (iii) a BD plus Toxins with 0.1% TOXO-XL (Toxins+XL1); and (iv) a BD plus Toxins with 0.2% TOXO-XL (Toxins+XL2), respectively, for 8 weeks. Compared with the control, dietary contamination of mycotoxins decreased (p<0.10) total egg weight and egg laying rate but increased (p<0.10) feed/egg ratio by 5.73-10.6% during week 1-4 and 5-8. Furthermore, dietary contamination of mycotoxins increased (p<0.10) mortality rate by 5.56% during week 5-8 and decreased (p<0.10) the settable eggs rate and hatch of total eggs rate at week 8. These changes induced by mycotoxins were mitigated by supplementation with TOXO-XL at both doses (p<0.10). Meanwhile, compared with the control, dietary supplementation of mycotoxins decreased (p<0.05) albumen height, Haugh unit, eggshell strength and eggshell thickness at week 4. These alterations induced by mycotoxins were alleviated by supplementation with TOXO-XL at 0.1% and (or) 0.2%. Moreover, dietary supplementation of mycotoxins reduced (p<0.05) the oviduct index (13.2%) and increased serum albumin (16.0%), compared with the control. Notably, these alterations caused by mycotoxins were mitigated by supplementation with TOXO-XL at both doses. In conclusion, this study demonstrated that TOXO-XL can mitigate the toxic effects of co-occurrence of AFB1, DON and OTA on laying and hatching performance, egg quality, and health status of broiler breeder hens.

CUTTING-EDGE STRATEGY TO MITIGATE THE EFFECT OF DEOXYNIVALENOL ON SWINE

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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, an in vivo trial in piglet was conducted. The study aimed to investigate the effects of 42 days exposure to naturally DON contaminated feed (~1000 ppb) on production performance, small intestine morphology and function, microbiota diversity in piglet. Results showed gender differences. Dietary exposure to DON impaired performance in males. Results obtained support the detrimental effects of low-level contamination by DON and indicate that supplementation with the immune-modulating yeast fraction increases piglet resilience to a subclinical challenge with the mycotoxin.
There is a large and progressively growing number of mycotoxins with new potential concerns and implications on consumer protection. The classical approach to risk management is to deal with each emerging hazard individually, leading to both overload and lack of coherence in terms of an overall risk-based approach. The development of mitigation strategies should prioritize mycotoxins that regularly occur at undesirable levels in commonly consumed commodities, wherein both the toxicological profiles and effectiveness of mitigation are understood with a reasonable degree of certainty. The ultimate goal of mycotoxin mitigation is to prevent adverse health effects caused by foodborne exposure to mycotoxins, while preserving nutritional and organoleptic quality of food. The International Life Sciences Institute Europe (ILSI Europe) Food Contaminants Task Force is firmly committed to contributing to the understanding of the issues of mycotoxins affecting the different points of the food chain.

This presentation will illustrate a recent new activity that is devoted to establishing a framework for the prioritization of mycotoxins found in food following a risk-based approach (decision tree). Based on the evidence and scale of risk to consumers, and the potential for risk mitigation, the framework will enable the differentiation between mycotoxins where risk management action is both warranted and likely to be effective based on available evidence. Through case-studies, this framework will also highlight potential knowledge gaps. The proposed activity is therefore devoted to delineating the right path for scaling and prioritizing mycotoxins in terms of risk-ranking and consequent mitigation opportunities.

References
MOULDY BREAD – A SPOILED FOOD WASTE OR A FUTURE FEEDSTOCK?

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Food waste is a major contributor to the global problems of climate change, biodiversity loss and pollution. Around 931 million tons of food go to waste each year, with a 61% of contribution coming from the households [1]. Bread, as one of the main staples represents a major part of disposed food either due to physicochemical processes such as the crumb firming, loss of flavor or microbial spoilage [2]. As soon as one part of a bread gets infested by moulds, it is recommended by the food safety and health risk authorities to discard the whole loaf immediately due to potential health risk [3]. However, part of the visible non-spoiled bread might be recycled and used as a feedstock for microbial production of fuels, chemicals and enzymes [4], unless it is contaminated by mycotoxins. Mycotoxins are toxic odorless and tasteless fungal metabolites which could be detected even in the bread slices lacking of moulds.

The aim of this study was to investigated the presence of fungal mycelium as well as mycotoxins in the individual slices of the mouldy whole meal spelt bread stored in a household. Three methodologies were chosen: DNA barcoding for identification of fungal species, multispectral imaging for macroscopic identification of mouldy regions on the bread slices and LC-MS/MS for multi-mycotoxin determination [5]. Chaetomium globosum, Penicillium chrysogenum and Scopulariopsis brevicaulis were identified on the visible mouldy bread slice 1. Chaetomium was confirmed by typical metabolite spectrum of chaetoglobosin A, chaetoglobosin B and chatoviridin A. Similarly, maleagrin, andrastins, roquefortine C and penicillin G were detected in P. chrysogenum. In the fungal spot of Scopulariopsis brevicaulis, no detectable levels of mycotoxins were found. Concerning the fungal penetration through the slices from the slice 1 to slice 2. However, no fungal presence was detected on the slices 3-5. This finding was confirmed by the non-detectable levels of mycotoxins [5]. Data for white bread, whole grain bread, sliced bread and whole loaf bread will be presented as well.

References
ENSURING THE SAFETY OF PLANT-BASED MEAT ALTERNATIVES: MYCOTOXIN OCCURRENCE, RISK-BENEFIT ASSESSMENT, AND CURRENT RESEARCH – WHERE WE ARE AND WHERE WE ARE SUPPOSED TO BE

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There is an ongoing shift towards more sustainable dietary schemes (i.e., flexitarian, vegan) due to recommendations by several international food-based dietary guidelines in regard to health and sustainability concerns associated with meat consumption. However, this shift can lead to a significant change in the exposure to natural contaminants such as mycotoxins. To provide a better picture regarding the research of mycotoxins in plant-based meat alternatives (PBMA) and potential human health risks we present, in the form of a workflow, the current research regarding the safety of meat alternatives and what we did to fill in the gaps so far.

In this study we present the current research regarding mycotoxins occurrence in the most used commodities for plant-based meat alternatives (PBMA) by means of a systematic review. Then, a case study is presented assessing the potential health impact of soy-based meat analogues contaminated with aflatoxin B1 by simulating a full replacement of meat with soy-based meat analogues for Italian consumers. The increased risk of liver cancer, due to exposure to aflatoxin B1, and lowered risk of colorectal cancer, due to elimination of processed meat from the diet, are weighted against each other. The final burden of disease is quantified in disability-adjusted life years (DALYs). Lastly, we developed a multi-mycotoxin method and determined the occurrence of 11 mycotoxins in 13 meat alternatives from the Italian market. By simulating a full replacement of meat with meat alternatives we assessed a preliminary dietary exposure of Italian consumers to mycotoxins. These works are proof of concepts and serve as a starting point for the MSCA-PF PRISMA (‘Providing risk-benefit insights of shifting to meat alternatives’) project won by the authors, which aims to evaluate the actual impact of PBMA consumption on the total mycotoxin exposure in non-traditional diets, with the eventual goal of assisting governing bodies and decision-makers in enhancing the regulatory system for meat alternatives.

THE ENVIRONMENTAL IMPACT OF MYCOTOXIN ANALYSIS: ON THE GREENNESS OF ROUTINE METHODS

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Numerous mycotoxin analyses are conducted worldwide to ensure the safety of food and feed. The utilization of liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) allows for simultaneous screening of multiple toxins, including all regulated mycotoxins. In fact, this approach has become widely adopted for confirmatory analysis. Enzyme-linked immunosorbent assays or other lateral flow assay formats are primarily employed where rapid screening in a cost-effective manner is needed. Despite the advantages offered by both methodologies, they also come with their limitations. LC-MS/MS necessitates expensive and complex equipment, as well as expert knowledge. On the other hand, assays are simpler to perform and cost-effective, but often suffer from cross-reactivities. Additionally, LC-MS/MS relies on the usage of organic solvents while assays require a significant amount of consumables. Considering the substantial number of measurements performed, these techniques may have a notable ecological footprint. Research on the environmental impact of the extensive volume of necessary mycotoxin analyses is scarce, primarily focusing on evaluating the sustainability of novel techniques. However, the escalating consequences of climate change have spurred an increasing interest in green analytical chemistry, underscoring the need to investigate the environmental burden imposed by routine mycotoxin analyses. In this presentation, the ecological impact of established analytical methods for mycotoxin analysis will be assessed. The greenness of commonly used methods will be evaluated and compared to potentially greener novel techniques. In the end, a critical discussion on the ecological footprint of both established and novel methods will be presented.
AN ACRONYM SOUP FOR BETTER RISK ASSESSMENT OF DON: HBM, NAMS, TK-TD, MCMC, HDMI, AND MORE!

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Deoxynivalenol (DON) is a mycotoxin frequently observed in cereals and cereal-based foods, with reported toxicological effects including reduced body weight, immunotoxicity and reproductive defects. The European Food Safety Authority (EFSA) used traditional risk assessment approaches to derive a deterministic tolerable daily intake (TDI) of 1 µg/kg-day. However, data from human biomarkers studies indicate widespread and variable exposure worldwide, necessitating more sophisticated and advanced methods to quantify population risk. The World Health Organization/International Programme on Chemical Safety (WHO/IPCS) has previously used DON as a case example in replacing the TDI with a probabilistic toxicity value, quantifying variability and uncertainty in the form of the human dose corresponding to an effect size M in the Ith percentile of the population (HDMI) of 2.9 [90% confidence interval: 0.44-19] µg/kg-day, for M=5% decrease in body weight and I=1%. In this study, we extend this case study by incorporating Bayesian modeling approaches through Markov chain Monte Carlo (MCMC) simulation, and both in vivo and new approach methods (NAM) data to quantify inter- and intraspecies toxicokinetic and toxicodynamic (TK-TD) differences. Combining these probabilistically, we improved both precision and accuracy of DON toxicity value, resulting in HDMI of 5.48 [1.37-23.81] µg/kg-day. Additionally, we converted the HDMI to biomonitoring equivalents, BE MI, in blood or urine to enable interpretation of human biomonitoring (HBM) data, with blood BE MI of 0.53 [0.17-1.62] µg/l and urinary excretion BE MI of 3.93 [0.98-16.37] µg/kg-day. Finally, we illustrated how this integrative approach can advance quantitative risk characterization using two HBM datasets, estimating both the fraction of population with an effect size M>5%, as well as using population dose-response functions to characterize the distribution of effect sizes. Overall, we demonstrate how an ‘acronym soup’ of state-of-the-art methods and approaches yields more accurate, precise, and comprehensive risk characterization for a common mycotoxin.

A NEW IN SILICO TOOL FOR THE PREDICTION OF MUTAGENICITY, GENOTOXICITY, AND CARCINOGENICITY OF OVER 4,000 MYCOTOXINS

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The regulation of genotoxic and carcinogenic mycotoxins is an important health concern since their effects may not be visible at short term, causing serious health problems at long-term [1]. Currently, several thousands of different mycotoxins have been reported, but only very few of them are regulated, mainly due the lack of data regarding their toxicity and mechanisms of action [2]. Thus, new, time-and cost-effective strategies for the prioritization of mycotoxins based on their genotoxicological profile are essential for the assessment of the emerging risks. In the last years, the performance of computational methods based on artificial intelligence to predict toxicological properties of chemical compounds has improved drastically [3]. However, most of these tools have not been validated/optimized for mycotoxins, which are mostly complex organic molecules with high molecular weights. There exist some very promising attempts to apply in silico approaches to mycotoxins [4,5], however, they are mainly focused on a small subgroup of mycotoxins.
In this work, we present a user-friendly webserver that provides predictions for genotoxicity, carcinogenicity and mutagenicity of a broad range of mycotoxins (https://chemopredictionsuite.com/MicotoXilico). For the prediction, specific robust QSAR models were generated, showing good accuracy, precision, sensitivity, and specificity when applied to external mycotoxin validation datasets [6]. Moreover, these models are compliant with the OECD criteria for in silico prediction tools and can be used for regulatory purposes. The webserver also incorporates a database of 4,360 different mycotoxins classified in 170 categories that can be interactively explored.

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PREDICTIVE MODELS TO MANAGE MYCOTOXIN OUTBREAKS IN THE USA

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Mycotoxin contamination of maize results in significant agroeconomic losses and poses serious health issues worldwide. We developed algorithms to confidently predict mycotoxin contamination of maize using machine learning and historical aflatoxin and fumonisin contamination levels. We used historical daily meteorological data from a 14-year period combined with corresponding aflatoxin and fumonisin contamination data from the State of Illinois to engineer input features that link weather to fungal growth and aflatoxin production. These features in combination with biogeochemical soil properties, and satellite acquired vegetation index were used for gradient boosting (GBM) and Bayesian network (BN) modelling. The GBM and BN models developed can predict mycotoxin contamination with overall 93% accuracy.Analyses for aflatoxin and fumonisin with GBM showed that meteorological and satellite-acquired vegetative index data between maize growing seasons significantly influence mycotoxin contamination levels at harvest. Prediction of high aflatoxin contamination levels was linked to high aflatoxin risk index (ARI) in weeks during the months of March/April/June/July. Similarly, high levels of fumonisin contamination were linked to high precipitation levels in week during February/March/September and high vegetative index in January. During maize flowering time in June, higher temperatures range increased prediction of high levels of fumonisin contamination, while high aflatoxin contamination levels were linked to high aflatoxin risk index. Meteorological events prior to maize planting in the field have high influence on predicting aflatoxin and fumonisin contamination levels at the end of the year. These early-year events detected by the models can directly assist farmers and stakeholders to make informed decisions to prevent or mitigate mycotoxin contamination of Illinois-grown maize.
Worldwide, providing safe food is a key topic on the agenda of food industry, policy makers and researchers. Mycotoxins are among the most important food safety hazards that threaten the safety of our food. Recently it was estimated that about 60-80% of our crops has detectable mycotoxin levels. Various drivers from within and outside the food supply chain have an impact on mycotoxin contamination. To mention one, climate change is expected to generally increase mycotoxin contamination. Finding feasible solutions to limit mycotoxin contamination is thus important for the global food supply chain to ensure a safe food supply. A recent way of thinking on how to improve the food safety performance of the food supply chain is via the strengthening the capacity of the chain to withstand disturbances, also called food safety shocks (i.e., unwanted disruptions related to the increasing presence or emergence of mycotoxins in this case). A resilient supply chain that has the capacity to adapt and manage possible mycotoxin contaminations is more suitable and practical than trying to achieve a state of zero food safety risks. The concept of resilience has been developed in other research domains, and recently has been adapted for food safety of supply chain. In this presentation, this concept will be presented with an example case on mycotoxins.
NUTRINUTS: SUCCESS STORY OF INDUSTRIAL PARTNERSHIP TOWARD SUSTAINABLE MITIGATION OF AFLATOXIN IN AFRICA

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In the last twenty years, the production of peanuts in Africa has doubled by two, reaching sixteen million tonnes in 2021 [1]. Peanuts represent the main cash crop for many smallholder farmers in Africa but, due to aflatoxin contamination, countries such as Ethiopia are banned from exporting peanuts to Europe and the UK. The NutriNuts project led by Cranfield University (2019-2023) in partnership with Hilina Enriched Foods PLC and Haramaya University lead to the development of applicable low-cost solutions for the full peanuts supply chain from farm to a new aflatoxin-safe peanut butter product launched in 2023. Example of successful results includes: (i) low-cost peanut dryer; (ii) low-cost sheller; (iii) new good agricultural practices; (iv) new training materials and courses; and (v) new process line including potential waste valorisation potential. Gender-equal implementation strategies are currently in development to further expand the results to other crops and countries following a similar industry/academic partnership model.

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References

MULTI-ACTOR COLLABORATION: EVERYONE AT THE TABLE FOR IMPROVED MYCOTOXIN RISK ANALYSIS – A PERSPECTIVE FOR THE EUROPEAN AND THE AFRICAN UNION

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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. To support these developments in risk analysis, a legitimate platform is needed to establish a dialogue between risk assessment, risk management, and risk communication stakeholders. This support can be achieved by developing tools for the evaluation of procedures and enforcement practices, and an analysis of Science-Policy-Society (SPS) collaboration systems. This is one of the many goals of FoodSafety4EU, a Horizon 2020 collaborative action focused to design, develop and release a multi-stakeholder platform for the future European Food Safety System. The developed strategies can also be useful to the African context, to pave the way to a strong African food safety system, including risk analysis procedures.
Currently, many challenges are directly and indirectly affecting European food safety regulations. Through in-depth interviews with European food safety authorities and risk assessors, issues regarding (emerging) mycotoxins were often highlighted as one of these challenges. Therefore, there is a need to identify stakeholders involved in mycotoxin risk analysis and how they interact and what challenges and/or constraints they perceive. This has been done through a Net-Map analysis. Net-Mapping is an interview-based mapping tool that can be used to visualise implicit knowledge and understand the interplay of complex formal and informal networks, power relations, and actors/stakeholders’ goals; uncover sources of conflicts as well as potentials for cooperation; facilitate knowledge exchange and learning processes; and develop visions and strategies to achieve common goals. Moreover, several multi-stakeholder co-creation workshops were organized, to find practical ways to help improve current risk analysis systems. The Net-Maps resulting from the analysis done in Italy and Czech Republic will be discussed, visualizing the current SPS collaboration system regarding mycotoxin risk analysis. Moreover, the results achieved from the co-creation workshops will be presented. Implementation of the same activities in Africa could unravel the interplay between several stakeholders in mycotoxin risk analysis, identify constraints in the current collaboration system and provide solutions to improve the food safety system.

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

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The Food Safety Coalition (FSC) brought together a group of experts from like-minded organizations to share data and knowledge about the challenge of global food safety – an area with existing and growing challenges, especially with climate change. Work focused on aflatoxins, a type of mycotoxins and poisonous chemical produced by mould that contaminates 25% of the world crops and impacts millions of people, given the serious health threats they pose. The aim of the coalition was to identify specific actions to progress actions at pace. Since the coalition began, contributing members have worked together to develop insights in a series of key areas including frameworks detailing accurate and efficient sample planning for accurate material analysis, risk communications required to enhance awareness among industry and the public on the dangers of aflatoxins, and options for tailoring outreach materials and approaches to specific stakeholders in the value chain to address aflatoxin risk.

At the World Mycotoxin Forum, an overview of the coalition work will be presented, together with peer-reviewed insights and methodologies which will be made publicly available to enable further collaboration and innovation in future.

MYCOTOXINS IN ASIA: IMPACT ON FOOD SAFETY AND INTERNATIONAL TRADE

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Mycotoxin contamination of foodstuffs is a cause of a range of diseases, especially in the developing world. The major classes of mycotoxins that are of the greatest agroeconomic importance are aflatoxins (AF), ochratoxins, fumonisins, zearalenone (ZEA), deoxynivalenol (DON) and patulin. This study outlines the occurrence of mycotoxins in agricultural commodities from Asian countries and analyzes the trends and impact on food safety, human health, economic losses, and international trade. Data on mycotoxin incidents in Asia were obtained from the Rapid Alert System for Food and Feed (RASFF)
portal from January 1, 2009, to December 31, 2019. Also, data from the BIOMIN Mycotoxin Survey on agricultural commodities (2017 to 2019) that are primary components for feed were compiled in this study. The findings revealed that AFs (n=1,711) was the major mycotoxin identified in exported products, and seeds, nuts and nut products imported from China (n=650), Iran (n=441) were more often susceptible to contamination by AFs and hence, subjected to frequent border rejections. Also, from the BIOMIN data, serious contamination was found in feed materials from China and Taiwan where DON was present in 90 and 92%, respectively, of all the samples tested. Assessing the impact of climate on the occurrence of mycotoxins is important in enabling effective combined mitigation strategies for improved health, income, and livelihoods in Asia and globally.

ESTIMATING THE PUBLIC HEALTH BURDEN OF AFlATOXINS – A GLOBAL EFFORT

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Aflatoxins are amongst the most poisonous mycotoxins and pose a major public health concern. It was estimated that each year aflatoxins cause approximately 21,800 liver cancers and 19,500 deaths, based on the WHO report published in 2015 [1]. The highest public health burden (disability-adjusted life years: DALYs) was observed in Africa and Asia. Based on the new World Health Assembly resolution [2], WHO re-established its technical advisory group in 2021 to update the estimates of foodborne disease burden, including aflatoxins M1 and B1. For this new round of estimation process, WHO aims to include other health outcomes other than hepatocellular carcinoma in order to quantify the real public health burden due to aflatoxins, nationally, regionally and globally.

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CHARACTERIZATION OF FILAMENTOUS FUNGI AND THEIR METABOLITES IN AQUAPONIC PRODUCTION OF HERBS

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While aquaponics is a sustainable and efficient food production approach performed in a closed system, it can be subjected to microbial and chemical contaminations. Filamentous fungi can enter aquaponic systems through various sources, including water or soil, air, infected plants or fish, or external sources like equipment or workers. Once inside the system, fungi can thrive in the warm and moist conditions typically found in aquaponic production. Fungal spores have a remarkable ability to disperse through water and air, leading to their widespread distribution across production and storage sites. This poses a high risk for spoilage and significant production losses in aquaponics. In addition to spoilage, filamentous fungi can produce toxic secondary metabolites so-called mycotoxins. Exposure to them can impair human and animal health.

Nowadays, a limited amount of comprehensive data is available regarding the characterization of the mycobiota and the presence of mycotoxins and other contaminants in aquaponics production. Thus, the main aim of this study was to characterize the mycobiota and mycotoxins present in the aquaponic production of herbs and to establish a source-tracking approach allowing to identify the main sources and migration routes of fungi found in aquaponics. In this study we used Fourier transform infrared (FT-IR) spectroscopy. This technique enables biochemical profiling it is used as a next-generation phenotyping technique in microbiology for chemical characterization and identification of
microorganisms. A set of samples including water, air, soil, plants and fish feed was obtained at critical points along the aquaponic production within different seasons. Approximately 400 isolates of filamentous fungi were obtained using the standard agar plating method on selective media - malt extract agar (MEA) and potato dextrose agar (PDA). These trials were accompanied by liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis to screen for the presence of secondary fungal metabolites and agrochemicals. The fungal isolates identification was performed by high-throughput screening (HTS) FT-IR spectroscopy. An in-house spectral library, combined with multivariate data analysis, laid the foundation for a source tracking concept. The source tracking concept involves tracing the origin and migration pathways for fungi along the aquaponic production system to identify the most critical control points for further monitoring. Few secondary metabolites have been found with LC-MS/MS, but not at hazardous levels. Future work is required to expand the library with isolated strains, expand the spectroscopic library, and establish robust machine learning models to enhance the effectiveness of the tracking method and detecting possible mycotoxin producers. In conclusion, this study provided evidence of the diversity of fungal strains in aquaponic production and for the first time established the initial framework for a fungal source tracking.
INFRARED SPECTROSCOPY IN FOOD SAFETY: HYPE OR HOPE?

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Photonic technologies have seen revolutionary developments in the past decades leading from conventional optics to on-chip photonic devices. In particular, mid-infrared (3-15 µm; MIR) laser technology and especially interband and quantum cascade lasers (ICLs, QCLs) as well as the first emerging MIR LEDs continue to evolve IR spectroscopy into a state-of-the-art tool for the molecularly selective and sensitive analysis of food and agricultural products. This enables the analysis and quantification of relevant target analytes in liquid, solid, and gaseous state in a wide variety of sensing and monitoring scenarios. High output power, narrow linewidths, single-mode operation, low power consumption, broad tunability and compact dimensions are just some of the most outstanding features facilitated especially by cascade lasers, which since their recent introduction have rapidly matured into the most important contemporary MIR laser light sources.

In this presentation, we will discuss state-of-the-art MIR sensing platforms in combination with innovative waveguide technologies and orthogonal sensing schemes providing direct access to molecularly selective information at yet unprecedented levels of sensitivity. We will discuss their utility in a range of food quality and safety scenarios such as but not limited to the detection of fungal infections and resulting mycotoxin contamination, food freshness, etc.

References
ALTERNATIVE APPROACHES TO THE ANALYSIS OF MYCOTOXINS BASED ON LUMINESCENT SENSING COUPLED TO BIOLOGICAL OR BIOMIMETIC MOLECULAR RECEPTORS

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The authors would like to dedicate this presentation to the late Prof. María C. Moreno-Bondi. Her impact on science will continue to be felt through her effect on us.

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The biorecognition event lies at the heart of all biosensing applications and directly impacts the platform's sensitivity and selectivity. Biomolecular engineering and phage display technologies have recently been introduced as a fascinating alternative in the quest for Nature-inspired recognition elements. In this regard, we have investigated the use of those techniques to select novel biorecognition elements and apply them to develop biosensing strategies to detect targets of food and clinical interest. For example, we have focused on applying peptidomimetics (mimopeptides) and recombinant antibody fragments (rAb) selected by phage display techniques for quantifying biotoxins. The latter small molecules are secondary metabolites of various origins including plants, fungi or bacteria that have a detrimental impact on human and animal health, on the environment, and on the economy worldwide. The functionality of the novel binders has been confirmed with phage-based ELISAs, nuclear magnetic resonance (NMR), and surface plasmon resonance (SPR) analysis. After identifying the sequence coding for the novel peptide or recombinant antibody, they have been chemically synthesized and modified with different binding tags or optically active nanoparticles. Alternatively, genetic engineering techniques have been used to fuse them to luminescent proteins, providing simple and cost-effective alternatives to traditional immunoassays. Heterogeneous and homogeneous bioassays based on the novel nature-inspired materials have been implemented in combination with different sensing schemes to yield improved sensitivity and simplified assay protocols compared to their natural counterparts [1-3].

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References

DETECTION OF T-2 TOXIN IN WHEAT AND MAIZE WITH A PORTABLE MASS SPECTROMETER

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direct methods for toxin measurement, such as mass spectrometry are generally confined to traditional laboratories, while indirect methods, such as Immunoassays, are often used for rapid estimation in the field. The potential of a portable scanning mass spectrometer was explored as a means to enable direct detection of T-2 and HT-2 toxins in settings outside of traditional laboratories. In order to detect T-2 and HT-2 toxins the inlet of the atmospheric pressure ionization source of a commercially available portable
linear ion trap mass spectrometer was modified. Modification allowed extracts of wheat or maize that had been cleaned up using MycoSep 225 columns to be infused into the instrument, using air to nebulize the samples. T-2 toxin was detected in soft white wheat, hard red wheat, and yellow dent maize with limits of detection of 20 to 28 μg/kg. The cut-off value in hard red winter wheat was 110 μg/kg. The appearance of an interfering compound at the same m/z as HT-2 prevented the measurement of that toxin, which undermined the use of the method for meeting EU performance criteria. Nevertheless, the results demonstrate that it is possible to develop rapid, field portable, mass spectrometric methods for detecting mycotoxins.

DEVELOPMENT OF A PORTABLE MICROARRAY LATERAL FLOW IMMUNOSORBENT ASSAY FOR MULTIPLE MYCOTOXINS DETECTION

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Mycotoxins cause both chronic and acute effects on human and animal health. This work successfully developed microarray lateral flow immunosassay (μLFIA) for detection of five regulated mycotoxins commonly found in food and feed samples, including aflatoxin B1 (AFB1), deoxynivalenol (DON), fumonisin B1 (FB1), T-2 toxin (T-2), and zearalenone (ZEA). Many parameters including concentrations of mycotoxin-BSA, specific anti-mycotoxin antibody, and reporter dye were optimized for fabrication of μLFIA. The optimal conditions enable μLFIA to detect single and multiple mycotoxins accurately and quantitatively. The ranges of detection were 0.05-0.5 ng/ml for AFB1, 5-500 ng/ml for DON, 10-500 ng/ml for FB1, 2.5-50 ng/ml for T-2, and 1-10 ng/ml for ZEA. The point-of-care μLFIA presents an alternative method for mycotoxin detection in agricultural commodities.

APPLICATION OF ELECTRONIC NOSE FOR FEED SAFETY AND ANIMAL NUTRITION

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Cereals in general, and maize in particular, is one of the most important food and feed commodities among cereal crops. However, maize diffusion, popularity, and, most of all, safety for consumption are threatened by mycotoxin contamination. Maize is often infected by mycotoxigenic fungi. Furthermore, global surveys indicate that more than 70% of the samples of feed and raw feed materials are positive for at least more than one mycotoxin, emphasizing co-contamination as a big issue for both food safety and public health. The co-occurrence of mycotoxins in food and feed is explained by three reasons: (i) most fungi are able to simultaneously produce several mycotoxins; (ii) commodities can be contaminated by several fungi simultaneously or in quick succession; and (iii) the complete diet comprises different commodities. Multi-mycotoxin contamination, however, is a topic of great concern, as co-contaminated samples might still exert adverse effects on animals due to additive/synergistic interactions of the mycotoxins. This scenario underlines the importance of multi-mycotoxin analysis methods that can also be rapid and user-friendly. Rapid methods for the determination of mycotoxins in cereals are hence highly needed in order to prevent the entry of mycotoxins into food and feed chains. In this scenario, electronic noses (e-nose) may represent a promising analytical tool for industry, also in combination with conventional rapid methods already widely used by the feed industry. An e-nose consists of an array of nonspecific chemical detectors that detect different volatile organic compounds (VOCs) and consequently provides a signal that can be used as a fingerprint of the specific volatile compounds in a sample. E-nose techniques for the detection of fungal infection are based on identifying
specific VOCs associated with the growth of fungi on cereal grains. At the current state E-nose has shown good potential in discriminating between non-contaminated and single-mycotoxin-contaminated grain. However, the predictive accuracy of e-nose is still limited and unsuitable for in-field application, where mycotoxin co-contamination occurs. Further research needs to be focused on the sensor materials, data analysis, pattern recognition systems, and a better understanding of the needs of the feed industry for a safety and quality management of the feed supply chain.

ACCURATE AND NON-DESTRUCTIVE MONITORING OF MOLD CONTAMINATION IN FOODSTUFFS BASED ON WHOLE-CELL BIOSENSOR ARRAY COUPLING WITH MACHINE-LEARNING PREDICTION MODELS

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Mould contamination in foodstuffs causes huge economic losses, quality deterioration and mycotoxin production. Thus, non-destructive and accurate monitoring of mould occurrence in foodstuffs is highly required. We proposed a novel whole-cell biosensor array to monitor pre-mould events in foodstuffs. Firstly, 3 volatile markers ethyl propionate, 1-methyl-1-H-pyrrole and 2,3-butanediol were identified from pre-mould peanuts using gas chromatography-mass spectrometry. Together with other 3 frequently reported volatiles from Aspergillus flavus infection, the volatiles at subinhibitory concentrations induced significant but differential response patterns from 14 stress-responsive Escherichia coli promoters. Subsequently, a whole-cell biosensor array based on the 14 promoters was constructed after whole-cell immobilization in calcium alginate. To discriminate the response patterns of the whole-cell biosensor array to mould-contaminated foodstuffs, optimal classifiers were determined by comparing 6 machine-learning algorithms. 100% accuracy was achieved to discriminate healthy from mouldy peanuts and maize, and 95% and 98% accuracy in discriminating pre-mould stages for infected peanuts and maize, based on random forest classifiers. 83% accuracy was obtained to separate mouldy peanuts from mouldy maize by sparse partial least square determination analysis. The results demonstrated high accuracy and practicality of our method based on a whole-cell biosensor array coupling with machine-learning classifiers for mould monitoring in foodstuffs.
COMMUNICATING WITH CONSUMERS: HOW TO TALK ABOUT FOOD RISK?

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The increasing availability of food and health information does not always improve people’s knowledge. However, it does present them with the obligation to understand in order to make choices. This creates a greater need for trust in food and health information, which can only come from presenting balanced information from credible sources that addresses the questions people have and communicating it in a way that the general public will understand.

Today’s digital environment characterised by the proliferation of mobile devices, makes possible an immediacy of the information, but also contributes to the information overload. Social media opens the doors for a direct dialogue with consumers and allows everyone to be a source of information. The consequence of all those elements is emotional discussions that start replacing fact-based debates, sensationalist headlines get prominence and uncredentialed sources of information succeed in undermining trust in science and increasing the uncertainty and complexity of consumer decision making.

A proactive approach to communicating about food would help to reassure the public about its safety, restore consumers’ trust in the authorities charged with regulating it, and help people understand how to eat safely and healthily. This presentation will provide best practices for good science communication with a specific focus on risk communication.

THE CHICKEN TIKKA MASALA AND THE IMPORTANCE OF DATA QUALITY: WHEN THE ANALYTICAL PERFORMANCE MAY BECOME A REAL HURDLE

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A comprehensive and updated dietary risk assessment of chemical substances is one of the major challenges for public health. In particular, the dietary exposure assessment relies on the combination of occurrence and consumption data, which should be as much accurate and updated as possible. Occurrence data collected for risk assessments consists of gathering data from food surveillance or food monitoring programs as well as some national projects. Protocols applied among different data providers are thus not harmonized, neither in terms of analytical workflow nor as for the final purpose. Instrumental techniques and target sensitivity may differ a lot, hence strongly affecting the overall uncertainty. Dietary exposure to contaminants that are not regulated presents additional challenges. At the time data are being collected, it might not be clear which foods contribute the most to the exposure, the samples might not be well characterized, there might not be enough analytical reference standards or reference materials, the method of analysis might not be harmonized or validated, and the sensitivity needed to rule out potential health risks might not be known.

In this already complex framework, one of the crucial steps is the handling of occurrence data below the LOD/LOQ. These data are reported as non-detects and the resulting distribution of values is called left-censored data. Although statistical methods may help in tackle some gaps, they usually overestimate the actual exposure and risk of humans to mycotoxins and contribute to the uncertainty of the risk assessment. Based on the precautionary principle, from a public health perspective an overestimation of risk is more appropriate than an underestimation. However, overestimation comes with regulatory and economic implications for the whole supply chain, from an increase in crop losses to more frequent food recalls from the market. Due to common misleading communication campaigns, this may end up
in a generalized lack of trust among consumers, with a strong impact on the agri-food market and even a higher risk for an increased mistrust into the food system and public authorities. The presentation will discuss the urgency of a harmonised workflow starting from project activities and somehow unexpected real examples, with the ultimate goal to promote discussion about current gaps and needs among analysts and risk assessors.

SHAPING THE FUTURE OF MYCOTOXIN MANAGEMENT IN SUB-SAHARAN AFRICA WITH PRAGMATISM

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Sub-Saharan Africa (SSA) presents unique challenges to mycotoxin management, distinct from the regulatory-driven approach of the developed world. Unlike Western countries which have stringent regulations governing food production, SSA relies heavily on self-produced or market-sourced foodstuffs often without much regulatory oversight. In this context, this paper advocates for a pragmatic paradigm shift, emphasizing education and social responsibility throughout the agricultural value chain in SSA. Equipping producers with mycotoxin knowledge foster informed choices and best practices, while promoting social responsibility within the value chain enhances food safety standards. Moreover, the discussion highlights the need to transition from a sole focus on aflatoxins to a more comprehensive approach encompassing multi-mycotoxins, particularly emphasizing the significance of fumonisins. Additionally, the paper underscores the importance of adopting risk-based regulatory limits for trade, rather than borrowing standards from the developed world. Therefore, this paper offers a comprehensive perspective on mycotoxin management in SSA, firmly grounded in the reality and future prospects of the region.

THINK GLOBAL, ACT LOCAL – ON-SITE MYCOTOXIN MANAGEMENT CHALLENGES IN THE LIGHT OF GLOBAL CLIMATE CHANGE

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The 2023 harvest season was more than challenging, presenting a blend of opportunities and obstacles for farmers and agricultural stakeholders. From the promising start with minimal Fusarium contamination risk during flowering of wheat in Europe to the unexpected challenge of mould contamination caused by excessive summer rains and the resulting late harvest, this presentation provides a detailed examination of the complexities that have shaped the outcome of Europe’s wheat harvest.

Optimal conditions during flowering in europe: Early promise. In large parts of Europe, the year commenced with favourable weather conditions during the flowering stage of wheat. Precipitation was notably scarce during this critical period, minimizing the potential for Fusarium infections and the risk of DON or other Fusarium toxins, setting a positive trajectory for yield projections.
Drought and heat in the US Corn Belt in early summer. At the same time, high temperatures and drought during June and July resulted in less favourable growth conditions in the Corn Belt in the US. However excessive rain in late summer in some parts of the United States caused additional problems with respect to mycotoxin risks. Also, the variability between or even within a field makes mycotoxin management extremely challenging.

Unforeseen consequences: Rainfall patterns as well as continuous drought. Yet, the trajectory was disrupted by an unforeseen change in weather patterns. Unusually heavy rainfall characterized the latter stages of summer in central and northern Europe, yielding a cascade of challenges for agricultural practitioners. While the initial rain was welcomed to alleviate dry conditions, the prolonged precipitation introduced complexities for bringing the harvest in. Due to delayed harvest, the quality of the wheat decreased, with lower falling numbers and mould infections. At the same time, other parts of Europe were still suffering from excessive heat and drought.

Impact of mould contamination: Late-harvested grains at risk. One of the pronounced challenges arising from the persistent moisture was mould contamination, particularly among late-harvested wheat and barley crops. Elevated humidity provided an optimal environment for mould proliferation, impacting both crop quality. *Fusarium* strains are ubiquitous in the field, so DON, T-2, and HT-2 toxin, Zearalenone may be the major culprits, yet *Alternaria* toxins might also play a role. This scenario underscores the delicate equilibrium within ecosystems and serves as a reminder of the repercussions of climatic deviations.

Implications for the agriculture sector: Resilience and insights. The variable trajectory of the 2023 harvest underscores the sensitivity of the agricultural sector to environmental dynamics. It emphasizes the intricate interconnectedness of weather variables, soil conditions, and plant disease management protocols. As stakeholders navigate the implications of local, extreme weather conditions, the importance of adaptability and diversified strategies comes to the fore.

Prospects ahead: Leveraging innovations. Amidst the challenges, opportunities for innovation emerge. Farmers are proactively employing technology to mitigate the effects of mould contamination. AI-enhanced, local forecasting models, plant disease and mould management, data-driven quality monitoring systems, and predictive analytics are contributing to the sector's resilience in the face of adversity.

To conclude, the narrative of the 2023 harvest highlights the complexities that can impact harvest outcomes but also demonstrates the sector's capacity to adapt. As this year's season draws to a close, the lessons gathered from the delicate dance between climate dynamics and agriculture will inform strategies and methodologies for years to come. Mycotoxins contaminations of crops are unavoidable, but mycotoxins can be managed. The use of mobile technologies and sharing the analytical data in the cloud has opened entirely new ways of mycotoxin data use. Analytical data about the quality of commodities can be available from all locations in real time. The analytical data in combination with other agricultural and environmental data, like weather conditions enable the creation of more precise predictive models and mycotoxin big data.

MYCOTOXINS AND CLIMATE CHANGE: MISSION IMPOSSIBLE FOR FEED AND FOOD SAFETY REGULATORS?

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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, to continue to ensure a high level of human health protection maximum levels of already regulated mycotoxins (such as for ochratoxin A and deoxynivalenol) have recently been strengthened or maximum levels have been set for mycotoxins for which previously no maximum levels were set (such as for T-2 and HT-2 toxin and ergot alkaloids) are discussed. 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 mainly 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. The presence of mycotoxins cannot be fully controlled by good
agricultural practices. This results in particular challenges from a regulatory point of view. The setting of maximum levels is based on the available data in the EFSA database, taking into account the year-to-year variation and geographical variation as reflected in the EFSA occurrence database, and thereby also taking into account the information provided by the stakeholders.

For certain mycotoxins, such as deoxynivalenol and ochratoxin A, EFSA has concluded that current exposure is a health concern for certain groups of consumers and/or certain animal species. Therefore, it is important that the current human and animal exposure is lowered and this is the objective of regulating mycotoxins in feed and food. On the other hand, it is observed that the presence of mycotoxins is steadily increasing due to climate change, despite application of good practices to minimize the presence of mycotoxins. Whilst regulating mycotoxins in feed and food has to ensure a high level of animal and public health protection, particular attention has to be paid by the regulators that the supply of major feed materials and staple foods is not endangered.

Finding the right balance is a challenge for feed and food safety regulators!
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P10 MYCOTOXIN SURVEY ON FLOURS IN THE MALTESE MARKET
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P13 MYCOTOXINS IN PLANT-BASED DIETS
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P16 PLANT-BASED FOOD – IDENTIFICATION AND QUANTIFICATION OF MYCOTOXINS
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P51 DETERMINATION OF THE RATE LIMITING STEP DURING ZEARALENONE HYDROLYSIS BY ZENA
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P52 EFFECTS OF SUPPLEMENTATION OF A MYCOTOXIN MITIGATION FEED ADDITIVE IN LACTATING DAIRY COWS FED FUSARIUM MYCOTOXIN-CONTAMINATED DIET FOR AN EXTENDED PERIOD
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P53 EVALUATION OF THE EFFICACY OF CINNAMON OIL ON ASPERGILLUS FLAVUS AND FUSARIUM PROLIFERATUM GROWTH AND MYCOTOXIN PRODUCTION: TOWARDS A MITIGATION STRATEGY
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P54 DETAILED ENZYME KINETICS OF FUMONISIN ESTERASES
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P55 PHYTOGENIC SUPPLEMENTATION WITH INEDIA® AND DAIRY CATTLE PERFORMANCES IN A CONTEXT OF THE PRESENCE OF NATURAL INFESTATION WITH MYCOTOXINS IN SILAGE
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P57 EFFECTS OF PHYTOGENIC SUPPLEMENTATION ON PIGLETS’ PERFORMANCE IN THE PRESENCE OF FEED CONTAMINATED WITH VERY HIGH LEVELS MYCOTOXINS
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P58 BIODEGRADATION OF ZEARALENONE IN SWINE AND CHICKEN DIGESTION SIMULATION
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P59 BIOLOGICAL CONTROL OF SILAGE MAIZE AFLATOXIN CONTAMINATION IN THE SOUTHWESTERN UNITED STATES
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P60 USE OF FUNGAL LACCASES FOR AFLATOXIN REDUCTION IN MAIZE SUB-PRODUCTS
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P61 BENTONITE MITIGATES THE NEGATIVE EFFECTS OF DIETARY AFLATOXIN B1 ON GROWTH PERFORMANCE, GUT MORPHOLOGY, AND IMMUNITY OF BROILERS
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P62 THE EFFECT OF A PULSED ELECTRIC FIELD ON THE FATE OF FUSARIUM MICROMYCETES AND THEIR MYCOTOXINS DURING MALTING
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P63 ANIMAL FEED MYCOTOXIN MITIGATION SOLUTION: IS IT AN EXTRA EXPENSE OR A DAILY DIET NECESSITY?
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P64 THE EFFECT OF CURCUMIN AND SILYMARIN IN MITIGATING THE OXIDATIVE STRESS INDUCED BY DEOXYNIVALENOL IN HEPATIC CELLS
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P65 THE ORAL BIOAVAILABILITY OF FUMONISIN B1 IS REDUCED BY AN ANTI-MYCOTOXINS AGENT IN BROILER CHICKENS IN A TOXICOKINETIC STUDY
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P67 EFFECTS OF A MULTI-COMPONENT MYCOTOXIN DETOXIFIER ON THE ANTIOXIDANT
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P68 THE EFFECTS OF A BROAD-SPECTRUM MYCOTOXIN ADSORBENT IN GESTATING AND
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P69 AN INTEGRATED MYCOTOXIN-MITIGATING AGENT CAN EFFECTIVELY MITIGATE THE
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P70 EFFECTS OF ANTI-BIOTOXINS SUPPLEMENTATION ON IMMUNOLOGICAL, BIO-
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P71 EVALUATION OF ESSENTIAL OILS AGAINST ASPERGILLUS CARBONARIUS AND THEIR
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P72 ADSORPTION OF AFLATOXIN B1 BY DIFFERENT ANTIMYCOTOXIN ADDITIVES:
BENTONITE, CLINOPTILLOLITE AND BETA-GLUCANS EXTRACTED FROM YEAST CELL
WALL
L.M.L. Schlösser1, Denize Tyska1, C.T. Simões1, J.A. Sarturi1, C.R. Da Silva1, I.F. Laber1,
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P73 ADSORPTION OF AFLATOXIN B1 BY DIFFERENT ANTIMYCOTOXIN ADDITIVES:
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WALL
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P74 BIOLOGICAL CONTROL OF AFLATOXINS AND FUMONISINS USING GREEK ENDEMIC
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P75 MITIGATION EFFECTS OF TOXO® IN BROILER CHICKENS WHEN EXPOSED TO MULTIPLE MYCOTOXINS
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M. Owen\textsuperscript{1}, P. da Silva\textsuperscript{1}, L. Jensen\textsuperscript{1}, S. Subramanian\textsuperscript{2} and Guan-Lin Wang\textsuperscript{2}
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Jessica M. Webster\textsuperscript{1}, T.J. Snelling\textsuperscript{1}, J.A. Huntington\textsuperscript{1}, D.R. Davies\textsuperscript{2}, N. Adams\textsuperscript{3}, J. Taylor-Pickard\textsuperscript{3}, H. Warren\textsuperscript{3} and L.A. Sinclair\textsuperscript{1}
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P88 WHO DOES WHAT ALONG THE CASSAVA VALUE CHAIN AND HOW DO THE PRACTICES ALONG THE CHAIN INFLUENCE MYCOTOXIN CONTAMINATION IN UGANDA?
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P95 MYCOTOXIN RISK MANAGEMENT IN MAIZE GLUTEN MEAL
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P100 ANALYSIS OF ERGOT ALKALOIDS IN A VARIETY OF SIMPLE AND COMPLEX MATRICES BY LIQUID CHROMATOGRAPHY-TANDEM QUADRUPOLE MASS SPECTROMETRY
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P106 VALIDATION OF THE AQUALOG® SYSTEM FOR RAPID AFLATOXIN QUANTIFICATION IN MAIZE SAMPLES
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P108 COMPREHENSIVE ASSESSMENT OF THE EFFECTIVE HOMOGENIZATION OF THE AGGREGATE SAMPLE PREPARED FOR TESTING MYCOTOXINS IN OFFICIAL CONTROL
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P110 DEVELOPMENT OF A MULTI-TOXIN UPLC-MS/MS METHOD FOR 50 MYCOTOXINS AND TROPANE ALKALOIDS IN CEREAL COMMODITIES
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P111 SUSTAINABLE ANALYSIS OF DEOXYNIVALENOL IN WHEAT VIA ATTENUATED TOTAL REFLECTION INFRARED SPECTROSCOPY
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P112 ON-SITE ANALYSIS OF MYCOTOXINS VIA INFRARED SPECTROSCOPY
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P115 DETERMINATION OF AFLATOXINS (AFB1, AFB2, AFG1, AFG2, AFB2) IN PISTACHIOS COLLECTED IN SOUTHERN ITALY (SICILY)
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P116 DEVELOPMENT OF AN ULTRA-RAPID LC-MS/MS SCREENING METHOD FOR THE DETERMINATION OF REGULATED AND MOST IMPORTANT EMERGING MYCOTOXINS IN THE FEED AND FOOD INDUSTRY
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P117 AUTOMATED MULTIPLEX MYCOTOXIN CONTROL FOR SAFE RAW MATERIAL INTAKE
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P118 ACCELERATION OF AN HPLC-MS/MS MULTI-CLASS METHOD FOR THE ANALYSIS OF >1,200 BIOTOXINS, PESTICIDES, AND VETERINARY DRUGS
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P120 SURFACE-ENHANCED RAMAN SPECTROSCOPY FOR THE RAPID DETECTION OF DEOXYNIVALENOL IN AGRICULTURAL PRODUCTS
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P121 DETECTION OF T-2 TOXIN IN WHEAT AND MAIZE WITH A PORTABLE MASS SPECTROMETER
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P122 COMPARATIVE PERFORMANCE OF RAPID TEST KITS FOR THE DETECTION OF T-2 AND HT-2 TOXINS IN OATS PRODUCED ON THE ISLAND OF IRELAND
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P123 USING SAMPLES COMPOSED OF DIFFERENT GRAIN TYPES TO ESTIMATE AFLATOXIN CONTAMINATION IN MAIZE LOADS
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P124 MULTI-TOXIN ANALYSIS: SINGLE EXTRACTION IMMUNOAFFINITY COLUMN CLEAN-UP FOR THE ANALYSIS OF 11 MYCOTOXINS IN PSEUDO CEREALS
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P125 THE ASSESSMENT OF 11 TOXINS IN MILK ALTERNATIVES USING IMMUNOAFFINITY COLUMN CLEAN-UP PRIOR TO LC-MS/MS DETECTION
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P126 AUTOMATED ANALYSIS OF OCHRATOXIN A IN HERBAL DRUGS AND HEALTH SUPPLEMENTS
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P127 ANALYTICAL METHODS FOR MONITORING MYCOTOXINS IN HUMAN URINE AND EXPOSURE ASSESSMENT – A REVIEW OF THE LAST 15 YEARS
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P128 ANALYSIS OF MYCOTOXIN OCCURRENCE AND FEED STORAGE PRACTICES IN SMALL-HOLDER BROILER FARMS IN KENYA
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P129 VALIDATION OF A FLOW-THROUGH RAPID TEST FOR THE QUICK AND EASY DETECTION OF OCHRATOXIN A IN WINE
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P130 VALIDATION OF TWO FLOW-THROUGH RAPID TESTS FOR THE QUICK AND EASY DETECTION OF OCHRATOXIN A IN GREEN AND ROASTED COFFEE WITH A CUT-OFF VALUE OF 3 mg/kg
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P131 ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY TO ADDRESS THE INDUSTRIAL NEEDS FOR THE DETECTION OF ERGOT ALKALOIDS IN SEVERAL CEREAL PRODUCTION CHAINS
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P132 DETERMINATION OF BIOMARKERS OF EXPOSURE OF T-2/HT-2 TOXINS IN HUMAN URINE
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P133 PENTAHELICENE DERIVATIVE COMPOUNDS: FLUORESCENT ORGANIC DYES THAT GIVE SIMPLICITY TO POINT-OF-NEED MYCOTOXIN DETECTION
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P134 MYCOSMART READER: MICROARRAY READER FOR MULTIPLE MYCOTOXIN DETECTION
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P143 AFlatoxins and ochratoxin a occurrence in dark chocolate bars marketed in southern Italy
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P145 Contamination by aflatoxins in different food matrices produced and consumed in Mozambique
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The contamination levels of six mycotoxins: aflatoxin (AF), deoxynivalenol (DON), fumonisins (FB), ochratoxin (OTA), T-2 and HT-2 toxin (T2HT2) and zearalenone (ZEA), in 5771 samples comprising of raw materials and complete feed collected from European region during 2022 were analysed. The median concentration of AF was 2 μg/kg for all types of commodities. The average concentration of AF varied between 2 and 6 μg/kg and the grain byproducts had the highest value. Among the feed categories, ruminant feed had the highest concentration of AF (3 μg/kg). DON is a major concern for European region. The median contamination of DON varied between 117 and 250 μg/kg, while its average concentration varied between 197 and 627 μg/kg. Grains were recorded with the highest level of DON (6,000 μg/kg). FUM has become another major concern, especially in southern Europe. The median concentration of FUM was up to 500 μg/kg, while its average concentration was between 390 and 2754 μg/kg. The highest concentration of FUM was recorded in grain byproducts (184,000 μg/kg). OTA contamination turned out to be insignificant in all samples, where its median and average concentration was up to 1 μg/kg. The median concentration of T2HT2 varied between 10 and 15 μg/kg, while its average concentration varied between 10 and 41 μg/kg. The median concentration of ZEA varied between 13 and 25 μg/kg, while its average concentration varied between 29 and 80 μg/kg and grain byproducts had the highest value. Monthly variation in the mycotoxin contamination in grains and complete feed were analysed, respectively. December recorded the highest average concentration of DON in grains (1096 μg/kg) closely followed by April (1094 μg/kg), while the lowest value occurred in August (296 μg/kg). The highest average concentration of DON in complete feed was observed in July (418 μg/kg) followed by December (402 μg/kg), while the lowest value in November (216 μg/kg). FUM contamination peaked both in grains and feeds during October with the average concentration of 3699 and 951 μg/kg, respectively. ZEA had the lowest contamination level in grains in August, where its average and median concentration was 19 and 3 μg/kg, respectively. A very weak positive correlation was observed for the monthly variation of DON, FUM, and ZEA between grains and complete feed (Spearman rank correlation coefficient ρ and p respectively for DON, 0.26 and 0.42; for FUM, 0.31 and 0.33; and for ZEA, 0.22 and 0.5).

The contamination levels of six mycotoxins: aflatoxin (AF), deoxynivalenol (DON), fumonisins (FB), ochratoxin (OTA), T-2 and HT-2 toxin (T2HT2) and zearalenone (ZEA), in 4319 samples comprising of raw materials and complete feed collected from Africa & Middle East region during 2022 were analysed. The median concentration of AF varied between 1 and 16 μg/kg, while its average concentration varied between 2 and 21 μg/kg. Both the highest median and average concentration of AF was found in pig feed. Grain byproducts (3 μg/kg) and pet food (2 μg/kg), on the other hand, had very low AF contamination. The median concentration of DON was up to 450 μg/kg. The average concentration of varied between 336 and 772 μg/kg and grains had the highest value (772 μg/kg) followed by poultry feed (726 μg/kg). The median concentration of FB was up to 450 μg/kg. FB average concentration varied between 178 and 1,346 μg/kg, where poultry feed had highest value and protein meal had the lowest one. The median concentration of OTA was up to 2.5 μg/kg, while its average concentration varied between 1 and 4 μg/kg. Regards T2HT2, grains had the highest average concentration (31 μg/kg). The median concentration of ZEA was up to 20 μg/kg. The average concentration of ZEA varied between 71 and 130 μg/kg and poultry feed had the highest value. Monthly variation of AF average concentration between grains, protein meal and feed were analysed, because AF is the most concerning mycotoxin for this region. The highest average concentration (45 μg/kg) of AF in grains was observed in December, while the lowest value (2 μg/kg) in both October and November. The highest average concentration (26 μg/kg) of AF in protein meal was observed in July, while the lowest value (1 μg/kg) in November. The average concentration of AF in complete feed peaked (18 μg/kg) in August and had the lowest value (5 μg/kg) in January.
The contamination levels of six mycotoxins: aflatoxin (AF), deoxynivalenol (DON), fumonisins (FB), ochratoxin (OTA), T-2 and HT-2 toxin (T2HT2) and zearalenone (ZEA), in 3,848 samples comprising of raw materials and complete feed from Asian region collected during 2022 were analysed. The median concentration of AF varied between 4 and 19 μg/kg. The average concentration of AF varied between 7 and 46 μg/kg and grains had the highest average contamination over other commodities. The median concentration of DON was up to 150 μg/kg, while its average concentration varied between 121 and 694 μg/kg. Among all samples, grain byproducts had the highest average concentration of DON. The median concentration of FB varied between 100 and 1,500 μg/kg, while its average contamination varied between 499 and 2,176 μg/kg. Poultry feed had the highest average concentration of FB (2,176 μg/kg), followed by grains (1,761 μg/kg). The median concentration of OTA varied between 1 and 18 μg/kg, while its average concentration was 2 and 25 μg/kg. Protein meals had highest median (18 μg/kg) and average (25 μg/kg) concentration of OTA. The median concentration of T2HT2 toxin were up to 5 μg/kg for all samples. Its average concentration varied between 1 and 7 μg/kg for most samples, except for grains (52 μg/kg). The median concentration of ZEA varied between 2 and 56 μg/kg, while its average concentration varied between 18 and 82 μg/kg. Grain byproducts had the highest average concentration of ZEA (82 μg/kg). Additionally, monthly variation of AF average concentration was analysed for both grains and complete feed, due to the fact that AF is the most concerning mycotoxin in this region. In grains, the highest average concentration of AF was observed in October (78 μg/kg) and the lowest one in May (17 μg/kg). In complete feed, the highest average concentration of AF occurred in August (40 μg/kg) and the lowest one in January (19 μg/kg). A weak positive correlation (Spearman rank correlation coefficient r and p respectively for AF, 0.61 and 0.04, was observed for the monthly variation of AF average concentration between grains and complete feed.

Latin America represents one of the world’s largest raw material suppliers for animal feed production. The contamination levels of six mycotoxins: aflatoxin (AF), deoxynivalenol (DON), fumonisins (FB), ochratoxin (OTA), T-2 and HT-2 toxin (T2HT2) and zearalenone (ZEA), in 3,328 samples comprising of raw materials and complete feed collected from Latin American region during 2022 were analysed. The median concentration of AF was between 2 and 3 μg/kg for all type of commodities. The average concentration of AF varied between 2 and 27 μg/kg and protein meals had the highest value. The median concentration of DON varied between 100 and 300 μg/kg and ruminant feed had the highest value. The average concentration of DON varied between 50 and 1,078 μg/kg and grain byproducts had the highest value. The median concentration of FB varied between 100 and 1,300 μg/kg, and its average concentration varied between 125 and 1,965 μg/kg. Both the highest median and average concentration of FB was found in poultry feed. The median concentration of OTA varied between 1 and 3 μg/kg. The average concentration of OTA varied between 2-13 μg/kg and grain byproducts had the highest value. Notably, both the highest median (16 μg/kg) and average concentration (28 μg/kg) of T2HT2 was also recorded in grain byproducts. The median and average concentration of zearalenone varied between 26.5 and 40 μg/kg, 48 and 125 μg/kg, respectively. Monthly variation of mycotoxin contamination was analysed for both grains and complete feed. The highest average concentration (14 μg/kg) of AF in grains was recorded in November, while the one (13 μg/kg) in complete feed occurred in June. Grains had the highest average concentration of DON in February (764 μg/kg), while the lowest value was recorded in December (195 μg/kg). For complete feed, the highest average contamination of DON was observed in March (644 μg/kg), while the lowest value in November (109 μg/kg). Both grains (1,774 μg/kg) and complete feed (2,110 μg/kg) had the highest average concentration of FB in December, while the lowest value in August (718 μg/kg for grains and 804 μg/kg for complete feed). A very weak positive correlations were observed for the monthly variation of DON and FB between grains and complete feed (Spearman rank correlation coefficient r and p respectively for DON, 0.32 and 0.31; and for FUM, 0.36 and 0.26).
P5 CLIMATE EFFECTS ON ERGOT AND ERGOT ALKALOIDS OCCURRENCE IN ITALIAN WHEAT
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Climate change in recent years has resulted in an increasingly hot and humid climate, with areas of the globe more prone to drought and others predisposed to increasing precipitation. Weather conditions during growing and harvesting seasons have increased the incidence and severity of mouldy grains and mycotoxin contamination. Torrential rains and wet conditions may delay grain drying, causing mould growth in the field. In July 2023, wheat crops in Como (Lombardy, Italy) were affected by torrential rains that led to the development of the fungus Claviceps spp. In the field, dark sclerotia were identified on some grain ears. Wheat ears, grains and sclerotia were collected and analysed by IZSLER, at the Food Chemical Department in Bologna, by LC-MS/MS. Wheat grains were analysed for mycotoxins (ochratoxin A, deoxynivalenol, zearalenone, fumonisins, T-2/HT-2 toxins and aflatoxins) after purification with immunoaffinity columns (IAC). Deoxynivalenol was the only one detected in wheat grains at concentration of 2,251 μg/kg. Wheat ears, grains and sclerotia were analysed for 12 ergot alkaloids (EAs) according to Commission Regulation (EU) 2021/1399 (ergocornine/ergocorninine; ergocristine/ergocristinine; ergocryptine/ergocryptinine; ergometrine/ergometrinine; ergosine/ergosinine; ergotamine/ergotaminine), after QuEChERS (Z-Sep/C18) purification. The analysed sclerotia showed significant differences in total alkaloids content that varies between 0.01-0.5% (w/w), according to the results of the EFSA scientific report (2017). Total alkaloids in freshly collected sclerotia ranged from 3 mg/kg to 4,951 mg/kg and the alkaloid profiles were very similar: analysis detected all 12 EAs and the levels of ergotamine/ergotaminine were the lower. EAs were detected in wheat ears up to 33 mg/kg; in line with this finding, the concentration of EAs in grain was 1 mg/kg. Climate change is expected to increase the frequency of extreme climatic events. In order to reduce the incidence of mycotoxin-producing fungi and protect human health, official controls should be implemented in the field.

P6 BIOTOXIN PROFILES OF TEA
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Tea is consumed globally and, specifically, in Nigeria across different climatic zones as a breakfast beverage. To date, there is scarce information on biotoxins in tea. Therefore, this study examined the biotoxins contaminating black, green and herbal tea vended in Nigeria. Tea samples (n=80) were purchased from supermarkets in Lagos, Nigeria, and composited into 20 samples based on production batch of tea type. The metabolites in each composite sample were profiled by a dilute-and-shoot liquid chromatography tandem mass spectrometric method covering over 500 metabolites. Altogether, 98 biotoxins consisting of metabolites from fungi (including mycotoxins), bacteria, lichens, and plants (phytoestrogens and phytotoxins) were detected. The most frequently occurring mycotoxins were citrinin, deoxynivalenol (DON), patulin (PAT), fumonisins and zearalenone (ZEN) that were found in 45, 40, 40, 35 and 35% of the samples, respectively. Additional mycotoxins in the tea samples include aflatoxin B1 detected in only one sample of herbal tea, alternariol, alternariol methyl ether, beauvericin, diacetoxyscirpenol, DHC, DON-3-glucoside, moniliformin, tentoxin, tenuazonic acid and ZEN-sulfate. PAT is documented for the first time in black tea, occurring in all its samples. A lichen metabolite, lecanoric acid, was detected in 80% of the samples. There were different co-contamination patterns in the tea samples. Thus, the diversity of biotoxins found in the tea samples including herbal tea, intended to ameliorate health challenges, question the mycotoxicological safety of tea. Further research is required to investigate the fate of biotoxins in the tea production chain.
Plant-based diets (PBD) continue growing in popularity in the world. The exponential rise in PBD is multifactorial and ranges from ethical, environmental, sustainability and animal welfare-related reasons to those seeking a healthier lifestyle and those wishing to improve health. Unfortunately, PBD products are not exempt from biological and chemical contamination, and consequently, it is crucial to understand the level of consumption exposure to these hazards. A recent publication showed that serum ochratoxin A (OTA) levels were two-fold higher in vegans than in omnivores diet [Penczynski et al., 2022]. Exposure to OTA is associated with liver failure and renal cancer, and it is commonly detected in cereals, spices, nuts, dried fruit and beverages, such as coffee. However, there is a lack of data about OTA contamination levels in highly processed plant-based products. Therefore, the main goal of this study was to assess microbial contamination and quantify the OTA occurrence in plant-based milk, energetic bars and vegetal burgers. Samples from three different brands of each product were purchased in conventional supermarkets. Microbial isolation from food samples was performed in potato dextrose and nutrient agars. Purified colonies were identified using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS/MS). OTA contamination was quantified by liquid chromatography (HPLC-FLD). A low level of microbial contamination was observed, as would be expected from processed food. Microorganisms were isolated from all the samples, although different species and contamination levels were found among different food categories. Only bacteria, *Micrococcus*, *Flavobacterium*, *Enterococcus* and *Actinomyces* were isolated from plant-based milk. On the contrary, fungi and bacteria species were isolated from energetic bars (*Bacillus* and *Mucor*) and vegetal burgers (*Bacillus*, *Enterococcus*, *Aspergillus*, *Penicillium* and *Fusarium*). Although in low concentration, OTA was detected in plant-based burger (0.78 ng/g), energetic bar (0.39 ng/g) and plant-based milk (0.24 ng/g). The results of this project will help to understand the OTA exposure through highly processed organic foods consumed in PBD and provide foundations for a risk assessment based on a cross-case analysis of the food chain, from farm to fork.

This study was intended toward evaluating the safety assessment of the food quality of cassava-based food (pupuru and lafun flour) collected from processing sites (P) (n=48) and open market sites (M) (n=48) respectively from different locations in South-West, Nigeria. Pupuru and lafun samples were assessed for mycotoxins contamination (aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), ochratoxin A (OTA), deoxynivalenol (DON), fumonisin B1 (FB1), fumonisin B2 (FB2), HT-2 toxin, T-2 toxin, STERIG, AME, and zearalenone (ZEN)) using a multi-mycotoxin liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Also, molecular characterization of moulds isolated from all the samples were detected using polymerase chain reaction and ITS sequencing of fungal species. Pupuru samples showed no contamination by any mycotoxin, except for STERIG and AME which was below the LOD (limit of detection). Lafun was contaminated with aflatoxin, *Fusarium* and other mycotoxins regardless of the agro-ecological zone. Molecular characterization of moulds isolated from all the samples showed mycobiota belonging to the genera: *Aspergillus*, *Fusarium*, *Penicillium*, and *Rhizopus*, which occurred more frequently from samples collected from open markets. More than 50% of lafun samples were contaminated with more than one mycotoxin which exceeded the maximum limits for food intended for consumption set in Nigeria. The contamination might be due to post-harvest storage and processing methods which might influenced mycotoxigenic mould formation. The information from the research shows that mould is dominant at each collection site of lafun and pupuru samples and good agricultural practices should be encouraged at the collection sites.
In 2021 several cat food products were recalled in the UK as they were implicated as a possible cause of an outbreak of feline pancytopenia, a rare bone marrow condition. At the time The Royal Veterinary College reported 565 cases of feline pancytopenia, with a mortality rate of 63%. Initially it was suspected the illness was potentially caused by the presence of mycotoxins in some cat foods, however this could not be definitively determined. As a result of the incident and subsequent recalls it was clear there was a lack of reliable data on the occurrence of mycotoxins in cat food. Therefore, Fera was commissioned to carry out a survey for a broad range of mycotoxins in cat food by the Food Standards Agency (FSA) and Food Standards Scotland (FSS). Forty samples consisting of a mixture of wet and dry products were collected following sampling guidelines for retail products from Commission Regulation (EC) No 401/ 2006. Samples were purchased from a representative cross section of brands and suppliers (including on-line suppliers). Aggregate samples were prepared to ensure the samples analysed were representative and homogenous. Samples were analysed using three different analytical methods allowing the analysis of approximately 70 analytes including some masked mycotoxins. Two of the methods (aflatoxins and ochratoxin A by HPLC, and a suite of 17 Fusarium mycotoxins by LC-MS/MS) were accredited to ISO17025 by Flexible Scope Accreditation. A follow up repeat survey of the same products sampled approximately six months later was also completed. The data from these two studies provide up to date information on the occurrence of a range of mycotoxins in cat food. The comparison of results from two sampling time points will allow the FSA and FSS to determine if there are seasonal differences in the potential mycotoxin contamination in the products. The results will be used to inform future UK policy decision making for pet food safety standards.

Mycotoxins, secondary toxic metabolites produced by fungi under favourable conditions, have long raised concerns as contaminants in various flours, including wheat, wholemeal, rye, and maize, due to their detrimental effects on both human and animal health. This study addressed a knowledge gap by conducting the first survey of mycotoxin contamination in flours available in the Maltese islands specifically flour being sold in local supermarkets, milling companies, and in bakeries to produce the Maltese bread and ftira. The Maltese ftira is a type of sourdough bread which was the first ever local product inducted on the UNESCO’s Intangible Cultural Heritage of Humanity list [Buttigieg et al., 2023]. A survey in 16 flour samples was performed, utilizing high-performance liquid chromatography coupled with fluorescent and photodiode detectors (HPLC-FLR-PDA) for the quantification of mycotoxin. Ochratoxin A, deoxynivalenol, aflatoxins, zearalenone, T-2 toxin, HT-2 toxin, fumonisin and patulin were the key mycotoxins examined. Notably, the co-occurrences of mycotoxins were widely observed; however, aflatoxins and fumonisins were absent from all samples. While common mycotoxins, including deoxynivalenol (190.30-324.55 μg/kg), T-2 (2.53-111.42 μg/kg), and HT-2 (2.45-9.75 μg/kg), were detected, the first was found below the maximum limits specified by the European Commission (EC) No. 1881/2006. Notably, the study revealed a significant exceedance of the permissible limit for OTA, with a concentration of 60 μg/kg detected, well surpassing the European Union threshold of 3.0 μg/kg. The presence of patulin (1.09-27.75 μg/kg) aligns with the prevalence of Penicillium spp. contamination on the Maltese Islands. This finding highlights the need for more awareness and targeted surveillance strategies to accurately assess the true extent of mycotoxin levels in products circulating within the Maltese market. In conclusion, this first study gives an indication of the types of mycotoxins present in local flours. The implications extend to both public health and food safety fields, prompting further investigations and the implementation of robust surveillance measures to ensure the accurate monitoring and control of mycotoxin levels within the local food supply chain.
OCCURRENCE OF MYCOTOXINS IN INFANT FOODS TRADED IN RIBEIRÃO PRETO, BRAZIL

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Mycotoxins are fungal toxic secondary metabolites, occurring in foods, is a major health concern, mainly for infants. Timely control measure and awareness among consumers are necessary, therefore a preliminary study was conducted to determine the co-occurrence of multi-mycotoxins in infant foods (n=34) traded in Ribeirão Preto, São Paulo, Brazil. In each city, samples of four types of food products intended for infant consumption, from the brands showing the greatest trade volume, were collected in supermarkets, as follows: infant formulae (n=8), follow-on formulae (n=4), cereal-based foods for babies (n=14), and fruit-based products (n=8). The first sampling procedure was conducted in May/2023, while the remaining three samplings will continue next over a one-year period (every 3 months). Identification and quantification of mycotoxins were performed by liquid chromatography coupled to mass spectrometry (LC-MS/MS). The study aimed to determine total aflatoxins (B1, B2, G1 and G2), fumonisins (FB1 and FB2), zearalenone (ZEN), T-2 toxin, deoxynivalenol (DON) and ochratoxin A (OTA) in the given food products. Higher frequencies were observed for ZEN, followed by DON, FBs, and OTA. Three samples of infant formula, two samples of dairy flour, eight samples of cereal-based and two fruit-based foods presented median ZEN levels of 3.4 (min-max: 3.1-3.4), 6.2 (3.4-9.1), 4.2 (3.0-7.8) and 6.3 (6.3-13.7) μg/kg, respectively. Moreover, DON with the median levels of 28.1 (28.1-185.3), 42.2 (33.5-129.0), and 207.9 (52.9-185.3) μg/kg were determined in two fruit-based, four cereal-based foods, and two samples of dairy flour, respectively. However, almost all cereal-based products presented total fumonisins (FB1 + FB2) at 37.1 (8.5-1,301) μg/kg, while 670.2 μg/kg of FB1 was determined in only one fruit-based sample. These findings indicate that cereal-based infant foods may pose a potential health risk, mainly to the infants, thus emphasizing the need for further preventive measures to avoid mycotoxin contamination in these products. Acknowledgements. FAPESP (Grants # 2019/21603-1, 2022/03952-1 and 2023/05989-2).

ALTERNARIA MYCOTOXINS IN TOMATO PRODUCTS: AN EMERGING OR LONG-STANDING PROBLEM?

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Although Alternaria toxins are considered emerging, their occurrence has been reported in different food, commodities and raw materials, for several years. Fruits and vegetables, as well as their derivatives are frequently contaminated with these toxins and tomato products have been identified among the most relevant contributors to human exposure to these mycotoxins. A study performed in Argentina in 2006 already showed a high incidence of Alternaria toxins in tomato products, with 49 % samples contaminated with either alternariol (AOH), alternariol monomethyl ether (AME) or tenuazonic acid (TeA). TeA and AME were the most frequent toxins (29 and 26%, respectively), while AOH was found in low frequency (6%) but significantly higher levels (up to 8756 μg/kg). The aim of the present study was to evaluate the evolution of the Argentinean market for tomato products in the last 15 years regarding the natural occurrence of Alternaria toxins. A total of 79 samples of tomato products from the Argentinean market were collected from 2019 to 2021. From these, 73 were of Argentinean origin and 6 Italian. Samples consisted of 38 tomato purees, 15 whole peeled tomatoes, 13 crushed tomatoes, 7 concentrated tomato pastes, 4 tomato sauces and 2 tomato pulps. The simultaneous quantification of altenuene (ALT), AOH, AME, altetroxin-I (ATX-I), tentoxin (TEN) and TeA was performed using a validated multi-mycotoxin LC-MS/MS method. From the 73 Argentinean samples, 29 (40%) were contaminated with at least one toxin, while no contamination was detected in the Italian samples. In terms of frequency, TeA and TEN were the predominant toxins (14%) followed by AOH (13%). AME was only detected in 3 samples (4%), in the same proportion than ALT, and ATX-I occurred in 2 samples (3%). Tomato purees showed the highest frequency of contamination (47%), followed by whole peeled tomatoes (38%), and crushed tomatoes and concentrated tomato paste at equal proportion (33%). None of the Alternaria toxins were detected in pulps or sauces. Tomato puree was the only product in which the six mycotoxins naturally occurred. As well, the highest concentration of TeA corresponded to a tomato puree sample (495.7 μg/kg). Comparisons between both surveys showed significant reduction in the levels of contamination. Regarding the three main mycotoxins, TeA remains being the predominant one, but the frequency of AME at present is significantly lower. Changes in agricultural and
processing practices and climate change are currently being explored as potential explanation for these changes.

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**MYCOTOXINS IN PLANT-BASED DIETS**

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Food consumption patterns are changing towards more sustainable and healthier diets. Part of this change is the rapid adoption of vegetarian or plant-based diets, including high-protein, fibre-rich, and whole grain food products. Plants are susceptible to fungal infection, causing economic losses and an associated health risk, since some fungi can produce mycotoxins: secondary metabolites causing toxic effects in humans and animals. Whether these diets contribute to a higher exposure to mycotoxins still needs to be evaluated. The aim of this study was to assess the mycotoxin distribution (i.e., profiles and contents) in vegetarian high-protein or fibre-rich food products in the Belgian market. Therefore, a total of 208 different commercially available vegetarian high-protein or fibre-rich food products were sampled in Belgium and analysed using in-house developed and validated liquid chromatography tandem-mass spectrometry (LC-MS/MS) methods for multiple-mycotoxins analysis. Quantifiable levels of alternariol, alternariol monomethyl-ether (AME), sterigmatocystin, deoxynivalenol (DON), DON-3-glucoside, 3-ADON, fumonisin B (FB) 1, FB2, FB3, zearalenone, citrinin, FUS-X, enniatin B, ergot alkaloids, T2 and HT2 toxin were detected in the samples. FB1 was the most frequent mycotoxin being present in 20% of the samples, followed by ENN B (19%) and AME (16%). It is of paramount importance to control mycotoxin exposure in these dietary transitions to ensure food safety. The gathered data contributes to explore whether the consumer needs to be further protected by expanding the maximum regulatory limit for some mycotoxins in vegetarian protein-rich and high-fibre food products. **Acknowledgements.** This research is funded by the Belgian Federal Public Service Health, Food Chain Safety and Environment through the contract RT 22/07 MYCOPROF.

**P14**

**MYCOTOXINS IN BREWING AND MALTING**

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Mycotoxins are secondary metabolites of fungi and represent a serious problem for human health. Due to the growing interest, they are widely studied by scientific groups in various aspects. One of these aspects is the food industry and the associated beer production. Mycotoxins can be present in beer, but also in the basic raw materials for beer production. Problematic mycotoxins that pose a serious risk for their toxicity and occurrence are aflatoxins especially aflatoxin B1 (AFB1), fumonisins (FBs), zearalenone (ZEN) and its metabolites, deoxynivalenol (DON) including its acetylated forms and also the masked form deoxynivalenol-3-glucoside (DON-3G), T-2 toxin, HT-2 toxin, ochratoxin A, and other mycotoxins such as nivalenol, diacetoxyscirpenol, fusarenon X, neosolaniol, sterigmatocystin, cytochalasin E and patulin. The Research Institute of Brewing and Malting has been dealing with the issue of mycotoxins since 2008 until now. During this time, more than 10,000 samples were analysed for mycotoxin content in various brewing and malting matrices. Fianovis, a French start-up biotechnology company, provides a state-of-the-art offer of standards for accurate and reliable mycotoxin detection, including isotopically labelled standards. The issue of mycotoxins in brewing and malting is a residual matter and the accurate quantitative determination of mycotoxins, correct interpretation of the results in connection with the toxicological values, and the maximum permissible value of mycotoxin have a key role for the world food safety and consumer protection. **Acknowledgements.** The authors would like to thank the Ministry of Agriculture of the Czech Republic, MZE- RO1923 for the financial support.
MYCOTOXIN CONTAMINATION AND NUTRITIONAL CONTENT OF MAIZE
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Mycotoxin-contaminated maize can pose a feed safety risk to poultry health and decrease production performance. Although mycotoxin regulatory guidelines are based on individual mycotoxin-contamination, feed and feed ingredients are often contaminated with multiple mycotoxins. Therefore, the objective of this study was to assess the mycotoxin co-contamination and its impact on the nutrient content of maize samples intended for poultry feed. We received 319 maize samples from different geographical regions in the Southeastern United States. Maize samples were analysed for aflatoxins (AFLA), fumonisins (FUM), deoxynivalenol (DON), and zearalenone (ZEA) using HPLC/MS and nutritional composition using Near-Infrared Spectroscopy. The statistical analysis of mycotoxins and nutritional values of samples was conducted by one-way analysis of variance (ANOVA) based on mycotoxin categories and the means were separated by Tukey's least-squares means comparison. Of the 319 samples analysed, 16.9% contained AFLA; 71.5% contained DON; 44.7% had detectable levels of ZEA. All tested samples had detectable levels fumonisins, and over 80% were contaminated with multiple mycotoxins. On co-contamination, 4.5% of the tested samples had all four mycotoxins, 39.5% of the samples had three mycotoxins, 38.1% of the samples had two mycotoxins, and 18.0 % of the samples had only one mycotoxin. When mycotoxins were grouped (not detected vs. detected) samples contaminated with AFLA and FUM had significantly higher protein values when compared to noncontaminated samples, while those contaminated with DON and ZEA had significantly lower (p<0.05) protein values when compared to noncontaminated samples. In terms of fat, AFLA contamination resulted in significantly lower (p<0.05) fat values when compared to noncontaminated samples while DON and ZEA contamination resulted in significantly higher (p<0.05) fat content. There was a significant increase (p<0.05) in moisture content in AFLA and FUM contaminated positive samples, while significant decrease (p<0.05) in moisture content in DON and ZEA contaminated samples. Both DON and ZEA- contaminated maize samples exhibited a significant increase (p<0.05) in fibre content. In terms of starch, AFLA and FUM contaminated samples had significantly lower (p<0.05) starch values, and DON and ZEA samples had significantly higher (p<0.05) values. In conclusion, mycotoxin contaminated maize samples had altered nutritional profiles, which can be caused by factors such as storage conditions, weather events, the year of harvest, and presumably infection of the maize by fungi producing different mycotoxins.

PLANT-BASED FOOD – IDENTIFICATION AND QUANTIFICATION OF MYCOTOXINS
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The growing popularity of alternative dietary choices, specifically vegan and vegetarian lifestyles, has become a notable trend in recent times. As a result, consumers are increasingly turning to plant-based substitute products ('alternative proteins') for animal-derived foods, such as fish, meat, and dairy products. These alternative products predominantly rely on plant-based proteins, including soy, pea, wheat, nuts, and oilseeds, as their primary protein source. Other constituents of these food items are derived from plant sources as well. However, plant-based raw materials often exhibit contamination by mycotoxins, i.e., harmful compounds produced by moulds. The presence of mycotoxins presents significant challenges to the food industry, necessitating careful control and mitigation strategies. Current estimates indicate that nearly 80% of food and feed samples are contaminated with mycotoxins [Eskola et al., 2020]. Furthermore, these toxins often withstand various food processing techniques, retaining their potency throughout the manufacturing process, and thus posing a concern even in processed foods. Earlier studies have even indicated higher levels of, e.g., mycotoxin ochratoxin A (OTA) in blood serum of vegans compared to non-vegans [Penczynski et al., 2022]. Therefore, within the scope of our work, various vegan and vegetarian substitute products were qualitatively and quantitatively analysed for mycotoxins. To achieve this, we optimized an LC-MS/MS multimethod tailored to the specific matrices and subsequently conducted thorough validation. This approach allowed us to undertake a comprehensive analysis of numerous commercial samples. Our investigation encompassed the identification of 15 mycotoxins, including deoxynivalenol (DON), DON-3-glucoside, 3-acetyl-DON, 15-acetyl-DON, HT-2 toxin, T-2 toxin, tenuazonic acid, alternariol, alternariol monomethyl ether, aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2, sterigmatocystin, and OTA.
OCHRATOXIN A PRODUCTION IN NOVEL PLANT-BASED FOODS

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When a mouldy spot develops on food during storage in a consumer’s fridge, she/he is faced with a dilemma: Avoid mycotoxins by discarding the entire product, or reduce food waste by salvaging the non-mouldy part? The few guidelines for safely salvaging certain products are not harmonized internationally and are often based on scant data from the 70s/80s. In particular, no data exist on potential mycotoxin production in new plant-based products, e.g., vegan cheeses, yogurt/creme fraiche and patés. Such products are becoming popular, even among non-vegans, for climate change and health reasons. However, the widespread use of coconut oil in vegan cheeses is potentially alarming from a mycotoxin perspective, because this substrate is known to stimulate mycotoxin production. In this study, we use an LC-MS/MS based multi-mycotoxin method to map the potential of moulds to produce toxins in vegan cheeses, creme fraiche and patés, compared with traditional products. Mould spores from ochratoxigenic *Penicillium* strains, *P. nordicum* and *P. verrucosum*, are point inoculated on the surface and the inoculated foodstuff is incubated at 8°C to mimic the temperature in a ‘warmer’ household fridge. After 21 days, sub-samples are taken directly from the mouldy spots (until a depth of 1.5 cm) and from underneath the spot to study the migration of the mycotoxins/fungal metabolites. Results from the first trials indicate that for many mycotoxins/ secondary metabolites, levels tend to be higher in dairy/meat products compared to their vegan analogues. Levels as high as 1 mg/kg ochratoxin A can be found in the mouldy spot-on pork liver paté, whereas concentrations in the vegan paté are 10-100-fold lower. At 1.5 cm below the mouldy spot, the concentrations of small and polar metabolites such as pestalotin and questiomycin A equal approx. 10% of the level found in the mouldy spot, whereas for more apolar compounds such as ochratoxin A the remaining level is 1% or even less.

NX-TOXINS FROM FUSARIUM GRAMINEARUM ISOLATES IN WESTERN CANADA

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*Fusarium graminearum* is considered an important plant pathogen, especially on the cereal crops, given the significance of the fusarium head blight it causes, severity, and accompanied economic losses. Every year significant quantities of grain becomes unfit for human and animal consumption due to mycotoxin contamination leading to huge economic losses world-wide. Over the past decade, newer mycotoxins known as NX toxins, were observed, produced by *Fusarium* species. Under the changing climate, where increased precipitation, moisture, temperature are a common occurrence, there is an increased risk of incidence of fungal diseases and subsequent accumulation of new and modified mycotoxins. Recently, a small percentage of *Fusarium graminearum* strains from Canada and USA were found to produce a novel NX-2 toxin in cereal crops. These new *F. graminearum* strains could pose a new threat to food safety in Canada and worldwide. However, the lack of reliable molecular and analytical methods to detect NX-2 isolates have limited the early detection and study of the novel NX-producing isolates in Canada. In the current study, *Fusarium graminearum* isolates and analytical methods targeting NX toxins were investigated. These methods will help in monitoring NX-producing isolates world-wide, characterizing their trends in the spatial and temporal dynamics, as well as promote early detection of emerging threats to plant, human, and animal health. Seven days old *F. graminearum* isolates were sub-cultured in sterile rice for 10 days. Ground rice samples were extracted for mycotoxins (NX-2, NX-3, deoxynivalenol, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, and nivalenol) with a solvent mixture containing acetonitrile, methanol, and water followed by sonication and centrifugation. The separated supernatant was filtered and an aliquot was evaporated to dryness under vacuum. Dried extracts were re-suspended in solvent matching the mobile phases used for separation of analytes on liquid chromatography column. A labelled internal standard was added followed by analysis using an ultra-high performance liquid chromatograph coupled with an Orbitrap Tribrid mass spectrometer. Preliminary results indicate a strong correlation between NX-2 toxins and other trichothecene mycotoxins. The details of this correlation and significance of these finding will be presented. These findings will help develop proactive mitigation strategies to increase resiliency in agriculture and achieve food security.
The avoidance of food—feed competition and the reduction of food and feed waste is highly important to meet our sustainability goals while nourishing a growing world population. Hence, using byproducts and other ingredients not suitable for human nutrition in livestock production is desirable. Some of these commodities are rich in nutrients but might be contaminated with undesirable substances as mycotoxins. To get a better picture about the contamination of byproducts and unusual feed materials with mycotoxins, DSM-Firmenich set a respective focus in their mycotoxin survey program 2022. To test the occurrence of multiple mycotoxin metabolites, a method based on Liquid Chromatography coupled with tandem Mass Spectrometry was used (LC-MS/MS method, Spectrum 380® and Spectrum Top® 50). The methods assess the levels of many more mycotoxins in addition to the major regulated and guideline aflatoxins (AFLA), zearalenone (ZEN), deoxynivalenol (DON), fumonisins (FUM), T-2 toxin (T-2) and ochratoxin A (OTA). Coconut samples as well as peanut samples from Asia were contaminated with extreme AFLA levels, showing a median of 61 ppb and 186 ppb, respectively. By having a closer look on brewery byproducts, a high contamination in terms of ZEN (mean of 109 ppb) and DON (mean of 1369 ppb) could be observed. Multi-mycotoxin analysis showed that Culmorin (occurrence of 100%) and its hydroxy-forms (occurrence of 75%) occurred at average levels of 448 ppb, 513 ppb and 1661 ppb, respectively. Culmorin is known to show severe synergistic effects with DON. Occurring both at quite high levels means that there is an enhanced risk for the animals by receiving that source of feed. In general, global contamination in lupins and pulses is quite low, except for beans—showing high ZEN contamination in Asia and Europe (mean of 247 ppb), as well as high AFLA contamination. Another highly contaminated byproduct is DDGS, especially in terms of FUM and DON. American samples showed an average contamination of 1,693 ppb DON and 1,875 ppb FUM. In Europe, average contamination is quite low, but there was a single sample found including 178,269 ppb ZEN and 445,956 ppb DON! By having a closer look on Asian DDGS samples, the contamination was incredible: all samples were contaminated with FUM and ZEN. Straw showed a global high contamination in ZEN (average of 189 ppb), B-trichothecenes (average 1,047 ppb) and A-trichothecenes (average 141 ppb). Furthermore, also maize byproducts as maize bran, hulls, gluten, etc., are representing products of high concern.

Maize holds significant global agricultural importance, serving various purposes from human and animal consumption to industrial applications. Fungal presence within maize grains, given favorable conditions, results in mycotoxin production, adversely affecting its quality. Maize is susceptible to fungal infestation and major mycotoxins. A study spanning 2022 and 2023 utilized Near-infrared Spectroscopy (NIRS) to conduct mycotoxicological and water activity (a_w) assessments on maize from Argentina, Bolivia, Brazil (stratified by regions), Colombia, Ecuador, Mexico, and Peru. A total of 24,300 spectra (118,201 analyses) were employed to quantify aflatoxin B1 (AFB1), fumonisins B1 + B2 (FBs), zearalenone (ZEN), deoxynivalenol (DON), and a_w. Maize samples were ground, homogenized, and subjected to NIRS scanning after passing through a 1 mm sieve. FBs were the most prevalent metabolites in Latin America maize, being detected in 87.3% and 85.8% of the samples; DON had the second highest positivity, 23.4% and 30.7%, followed by ZEN, 28.2% and 27.4% (2022 and 2023, respectively). FBs also had the highest incidence in Brazilian maize, 86.1% and 80.0%, followed by ZEN, 44.5% and 44.9%, and DON, 12.8% and 15.6% (2022 and 2023, respectively). In this 2-year mycotoxicological evaluation, 94.5% (2022; n=14,930) and 92.7% (2023; n=9,370) of the spectra predicted for FBs, AFB1, ZEN and DON were contaminated with at least one mycotoxin. The mycotoxins found co-contaminating the maize belong to the genera Fusarium and Aspergillus; FBs, followed by ZEN and AFB1, were the most prevalent toxins in the associations. In total, 10.8% of the analysis showed aw results above 0.69. In Brazilian regions, FBs showed the highest positivity in maize (90.4% and 90.1%), followed by ZEN (28.2% and 25.4%) and DON (9.5% and 26.2%) throughout 2022 and 2023. Insights into maize prevalence, mycotoxicological contamination, and storage conditions significantly impact the utilization...
of raw materials. Within this context, NIRS perfectly aligns with industry needs by offering real-time insights across various parameters.

**FOCUS ON MYCOTOXIGENIC FUNGI**

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**P21**

**DEVELOPMENT OF AN EFFICIENT CRISPR/Cas9 SYSTEM IN *FUSARIAUM VERTICILLIOIDES* AND ITS APPLICATION IN REDUCING MYCOTOXIN CONTAMINATION**

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The filamentous fungus *Fusarium verticillioides* is a significant pathogen affecting maize crops and capable of producing fumonisins (FBs). These mycotoxins can have adverse effects on human and animal health when they contaminate food, feed, and agricultural products worldwide. Understanding the biological mechanisms of this fungus is crucial for effective management and applications. However, traditional reverse genetic methods relying on homologous recombination can be laborious and time-consuming. In this study, we established an efficient CRISPR/Cas9 genome-editing system in *F. verticillioides* and explored multiple forms of genome editing. Initially, we validated the feasibility of the developed CRISPR vector by successfully disrupting the *pyrG* and *pyrE* genes, representing the first reported example in this species. Furthermore, we demonstrated the utility of this strategy for gene deletion by co-delivering the CRISPR vector targeting single or double cleavage sites along with donor DNA into protoplasts. Building upon these findings, we employed the *FUM1* as a target to develop an effective uracil auxotrophic selection system. This system also facilitated the deletion of the approximate 38-kb fumonisin biosynthetic gene cluster with high efficiency. Finally, we utilized the resulting atoxigenic strain, in which the *ΔFUM1* and *ΔFUM_cluster* were replaced, as a biocontrol agent to mitigate the production of Fumonisin B1 (FB1) contamination. The genetic methods developed in this study open up new avenues for studying and manipulating *F. verticillioides* at the molecular level. Moreover, they provide a potential solution for reducing mycotoxin contamination in the food industry.

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**P22**

**THE INFLUENCE OF CO₂ ON ASPERGILLUS FLAVUS AND FUSARIAUM PROLIFERATUM GROWTH AND MYCOTOXIN PRODUCTION DURING PADDY STORAGE**


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Mycotoxigenic fungi are responsible for the quality losses during storage. Of those, *Fusarium proliferatum* and *Aspergillus flavus* usually occurred during rice production, especially during medium to long term rice storage in tropical countries. Since temperature, water activity and gas composition are considered as the most abiotic conditions that impact the fungal growth and mycotoxin production. The purpose of this study is to assess the influences of temperature, water activity and CO₂ on the growth of *F. proliferatum* 01 (FP01) and *A. flavus* 01 (AF01). The study showed that at 0.80, 0.85 and 0.90 aw, both these fungal strains were not able to grow at all surveyed temperature levels. AF01 grew optimum at 0.99 aw, 35°C, with average growth rate of 6.44±0.02 cm³/day, while the optimal growing conditions of FP01 were 0.99 aw and 30°C with average growth rate of 4.97±0.03 cm³/day. At CO₂ 17%, the maximum inhibition of AF01’s growth rate at 0.95 aw, 30°C and 35°C, was 76 and 80%, respectively. Meanwhile, at CO₂ 19%, the maximum inhibition of FP 01 growth rate at 25°C and 30°C, 0.99 aw, was 44 and 81%, respectively. This study supplied valuable information for rice companies during rice storage.
Filamentous fungi, although producing noxious molecules, such as mycotoxins that are toxic for humans and animals, have been reported and used to produce numerous drugs active against human diseases. Indeed, fungal-derived products such as paclitaxel, statins or penicillin saved millions of human lives. Cyclic fungal peptides, such as depsipeptides, are a good example of molecules produced by fungi with potential adverse and positive effects on human health. Although these peptides are not recent and that some are clinically used to treat bacterial infections, comparative study of their activity and toxicity are missing and the mechanism of action involved in their antimicrobial activity is still to identify. We evaluated the antimicrobial activity and toxicity of different fungal cyclodepsipeptides and found that, although very close structurally, these peptides differ in terms of (i) antimicrobial activity and selectivity, (ii) toxicity against human cells, and iii) mechanism of action. Taken together, these results demonstrate that fungal cyclodepsipeptides could be considered either as mycotoxins or potential therapeutic drugs to treat human diseases caused by bacterial infections.

OCHRATOXINS AND OTHER SECONDARY METABOLITES PRODUCED BY *PENICILLIUM* IN WHEAT UNDER INTERACTING ABIOTIC FACTORS AND USE OF CO$_2$ FUNGAL ACTIVITY SENTINEL

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Mycotoxigenic fungi inhabiting grains pre- and post-harvest engage in competition with a variety of other colonizing fungi. The results of these interactions are shaped by the prevailing environmental conditions and the competing species. *Penicillium verrucosum* mostly found in cool damp northern European climates produce Ochratoxins and other metabolites in stored grains. Previous research shows that CO$_2$ production can be a sensitive indicator of the onset of mould spoilage and mycotoxins contamination in stored grains. However, the concentrations of ochratoxins, its conjugates, other secondary metabolites, in relation to the CO$_2$ produced during storage of wheat and how they are impacted by different interacting environment conditions are not well documented. Hence, the objectives of this study were to examine how different water activities (0.70-0.95 $a_w$) and temperature (15-20°C) conditions impact on the concentrations of ochratoxins, its conjugates and other *Penicillium* secondary metabolites using the respiration rates produced in stored natural and *Penicillium verrucosum* inoculated wheat to determine the earliest time and storage conditions that would represent a risk regarding contamination with fungal secondary metabolites, especially ochratoxin A. Wheat grains were stored for 20 days in mini-silo flasks with centrally placed CO$_2$ sensors recording every five minutes throughout the experiment. Fungal diversity and populations were analysed in contaminated wheat samples from both treatments (natural and inoculated) on day 10 and day 20. Finally, 48 samples of wheat were analysed using LC-MS/MS. The mycotoxin profile analysis showed that grains were contaminated with regulated, masked, emerging mycotoxins and other fungal metabolites. Secondary metabolites of *Penicillium*, *Fusarium*, *Alternaria*, and *Aspergillus* were present at levels higher than the regulated mycotoxins at wettest conditions, with chances of increasing the toxicity of the wheat grains. In the natural wheat grain, the earliest time of ochratoxin A (OTA) detection was at day 10 at 0.90 $a_w$, 20°C and concentrations exceeded legislative limit at day 20. Respiration rate was eight times higher at day three in the *P. verrucosum* inoculated wheat at 0.95 $a_w$ than in the natural wheat due to inoculum load. Therefore, at storage conditions >0.80 $a_w$ (16% moisture content) with respiration rate >0.02 mg CO$_2$/kg/h, grains are at risk of spoilage mould growth and OTA contamination exceeding legislative limit. Findings would be used to develop a decision support tool for grain farmers/companies to improve post-harvest management of stored grains thereby supporting Sustainability Development Goals 2 and 3.
THE INTERACTION OF DEOXYNIVALENOL AND NATURAL MICROBIAL FLORA

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Deoxynivalenol (DON) is a secondary metabolite produced by several Fusarium species, including F. graminearum and F. avenaceum and can be found in maize, wheat, and other cereal crops. A warm and wet environment aggravates DON production and its accumulation in grains. It also reported that DON can enhance the survival and competitiveness of the toxin-producing fungi in the soil, alter nutrient availability, and impact the composition and activity of soil microbiome, potentially favouring the growth of the pathogens and affecting microbial interactions. However, the intricate and multifaceted effects of Fusarium toxins on the soil microbiome flora remain largely unexplored. As a first step to this question, the interactions between DON and soil bacterial flora were studied by using four samples from different natural niches, including a chicken stable (expJ), a sheep stable (expY), a wheat field (expT) and a horse stable (expM). The collected soil samples were treated with DON. After being co-incubated at 30°C with 130 rpm shaking for 96 h, DON was reduced by 74.5, 43.0, 46.7, and 86.0% by expJ, expY, expT, and expM, respectively. After DON (0.8 ml of 100 μg/ml) was co-cultivated with 0.2 ml of the supernatant of each sample (i.e., suspensions of microbial communities) at 30°C for 96 h, DON was reduced by 98.9, 99.8, 79.5, and 78.9% in expJ, expY, expT, and expM, respectively, and was completely degraded after 8 days by all samples except of expM. The bacterial flora of the samples was examined through 16S rDNA flux sequencing and was compared pre- and post the addition of DON. The results confirmed that the diversities of bacterial flora were affected by DON. After DON treatment, the most abundant bacteria belong to Galbibacter (16.1%) and Pedobacter (8.2%) in expJ; Flavobacterium (5.9%) and Pedobacter (5.5%) in expY; Microscillaceae (13.5%), B1-7BS (13.4%), and RB41 (10.5%) in expT; and Acinetobacter (24.1%), Massilia (8.8%), and Arthrobacter (7.6%) in expM. This first study on the interactions between DON and natural microbial flora provides useful information and a methodology for further development of microbial consortia for mycotoxin detoxifications.

ESTABLISHMENT OF AN EFFICIENT GENE TARGETING SYSTEM FOR OCHRATOXIN-PRODUCING FUNGUS ASPERGILLUS WESTERDIJKIAE

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Aspergillus westerdijkiae, the producer of ochratoxin A (OTA), which is of worldwide concern, is an import fungal species in agriculture, food, and industry. Here, we got the uridine auxotrophic mutant of A. westerdijkiae by deleting AwpyrG. The ΔAwpyrG could be used for biotransformation with exogenous ApyrG expression cassette as a selection marker. In order to enhance the efficiency of gene targeting, Awku70 and Awig4 were homologously deleted from ΔAwpyrG. The efficiencies of homologous replacement for ΔAwku70 and ΔAwig4 were 95.7 and 87.0% in the deletion of AwaReA, respectively, demonstrating a drastic increase from 4.3% of the wild type (WT) strain. Furthermore, the function of AwaReA was identified with AwaReA deletion mutant and the control strain ΔAwku70. AwaReA regulated the growth and conidiation of A. westerdijkiae in response to nitrogen sources. The concentration of OTA in the range of 19.4 to 186.9 ng/cm² on all kinds of nitrogen sources. The OTA production influenced by the deletion of AwaReA was different based on nitrogen sources. Pathogenicity assays on pears, grapes, salted meat, and cheese showed that AwaReA acted as a negative regulator in the infection of food substrates. Therefore, the genetic methods and engineered strains enable us to substantially expand the use of A. westerdijkiae, one of more than twenty OTA-producing fungi, in the study of mycotoxin biosynthesis and regulation, and consequently to aim at providing new ways for controlling this pathogen.
EXPLORING TOXICITY USING FUNGAL GENOMES: A CASE STUDY ON PSEUDOPITHOMYCES CHARTARUM, THE CAUSAL AGENT OF FACIAL ECZEMA IN CATTLE

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Facial eczema (FE), also known as pithomyccotoxicosis, is a secondary photosensitization disease in ruminants caused by sporidesmin A. This mycotoxin is classified as an epipolythiodioxopiperazine (ETP) toxin and is produced by the fungus Pseudopithomyces chartarum (previously known as Pithomyces chartarum). This fungus mainly colonises leaf litter within pastures. First documented in New Zealand in 1894, FE has expanded its presence to countries such as Argentina, Australia, France, the Netherlands, Portugal, South Africa, Spain, Türkiye, USA, and Uruguay. The occurrence of outbreaks is influenced by weather conditions and typically occurs during warm and humid periods in summer and autumn. A previous New Zealand study identified in a P. chartarum genome an ETP biosynthetic gene cluster (BGC) that encodes the necessary enzymes for sporidesmin biosynthesis. In this study, we analysed four additional P. chartarum isolates from South Africa and compare them with P. sacchari and P. maydicus, non-sporidesmin-producing species, as well as the New Zealand genome of P. chartarum. The study involved the phylogenetic dereplication of Non-Ribosomal Peptide synthetases (NRPSs) across the Pseudopithomyces genomes. A maximum likelihood phylogenetic tree was constructed using characterized fungal NRPSs from the MIBiG database and existing literature as references, which retrieved the candidate BGC for sporidesmin production. Comparison of the locus in the different genomes revealed a partially conserved ETP BGC. This is the first example of comparison of the potential sporidesmin BGC within the genus Pseudopithomyces. Confirmation of the role of this BGC in sporidesmin biosynthesis requires further functional characterization.

EXPOSURE ASSESSMENT AND HUMAN HEALTH

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ANALYSING EPIGENETIC TOXICITY CAUSED BY MULTI-MYCOTOXIN EXPOSURE USING AN INTESTINAL AND HEPATIC CELL CULTURE MODEL

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During the past decades, awareness of mycotoxin contamination in food and feed has increased. Previous European cohort-based research, the European Prospective Investigation into Cancer and Nutrition (EPIC), has shown chronic low-dose intake of multiple mycotoxins (i.e., deoxynivalenol (DON), patulin (PAT), sterigmatocystin (STC)) to be associated with an increased risk of colorectal carcinoma (CRC). Furthermore, a Malawian cohort investigated the association between multi-mycotoxin biomarker profiles and hepatic illness (cirrhosis and hepatocellular carcinoma (HCC)) and observed an association among ochratoxin (OTA), citrinin (CIT) and HCC. The paradigm of cohort-based association is causality which needs to be further resolved. Therefore, this study aims to clarify the toxicological impact of DON, PAT, STC, CIT, OTA and Alternaria mycotoxins through heritable non-genetic histone post-translational modifications (hPTMs). This will allow us to gain a more in-depth understanding of crucial toxicological aspects as well as the potential mechanism behind carcinogenesis. As the genomic sequence does matter in cancer so do epigenetic alternations, which are reversible changes in gene expression without permanently changing the genome sequence. Caco-2 (Cancer coli-2) and HepG2 (Human hepatocellular carcinoma) cells will be used as in vitro models for human intestine and hepatic, respectively. The hPTMs which are covalent enzymatic modifications of histones and important determinants of the epigenetic status (e.g., acetylation and methylation of lysine’s and arginine’s residues), will be analysed by liquid chromatography–tandem mass spectrometry, thoroughly validated for purpose. As a pioneer in the field, this research will highlight results regarding mycotoxin-induced cytotoxicity and hPTM induction, to investigate causal links between multiple mycotoxins and carcinogenesis. Acknowledgements. This project has received funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation program (grant agreement No 946192, HUMYCO).
A MOLECULAR MODELING STUDY TO GRASP THE MECHANICS OF ZEARALENONE ESTROGENICITY: SPOTLIGHTING AROMATASE FROM A ‘PERSONALIZED’ STANDPOINT
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Zearalenone (ZEN) is a Fusarium mycotoxin that contaminates food commodities threatening humans and animals’ health because of its endocrine disrupting effects. Although regulated in many countries, effects impairing human health attributable to its xenoestrogenicity have been reported over the years, including precocious puberty, telarche/mastopathy development and carcinogenicity. From a mechanistic standpoint, ZEN xenoestrogenicity may rely on its concerted capability to activate estrogen receptors (ERs) and inhibit aromatase (CYP19A1). ERs elicit an estrogenic response, while aromatase inhibition has an opposite effect being the aromatase responsible for estrogens production. The final balance of these two apparently contradictory effects concurs determining the final estrogenic potency of ZEN. ERs and aromatase show many single nucleotide variations among individuals reasonably varying the capability of ZEN to interact with them and eventually exert its endocrine disruption among the human population. This work focused on aromatase investigating via 3D molecular modelling whether single nucleotide variations reported to occur in the human population may affect the inhibitory potential of ZEN. UniProt (https://www.ncbi.nlm.nih.gov/snp) was browsed, and the 434 missense-mutated aromatase variants were analysed. Then, docking simulations and molecular dynamics served to study from a molecular level the punctual effects of a selection of mutations on the inhibitory potential of ZEN. The study showed that mutations significantly modifying the aromatase susceptibility to ZEN exist. As an example, depending on the amino acid substitution, mutation of T310 or D309 may have opposite effects either increasing ZEN-aromatase interaction (likely leading to an enhanced inhibitory activity) or in a loss of interaction. Importantly, some of those mutations have been previously described relevant from a clinical perspective being found in cancer and other pathologies. In conclusion, mutated variants of aromatase either with an enhanced or reduced susceptibility of being inhibited by ZEN were described. Both cases, ZEN may reasonably have a diverse effect at an individual basis depending on the actual variant expressed. This work spotted those most worthy of further research. Moreover, the clinical relevance of some of those variants suggests questioning whether ZEN may have a greater impact on certain subjects. This could partially explain the mechanics of ZEN-dependent pathogenesis and related inter-individual variability, which are still almost unveiled.

HUMYCO – MAPPING THE HUMAN MYCOBOLOME AND CANCER-RISK ASSOCIATION THROUGH EPIDEMIOLOGIC AND MECHANISTIC STUDIES
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To date, the research field on mycotoxin biomarkers of exposure and associated cancer risk is a poorly unexplored territory: validated biomarkers are scarce, epidemiological studies are lacking and mechanistic studies to determine causality based on (epi-)genetic toxicity are non-existent. HuMyco aims to comprehensively investigate the human mycobolome and the associated health effects through intervention trials and large-scale epidemiological cohorts, as well as unravel causal relationships via (epi-)genetic toxicity. First, the human toxicokinetic profile of emerging mycotoxins (i.e., Alternaria mycotoxins, citrinin and patulin) are studied by monitoring urine, blood & faeces with subsequent multi-biomarker of exposure analyses based on ultrahigh performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS). Second, in vitro samples, resulting from liver microsome experiments, and in vivo trial samples, will be submitted to high-resolution mass spectrometry to elucidate the metabolic profile and identify as well as validate new biomarkers of exposure. Third, associations between estimated external and internal dietary mycotoxin exposures (single & multi-mycotoxin exposures), and the development of renal illnesses, as well as hepatocellular & colorectal carcinomas, will be investigated within 3 large-scale epidemiological cohorts, namely Europe (European Prospective Investigation into Cancer and Nutrition, EPIC, chronic-low exposure), Groningen (the Netherlands, chronic-low exposure) and Malawi (Africa, chronic-high exposure). Multiple supervised machine learning techniques (e.g., K nearest neighbours, Partial Least Squares Discriminant Analysis, Naïve Bayes, Support Vector Machine, Decision Tree and Random Forest) will be used to address the issues of high dimensionality and highly correlated data. Finally, to determine causality, mycotoxin-induced damage
at the (epi-)genetic level will be analysed in human intestinal Caco-2 and liver HepG2 cells, priorly exposed to Alternaria mycotoxins, patulin, deoxynivalenol and sterigmatocystin. Genotoxicity testing will be performed by γ-H2AX immunofluorescence to detect DNA-double strand breaks. Post-translational histone modifications (PTMs), which are covalent enzymatic modifications of proteins and important determinants of the epigenetic status (e.g., acetylation and methylation of lysine’s and arginine’s residues), will be analysed by UHPLC-MS/MS, thoroughly validated for purpose. As such, HuMyco provides a unique, holistic approach to determine the overarching health burden of mycotoxins in humans. Results of this research will be presented at the conference. Acknowledgements. HuMyco was funded by the European Research Council (HuMyco, ERC 946193).

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DIETARY MYCOTOxin EXPoSURE AND HUMAn HEALTH RISKS: A PROTOCOL FOR A SYSTEMATIC REVIEW
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Over the past decade, efforts have been made to evaluate human health effects of dietary mycotoxins through intervention trials, cohort studies, case-control studies, cross-sectional studies, etc. Results are often study-specific, related to the measured health effects and the studied mycotoxin, and little attention is paid to multiple mycotoxin exposure as well as evidence appraisal. In contrast to existing mycotoxin-related literature reviews, this protocol aims to cover all existing scientific information relevant to the overarching burden of dietary mycotoxins on human health, and addressing co-exposure risks, as well as providing evidence appraisal, in a systematic manner. A state-of-the-art, all-comprehensive search string was developed for Pubmed, Embase and Cochrane Library, in an iterative process during scoping (i), building on the advice of experts in mycotoxicology, human health, nutrition and systematic review (SR) methodologies (ii), and the exploration of MeSH and Emtree databases (iii). Based on the expected extent of the SR, a semi-automatic workflow was established by means of Covidence, integrated with reference management software. Within the workflow, primary research on any measured or modelled dietary exposure to a single or multiple mycotoxins, and adverse human health outcomes (i.e., cancer, non-carcinogenic diseases, and reproductive and developmental conditions) will be included. Two independent reviewers will screen titles and abstracts, and review full texts. Any disagreements will be resolved by a third reviewer. Eligible studies will be extracted and evaluated for risk of bias and certainty of evidence, and a meta-analysis may be performed based on the variability in predefined Population, Exposure, Comparator, Outcome (PECO) elements and depending on the heterogeneity of studies. This protocol describes the methodology for the conduct of a SR on mycotoxin-related human health risks, the latter providing a thorough synthesis of the current knowledge (gaps) that could guide future research and inform regulatory decisions, as emphasized by the European Commission within the field of regulatory risk assessment for emerging chemicals. The established protocol will serve as a working template for the upcoming years. Acknowledgements. This research was funded by the European Research Council (ERC 946193).

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PREDICTORS OF KNOWLEDGE, ATTITUDES, AND PRACTICES ASSOCIATED WITH OESOPHAGEAL CANCER RISK OR PREVENTION IN MALAWI
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Oesophageal cancer (EC) is the seventh most common cancer and the sixth leading cancer-causing death globally. EC is a multi-factorial disease influenced by internal and external exposome factors. Specifically for external exposome factors, mycotoxin exposure, alcohol consumption, tobacco smoke, poor oral hygiene, hot-beverage consumption, geophagia, and poor diet have all been linked to increased EC cases in high-risk regions. Worldwide, Malawi has the highest number of EC cases, with a substantial proportion of new cases observed in the young population (<45 years). Contrary to the etiological evidence pointing to the vital contribution of the modifiable EC external exposome factors (including mycotoxin exposure), data on individuals’ knowledge, attitude, and practices (KAP) regarding EC risk factors or its prevention are limited in Malawi. A descriptive cross-sectional study (n=310) was
conducted in Blantyre and Chiradzulu districts to provide a snapshot of predictors of KAP associated with EC risk or prevention in Malawi. The study hypothesized that participants’ knowledge, attitudes, and practices towards EC risk factors (including mycotoxin exposure) or their prevention would vary among age groups, gender, monthly income levels, education backgrounds, marital status, and occupation status in logistic regression models and sample t-tests. Preliminary analysis shows that being a male increased the chances of being knowledgeable about EC prevention two times higher than female counterparts (OR=2.303, 95% CI: 1.455-3.644 1.45). Similarly, married individuals were 18 times more likely to be knowledgeable about EC risk factors in a fully adjusted multivariate model (OR=17.387, 95% CI: 1.160-260.530). One unit increase in age increased participants’ chances of having good lifestyle behaviours or practices concerning EC prevention by a factor of 1 in the fully adjusted model (OR=1.061, 95% CI: 1.003-1.123). However, there were no significant differences in KAP mean scores between young individuals (18-44 years) versus adults (45-70 years) in independent sample t-tests. In conclusion, Malawi’s current EC incidence trends place a strenuous demand on an already dilapidated healthcare system. Regardless, this presents an opportunity to awaken stringent primary prevention strategies to contain EC cases. The preliminary data from this study suggests a vital contribution of masculinity, matrimony, and increased age in EC prevention. While the direct relations of these predictors to EC risk reduction have not been ascertained at a large scale, their prospects (together with other possible explanatory variables) warrant further research to inform policy in national cancer control programmes.

P33 BUILDING A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR A CARCINOGENIC FOOD CONTAMINANT

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Mycotoxins, secondary metabolites produced by fungi, can be present in food commodities [WHO, 2018]. Mycotoxins are a global health issue, however, some regions are more affected due to climatic conditions and lack of controlling monitoring mechanisms, e.g., Sub-Saharan Africa. Aflatoxin B1 (AFB1) is a human carcinogenic, immunotoxic and hepatotoxic mycotoxin, produced by Aspergillus strains [IARC, 2018; Smith et al., 2016; Rotimi et al., 2019; Claeyss et al., 2020]. A physiologically-based pharmacokinetic (PBPK) model was built using SimCYP®(v21). The model was based on in-house performed in vitro experiments and literature data [Lootens et al., 2022]. The verification of the model was executed via comparison with in vivo human PK data [Jubert et al., 2009]. The developed model was used to simulate exposure in different populations and interactions with commonly used medicinal drugs in South Africa. The PK of AFB1 was simulated in the black South African, Sim-NEur Caucasian population and Sim-Chinese healthy volunteer population, available from SimCYP. Simulations between commonly prescribed medicinal drugs in South-Africa, using the Essential Medicine List of the World Health Organization, and the developed AFB1 compound were simulated in a black South African population. Drugs that are CYP1A2/3A4 substrates or impact at least one of the two enzymes were used. Performed simulations showed no influence of AFB1 on drug disposition of commonly prescribed drugs that are metabolized by CYP1A2/CYP3A4 in the black South African population. Nonetheless, acute high levels of AFB1 might impact drug disposition but it also leads to liver failure, which was not further investigated since this could not be taken into account in the model. It was clear that CYP1A2/CYP3A4 inducer/inhibitor drugs did impact the disposition of the daily administered AFB1. The different metabolites of AFB1 exert different health effects, formation of higher aflatoxin-8,9-endoxoepoxides (AFBO) levels lead to higher carcinogenicity. Impact of drugs on the PK of AFB1 may lead to more severe health effects by AFB1 metabolites when exposed to AFB1 alone. Building a PBPK-model for food-contaminants might be helpful to predict possible interactions with commonly prescribed drugs. Noteworthy the chronic exposure to food-contaminants is much lower compared to drugs, therefore it is not likely that contaminants will influence drug disposition in the developed model. The developed food-contaminant compound files can also be used to simulate food-contaminant – food-contaminant interactions, giving insight to what might happen when co-ingested.
THE EPIGENETIC INTERPLAY OF MYCOTOXINS AND EPSTEIN BARR VIRUS TOWARDS CHILDHOOD CANCER
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Vulnerable populations in low- and middle-income countries (LMICs) are daily exposed to dietary carcinogens such as mycotoxins. Infection by Epstein Barr virus (EBV) is linked to a childhood cancer called Burkitt lymphoma (BL) which is endemic in parts of sub-Saharan Africa where chronic mycotoxin exposure co-exists. It was recently demonstrated in vitro that aflatoxin B1 (AFB1) modifies the DNA methylome and promotes EBV infection in B-cells. In addition, both AFB1 and EBV may alter DNA methylation levels and deregulate the expression of cancer-related genes. The aim of this study is to assess the epigenetic interaction between mycotoxins and EBV (and other infections) in affected populations and validate the underlying mechanisms using in vitro and in vivo models. Accurate exposure assessments of mycotoxins and EBV were conducted in an established cohort (n=300) of African infants and children, embedded in the MISAME-III cohort. Multiple mycotoxins were detected in 30% of the samples with ochratoxin A (OTA) being the most prevalent. Additionally, EBV was detected in 8% of the samples. Subsequently, DNA methylation status was assessed in the blood of the children and was associated with the levels of mycotoxin exposure and EBV infection status. Preliminary results have uncovered significant alterations in the DNA methylation status of 16 genes associated with EBV infection. To delve into the role of the co-exposure on the development of BL this study aims to perform identical analyses in tissue samples collected from patients within the same region. The outcome of this research will elucidate the mechanistic pathway(s) of environmentally induced cancer. Understanding the mechanisms by which mycotoxins and viruses interact to deregulate the epigenome and induce tumours will provide insights to both the scientific community and governmental officials on how to overcome this public health challenge with focus on LMICs.

INVESTIGATING THE PROGRESSIONS OF HEPATOCELLULAR CARCINOMA AND POST-KIDNEY TRANSPLANT FAILURE CAUSED BY MULTIPLE MYCOTOXIN EXPOSURE THROUGH UNITING EPIDEMIOLOGICAL AND MULTI-OMICS STUDIES
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For decades, mycotoxin contamination has posed a persistent global challenge to both food safety and population health. The spectrum of adverse effects on human health, including organ damage, immune disorders, and carcinogenesis, can be attributed to both acute and chronic exposure to mycotoxins. By investigating large-scale cohorts on mycotoxin intakes across Europe and Africa, the deleterious impacts of mycotoxins become even more pronounced. In this study, ultra-high performance liquid chromatography tandem mass spectrometry has been used to assess the multiple mycotoxin exposure in the patients at the ending stage of cirrhosis and hepatocellular carcinoma within a Malawian cohort (n=420). The preliminary results indicate a higher exposure level of ochratoxin A (OTA) and citrinin (CIT) in the serum samples of the patients, as compared to the control group. Concomitantly, an ongoing investigation within the Dutch population (n=932) is underway to uncover external risk factors contributing to post-kidney transplant morbidity and mortality. This study has revealed the presence of OTA, tenuazonic acid (TeA), enniatin B (EnnB), CIT, deoxynivalenol (DON), T-2 toxins (T-2), and zearalenone (ZEN) within the patient group. Recognizing that conventional regression models using epidemiological data may not always sufficiently elucidate the causal relationship between exposures and diseases, our research integrates multi-omics approaches, including metabolomic and metagenomic features. These approaches allow us to gain a deep insight into the intricate mechanisms underlying the progression of hepatocellular carcinoma and post-kidney transplant failure due to exposure to multiple mycotoxins. Although the complexity introduced by the high dimensionality of epidemiological and multi-omics data presents a significant challenge to conducting robust analyses, the evolving fields of machine learning and deep learning offer promising solutions. Consequently, this research leverages these advancements to extract critical features and construct prognostic prediction models.
models for hepatocellular carcinoma and post-kidney transplant failure. **Acknowledgements.** This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement No 946192, HUMYCO).

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**THE MYCOTOXINS, A PART OF THE DIETARY EXPOSOME: A CHALLENGE FOR TOXICOLOGIST**

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Throughout our lives, humans are exposed to a wide range of food contaminants, bacterial toxins and neoformed products from our diet: this is the concept of the dietary exposome. Among natural food contaminants, mycotoxins being the most frequently occurring natural ones. Mycotoxins are secondary fungal metabolites produced mainly by Aspergillus, Penicillium and Fusarium. Mycotoxins co-contamination is confirmed by the co-occurrence of these toxins in food and by co-exposure monitoring survey. The co-occurrence of mycotoxins in food is explained by three different reasons: (i) most fungi are able to simultaneously produce several mycotoxins; (ii) commodities can be contaminated by several fungi simultaneously or in quick succession; and (iii) the complete diet comprised different commodities. In practice, the co-occurrence of mycotoxins represents the rule and not the exception. Unfortunately, the data on the combined toxic effects of mycotoxins are limited and therefore, the health risk from exposure to a combination of mycotoxins is incomplete. Most of the studies concerning the toxicological effect of contaminant have been carried out taking into account only one compound. A synergistic effect between mycotoxins of the trichothecene family mycotoxins was observed both for intestinal cytotoxicity and inflammatory response and the synergy was already seen at low doses. Besides mycotoxins, other contaminant can be found in our diet, such as heavy metals, bacterial toxins, pesticides or neoformed products. Thus, the combined exposure to the mycotoxin deoxynivalenol and cadmium was also studied in several human cell lines and interactions were specific to the target organ. Moreover, the interaction between deoxynivalenol and genotoxins, present either as food contaminant or as toxins from our microbiota, was also investigated. Although not carcinogenic, DON exacerbates DNA damage induced by genotoxins such as bacterial toxins, pesticides or reference genotoxic compounds, suggesting a role in colorectal cancer. Altogether, these data demonstrated that (i) mycotoxin cocktails can lead to synergistic interaction and (ii) mycotoxins also interact with other food contaminants (heavy metal, genotoxins, ...) and with the intestinal microbiota. Thus, contaminations should be taken in the global context of the dietary exposome.

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**A (PROCESSED) APPLE A DAY, KEEPS THE DOCTOR AWAY? NATURAL OCCURRENCE, EXPOSURE ASSESSMENT AND RISK CHARACTERIZATION OF ALTERNARIA MYCOTOXINS IN APPLE BY-PRODUCTS IN ARGENTINA**

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Apples are susceptible to infection with Alternaria species, mainly causing mouldy core disease, hinder the detection of infected fruits by processing industries. Data on the natural occurrence of Alternaria mycotoxins in apple by-products is lacking in Argentina and the risk of exposure to these mycotoxins has not been characterized before. The levels of alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT), tenuazonic acid (TeA), tenu toxin (TEN), altertoxin-I (ATX-I), altertoxin-II (ATX-II), alternariol 3-sulfate (AOH-3-S), alternariol 3-glucoside (AOH-3-G), alternariol monomethyl ether 3-sulfate (AME-3-S), and alternariol monomethyl ether 3-glucoside (AME-3-G) were determined in clarified and cloudy apple juices, marmalades, and apple-based infant food from the Argentinean market using a validated multi-mycotoxin LC-MS/MS method. As well, the risk of exposure was characterized. This is the first report of Alternaria mycotoxins and their modified forms in commercial apple by-products from the Argentinean market. All the different food categories analysed were contaminated with Alternaria toxins, but higher levels were found in non-clarified products destined to infants. Detectable levels of AME, TEN, TeA, AME-3-S and AOH-3-G were found in clarified juices (range 0.9-28 μg/kg), while the same mycotoxins plus AOH were found in cloudy apple juices in higher concentrations (range 0.9-79.8 μg/kg). AME, TEN, TeA and AOH-3G were detected in marmalades
(range 3.9-144.3 μg/kg), and AOH, AME, TEN and TeA in apple infant food (range 1.7-225.7 μg/kg). Probabilistic exposure assessments and risk characterizations were carried out for children between 6 months and 5 years old in Argentina. The highest risk of exposure affected children between 6 and 23 months and it is mainly associated with the alternariols. Over 90% of these children are exceeding the threshold of toxicological concern for AME from the consumption of apple infant food. Even though the risk posed for TeA was low in the present analysis, it should not be underestimated, since this mycotoxin was found in higher concentrations and is broadly distributed in other food commodities. Better control strategies to prevent the incorporation of *Alternaria* infected apples into the process line and the establishment of legislation for *Alternaria* mycotoxins are needed in Argentina.

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**AFLATOXIN B1 EXPOSURE AND LIPID LEVELS OF PREGNANT WOMEN IN ABEOKUTA, NIGERIA**

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Aflatoxin B1 (AFB1), the most toxic mycotoxin, has been linked to adverse maternal and birth outcomes, when exposure occurs during pregnancy. Previous studies, in animals, indicated that AFB1 exposure could perturb the lipid levels. However, few data exist concerning the association between AFB1 and lipid levels in humans. The aim of this study was to investigate the association between AFB1 and lipid profiles of pregnant women in their first and third trimesters. Pregnant women (n=66) were recruited and followed up till third trimester. Plasma cholesterol and triglycerides were analysed spectrophotometrically, while AFB1-albumin adduct levels were assayed using an ELISA kit. The mean ± standard deviation values of total cholesterol and total triglycerides showed a significant increase (p<0.05) from 75.38±29.15 mg/dl and 73.77±23.70 mg/dl in the first trimester to 98.91±26.87 mg/dl and 126.24±29.281 mg/dl in the third trimester, respectively. HDL1 cholesterol levels decreased from 55.73±18.32 mg/dl in the first trimester to 52.79±16.12 mg/dl in the third trimester, while HDL2 cholesterol levels increased from 48.12±18.52 mg/dl in the first trimester to 51.24±14.99 mg/dl in the third trimester. HDL1 and HDL2 triglyceride both increased from 40.39±15.93 mg/dl and 28.99±7.67 mg/dl in the first trimester to 47.07±20.96 mg/dl and 30.91±10.16 mg/dl in the third trimester, respectively. AFB1-albumin adducts significantly (p<0.05) decreased from 0.85±0.40 ng/ml in the first trimester to 0.66±0.27 ng/ml in the third trimester. The association between AFB1-albumin adducts and total cholesterol was negatively weak (p=0.096) in the first trimester and positively weak (p=0.463) in the third trimester. Likewise, that of AFB1-albumin adducts and total triglyceride was negatively weak (p=0.438) in the first trimester and positively weak in the third (p=0.051). The results presented in this study showed weak associations between AFB1-albumin adducts and total cholesterol, and total triglyceride in the first and third trimesters of pregnancy. Further studies are required to investigate more on this association.

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**MYCOLON – A MECHANISTIC APPROACH TO MAP THE INTERPLAY OF MYCOTOXINS AND GUT EPITHELIUM TOWARDS COLORECTAL CANCER RISK**

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Mycotoxins are toxic fungal secondary metabolites that contaminate a wide spectrum of essential foods worldwide, estimated between 60 to 80% of the world’s food crops. Therefore, human exposure to one or more mycotoxins is widespread. Potential associations between (multiple) mycotoxin exposures and colorectal cancer (CRC) risk were investigated in the European Prospective Investigation into Cancer and Nutrition cohort (EPIC, n=476,160). Results indicated that exposure to deoxynivalenol (DON) and patulin (PAT) significantly increases the risk for CRC. Although in vivo evidence for causal relationships is still lacking, exposure to these mycotoxins is hypothesized to have a profound impact on the intestinal morphology by compromising the epithelial barrier and genomic integrity of intestinal epithelial cells, hereby promoting the initiation and/or development of CRC. In this research, two mice experiments were performed to investigate the effect of mycotoxins on gut homeostasis. DON and PAT were administered in drinking water at a single high and low concentration, or as a combination (10 mg/l and 1.75 mg/l for DON; 1 mg/l and 50 μg/l for PAT and 1.75 mg/l DON+50 μg/l PAT for the mix) for 3 months. Gut morphology, epithelial DNA damage, as well as intestinal and systemic inflammation were evaluated by means of H&E staining, γH2AX staining, MPO assays and full blood analysis ( Vetscan®), respectively.
In parallel, an extraction and ultrahigh performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method was developed and optimized for the detection of DON in murine whole blood. Preliminary results of the mice experiments indicated that both single as well as combined exposure of DON and PAT induces an elevation in monocytes and neutrophils, suggestive of systemic inflammation. Additionally, murine intestinal organoids were cultured and subjected to mycotoxin-induced cytotoxicity assessments using a CellTiter-GloTM 3D Cell Viability assay. Cytotoxicity was found at 5, 10 and 20 μM, both for DON and PAT. Further cytotoxicity testing will be performed at lower concentrations. Building on these results, the phenotypic and molecular effects of single as well as combined mycotoxin exposure on murine gut homeostasis, tumorigenesis and tumour organoids will be mapped. Results of this research will form a basis for future strategies in early cancer detection and prevention by identification of risks related to mycotoxin exposure.

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THE EFFECT OF NATURALLY CONTAMINATED DIETS ON PIG PERFORMANCE AND SELECTED BLOOD BIOCHEMICAL PARAMETERS – ASSESSMENT AFTER THE TRANSITION TO MYCOTOXIN-FREE FEED
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Mycotoxins are among the most common contaminants in feedstuffs, and their ingestion causes poisoning in livestock. Pigs are fed cereal-based diets, and therefore they are exposed and particularly susceptible to mycotoxins. Studies conducted to date have investigated exposure to a single mycotoxin for an extended period of time. However, mixed mycotoxicoses and long-term effects of mycotoxin exposure (evaluated after mycotoxin removal from feed) remain insufficiently researched. To fill this knowledge gap, an experiment was performed on 24 pigs divided into three equal groups: E1, fed a high dose of mycotoxins; E2, fed half of the mycotoxin dose administered in group E1; and C, control. Diets for groups E1 and E2 were balanced based on naturally contaminated ingredients containing deoxynivalenol (DON), zearalenone (ZEN) ochratoxin A (OTA), and citrinin (CIT). Blood biochemical parameters (glucose, triglycerides, alanine aminotransferase, aspartate aminotransferase, urea, creatine kinase, urea nitrogen, and cholesterol) and performance parameters (body weights, weight gains, and feed conversion ratio - FCR) were used as diagnostic indicators of intoxication. The analysed parameters were determined at seven-day intervals for four weeks. The experiment consisted of two stages: pigs in groups E1 and E2 received contaminated diets for the first two weeks, and they were fed a control diet for another two weeks. The control group was administered mycotoxin-free feed throughout the experiment. An analysis of biochemical and performance parameters, conducted when the animals were fed contaminated and control diets, revealed no differences between groups. The observed situation can be interpreted in two ways: (i) antagonistic interactions occurred between the components of the applied mycotoxin mixture; and (ii) the diagnostic indicators used for the macro evaluation were not sensitive enough to identify subtle changes at the level of cellular and humoral immune responses. The results of the study suggest that further research is needed to detect potential homeostatic imbalance in animals and determine the extent of disruptions.

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OCURRENCE OF ZEARALENONE AND ITS METABOLITES IN THE BLOOD OF HIGH-YIELDING DAIRY COWS AT SELECTED COLLECTION SITES IN VARIOUS DISEASE STATES
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Zearalenone (ZEN) and its metabolites, alpha-zearalenol (α-ZEL) and beta-zearalenol (β-ZEL), are ubiquitous in plant materials used as feed components in dairy cattle diets. The aim of this study was to confirm the occurrence of ZEN and its selected metabolites in blood samples collected from different sites in the hepatic portal system (posthepatic-external jugular vein EJV; prehepatic–abdominal subcutaneous vein ASV and median caudal vein MCV) of dairy cows diagnosed with mastitis, ovarian cysts and pyometra. The presence of mycotoxins in the blood plasma was determined with the use of combined separation methods involving immunoaffinity columns, a liquid chromatography system and
a mass spectrometry system. The parent compound was detected in all samples collected from diseased cows, whereas α-ZEL and β-ZEL were not identified in any samples, or their concentrations were below the limit of detection (LOD). Zearalenone levels were highest in cows with pyometra, where the percentage share of average ZEN concentrations reached 44%. Blood sampling sites were arranged in the following ascending order based on ZEN concentrations: EJV (10.53 pg/ml, 44.07% of the samples collected from this site), ASV (14.20 pg/ml, 49.59% of the samples) and MCV (26.67 pg/ml, 67.35% of the samples). The results of the study indicate that blood samples for toxicological analyses should be collected from the MCV (prehepatic vessel) of clinically healthy cows and/or cows with subclinical ZEN mycotoxicosis. This sampling site increases the probability of correct diagnosis of subclinical ZEN mycotoxicosis.

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EFFECT OF AN ALGOCLAY-BASED DECONTAMINANT ON THE PERFORMANCE, INTESTINAL INTEGRITY, AND LIVER OXIDATIVE STRESS IN BROILER CHICKENS FED A DIET NATURALLY CONTAMINATED WITH DEOXYNIVALENOL
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Dietary deoxynivalenol (DON) exposure severely impairs broiler chicken performance and intestinal integrity, even at levels far lower than the recommended 5,000 ppb in the final diet. Therefore, dietary interventions should efficiently counteract the intestinal and liver harm caused by DON. This study aimed to evaluate the effect of an algoclay-based decontaminant on the performance, intestinal integrity, and liver oxidative stress in broiler chickens fed a DON-contaminated diet. One-day-old male Ross broilers (n=600) were divided into 3 treatments with 10 replicate pens each, and a pen containing 20 birds was the experimental unit. The animals were fed a control diet with marginal levels of DON (189-249 ppb) or diets naturally contaminated with DON (2,690-2,910 ppb) either supplemented or not with an algoclay-based decontaminant (2 g/kg diet). No differences in production performance were observed during the starter (d0-14), grower (d14-28), or finisher (d28-37) feeding periods. Days 14 and 28 showed no villus morphometry changes. The jejunum villus height (VH) of broiler chickens fed the DON-contaminated diet decreased significantly on d37. After adding algoclay-based decontaminant to the DON diet, VH was equivalent to the control diet. During the grower period, broiler chicks fed the DON diet had a significant increase in villus tip injury, and this damage was not present in birds fed the DON diet with the algoclay-based decontaminant. On day 37, the mRNA expression of glutathione synthetase was significantly upregulated in the liver of broiler chickens fed the DON-contaminated diet. The expression of this marker was similar to that of control when the DON-contaminated diet was supplemented with the algoclay-based decontaminant. In conclusion, dietary contamination with DON at levels between 2,690 and 2,910 ppb did not impair production performance but had a negative impact on broiler chicken welfare. This was characterised by intestinal damage and oxidative stress in the liver. When the algoclay-based decontaminant was added to the diet, the harm caused by DON was no longer observed.

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INDIVIDUAL AND COMBINED EFFECTS OF DEOXYNIVALENOL (DON) WITH OTHER FUSARIUM MYCOTOXINS ON RAINBOW TROUT (ONCORHYNCHUS MYKISS) GROWTH PERFORMANCE AND HEALTH
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This study assessed whether the toxicological effects of deoxynivalenol (DON) produced by Fusarium graminearum in rainbow trout (Oncorhynchus mykiss) are altered by the co-exposure to a mixture of toxins produced by Fusarium verticillioides (FUmix). This FUmix contained fusaric acid and fumonisins B1, B2 and B3. Four diets were formulated according to a 2×2 factorial design: CON-CON; CON-FUmix; DON-CON; and DON-FUmix. Diets with and without DON contained on average 2,700 and 0 μg/kg feed, respectively. The sum of the analysed FUmix toxins was 12,700 and 100 μg/kg feed in the diets with and without FUmix, respectively. The experiment consisted of a 6-week restrictive feeding period immediately followed by a 2-week ad libitum feeding period. Growth performance measurements were taken per feeding period. Histopathological measurements in the liver and gastrointestinal tract (pyloric caeca, midgut and hindgut) were assessed at the end of week 1 and week 6 of the restrictive feeding period and at week 8, the last day of the ad libitum feeding period. During both restrictive and ad libitum feeding, the effects of FUmix and DON on growth performance were additive (no interaction effect; p>0.05). During the restrictive feeding period, exposure to DON (p≤0.001) and FUmix (p≤0.01)
inhibited growth and increased feed conversion ratio (FCR). During this period, DON exposure decreased the protein (ps0.001) and energy retention (ps0.05) in the trout. During the *ad libitum* feeding period, FUMix affected HSI (ps0.01), while DON exposure reduced feed intake (ps0.001) and growth (ps0.001) and increased FCR (ps0.01). In general, for both liver and intestinal tissue measurements, no interaction effects between DON and FUMix were observed. In the liver, histopathological analysis revealed mild alterations, increased necrosis score by DON (ps0.01), increased glycogen vacuolization by FUMix (ps0.05) and decreased percentage of pleomorphic nuclei by FUMix (ps0.01). Overall, the co-exposure to FUMix and DON gave rise to additive effects but showed no synergistic or antagonistic effects for the combination of DON with other *Fusarium* mycotoxins.

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**EFFECTS OF FEEDING MAIZE NATURALLY CONTAMINATED WITH AFLATOXIN B1, DEOXYNIVALENOL, AND ZEARALENONE ON REPRODUCTIVE PERFORMANCE OF BROILER BREEDERS AND GROWTH PERFORMANCE OF THEIR PROGENY CHICKS**

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To evaluate the toxic effects of mycotoxin-contaminated maize (MC) on the breeders and their progeny chicks, a total of 480 50-weeks-old Cobb broiler breeder hens were fed the following diets: (i) a maize-soybean meal diet (control; containing 70.35% maize); (ii) MC substituting for 50% of maize in control (LM); (iii) LM diet plus 2 kg/mc one mycotoxin sequestrant (TNP) (LMT2.0); (iv) MC substituting for 100% of maize in control (HM); (v) HM diet plus 2 g/kg TNP (HMT2.0); and (vi) MC substituting for 100% of maize in control (HM). The MC contained 69.25 μg aflatoxin B1 (AFB1)/kg, 4.875 μg deoxynivalenol (DON)/kg, and 2,262 μg zearalenone (ZEN)/kg. At week 4 after MC inclusion, all eggs laid were used for hatch, and all progeny chicks were fed the same mycotoxin-untreated diet for 14 days. Dietary MC inclusion decreased the hatchability of set eggs and increased embryo mortality during day 18-21.5. The TNP addition increased these aforementioned indices in MC-included diets. Maternal HM treatment decreased the BW of progeny chicks at age of 14 days and BWG of progeny chicks during days 1-14, whereas maternal LM treatment did not affect these indices. In parallel, maternal HM treatment decreased the concentrations of serum IgA, IgG, and lysozyme in the progeny chicks on day 14, but maternal LM treatment did not affect these indices. Overall, maternal dietary TNP treatments increased the growth of progeny chicks and had a trend to increase the concentrations of serum IgA and IgG on day 14 compared to maternal MC treatments. It was concluded that the feeding of relative high ratio of maize contaminated with AFB1, DON, and ZEN negatively affected the reproductive performance of breeders and the growth performance of their progeny chicks, and TNP addition alleviated these toxic effects.

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**CHRONIC EXPOSURE TO MULTIPLE MYCOTOXINS IN LAYING HENS ALTERS BIOMARKERS AND AFFECTS EGG QUALITY**

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Mycotoxins are the secondary chemical metabolite produced by fungi and are globally distributed contaminants in cereal grains. The adverse effects of these compounds on animal and human health have been reported. The prevention of mycotoxicosis with mycotoxin adsorbents has also been demonstrated in previous studies. The objectives of the current study were to evaluate the effects of two chronic mycotoxin challenges on the health and performance of laying hens and the efficacy of an absorbent, containing bentonite, yeast cell wall components and a mixture of phytogenics, on the mitigation of those effects. A total of 270 Lohmann, 28-week-old brown laying hens with 3 birds per replicate and 15 replicates were randomly assigned to each of 6 diets, including: (i) control; (ii) control + mycotoxin mitigation product (Solis Max); (iii) mycotoxin challenge 1 (100 ppb aflatoxin B1 + 9,000 ppb fumonisin B1; (iv) mycotoxin challenge 2 (1,400 ppb deoxynivalenol + 300 ppb T-2 toxin); (v) mycotoxin challenge 1 + 0.2% Solis Max; and (vi) mycotoxin challenge 2 + 0.2% Solis Max for a 12-week period at Kasetsart University, Thailand, from June to August of 2022. Experimental parameters analysed were performance, egg quality, haematology, and plasma biochemistry. Feeding diets naturally contaminated with both mycotoxin challenges tended to decrease feed-intake and increase FCR. The mycotoxin contamination in both the challenges significantly reduced egg weight throughout the study. A significant degradation of egg quality (Haugh units, albumin height and eggshell breaking strength) as well as an alteration of some plasma biochemistry parameters were observed in the hens.
from the group with challenge 2, compared to the control. However, challenge 1 had a major effect on blood phosphorous content and in liver-health related parameters, as expected in challenges including aflatoxins. It could be concluded that chronic exposure to *Fusarium* mycotoxins can adversely affect egg quality in laying hens. The in-feed product was effective in mitigating these adverse effects.

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**AGE-ASSOCIATED CHANGES IN AFLATOxin B1 DEPOSITION IN THE LIVERS OF GROWING PIGS AND BROILER CHICKENS**

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We investigated the uptake and deposition of aflatoxin B1 (AFB1) in liver over time in growing pigs and broiler chickens and impact on zootechnical performances. Poultry trial: Experimental treatments comprised a wheat and soy-based control diet (F0p) and a challenge diet (F1p) containing AFB1 at 20 μg/kg of feed. Starter and grower formulations were fed prior and post-21 days. Forty-eight chickens were divided into two groups, each with eight pens, and received either the F0p or the F1p diet. Birds were euthanised on days 4, 7, 14, 28, 35 and 42, and their livers were collected. Swine trial: Experimental treatments included a control (F0s) and challenge diet (F1s) containing AFB1 at 20 μg/kg of feed. Thirty-two pigs, averaging 19.3kg weight, housed in pairs in 16 pens, were fed F0s during an adaptation week and then fed the F1s diet from day 7 to 35. Pigs were sacrificed at 0, 3, 7, 14, 21 and 28 days intervals under F1s treatment, and their livers were collected. Entire livers were homogenised individually, and 10 g subsamples were extracted with and without enzymatic glucuronide deconjugation, and further purified via immunoaffinity chromatography before analysis for AFB1 and aflatoxin M1 (AFM1) using HPLC-FLD. Poultry results: AFB1 (at 20 μg/kg) significantly reduced body weight gain in young broiler chickens. No change was observed in feed consumption, and accordingly, the feed conversion ratio (FCR) was impaired. The AFB1-challenged birds recovered the performance values of the control birds over time, with no significant differences detected at day 42. The liver concentration of AFB1 increased during the starter diet period and reached a maximum of 110 ng/kg at two weeks of age. Before starter-to-grower feed change at three weeks, the concentration of AFB1 dropped to ~75 ng/kg. From day 28 onwards, AFB1 concentration in the liver tended to decrease continuously, reaching 40 ng/kg on day 42. All AFB1 were unconjugated, and no AFM1 was detected. Swine results: No significant zootechnical differences were observed in pigs. The maximal concentration of AFB1 was detected in the livers of pigs sacrificed after 3 under AFB1-containing diet. Approximately 60% of the toxin was metabolised in AFM1. Inter-animal variability decreased after two weeks. The concentration of aflatoxins was over 80% of that detected after 3 days under AFB1 treatment, before decreasing to less than 50% at day 21 and 25% at the end of the study. We concluded that mature animals could metabolise AFB1 more effectively, and that two weeks is an optimal time point for observing a significant accumulation of AFB1 in the livers of chickens and pigs.

**MITIGATING THE NEGATIVE IMPACT OF MYCOTOXINS**

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**EFFECTIVE DRY SORTING OF PISTACHIO ON REDUCTION OF AFLATOxin CONTAMINATION LEVELS**

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Aflatoxins are one of the most important mycotoxins as natural pollutants of agricultural products, which, in addition to the risks they have on the health of human, could bring enormous economic costs to producers and exporters. Tree nuts in general and pistachio particularly are highly susceptible to aflatoxin contamination which is a serious challenge for major producing countries. Physical sorting is the usual method to remove mycotoxin-contaminated crops, and this method has been used for many years for products contaminated with mycotoxins. Normally, in good sorting facilities, they take one small to medium size samples per entry goods and, if the level of contamination is not high based on their own experience, they accept these to be sorted. In our facility, as subject of research, after taking the samples based on the weight of received goods, the samples were subjected to hand sorting and subsequently divided into two parts, i.e., ‘sorted pistachios’ and ‘rejected pistachios’. Then, both parts
were made separately into slurries and subsequently analysed for aflatoxin contamination using an HPLC method after immunoaffinity clean-up. The two parts ‘sorted pistachios’ and ‘rejected pistachios’ could be assigned to 3 different groups on the basis of the level of aflatoxin contamination. Group 1, the majority, the ‘sorted pistachios’ were conform the level of contamination of Regulation (EC) No 1881/2006 of 8 μg/kg for aflatoxin B1 and 10 μg/kg for total aflatoxins intended for direct human consumption; the ‘rejected pistachios’ had moderate to high levels of contamination. Group 1 was considered as sortable consignment and subsequently purchased from the supplier. Group 2, both ‘sorted pistachios’ and ‘rejected pistachios’ were conform the level of contamination of Regulation (EC) No 1881/2006, such consignment was also purchased from supplier with the view that further sorting would only have effect on its appearance and no effect on reduction of contamination. In rare occasions, group 3, it had been noticed that regrettably both ‘sorted pistachios’ and ‘rejected pistachios’ were not conform Regulation (EC) No 1881/2006; such consignment was labelled as ‘unsortable’ and returned to the supplier. In conclusion, if contamination occurs at different levels of production from farm to wet sorting, it correlates with some physical abnormalities that can be diagnosed by naked eyes or sorting machines, such as early splits; such consignment is sortable. However, if contamination happens after wet sorting, no obvious physical abnormalities are associated and, therefore, they are grouped as ‘unsortable’.

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YEAST CELL WALL IMPROVES IMMUNE MODULATION RESPONSE IN AFLATOXIN CHALLENGE BROILERS
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The objective of this study was to evaluate the effect of a yeast cell wall (source of β-glucans and MOS) to modulate the immune responses in aflatoxin-challenged broilers. It was used 152 one-day-old chicks randomly distributed in 4 treatments: (i) NC (negative control, basal diet without contamination); (ii) AFB (positive control, basal diet with 2.5 ppm of aflatoxin in diet administration); (iii) YCW (basal diet with 0.5 kg/MT from Saccharomyces cerevisiae cell wall, ImmunoWall®); and (iv) YCW+AFB (yeast cell wall diet with 2.5 ppm contamination of aflatoxin). The experimental period was 35 days with the diets and on the 14th day the Newcastle vaccine was administered. On days 1 and 14, blood samples were collected with anticoagulants for ELISA antibodies against Newcastle Disease (NDV) to determine maternal titer (10 birds/treat). ELISA for antibodies against NDV was assessed in blood samples at days 28 and 35 (8 birds/treat). The blood collection samples with anticoagulant for determination of cell profile by flow cytometry, the phagocytic capacity of peripheral blood lymphocytes, and the assessment of intestinal permeability (using dextran-FITC, 3-5 kD) were performed at days 7, 28, and 35 (8 birds/treat). The data from each analysis were submitted to the D’Agostino-Pearson omnibus K2 normality test. However, the data that failed this test were submitted to non-parametric analyses, the Kruskal-Wallis test (GraphPad Software). The challenge with aflatoxin was verified and resulted in 2.24±0.28 ppm in the challenged groups. The aflatoxin challenge promoted the depletion of cytotoxic T lymphocytes on day 14. YCW+AFB promoted an increase (p<0.05) in diverse populations of immune cells, including total lymphocytes, T and B lymphocytes, in addition to phagocytic macrophages and heterophils (at day 28). Even without an aflatoxin challenge, animals treated with YCW showed higher production of NDV antibodies (p<0.05) at day 35. No differences between the treatments were found in intestinal permeability. The YCW supplementation in broilers challenged with 2.5 ppm of aflatoxin showed an immune modulation effect; in non-challenged birds, it was observed an impact in higher production of antibodies, probably through the trained immunity mechanism (cytotoxic T lymphocytes).

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AFLATOXIN B1 DEGRADATION BY ERY4 LACCASE: ASSESSMENT OF DEGRADATION PRODUCTS AND EFFICACY IN MAIZE
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Aflatoxins (AFs) are toxic secondary metabolites produced by Aspergillus spp. found as food and feed contaminants worldwide. AFs cause liver cancer, inflammation and necrosis, immune depression, stunting, growth and development impairment, reproductive dysfunction, and even death when consumed at high dosages. Due to their toxic potential and occurrence, they represent a critical issue for the global health and economy. Maize is amongst the most susceptible commodity to be contaminated by AFs and represent the staple food of human and animal diet. Therefore, to ensure food
and feed safety, it is mandatory to develop green technologies for AFs reduction in contaminated matrices. With this regard, enzymatic degradation is an effective and environmentally friendly approach, which reduces mycotoxin contamination under mild operational conditions and with minor impact on the food and feed matrix. Therefore, in this work Ery4 laccase and different redox mediators were investigated in vitro and then applied in artificially contaminated maize for AFB1 reduction. The degradation products were also investigated in vitro by UHPLC-HRMS, to assess the detoxification potential of this degradation method. AFB1 (0.1μg/ml) was completely removed in vitro after 30 min of incubation and reduced by 26% in artificially contaminated maize (50 μg/kg) after 3 h. Several degradation products were detected in vitro after laccase treatment, and likely corresponded to AFQ1, epi-AFQ1, AFB1-diol or AFB1 dialdehyde, AFB2a, and AFM1. All detected degradation products showed a higher polarity and a higher excretion rate via urine and faeces, and likely a lower toxicity than AFB1. Although further studies are needed to improve AFB1 reduction and further characterize the degradation products also in maize, the results of this study are promising and suggest that Ery4 laccase can be effectively applied for the reduction of AFB1 in maize.

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DETOXIFICATION OF PATULIN BY AN EPIPHYTIC YEAST OF APPLE FRUIT
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Patulin is a mycotoxin with mutagenic, carcinogenic, immunotoxic, neurotoxic, genotoxic, and teratogenic effects, commonly produced by Penicillium expansum, one of the most important postharvest pathogens of fruits. Although patulin contamination should be avoided/reduced with the control of its producer, often synthetic fungicides do not affect fungal secondary metabolism. Furthermore, the reduction/lack of fungicides to be used after harvest and the consumers’ need for pesticide-free agri-foods require sustainable alternatives, such as biocontrol agents, which might both control the disease and prevent/degrade the mycotoxin if contamination occurs. In this study, the patulin degradation potential of the yeast strain T1 of Meyerozyma caribbica was tested. This strain was isolated from the surface of the apple fruit and it proved ability to control P. expansum both in vitro and in vivo on apple fruits. The ability to reduce patulin contamination was determined by HPLC from a liquid culture of the biocontrol strain grown in presence of a pure standard concentration of patulin. Patulin concentration proved to be reduced for up to 74%. Although further research is necessary, the selected yeast strain with its ability to suppress the pathogen growth and degrade patulin is a promising potential biocontrol agent for safe apple fruit production. Acknowledgements. This study was funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3 – Call for tender No. 341 of 15 March 2022 of Italian Ministry of University and Research funded by the European Union – NextGenerationEU; Project code PE00000003, Concession Decree No. 1550 of 11 October 2022 adopted by the Italian Ministry of University and Research, CUP D93C22000890001, Project title ‘ONFOODS – Research and innovation network on food and nutrition Sustainability, Safety and Security – Working ON Foods’.

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DETERMINATION OF THE RATE LIMITING STEP DURING ZEARALENONE HYDROLYSIS BY ZENA
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Zearalenone (ZEN) is a mycotoxin produced by various phytopathogenic members of the genus Fusarium, particularly by F. graminearum, which infects major crop plants worldwide. Biological decontamination is a favourable method to reduce ZEN concentration in contaminated feed. Previously we identified, cloned, and produced a ZEN hydrolysing alpha/beta hydrolase from a gram-positive soil bacterium, taxonomically assigned to the species Rhodococcus erythropolis. This enzyme was named ZenARE. ZenARE hydrolyses zearalenone to hydrolysed zearalenone (HZEN) which then decarboxylates further to decarboxylated hydrolysed zearalenone (DHZEN). Due to low heat stability and susceptibility to low pH it was considered unfit for industrial application as feed additive active in the animal gastrointestinal tract. The homologous enzyme from Streptomyces coelicoflavus (ZenAScfl) showed higher thermostability, but with lower substrate affinity and turnover rate. It was used as starting point for enzyme engineering, resulting in a variant with higher substrate affinity and increased catalytic
efficiency (ZenAScfl041). All three enzymes were subjected to pre-steady state enzyme kinetics to elucidate their reaction mechanism and identify the rate limiting step as a basis for further targeted engineering. It was shown that substrate binding and affinity of all variants is significantly driven by conformational change (enzyme closure). ZenARc revealed the highest affinity to the substrate. This is partially related to increased binding affinity but also to the fast kinetics of the consecutive enzyme isomerization (closure) which brings the complex to the reactive mode. A six-step kinetics mechanism, including the enzyme isomerization steps, was proposed for all three variants. The rate-limiting step differs between ZenAScfl variants and ZenARc. Both, ZenAScfl and ZenAScfl041, are limited by the first chemical step forming the intermediate form. ZenARc is limited by the second chemical step (hydrolysis of the intermediate) at a temperature above 298 K. The rate-limiting step is shifted to the enzyme opening at the temperature below this temperature threshold. The results showed that different ZenA variants may need different approaches for improvement of kinetics by enzyme engineering.

P52 EFFECTS OF SUPPLEMENTATION OF A MYCOTOXIN MITIGATION FEED ADDITIVE IN LACTATING DAIRY COWS FED FUSARIUM MYCOTOXIN-CONTAMINATED DIET FOR AN EXTENDED PERIOD

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The Fusarium mycotoxins are inactivated by rumen flora. However, certain amount can pass the rumen-reticulum compartment unchanged or converted into metabolites with biological activity. Limited scientific evidence is available on the impact and mitigation of Fusarium mycotoxins on dairy cow’s performance and health, in particular when cows are exposed for a long period, being more than 2 months. The information available related to the effects of these mycotoxins on milk cheese-making parameters is also very poor. The objective of this study was to evaluate a commercially available mycotoxin mitigation product (MMP, i.e., TOXO® HP-R, Selko, Tilburg, the Netherlands) in lactating dairy cows fed a Fusarium mycotoxin contaminated diet and the repercussions on dry matter intake, milk yield, milk quality, cheese-making traits and health status of cows. The MMP contains smectite clays, yeast cell walls and antioxidants. In the study, 36 lactating Holstein cows were grouped based on days in milk, milk yield, body condition score and randomly assigned to specific treatments. The study ran over 2 periods (March/May-May/July 2022). In each period, six animals/treatment were considered. Experimental periods consisted of 9 days of adaptation and 54 days of intoxication. Physical activity, rumination time, daily milk production and milk quality were measured. Cows were fed once daily with the same TMR composition. Experimental groups consisted of CTR diet, TMR with low contaminated high moisture corn (HMC), C-B mix and beet pulp; MTX diet, TMR with high contaminated HMC, C-B mix, and beet pulp; MMP HP-R diet, MTX diet supplemented with 100 g/cow/day of MMP. The use of MMP reduced mycotoxin negative effects on milk yield and quality (protein, casein, lactose, and clotting parameters). MTX diet had a lower milk yield and feed efficiency than the CTR and MMP HP-R diets. These results provide a better understanding of mycotoxin risk on dairy cows’ performances and milk quality. Further analyses should be carried out to evaluate MMP outcome on immune-metabolic responses and diet digestibility.

P53 EVALUATION OF THE EFFICACY OF CINNAMON OIL ON ASPERGILLUS FLAVUS AND FUSARIUM PROLIFERATUM GROWTH AND MYCOTOXIN PRODUCTION: TOWARDS A MITIGATION STRATEGY

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Paddy and polished rice are usually stored in a warehouse from three to twelve months under poor technical conditions and/or in inappropriate facilities, that is a favourable environment for fungi to infect and grow, especially in high moisture and temperature conditions. Of these, Aspergillus spp. and Fusarium spp. were frequently determined in paddy rice in Mekong Delta, Vietnam, spoiling agricultural products and producing mycotoxins. Cinnamon has been approved by the Food and Drug Administration in the GRAS-category as a food additive. It has been widely used in food to inhibit the growth of such
as *F. verticillioides*, *A. ochraceus*, *P. expansum* and *A. flavus*. Hence, in this study, the effects of cinnamon oil (CO) concentration (10, 30, 50 and 70%) on the growth rate (mm/day) and aflatoxin B1 (AFB1) and fumonisin B1 (FB1) production of *A. flavus* (AF01) and *Fusarium proliferatum* (FP01) isolates respectively at optimum water activities (0.95 and 0.99 aw) and temperatures (25, 30 and 35°C) on paddy and white rice grains were determined. The results showed that the growth rate and AFB1 and FB1 production of all the fungal isolates decreased with an increase in CO concentrations on both matrices. AF01 failed to grow at 30% of CO (except for 0.99 aw/30°C) while no growth was also found at 30% of CO all conditions in regard to FP01 on paddy (except for 0.99 aw) and both fungi did not grow at 50% of CO. However, for white rice, both fungi were completely inhibited at higher CO concentrations (50 or 70%) depending on fungi, aw and temperature determined. Regarding mycotoxin production, 30 and 50% of CO could be suggested as a minimum inhibitory concentration for inhibition of AFB1 production on paddy and white rice respectively, whereas such contents were lower such as 10% and 30% of CO, respectively for FB1 production. Production of mycotoxins was significantly influenced by cinnamon oil compared to growth of both fungi. Prospective application of cinnamon oil as a mycotoxin mitigation strategy in agricultural commodities could be recommended and merits further research.

**P54**
**DETAILED ENZYME KINETICS OF FUMONISIN ESTERASES**

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Fumonisins are one of the most prevalent mycotoxins produced by certain *Fusarium* species, which contaminate crops globally, predominantly maize. The family's most important and prominent substance is fumonisin B1 (FB1). FB1 inhibits ceramide synthase, an important enzyme of sphingolipid biosynthesis in mammals. The altered sphingolipid metabolism can lead to different serious adverse health effects in humans and animals, therefore crops contaminated with high levels of FB1 should be removed from the food or feed chain. Physical or chemical decontamination of crops is often not effective enough; however, enzymatic detoxification can be a solution to the problem. Fumonisin esterases cleave the two tricarballylic acid groups of FB1, leading to partially and fully hydrolysed FB1 (pHFB1 and HFB1, respectively). These metabolites don’t inhibit ceramide synthase, therefore possess significantly lower toxicity. Fumonisin esterases are excellent candidates for food or feed additives against FB1 contamination, however, the kinetics and mechanism of these enzymes are not well characterized yet. The study aims to understand the detailed enzymatic mechanism of two of these biocatalysts, to fine-tune already existing product. For that reason, the enzymes were expressed in *Pichia pastoris* expression system, and kinetic constants were evaluated, with both FB1 and pHFB1 as substrate. It was found that fumonisin esterases selectively produce one of the two possible pHFB1, and the two enzymes significantly differ in their affinity towards pHFB1. A reaction mechanism was proposed based on our results, although further structure analyses are necessary. A selective preparation and isolation method of pHFB1_7 was also developed for further toxicologic studies. Furthermore, under various circumstances, the kinetics of the non-enzymatic acyl migration reaction between the two pHFB1s were examined.

**P55**
**PHYTOGENIC SUPPLEMENTATION WITH INEDIA® AND DAIRY CATTLE PERFORMANCES IN A CONTEXT OF THE PRESENCE OF NATURAL INFESTATION WITH MYCOTOXINS IN SILAGE**


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Mycotoxins can increase the incidence of disease and reduce production efficiency in cattle. Symptoms are often nonspecific and the result of a progression of effects, making a diagnosis difficult or impossible because of the complex clinical results with a wide diversity of symptoms. Further research is needed to identify conditions that facilitate the growth of mycotoxin-producing fungi and also to try to recognize possible synergistic effects of different mycotoxins in dairy cattle organism. This trial conducted in two conventional facility for 20 weeks in the first facility (TC. TER-T.T.1) and 10 weeks in the second facility (TC. TER-T.T.2) with a mycotoxins polycontaminated feed. The aim of this study is to evaluate the efficiency of a phytogenic supplementation on zootechnical performances in comparison to the situation before treatment. 100 dairy cattle were treated with a phytogenic supplementation in the first facility (T.T.1) with 40 primiparous and 60 multiparous (lactation stage 6, dairy milk production 32.81 l/dairy cattle (dc)) where the analysis of the feed highlighted the presence of the mycotoxins naturally produced by a rate of 1,559 ppb of deoxynivalenol, 1,515 ppb of zearalenone and 119 ppb fumonisins. The results
show that the milk production increased by 1.5 l/dc/day compared to the period before introduction of the phytogenic supplementation. In the same way, interesting results were obtained in the second facility (T.T.2) with 88 dairy cattle, 32 primiparous (lactation stage 7.4) and 56 multiparous (lactation stage 5.9), and dairy milk production 26 l/dc was supplemented for 10 weeks with the same phytogenic mix. The analysis of the feed shows the presence of mycotoxins naturally produced at a level of contamination of 2,672 ppb of deoxynivalenol, 931 ppb zearalenone and 173 ppb fumonisins. The results show a spectacular increase in milk production, 30.1 l/dc/day compared to the period before supplementation product, 22.6 l/dc/day. The phytogenic additive, incorporated at 10 g/dc/day in top feeding in a mycotoxins polycontaminated naturally feed, allowed the animals to have better zootechnical performances than a reference period (without supplementation), even though they consumed mycotoxins.

P56
EFFECTS OF A PHYTOGENIC ADDITIVE IN MYCOTOXINS MANAGEMENT IN COMPARISON WITH A KNOWN BINDER: AN APPROACH BY INCREASING THE TOLERANCE THRESHOLD OF FEED CONTAMINANTS
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Mycotoxins have an impact on global performances in any animal production. This trial was conducted in an experimental facility for 21 days with a polycontaminated feed aims to evaluate the efficiency of a phytogenic additive (clay-free) on zootechnical performances in comparison to a reference clay-based binder (including enzymes and phytotherapeutics) available on the market. 240 one-day-old chicks with an average weight of 45.7 g are randomly divided into four groups. Each group comprises six replicates of 10 animals. The first group is the control group of which the feed is not contaminated. For the 3 other groups, the feed is contaminated by way of experiment with three mycotoxins produced naturally by fungi at a rate of 1 ppm of aflatoxins, 50 ppm of fumonisins and 40 ppm of deoxynivalenol. Among these 3 groups: (i) one is the positive control which only received the contaminated feed; (ii) one is the ‘binder’ group and received the reference binder incorporated at 0.1% in the feed; and (iii) the last is the trial group which received a phytogenic additive (a mix extracts including Foeniculum vulgare, Rosmarinus officinalis, and others) incorporated at 0.025% in the feed. At day 21, zootechnical performances such as weight, feed conversion ratio (FCR) is calculated. On 5 birds per treatment, villi height/crypt depth ratio and relative weight of liver are measured. The differences in the averages were compared by PHDQVRID%RQIHUURQLWHVWS". The results show that the trial group has a different weight (764 g) than the negative control group (747.7 g) and a higher weight than the binder group (714.2 g). The FCR of the trial and negative control groups are equal (1.57). The villi height/crypt depth ratio of the trial group is higher (10.03) than the other groups (negative control 8.34, positive control 8.64 and ‘binder’ group 7.81). No difference between negative control, the binder group and the trial group were found regarding the relative weight of liver. The phytogenic additive, incorporated at 0.025% in a mycotoxins polycontaminated feed, allowed birds to have similar and in some case better zootechnical performances than a reference binder.

P57
EFFECTS OF PHYTOGENIC SUPPLEMENTATION ON PIGLETS’ PERFORMANCE IN THE PRESENCE OF FEED CONTAMINATED WITH VERY HIGH LEVELS MYCOTOXINS
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The presence of food contamination by mycotoxins remains a topical issue whose impacts on performance are often difficult to quantify. This is the case for pork, which is a particularly sensitive species to mycotoxins contamination. The purpose of this trial was to evaluate effects of a dietary supplementation of phytotherapeutic extracts on the performance of piglets in the presence of feed contaminated with a mycotoxins blend and compare them to a clay-based binder and a control lot. 24 piglets, randomly divided in 3 lots of 8 piglets were followed during 28 days. The control lot (T) has a feed with mycotoxin contaminations and without supplementation. The feed level contamination was 0.5 ppm aflatoxins+25 ppm fumonisins+4 ppm deoxynivalenol during the whole trial. Both other groups were fed with the same co-contaminated feed supplemented: a clay-based binder has been added at the recommended dose of 0.1% to lot (C) and the phytogenic product has been added at 0.025% to lot (E). Results showed that the mycotoxin cocktail without supplementation has a negative effect on piglets’ performance with a statistical difference in DFI (daily feed intake) (kg/piglet/day) after 28 days contamination (0.667) in comparison with piglets with phytogenic supplement product (E) (0.733)
Animal production can be significantly impacted by mycotoxin contamination. These mycotoxins can lead to various pathologies, including fatal outcomes. Among these mycotoxins, zearalenone (ZEA) stands out due to its estrogenic toxicity. Its effects can encompass reduced fertility in both males and females, decreased zootechnical parameters, and even cancer. To address this issue, numerous strategies are being developed, with biodegradation standing out due to its cost-effectiveness and high efficiency. The aim of this study was to assess the degradation potential of ZEA by four bioprotective bacteria during simulated swine and poultry digestion. The selected bacteria were Bacillus amyloliquefaciens plantarum CICC 23985, Bacillus subtilis CECT 499, Bacillus velezensis CL197, and Streptomyces griseus CECT 3276. Initially, these bacteria were encapsulated via spray-drying in a 20% maltodextrin solution, to enhance their viability throughout gastrointestinal transit. Subsequently, the encapsulated material was suspended in sterile water (50 g/L), contaminated with 2 μg/ml of ZEA. This mixture was then subjected to simulated swine digestion lasting 6 h, covering both stomach and intestinal phases, as well as simulated chicken digestion lasting 4 h and 20 min, covering crop, proventriculus, gizzard, and small and large intestine phases. ZEA concentrations and metabolites produced were monitored throughout the process using UHPLC-qTOF/MS. In the simulated swine digestion, the most effective bacteria was B. subtilis CECT 499, which achieved complete degradation of ZEA during the stomach phase (2 h of digestion). B. amyloliquefaciens plantarum CICC 23985, B. velezensis CL197, and S. griseus CECT 3276 exhibited degradation rates of 80.14±5.71%, 60.41±7.23% and 76.18±2.28%, respectively, by the end of the stomach phase. At the conclusion of the intestinal phase (6 h of digestion), only the treatment with B. velezensis CL197 retained detectable ZEA, with a degradation rate of 96.74±2.13%. In the simulated chicken digestion, the most favourable outcomes were observed in treatments with B. subtilis CECT 499 and B. velezensis CL197. These treatments achieved complete ZEA degradation by the end of the gizzard phase (2 h of digestion). In gizzard phase conclusion, B. amyloliquefaciens plantarum CICC 23985 and S. griseus CECT 3276 had achieved degradation levels of 97.12±4.36% and 92.44±6.88%, respectively. At the end of the large intestine phase (4h 20 min of digestion), ZEA was undetectable in any of the treatments. In the analysis of produced metabolites, no compounds of greater toxicity, such as a-zearalenol, were detected. The study concluded that the bacteria effectively degraded ZEA, yielding satisfactory results even in treatments with residual toxin concentrations.
quantified. In 2021 and 2022, 14 silage maize fields in Arizona were selected, and soil was sampled both prior to application of a biocontrol product and following harvest. Biocontrol products (AF36 Prevail or Afla-Guard) were applied prior to tasselling of the maize crop at the labelled rate. At harvest, multiple subsamples of chopped maize silage and soil were collected from each field. Samples were dried, homogenized, and plated on agar media for isolation of *A. flavus*. Frequencies of applied biocontrol strains from the soil and crop were determined using DNA-based methods. Crop aflatoxin concentrations were measured using a commercial kit that has been validated for maize silage. Approximately monthly following harvest, representative sub-samples from multiple locations and depths of silage piles were sampled, and composite samples were analysed using the methods described above. At harvest, biocontrol strains made up 98% (AF36) and 25% (Afla-Guard) of the *A. flavus* population in soil and 88% (AF36) and 57% (Afla-Guard) in the crop. Whereas a single isolate of Afla-Guard was recovered from untreated fields, AF36 made up an average of 32% and 16% of *A. flavus* in soil and on the crop, respectively, in fields that did not receive an AF36 application. Relatively high frequencies of AF36 in non-treated fields may be due to widespread application of AF36 in Arizona in multiple crops over several decades. Few propagules of *A. flavus* were recovered from samples post-ensiling, and all isolates were biocontrol strain genotypes. Aflatoxin concentrations in the pre-and post-ensiled crop from all treated fields were below 10 ppb, demonstrating the effectiveness of biocontrol products for mitigating aflatoxin contamination in maize silage.

**P60**

**USE OF FUNGAL LACCASES FOR AFLATOXIN REDUCTION**

**IN MAIZE SUB-PRODUCTS**

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Fungal contamination is a phytosanitary problem of concern in maize and by-products. *Aspergillus* section *Flavi* strains can generate important problems due to contamination with aflatoxins (AFs). During the bioethanol production from maize, AFs levels can increase up to three times in the final co-product (DDGS or WDGS), intended for animal feed. Moso is the milled maize grains with the addition of water and amylase enzymes that enter the fermentation stage. One strategy to reduce the mycotoxin contamination levels is the use of microorganisms or enzymes able to metabolize, destroy or inactivate those compounds. Laccases are enzymes with biocatalytic capacity and wide biotechnological application, including food industry. Particularly, laccases from fungi that cause white rot have been proposed for mycotoxin biotransformation. In the present work, the effectiveness of different fungal laccases in reducing AFs levels (AFB1, AFG1, AFB2 and AFG2) was evaluated under an *in vitro* assay.

Laccase production by Phylum Basidiomycota strains was evaluated and 9 of them were selected to carry out *in vitro* decontamination tests. A concentration of 1 μg/ml of AFB1 and AFG1, and 0.25 μg/ml of AFB2 and AFG2 were used at concentrations of 5, 10, 15 and 20 U/ml of laccases contained in enzyme extracts, in addition to vanillic acid as redox mediator (1 and 10 mM). The strain *Trametes* sp. B7-IMICO-RC was able to degrade the 4 AFs at high levels under all the evaluated conditions. The highest degradation percentage was reached in presence of 1 mM vanillic acid and 20 U/mL laccase (88, 99, 87 and 70%, for AFB1, AFG1, AFB2 and AFG2, respectively). An additional *in vitro* degradation assay of AFs was carried out using moso as the substrate. The degradation percentages observed under these same conditions were 26% for AFB1 and AFG2, and 54% for AFG1. Currently, studies are being carried out in order to determine the degradation products toxicity. These results would contribute to the development of an eco-friendly strategy to reduce mycotoxin contamination, ensuring food safety.

**P61**

**BENTONITE MITIGATES THE NEGATIVE EFFECTS OF DIETARY AFLATOXIN B1 ON GROWTH PERFORMANCE, GUT MORPHOLOGY, AND IMMUNITY OF BROILERS**

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The use of clay minerals has been widely recognized as one of the most effective methods to mitigate the negative effects of dietary aflatoxins in broilers, due to their reported capability of binding aflatoxins. Commercial products, however, may contain different clay minerals (e.g., bentonite, sepiolite, and zeolite), leading to different binding capacities and affinity for aflatoxins. Therefore, it is crucial to validate commercial products via the same broiler model. This aflatoxin B1 (AFB1) challenged study was
designed to evaluate the effects of two commercial products based on 100% bentonite (TOXO®-MX) compared with a blend of bentonite, sepiolite, and propionic acid (BSP) on growth performance, gut morphology, and health of broilers. One-day-old male broiler chicks (n=600) were randomly allocated to four treatments with 10 pens of 15 birds each. The treatments included: (i) negative control (NC, a basal diet); (ii) positive control, (PC, as NC supplemented with 500 μg/kg AFB1); (iii) PC+TOXO-MX (TMX, as PC supplemented with 2 kg/t TOXO-MX); and (iv) PC+ BSP (BSP, as PC supplemented with 2 kg/t BSP). After a 42-day exposure to dietary AFB1, the ADG (-28%) and FCR (+33%) of the PC birds were significantly inferior compared with the NC birds (p<0.05). Birds supplemented with TMX (+21%) and BSP (+9%), however, had significantly higher ADG compared to the PC birds. The FCR of broilers supplemented with TMX was significantly better (-18 points) than the PC (p<0.05), while no difference was observed between PC and BSP. The villous height measured in the duodenum of broilers with TMX supplementation was higher compared to BSP and PC (p<0.05). In comparison with the PC, the addition of TMX and BSP improved the antioxidant status of broilers, as indicated by higher superoxide dismutase (SOD) and lower malondialdehyde (MDA) concentrations (p<0.05). In addition, TMX and BSP increased the production of antibody titer against Infectious bursal disease virus (IBDV) at days 21 and 42 (p<0.05). Furthermore, TMX and BSP decreased alanine transaminase (ALT) and aspartate aminotransferase (AST) activities (p<0.05), indicating an improvement in liver health. The study concluded that TMX and BSP are equally effective in supporting the immunity, antioxidant status and liver health of broilers under aflatoxin-challenging conditions. TMX, however, is much better than BSP in improving growth performance and intestinal morphometry.

P62
THE EFFECT OF A PULSED ELECTRIC FIELD ON THE FATE OF FUSARIA
MICROMYCETES AND THEIR MYCOTOXINS DURING MALTING
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Since contamination of cereals and cereal-based products by micromycetes and their secondary toxic metabolites, mycotoxins, is a problem of a global scope, development of strategies to minimize their occurrence at different levels of the food production chain is of high importance. Currently, there are various more or less sophisticated physical, chemical or biological strategies, which can be applied pre- or post-harvest. Pulsed electric field (PEF) represents an innovative technology with potential to damage micromycetes. This study deals with the characterization of PEF influence under various conditions (pre-soaking time of barley before PEF, electrolyte medium, input voltage, current, wavelength and number of pulses) on four species of Fusarium fungi (F. culmorum, F. graminearum, F. sporotrichioides and F. poae) and their 15 mycotoxins (deoxynivalenol, deoxynivalenol-3-glucoside, 3-acetyldeoxynivalenol, 15-acetyldeoxynivalenol, nivalenol, zearalenone, neosolaniol, diacetoxyscirpenol, HT-2 toxin, T-2 toxin, enniatins A, A1, B, B1 and beauvericin) in barley after malting. The content of individual Fusarium species was determined using polymerase chain reaction in real time (RT-PCR), and determination of mycotoxins was performed using ultra-performance liquid chromatography and tandem high-resolution mass spectrometry (U-HPLC-HRMS/MS). It has been shown that for each Fusarium species, the optimum PEF conditions were slightly different, nevertheless, significant decreases were encountered for all of the species. The greatest reduction was achieved in the case of F. sporotrichioides and F. poae, and their content in PEF-treated malt was approximately four and six times lower, respectively, when compared to the control malt. Regarding F. culmorum and F. graminearum, their content was two times lower in PEF-treated malt as compared to control malt. The micromycetes reduction always correlated with the reduction of relevant mycotoxins, therefore the highest reduction was achieved for mycotoxins produced by F. sporotrichioides and F. poae, specifically for type A trichotheccenes and enniatins.

P63
ANIMAL FEED MYCOTOXIN MITIGATION SOLUTION: IS IT AN EXTRA EXPENSE OR A DAILY DIET NECESSITY?
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It is a well-known fact that the presence of mycotoxins in pig's feed can and will make a negative impact on the animals' health and well-being. A standard maize-based diet was fed to post-wean pigs: maize was obtained from one of the area's maize storage facilities used by the area feed mills and analysed for the presence of mycotoxins. Analysis revealed the presence of increased levels of zearalenone,
fumonisins B1 and B2, as well as deoxynivalenol. Post-wean pigs were fed with the diet containing contaminated maize only (control group) as well as added modified clinoptilolite-based product, MultiSHIELD (groups 1 and 2). A significant positive impact on the animals’ health and economic performance has been demonstrated when MultiSHIELD was used for the pigs fed a mycotoxin-contaminated diet. The presented study demonstrates the need to include a mycotoxin mitigation solution in post-wean pigs’ daily diets in order to ensure animal health and performance.

**P64**

**THE EFFECT OF CURCUMIN AND SILYMARIN IN MITIGATING THE OXIDATIVE STRESS INDUCED BY DEOXYNIVALENOL IN HEPATIC CELLS**

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

**P65**

**THE ORAL BIOAVAILABILITY OF FUMONISIN B1 IS REDUCED BY AN ANTI-MYCOTOXINS AGENT IN BROILER CHICKENS IN A TOXICOKINETIC STUDY**

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Toxicokinetic studies are necessary to evaluate the efficacy of mycotoxin detoxifiers, considering the possible effects on the oral absorption and disposition of the mycotoxins in broiler chickens. The detoxification capacity of mycotoxins binder for fumonisin B1 is rather limited [Antonissen et al., 2020]. Consequently, a toxicokinetic study was performed to determine the effects of an anti-mycotoxins agent based on minerals, phytogenics and organic components on the plasma concentration-time profile of FB1 in broiler chickens. The study was carried out with six 21 day-old-broiler chickens Ross 308. Diet was evaluated on possible contamination by a multi-mycotoxin LC-MS/MS method. After one week of acclimatization, the mycotoxin FB1 at a dose of 2.5 mg/kg BW and the anti-mycotoxins product at 3 mg/kg BW were given as a single intracrop oral bolus in a cross-over study design. Blood samples were collected at 0 h (just before administration) and 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 8 and 12 h post administration, and plasma was obtained. The toxicokinetic parameters were analysed using non-compartmental analysis (WinNonlin 6.3, Pharsight Corporation, USA). The area under the concentration–time curve from time zero to the last time point (AUC₀–t), maximum plasma concentration (C_max), time at maximal plasma concentration (T_max), elimination half-time (T_{1/2el}) and elimination rate constant (k_e) were determined. The effect of the anti-mycotoxins agent on the oral...
absorption of FB1 was evaluated by comparing toxicokinetic parameters based upon the FB1 plasma concentrations measured in the FB1 and FB1+ anti-mycotoxins product samples, with special emphasis on AUC0−12 and Cmax. A student t-test was performed with SPSS 24.0 (IBM, USA) to evaluate possible significant differences. The feed was contaminated with low levels of deoxynivalenol (152 μg/kg), zearalenone (19.9 μg/kg) and FB1+FB2 (51.2 μg/kg). The contamination levels were within the acceptance criteria of the EU (2006/576/EC). The systemic exposure of FB1 was statistically significantly lower (p< 0.05) in the birds receiving the anti-mycotoxins agent (AUC0−12 6.17±4.881 h.ng/ml), compared to birds that only received the mycotoxin (AUC0−12 34.30±22.760 h.ng/ml). Nonetheless, the Cmax was not significantly lower in the supplemented group (5.09±2.937 ng/ml) in contrast to FB1 group (19.63±26.103 ng/ml) due to the high SD. It can be concluded that the anti-mycotoxins agent tested in the present study is efficient in reducing the total systemic exposure to FB1 in broiler chickens.

P66
ANTIFUNGAL EFFECT AND MODE OF ACTIVITY OF ZINC CHLORIDE AGAINST THE TOXIGENIC FUNGUS ASPERGILLUS FLAVUS
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Fungal plant pathogens cause considerable losses in the yield and quality of field crops worldwide. In addition, under specific environmental conditions, many fungi, such as Aspergillus spp., are further able to produce mycotoxins while colonizing their hosts during the pre- and post-harvest stages. These toxic metabolites accumulate in human and livestock and pose a serious threat to consumer health. Extensive and prolonged use of fungicides in crop protection has stimulated the emergence of acquired drug resistance in some plant and human fungal pathogens. The use of metal compounds as antimicrobial agents offers an alternative strategy for managing potentially resistant toxigenic fungi and reducing the required dosage of specific drugs. In the current study, we investigated the effect of zinc chloride (ZnCl2) on Aspergillus flavus contamination and aflatoxin biosynthesis in cereal grains (wheat and maize) and legume crops (groundnuts and chickpea). Following ZnCl2 treatment, quantitative PCR analysis showed a significant decrease in the fungal DNA content in cereal grains and legumes samples contaminated by A. flavus. Chitin content in A. flavus was dramatically reduced following ZnCl2 treatment, suggesting a possible mode of activity of the zinc compound through perturbation of the fungal cell wall by inhibition of chitin synthesis. Moreover, after 5 days of treatment, ZnCl2 at a concentration of 10 mM reduced aflatoxin production by A. flavus in the tested samples by up to 43%, while higher concentrations of the compound (20 and 40 mM) resulted in a marked decrease of the toxin synthesis, with inhibition rates ranging from 87.4 to 99.7%. These findings were supported by qRT-PCR analysis, showing down-regulation of key genes involved in the aflatoxin biosynthetic pathway under ZnCl2 treatment. Our results provide evidence for antifungal and antimycotoxigenic effect of ZnCl2 against the filamentous fungus A. flavus. Future application of these findings may allow sustainable use of the compound in agricultural settings, while reducing potential concerns of over exposure to high doses of fungicides that are harmful to the environment.

P67
EFFECTS OF A MULTI-COMPONENT MYCOTOXIN DETOXIFIER ON THE ANTIOXIDANT STATUS AND THE PERFORMANCE OF SOWS
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This study was conducted to investigate the effects of an anti-mycotoxins agent on the antioxidant capacity, health and reproductive performance of gestating-lactating sows challenged by mycotoxins. Two trials were performed to evaluate the use of the multi-component mycotoxin detoxifying agent, containing adsorbent material, phytochemicals and a combination of selected yeasts, one month before farrowing and during the lactation period. A total of 80 primiparous sows were distributed into two groups (2 replicates/treatment) in each experiment: (i) the control group received the contaminated feed; and (ii) the experimental group received the contaminated feed supplemented with the anti-mycotoxins agent. Thiobarbituric acid reactive substances (TBARS), protein carbonyls (CARBS) and the total
antioxidant capacity (TAC) were evaluated in plasma, as oxidative stress biomarkers. Clinical and reproductive parameters including the rectal temperature, the clinical examination of mammary glands and the litter characteristics were recorded. In the first experiment, sows (Large White x Landrace, DanBred) fed the control diet contaminated with 2,767.5 ppb fumonisin B1, 700.5 ppb fumonisin B2, 14.6 ppb zearalenone and 4.2 ppb T2-toxin. In the second treatment, sows received the natural contaminated diet supplemented with the anti-mycotoxins agent at 1.5 kg/t. The results revealed that the inclusion of the anti-mycotoxins agent reduced the TBARS (p=0.009) and CARB levels (p<0.001) and increased the TAC (p<0.001) in plasma, suggesting a better antioxidant status. There were improvements (p<0.001) in the mammary gland development, health status and milk production. Furthermore, the number of piglets born alive and weaned per litter increased (p<0.001), as well as the stillbirths and mummies were reduced (p<0.001). In the second experiment, the sows (Large White x Landrace, Topigs Norsvin) were naturally challenged by 5,109.4 ppb fumonisin B1, 1,380.1 ppb fumonisin B2 and 5.1 ppb aflatoxin B1. The supplemented group received 2.5 kg/t of the additive one month before farrowing and 1 kg/t during the lactation period. The level of TBARS and CARB were significantly lower (p<0.001), meanwhile the TAC was increased (p=0.007) in the supplemented sows. The rectal temperature was reduced (p<0.001) and improvements (p<0.001) in the mammary glands and the litter characteristics were observed in sows fed the anti-mycotoxins agent. In conclusion, the use of the multi-component mycotoxin-detoxifying agent promotes the health and the performance of sows and improves the antioxidant status under mycotoxin multi-contamination challenge.

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THE EFFECTS OF A BROAD-SPECTRUM MYCOTOXIN ADSORBENT IN GESTATING AND LACTATING SOWS
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Pigs are considered to be among the most sensitive species to mycotoxins, especially sows and piglets. This highlights the need for solutions to reduce the negative effects of mycotoxins on pig performance. The aim of this study was to evaluate the effects of two broad spectrum mycotoxin adsorbents on sows and piglet performance, when fed to sows from late gestation until farrowing, and throughout lactation. A total of 100 primiparous and multiparous sows (day 84 of gestation) were divided over 3 treatments: (i) control, normal gestation and lactation diet without mycotoxin binders; (ii) treatment 1, control + mycotoxin adsorbent A at 0.15% inclusion; and (iii) treatment 2, control + mycotoxin adsorbent B (Excential Toxin Plus, Orffa Additives BV) at 0.15% inclusion. Both mycotoxin adsorbents were broad spectrum products but contained different ingredients. The trial started at day 107 of gestation and lasted until weaning. The following parameters were recorded: Knauer Caliper score, farrowing time, piglet expulsion time, sow performance (daily feed intake, feed conversion ratio (FCR), mortality), piglet performance (average weaning weight, averaged daily gain (ADG), litter size at weaning, mortality), diarrhoea score and incidence, and wean-to-service interval. The diets were analysed for mycotoxins. The following mycotoxins were detected in the feed samples: aflatoxins (6.85 ppb), deoxynivalenol (103.65 ppb), beauvericin (8.5 ppb), moniliformin (5.53 ppb), enniatins (18.80 ppb), fusaric acid (35.55 ppb), fumonisins (217.10 ppb), mycophenolic acid (9.15 ppb), and zearalenone (27.10 ppb). For feed intake of the sows, a trend was observed where sows from treatment 2 had a higher feed intake (6.32 kg/d) compared to the control (6.03 kg/d) and treatment 1 (5.78 kg/d) (p<0.10). Treatments 1 and 2 showed an improved FCR (lactation feed/litter weight gain) of 2.99 and 2.97, respectively compared to the control (3.83) (p=0.033). The results in piglets showed a significantly higher average weaning weight for treatment 1 (7.25 kg/piglet) and 2 (7.14 kg/piglet) compared to the control (6.17 kg/piglet) (p<0.01). Furthermore, the pre-weaning ADG was improved for treatment 1 and 2 (both 206 g/d) compared to the control (170 g/d) (p<0.01). The litter growth rate was significantly higher for treatment 2 (2.02 kg/d) compared to the control (1.68 kg/d) (p=0.04). Overall, it can be concluded that the inclusion of a broad-spectrum mycotoxin adsorbent in the diet of sows during gestation and lactation can improve sow performance (feed intake, FCR) as well as piglet performance (average weaning weight, ADG and litter growth rate).
P69
AN INTEGRATED MYCOTOXIN-MITIGATING AGENT CAN EFFECTIVELY MITIGATE THE COMBINED TOXICITY OF AFB1, DON AND OTA IN BROILER BREEDER HENS
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The objective of this study was to evaluate the efficacy of an integrated mycotoxin-mitigating agent in reducing the adverse effects of co-occurring dietary AFB1, DON and OTA on broiler breeder hens. Three hundred and sixty 30-week-old broiler breeder hens were randomly allocated into four experimental groups with 10 replicates of 9 birds each. The four groups received: (i) a basal diet (BD; Control); (ii) a BD supplemented with 0.15 mg/kg AFB1 + 1.5 mg/kg DON + 0.12 mg/kg OTA (Toxins); (iii) a BD plus Toxins with 0.1% TOXO-XL (Toxins+XL1); and (iv) a BD plus Toxins with 0.2% TOXO-XL (Toxins+XL2), respectively, for 8 weeks. Compared with the control, dietary contamination of mycotoxins decreased (p<0.10) total egg weight and egg laying rate but increased (p<0.10) feed/egg ratio by 5.73-10.6% during week 1-4 and 5-8. Furthermore, dietary mycotoxins increased (p<0.10) mortality rate by 5.56% during week 5-8 and decreased (p<0.10) the settable eggs rate and hatch of total eggs rate at week 8. These negative alterations induced by mycotoxins were mitigated by the supplementation of TOXO-XL at both dosages (p<0.10). Compared with the control, dietary mycotoxins reduced (p<0.05) the oviduct index (13.2%), increased (p<0.05) serum albumin (16.0%) and induced histopathological lesions and apoptosis in liver and oviduct; it also increased (p<0.05) PC concentration but decreased (p<0.05) T-AOC, GPX and CAT activities, and increased (p<0.05) IL-1β and IL-6 expression in liver and/or oviduct. Notably, these negative alterations caused by mycotoxins again were mitigated by the supplementation with TOXO-XL at both dosages. In conclusion, this study demonstrated that TOXO-XL can mitigate the toxic effects of co-occurrence of dietary AFB1, DON and OTA on laying and hatching performance, egg quality, and health status of broiler breeder hens.

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EFFECTS OF ANTI-BIOTOXINS SUPPLEMENTATION ON IMMUNOLOGICAL, BIOCHEMICAL PARAMETERS AND PERFORMANCE OF DAIRY COWS
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Mycotoxins and more generally biotoxins, represent a real but often underestimated risk in ruminants. These can be involved in chronic syndromes, with impaired rumen function or increased predisposition to infectious diseases and, less frequently, acute toxicoses with severe illness and death. Although the presence of mycotoxins is practically unavoidable in feed rations, there is limited scientific evidence regarding the effects of low and multiple mycotoxin ingestion on the health status and performance of dairy cows. This may partly explain by the complexity of animal responses, diversity of environmental conditions, and the difficulty of diagnostic due to ambiguous subclinical disorders. The aim of the present study was to evaluate the effect of an anti-biotoxins solution supplementation in feed containing naturally mycotoxins contamination on physiologic and metabolic parameters in dairy cows. The trial was investigated in a commercial French farm of 70 lactating Holstein dairy cows, receiving diet supplemented by anti-biotoxin (MPY) during 60 days at 50 g/cow/day. Mycotoxins and metabolites analysis were performed in total mixed ration (TMR) and urine. Blood samples and urine were realized every 2 weeks. Milk production, immunological, blood serum chemistry, biomarkers of oxidative status and liver and kidney functions, serum immunoglobulins concentration were followed. TMR analysis showed low but multiple mycotoxins contamination, with a predominance of DON, ZEA, FUMs, HT-2 and tenuazonic acid with levels above 0.24, 0.015, 0.176, 0.01, and 0.065 mg/kg, respectively. Supplementation was associated to significant changes of total proteins and globulins levels, a decrease of liver/kidney functions such as urea, bilirubin and the increase of tenuazonic acid excretion. Immunoglobulin G (IgG) concentration was in average 35 mg/ml at T0 and decrease in a range of 15-30 mg/ml after diet supplementation. In addition to these effects on immunity and liver/kidney functions, improvement of zootechnical performance have been observed with a calculated gain of 0.8 kg milk/cow/day considering milk production persistency at 93.7% during the trial period (vs. milk persistency during pre- and post-trial period). These results confirm the regular animal exposition to low and multiple contamination by mycotoxins, that can adversely affect their health and performance. Moreover, supplementation with anti-biotoxin product (MPY) demonstrated evidence to support animal to counteract most of these negative effects. Finally, together with results obtained from previous trials,
this study highlighted some interesting biomarkers that can be followed for a better exposure evaluation and understanding of mycotoxins effects in dairy cows.

P71
EVALUATION OF ESSENTIAL OILS AGAINST ASPERGILLUS CARBONARIUS AND THEIR EFFECTS ON OCHRATOXIN A AND AclaeA GENE EXPRESSION
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Aspergillus carbonarius is a mycotoxigenic fungus that infects grapes and produces ochratoxin A (OTA) which is a hazard for human and animal health. Chemical and physical treatments have been proven inefficient at removing OTA from grapes and wine without effecting their organoleptic properties. The increasing use of pesticides in vine cultivation during the last decade, combined with the low maximum residue levels (MRLs), demands alternative applications of natural antimicrobials to control sour rots and OTA contamination in vineyards. Essential oils contain diverse bioactive compounds that can prevent mould growth and their toxic metabolite production, while they are not phytotoxic and biodegradative. The aim of this study was to evaluate the impact of ten essential oils, (cinnamon, thyme, mint, lavender, marjoram, tea tree, rosemary, sage, citronella and geranium) on growth of A. carbonarius (strains Ac29 and 5010) and OTA production. Experiments of fungal growth and OTA production were performed on Malt Extract Agar (MEA) and Czapek Yeast Extract Agar (CYA) solid cultures, and the impact of essential oils was measured after seven days of incubation at 25°C. In order to start unravelling their mode of action, the most efficient essential oils were further evaluated for their role in the relative expression of the AclaeA gene (a global fungal regulator of mycotoxins in fungi), by using real-time PCR on synthetic grape medium (SGM) liquid cultures. The results showed that several essential oils significantly reduced the expression of AclaeA in comparison with the WT 5010.

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ADSORPTION OF AFLATOXIN B1 BY DIFFERENT ANTIMYCOTOXIN ADDITIVES: BENTONITE, CLINOPTILOLITE AND BETA-GLUCANS EXTRACTED FROM YEAST CELL WALL
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The present study aims evaluate and compare three different antimycotoxin additives (AMAs) with respect to their ability to bind aflatoxin B1 (AFB1), using the adsorption isotherms mathematical models. Three AMAs of them were selected for an in vitro adsorption experiment with AFB1 in seven concentrations (0.05-4 mg/l), using simulated solutions of gastric (pH 3) and intestinal (pH 6) juices, with an inclusion rate of 0.5% and analysed by high performance liquid chromatography coupled with mass spectrometry (HPLC-MS/MS). The products were individually composed of bentonite (AMA 1), clinoptilolite (AMA 2), and beta-glucans extracted from yeast cell wall (BGYCW) (AMA 3). The AMAs will be characterized by X-ray diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR). The equilibrium isotherm functions were fitted to the data by non-linear regression analysis using MatLab, applying the Langmuir, Freundlich, and BET models (Brunauer, Emmett and Teller) and adsorption data obtained for each material was analysed by Statgraphics Centurion XV. At pH 3, AMA 1 obtained better adsorption rates (99.69 to 99.98%) in all AFB1 concentrations tested when compared to the other AMAs (p<0.05). At pH 6, concentrations of 0.05 to 0.75 of AFB1 (mg/l) demonstrated no statistical differences between the AMAs tested (p>0.05). At concentrations of 1-4 mg/l of AFB1, AMA 1 obtained the best adsorption result (99.26 to 99.86%) (p<0.05). The Freundlich model best fitted the AMA 1 adsorption process data (r² of 0.8518 and 0.9484 at pH 3 and pH 6, respectively). For the other additives, the Langmuir model obtained the best fit, demonstrating an r² in AMA 2 at pH 3 of 0.9813 and at pH 6 of 0.9660, with qm (mg/g) of 8.6 at pH 3 and 2.3 at pH 6; and for AMA 3 an r² at pH 3 of 0.9737 and at pH 6 of 0.9942, with qm (mg/g) of 3.4 at pH 3 and 2.3 at pH 6. In summary, we can conclude that the bentonite-based AMA showed the best adsorption rates among the AMAs tested and the Freundlich model best fitted the data of the AFB1 adsorption process with the additive composed by bentonite and the Langmuir model, for the additives composed by clinoptilolite and BGYCW. The analysis of the individual results of the isotherm models, show that the maximum adsorption capacity varies according to the adsorbent, pH and mycotoxin concentration.
The present study aims evaluate and compare three different antymycotoxin additives (AMAs) with respect to their ability to bind aflatoxin B1 (AFB1), using the adsorption isotherms mathematical models. Three AMAs of them were selected for an in vitro adsorption experiment with AFB1 in seven concentrations (0.05-4 mg/l), using simulated solutions of gastric (pH 3) and intestinal (pH 6) juices, with an inclusion rate of 0.5% and analysed by high performance liquid chromatography coupled with mass spectrometry (HPLC-MS/MS). The products were individually composed of bentonite (AMA 1), clinoptilolite (AMA 2), and beta-glucans extracted from yeast cell wall (BGYCW) (AMA 3). The AMAs will be characterized by X-ray diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR). The equilibrium isotherm functions were fitted to the data by non-linear regression analysis using MatLab, applying the Langmuir, Freundlich, and BET models (Brunauer, Emmett and Teller) and adsorption data obtained for each material was analysed by Statgraphics Centurion XV. At pH 3, AMA 1 obtained better adsorption rates (99.69 to 99.98%) in all AFB1 concentrations tested when compared to the other AMAs (p<0.05). At pH 6, concentrations of 0.05 to 0.75 of AFB1 (mg/l) demonstrated no statistical differences between the AMAs tested (p>0.05). At concentrations of 1-4 mg/l of AFB1, AMA 1 obtained the best adsorption result (99.26 to 99.86%) (p<0.05). The Freundlich model best fitted the AMA 1 adsorption process data (r² of 0.8518 and 0.9484 at pH 3 and pH 6, respectively). For the other additives, the Langmuir model obtained the best fit, demonstrating an r² in AMA 2 at pH 3 of 0.9813 and at pH 6 of 0.9680, with qm (mg/g) of 8.6 at pH 3 and 2.3 at pH 6; and for AMA 3 an r² at pH 3 of 0.9737 and at pH 6 of 0.9942, with qm (mg/g) of 3.4 at pH 3 and 2.3 at pH 6. In summary, we can conclude that the bentonite-based AMA showed the best adsorption rates among the AMAs tested and the Freundlich model had the best fit to the data of the AFB1 adsorption process with the additive composed by bentonite and the Langmuir model, for the additives composed by clinoptilolite and BGYCW. The analysis of the individual results of the isotherm models, show that the maximum adsorption capacity varies according to the adsorbent, pH and mycotoxin concentration.

Mycotoxins are one of the major threats to food and feed safety and quality worldwide. Aflatoxin AFB1 and its metabolite AFM1, produced by fungi belonging to Aspergillus flavus and A. parasiticus, have been classified by the International Agency for Research on Cancer as the most carcinogenic compounds for humans. In maize, aflatoxins are often co-occurring with fumonisins, produced by several Fusarium species, such as Fusarium verticillioides, which can cause several diseases, including cancer. Due to the inability of chemical methods to control mycotoxin levels in maize, the use of non-aflatoxigenic strains of Aspergillus flavus has been characterized by numerous studies as the most effective control strategy against aflatoxins. The purpose of the present study was initially to evaluate 14 and 35 endemic non-aflatoxigenic strains isolated from pistachios and maize respectively, in terms of their ability to reduce aflatoxin production in situ, on artificially inoculated maize seeds by endemic highly aflatoxigenic A. flavus strains. Previous experiments indicated the high efficacy of the 14 non-aflatoxigenic strains isolated from pistachios, in inhibiting the biosynthesis of aflatoxins on pistachios, with aflatoxin levels reduction rates ranged between 80-90%, both in laboratory and field experiments. Additionally, in situ experiments on maize seeds indicated high inhibition rates that exceeded 90%. Subsequently, the non-aflatoxigenic strains were subjected to Vegetative Compatibility Groups (VCGs) aiming to find genetically stable isolates, suitable to serve as biological control agents against aflatoxin contamination in Greece. The total of 49 isolates were further applied on maize plants, to evaluate their effectiveness in reducing aflatoxins levels under field conditions. 14 out of 49 isolates were able to minimize aflatoxin levels by 95-100% and were included in large VCG groups, indicating that they are also well adapted at the local environment. Finally, 4 of the most effective non-aflatoxigenic isolates, from distinct VCGs, were tested for their ability to decrease fumonisins levels on artificially inoculated maize seeds, with a highly toxigenic Greek isolate belonging to F. verticillioides. The results of the
Present study could potentially provide useful biocontrol agents, for the control of mycotoxins in maize, in Greece.

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MITIGATION EFFECTS OF TOXO® IN BROILER CHICKENS WHEN EXPOSED TO MULTIPLE MYCOTOXINS

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Exposure to mycotoxins in broiler chickens can have significant negative consequences on their health, growth, and overall performance. Among more than hundreds of mycotoxins, Aflatoxins (AFLA), Ochratoxins (OCHRA) and T-2 toxins (T-2) have been proven to be very toxic to broilers and their co-occurrence in the feed are observed and rising. It is common for broiler producers to choose commercial feed additives for mycotoxin mitigation. The aim of this study is to establish a broiler model with the challenge of a combined AFLA, OCHRA, and T-2, and then to validate the mitigation efficacy of TOXO® which is a broad-spectrum product and globally available. A total of 600-day-old male broiler chicks (Cobb) were randomly assigned to four treatments with 10 replicates of 15 birds each. The treatments include: (i) negative control (NC, as basal diet); (ii) positive control (PC, as NC diet with mycotoxins, 125 µg/kg AFLA + 100 µg/kg OCHRA + 100 µg/kg T-2); (iii) low dose of TOXO (TOXO-L, as PC diet with 1.5 kg/ton TOXO); and (iv) high dose of TOXO (TOXO-H, as PC diet with 3.0 kg/ton TOXO). The birds were housed in a research facility but with a set up like a local commercial farm. After a 42-day exposure to mycotoxins, the average daily gain (ADG) of birds significantly reduced (54.47 g for NC vs. 40.90 g for PC; p<0.05), leading to a reduced body weight (bw, 1.74 kg for PC vs. 2.33 kg for NC; p<0.05). The feed conversion ratio (FCR) was also substantially compromised (1.810 for NC vs. 2.386 for PC; p<0.05). Compared to PC, TOXO-L improved ADG, BW, and FCR by 11, 13, and 10%, respectively (p<0.05), while TOXO-H by 20, 21, and 15%, respectively (p<0.05). Liver health was also compromised by mycotoxin exposure, where the liver index increased from 2.53 to 3.37 and ALT activity increased from 21.52 to 41.94 U/L on day 42 (p<0.05). Comparing to PC, TOXO addition in feed again improved both liver index (3.20 for TOXO-L; 3.00 for TOXO-H, p<0.05) and ALT activity (31.36 U/L for TOXO-L, p<0.05; 28.72 U/L for TOXO-H, p<0.05). To conclude, a 42-day exposure to dietary mycotoxins caused significant negative consequences on the liver health and growth performance of broiler chickens, while the addition of TOXO in feed at both 1.5 and 3.0 kg/ton showed significant mitigation efficacy.

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EFFICACY OF BENTONITE AGAINST VIBRIO PARAHYDROMELYTICUS CAUSING ACUTE HEPATOPANCREATIC NECROSIS DISEASE (AHPND) IN PACIFIC WHITE SHRIMP, PENAEUS VANNAMEI

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Vibrio parahaemolyticus is a bacterial pathogen that becomes lethal to Penaeus shrimps when acquiring the pVA1-type plasmid carrying the PirABvp genes which secrete PirA and PirB toxins, causing acute hepatopancreatic necrosis disease (AHPND). This disease has caused serious global economic losses in the shrimp farming industry, with outbreaks reported in Southeast Asia, Mexico, and South America. Consequently, the degradation and/or elimination of the PirAB toxins might be a valid strategy to control or mitigate AHPND. Bentonite is a feed additive authorised in the EU as an adsorbent substance for the reduction of the contamination of feed by mycotoxins. The efficacy of these type of adsorbents on the inactivation of bacterial endotoxins from the gut of animals has been extensively tested by in-vitro methodologies. However, so far, none of these adsorbents could be clearly assigned to endotoxin adsorption while fed to penaeid shrimp. The present study explored the application of dietary bentonite TOXO®-MX, a commercial mycotoxin absorbent, and its abilities as potential adsorptive agent to PirA and PirB toxins while lowering the burden of AHPND’s causative agent. A feeding trial was conducted with specific pathogen free Penaeus vannamei having an initial body weight of 1.0±0.5 g per shrimp. Animals were reared in tanks with seawater water at 30 ppt salinity and 28.5±1°C temperature. Triplicate tanks of twenty shrimp were fed either an un-supplemented control diet or a diet containing 0.2% TOXO-MX, for 21 days prior to challenge with VP AHPND. Shrimps were challenged by immersion with a V. para haemolyticus virulent strain (VP Strain 13-028A/3) causing AHPND. The survival of shrimps was monitored and recorded for seven consecutive days after the challenge event. The results shown that 0.2% TOXO-MX dietary inclusion has significantly improved shrimp survival, when compared to the values obtained with the un-supplemented control diet (p<0.001). Five days after the challenge event, shrimp group fed 0.2% TOXO-MX reached 56.82% mortalities with
a relative percent survival of 40.18%. Overall, this study suggests the efficacy and extended benefit of using mycotoxin adsorbent, bentonite (TOXO-MX), in shrimp feed to improve survival of shrimp infected with VP_AHPND.

P77
MITIGATION EFFICACY OF THREE COMMERCIAL PRODUCTS WHEN BROILER FEED IS CONTAMINATED BY MULTIPLE MYCOTOXINS
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The co-contamination of multiple mycotoxins in animal feed has become a rising concern for broiler production. Among more than hundreds of mycotoxins, Aflatoxins (AFLA), Ochratoxins (OCHRA), and T-2 toxins (T-2) have been proven to be highly toxic to broilers. This study aims to establish a model where broiler feed is contaminated by AFLA, OCHRA, and T-2, and then to validate the mitigation efficacy of three commercial products (TOXO®-XL, TFN 360, and MF Plus) that are globally available.

A total of 750-day-old male broiler chicks (Cobb) were randomly assigned to five treatments with 10 replicates of 15 birds each. The treatments include: (i) negative control (NC, as basal diet); (ii) positive control (PC, as NC supplemented with mycotoxins: 125 µg/kg AFLA + 100 µg/kg OCHRA + 100 µg/kg T-2); (iii) PC + TOXO-XL (TXL, as PC supplemented with 1.5 kg/ton TOXO-XL); (iv) PC + TFN 360 (TFN, as PC supplemented with 1.5 kg/ton TFN 360); and (v) PC + MF Plus (MFP, as PC supplemented with 1.5 kg/ton MF Plus). After a 42-day exposure to dietary mycotoxins, the average daily gain (ADG) of birds significantly reduced (54.47 g for NC vs. 40.90 g for PC; p<0.05), leading to reduced body weight (BW, 1.74 kg for PC vs. 2.33 kg for NC; p<0.05). The feed conversion ratio (FCR) was also substantially compromised (1.810 for NC vs. 2.386 for PC; p<0.05). Compared to PC, TXL improved ADG, BW, and FCR by 16, 17, and 15%, respectively (p<0.05), while both TFN and MFP by 13, 14, and 11%, respectively (p<0.05). Liver health was also compromised by mycotoxin exposure, where the alanine aminotransferase (ALT) activity increased from 21.52 to 41.94 U/l on day 42 (p<0.05). Compared to PC, all three products improved ALT activity (29.82 U/L for TXL, p<0.05; 32.51 U/l for TFN, p<0.05; 32.31 U/l for MFP, p<0.05). The antioxidant status of broilers, expressed as the concentration of superoxide dismutase (SOD), was impaired by mycotoxin exposure (178.38 U/ml for NC vs. 125.95 U/ml for PC, p<0.05). Compared to PC, the SOD concentration got significantly improved by TXL (149.97 U/ml, p<0.05) and MFP (147.70 U/ml, p<0.05). To conclude, a 42-day exposure to dietary mycotoxins caused significant negative consequences on the liver health, antioxidant status, and growth performance of broiler chickens, while the addition of three commercial products in feed at 1.5 kg/ton showed significant mitigation efficacy and TOXO-XL delivered the numerically highest results.

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DEGRADATION OF DEOXYNIVALENOL BY A MICROBIAL CONSORTIUM C1 FROM DUCK CECUM IN VITRO AND IN VIVO
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Deoxynivalenol (DON) is a mycotoxin commonly found in food and feed posing a significant health risk to both humans and animals. To address this issue, researchers have explored microbial degradation as a potential strategy for removing DON from contaminated samples. In this study, a microbial consortium was obtained from the cecum contents of ducks and tested for its ability to remove DON from various mediums over a 24 h period at 37°C. The consortium was also evaluated for its decontamination activity in DON-contaminated corn steep liquor (CSL). Moreover, an experiment was conducted with ICR mice, in which the mice were orally administered DON (at doses of 5 and 10 mg/kg bw/d) for 3 days followed by oral administration of the microbial consortium (C1) for 6 days. The residual concentration of DON in CSL and mice was determined using ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) analysis. The results showed that the microbial consortium C1 primarily consisted of three genera: *Escherichia*, *Enterococcus*, and *Clostridium*. C1 showed the ability to convert DON to de-epoxy DON (DOM-1). After three generations of subculture, C1 was able to remove up to 100% of DON in the BHI medium, which was higher than that in the AIM medium (14.59-95.07%) and the mixed AIM/BHI medium (28.71-91.43%). Additionally, C1 effectively degraded DON in CSL, with an efficiency of 49.44% after a 14-day incubation period. DON administration induced liver, spleen, and kidney damage in mice, including inflammation and necrosis. However, oral administration of C1 significantly alleviated the symptoms of poisoning in mice. Furthermore, the levels of total protein...
Mycotoxins, such as aflatoxin B1 (AFB1) and zearalenone (ZEN), frequently co-occur in cereal-based feed and food products, posing a significant health risk. Existing mycotoxin-degrading enzymes primarily target a single type of mycotoxin. Therefore, it is crucial to identify enzymes capable of simultaneously degrading multiple mycotoxins. This study focuses on the \textit{cotA} gene from \textit{Bacillus subtilis} ZJ-2019-1, which is involved in the degradation of AFB1 and ZEN. The aim is to investigate the potential of the CotA enzyme for simultaneous degradation of AFB1 and ZEN through a mediator-assisted laccase interaction system. The \textit{cotA} gene was cloned and expressed in \textit{Escherichia coli} BL21/pEASY-Blunt. Recombinant CotA protein was obtained through induction with 0.2 mM isopropyl \(\beta\)-D-1-thiogalactopyranoside (IPTG) at 16°C, followed by purification using a Ni\(^2+\) column. The molecular mass (MW) of the purified CotA protein was confirmed as approximately 58 kDa. The optimal conditions for mycotoxin degradation were determined at 70°C and pH 4.0. The degradation efficiency of CotA towards AFB1 and ZEN was evaluated by incubating the mycotoxins with the enzyme for 12 h at 37°C in the absence of mediators. Additionally, various natural mediators, including vanillin, acetosyringone, syringaldehyde, sophoridine, and matrine, were tested to enhance the degradation of AFB1 and ZEN by CotA. Recombinant CotA protein exhibited high degradation activity, achieving reductions of 96% for ZEN and 79.28% for AFB1 after a 12-h reaction at 37°C in the absence of mediators. The presence of natural mediators significantly improved the degradation efficiency of CotA. When sophoridine (4 \(\mu\)M) and matrine (4 \(\mu\)M) were added, AFB1 degradation increased by 46.76 and 33.71%, respectively. Syringaldehyde (10 \(\mu\)M) increased the degradation of ZEN and AFB1 by 50 and 90%, respectively. In the presence of vanillin (10 \(\mu\)M), CotA achieved complete degradation of AFB1 and a 69.5% reduction in ZEN levels. CotA completely degraded both AFB1 and ZEN in the presence of acetosyringone (10 \(\mu\)M). In conclusion, these findings demonstrate that the CotA enzyme from \textit{B. subtilis} shows promising potential for the simultaneous degradation of AFB1 and ZEN. The mediator-assisted laccase interaction system, particularly involving vanillin and acetosyringone, significantly enhanced the degradation efficiency of CotA. This research contributes to developing strategies for the effective detoxification of mycotoxin-contaminated feed and food, reducing the health risks associated with AFB1 and ZEN co-occurrence.
the final 5 days of each period, cows received one of five treatments: (i) control silage (C); (ii) spoiled silage (S); (iii) control silage + mycophenolic acid (CM); (iv) spoiled silage + mycophenolic acid (SM); or (v) spoiled silage + mycophenolic acid + binder (SMB). Mycophenolic acid was added at 5 mg/kg dm of the total diet and the binder at 150 g/cow/day. Data were analysed as a Latin-square with repeated measures analysis of variance. Rumen fluid ammonia concentration was unaffected by treatment, with a mean value of 190 mg/l (p>0.05), however, the acetate:propionate ratio was between 0.17-0.33 greater in cows fed diets containing the spoiled silage (p>0.05). Despite this, there was no effect of treatment (p>0.05) on cow dry matter intake or milk yield (means of 19.5 and 22.9 kg/d, 42.4 and 33.9 g/kg, respectively). Similarly, body temperature, and red and white blood cell count were unaffected by dietary treatment (p>0.05). In conclusion, short-term feeding of spoiled grass silage high in mycotoxins increased the proportion of acetate to propionate in the rumen fluid of lactating dairy cows.

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MITIGATING MYCOTOXIN UPTAKE IN MAIZE PORRIDGE USING ENZYME-FORTIFIED FLOUR
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Mycotoxins are regularly found in food and pose a health risk to consumers. Occurrence of mycotoxins mainly depends on the crop and climatic conditions. Especially maize is frequently contaminated with mycotoxins. Once these toxic metabolites are formed, they are hard to get rid of as they are thermally and chemically stable. While good manufacturing practices, rigid quality management systems and frequent testing are implemented in industrialized countries, the exposure to mycotoxins can be expected to be much higher in low-income countries. In addition, climatic conditions in these regions, e.g., in Southern Africa (where maize is a major source of nourishment), can favour the occurrence of certain mycotoxins, such as fumonisin (FUM) or zearalenone (ZEN). Biotransformation of these toxins can be an effective strategy to improve food and feed quality and reduce health risks. In this talk, a FUM- and a ZEN-degrading enzyme and different approaches for their application in food and feed production are presented. The emphasis is made on maize processing, as it is most frequently contaminated. The presented solutions are not limited to low-income countries but should also be transferred to industrialized countries to achieve even lower mycotoxin levels than reasonably achievable (ALARA) with current practices.

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IN VITRO DETOXIFICATION CHARACTERISTICS OF YEAST CELL WALL EXTRACT TOWARD EMERGING MYCOTOXINS
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Recent data highlighted the potential importance of emerging mycotoxins as recurring contaminants of cereals and feedstuffs. Analytical surveying work (37™, Alltech laboratory, Nicholasville KY) confirmed the large distribution and occurrence of beauvericin (30%) and enniatins (>50%) that often compares to the ones of trichothecenes B. To gain insight into the extended applicability of a yeast cell wall extract (YCWE, Mycosorb®, Alltech, Inc.) at mitigating mycotoxins, the in vitro adsorption characteristics toward mycotoxin identified as emerging mycotoxins, comprising citrinin, citreoviridin, beauvericin, enniatins A/B, mycophenolic acid, and phomopsin A were evaluated. An adsorption study was conducted to determine isothermal adsorption kinetics of YCWE, calculated using a differential analysis of supernatant comparing YCWE vs non-YCWE treated toxin mixture by means of free toxin analysis and multiple regression fitting used in the art (Langmuir, Freundlich, Hill’s equation with n sites). Five concentrations of an emerging mycotoxin mixture (0.5, 1.0, 1.25, 2.5, 5.0 μg/ml) were prepared in a 10mM ammonium citrate buffer maintained at pH3. A slurry sample of YCWE was applied to each concentration in a Pierce Spin Column cassette equipped with a frit and filter disc. The samples, with or without addition of YCWE, were incubated for 60min under agitation at 37°C. After reaction, the cassettes were centrifuged and 200 μl of sample was transferred to silanized chromatography vials. Mycotoxin quantification was performed using a matrix-matched calibration curve by means of a UPLC-high-resolution hybrid mass spectrometer in full scan mode (Vion™, Waters Corp.) fitted with an electrospray ionization source operating in positive mode. Hill’s equation with n sites demonstrated best fit (r²>0.95) for all toxins tested. Beauvericin had the highest maximal amount of toxin bound amongst toxin tested, with an extrapolated maximum of 122 μg/ml. Enniatin A, citreoviridin and enniatin B found maximas tested concentration with Tmax of 12 for the two former and 31 μg/ml for the latter. Other toxins, such as mycophenolic acid, citrinin and phomopsin A, saturated above concentrations of 3.2 μg/ml. Cooperativity (n>1) was found for all toxins. Average adsorption affinity ranked from 100 down
to 20%, in the order beauvericin>>enniatins A/B>citrinin>citreoviridin>mycophenolic acid>phomopsin A. Beauvericin adsorption remained at 100% across concentrations tested. Steady increase of adsorption for enniatins was seen from 30 to 80%. For other toxins, adsorption steadily increased to reach in-between 50 and 60% for the final 5.0 μg/ml concentration. This study further demonstrated the positive large spectrum properties of YCWE toward the adsorption of multiple mycotoxins, including emerging mycotoxins in addition to previously reported mycotoxins.

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META-ANALYSIS WITH META-REGRESSION TO ASSESS THE USE OF YEAST CELL WALL EXTRACT SUPPLEMENTATION ON PIG PERFORMANCE DURING MYCOTOXIN CHALLENGES BELOW OR ABOVE REGULATORY GUIDELINES
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A random-effects meta-analysis was conducted to evaluate the effect of mycotoxins (MT) on the performance of growing pigs, without or with the inclusion of yeast cell wall extract (YCWE, Mycosorb®, Alltech, Inc.) and in contrast to control (CTRL) animals not consuming mycotoxins. Data was extracted from 23 research experiments (30 mycotoxin treatments) and performance parameters investigated were average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G:F). Overall, pigs fed MT had lower ADG (p<0.001) and ADFI (p<0.0001) from CTRL by -84 and -165 g, respectively. Use of YCWE during mycotoxin challenges (YCWE+MT) tended to result in greater ADG (+17 g, p=0.068) compared to MT. The gain to feed ratio (G:F) was not impacted by any treatment. Meta-regression was employed to further explore the impacts of feeding MT to pigs at different levels, either at/below (category 1), or above (category 2), regulatory guidelines suggested by the European Union or United States. For category 1, pigs fed MT had lower ADG (-78.5 g, p<0.001) from CTRL, while YCWE+MT had higher ADG (+48 g, p<0.001) over MT and was similar to CTRL. Although ADFI was not impacted, YCWE+MT had ADFI values similar to the CTRL. In category 2, pigs fed MT had lower ADG and ADFI than CTRL (-85.1 and -166 g, respectively, p<0.0001), with tendency for higher ADFI (+25.3 g, p=0.062) with YCWE+MT. This meta-analysis with meta-regression showed mycotoxin consumption can negatively impact pig performance. Furthermore, swine performance was limited when mycotoxins were both below and above regulatory guidelines. The inclusion of YCWE during mycotoxin challenges resulted in performance equal to the level of unchallenged animals, particularly at mycotoxin levels commonly found below regulatory guidance. As such, this meta-analysis provides useful information to swine nutritionists and producers on not only the impacts of mycotoxins but also a beneficial method for managing mycotoxin.

MANAGING MYCOTOXIN RISKS
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P94
TARGETED INOCULATION WITH _FUSARIUM CULMORUM_: TRACING _FUSARIUM_ TOXINS FROM BARLEY TO BEER
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_Fusarium_ species can infect nearly all types of cereal crops such as barley which is commonly used in beer production. The presence of these fungi results in decreased grain yields and quality issues, and in a potential risk to consumer safety due to the production of mycotoxins. These toxins are known to persist throughout malting and brewing processes and end up in the final beer product. Besides the potential health hazard, fungal infestation can impact the flavour profile of beer. Due to beer's widespread global significance, it is imperative for manufacturers to rigorously implement quality control protocols to ensure the absence of contaminants in raw materials. To enhance the surveillance of mycotoxin levels, the deployment of comprehensive cost-effective methods is pivotal. These methodologies are essential in facilitating appropriate interventions aimed at mitigating potential hazards to consumer health. To further investigate _Fusarium_ infestation of barley during malting, a micro-malting model system was developed. Therefore, targeted inoculation with _Fusarium culmorum_ before germination was used to produce defined, infected material. Mycotoxin production was monitored over malting and following beer brewing processes. Malting process was performed using a standard micro-malting procedure and beer was brewed afterwards on a microbrewery scale. Samples were taken at key steps during malting and beer brewing and analysed for 14 _Fusarium_ toxins using LC-MS/MS.
methods that included the modified mycotoxin deoxynivalenol-3-glucoside (DON-3-glc). To ensure precision in quantification, a stable isotope dilution assay and matrix-matched calibration were employed. Inoculation with *F. culmorum* did not yield in visible fungal growth across all approaches, yet it resulted in toxin biosynthesis in the germinating barley. The present investigation demonstrated an increase in the levels of deoxynivalenol (DON) and its glycosylated derivative throughout the course of the malting procedure, with DON-3-glc manifesting in concentrations surpassing those of DON itself. Substantial quantities of DON, 3-acetyl-DON, and notably, DON-3-glc, were also identified in the subsequently brewed beer. These outcomes signify the concurrent presence of the altered mycotoxin form, underlining its significance in the evaluation of mycotoxin levels within cereals, malt, and beer products.

**P85**

**GRADIENT BOOSTING MACHINE LEARNING MODEL TO PREDICT AFLATOXINS IN IOWA MAIZE**

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Aflatoxin (AFL), a secondary metabolite produced from filamentous fungi, contaminates maize, posing significant health and safety hazards for humans and livestock through toxigenic and carcinogenic effects. Maize is widely used as an essential commodity for food, feed, fuel, and export markets; therefore, AFL mitigation is necessary to ensure food and feed safety within the United States (US) and elsewhere in the world. In this case study, an Iowa-centric model was developed to predict AFL contamination using historical maize contamination, meteorological, satellite, and soil property data in the largest maize-producing state in the US. We evaluated the performance of AFL prediction with gradient boosting machine (GBM) learning and feature engineering in Iowa maize for two AFL risk thresholds for high contamination events: 20 and 5 ppb. A 90%-10% training-to-testing ratio was utilized in 2010, 2011, 2012, and 2021 (n=630), with independent validation using the year 2020 (n=376). The GBM model had an overall accuracy of 96.77% for AFL with a balanced accuracy of 50.00% for a 20 ppb risk threshold, whereas GBM had an overall accuracy of 90.32% with a balanced accuracy of 64.88% for a 5 ppb threshold. The GBM model had a low power to detect high AFL contamination events, resulting in a low sensitivity rate. Analyses for AFL showed satellite-acquired vegetative index during August significantly improved the prediction of maize contamination at the end of the growing season for both risk thresholds. Prediction of high AFL contamination levels was linked to aflatoxin risk indices (ARI) in May. However, ARI in July was an influential factor for the 5-ppb threshold but not for the 20 ppb threshold. Similarly, latitude was an influential factor for the 20-ppb threshold but not the 5 ppb threshold. Furthermore, soil-saturated hydraulic conductivity (Ksat) influenced both risk thresholds. Developing these AFL prediction models is practical and implementable in commodity grain handling environments to achieve the goal of preventative rather than reactive mitigations. Finding predictors that influence AFL risk annually is an important cost-effective risk tool and, therefore, is a high priority to ensure hazard management and optimal grain utilization to maximize the utility of the nation’s maize crop.

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**FATE OF AFLATOXINS, FUMONISINS AND ZEARALENONE DURING GLUTEN-FREE PASTA PRODUCTION**

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A FoodSafeR project aims to conduct in-depth research on the fate of mycotoxins during industrial processing [https://foodsafer.com](https://foodsafer.com). The aim is to investigate and understand how the fungal toxins behave during various food production chains. Based on the current knowledge in this area, it is assumed that the toxins could degrade or be modified due to exposure to energetic and mechanical conditions during the process, and thus form novel modified mycotoxins. Currently, the information on the degradation products of mycotoxins is lacking. Therefore, identification and investigation of toxicity potential of the novel degradation products of mycotoxins is of a high interest risk assessing bodies. Besides rice, mainly maize is being used as a raw material for the production of a gluten-free products. However, maize is often contaminated with mycotoxins, especially aflatoxins, fumonisins, trichothecenes and zearalenone. According to the recent study on mycotoxin occurrence in gluten-free
pasta, 90% and 71% of samples were contaminated with fumonisins and zearalenone, respectively [Tolosa et al., 2021]. This might pose a high health risk to consumers with celiac disease and/or gluten intolerance. The aim of this study was to investigate the fate of aflatoxins, fumonisins and zearalenone during gluten-free pasta production. Preparation of the gluten-free pasta was performed in Barilla. There, pastas from mycotoxin-free maize and maize containing aflatoxins, zearalenone and fumonisins were produced. All stages of the production line were sampled for investigation of degradation products. The preliminary experiments were aimed to a development of a reliable LC-MS/MS method with a focus on estimation of limits of quantification, matrix effects and extraction recovery of the respective toxins. In order to simulate the formation of the degradation products, pure analytical standards of the toxins were dissolved in water and heated under the conditions of gluten-free pasta production. The heated solutions of standards were investigated on novel degradation products which were then screened in the gluten-free pasta produced from mycotoxin contaminated material as well. Performance characteristics of the LC-MS/MS method as well as the preliminary data on novel degradation products of aflatoxins, fumonisins and zearalenone will be presented.

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THE FOODSAFETY4EU STRATEGIC RESEARCH AND INNOVATION AGENDA: BOOSTING FOOD SAFETY IN THE TRANSITION TOWARDS SUSTAINABLE FOOD SYSTEMS

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Funded by a H2020 policy driven call, the FoodSafety4EU project is committed to provide input for the future Food Safety R&I framework and policies. To this scope, the project built a multi-stakeholder collaborative space (platform), where actors of the EU food safety system can meet to interact and network, to create new partnerships and joint activities. The overall ambition of the FS4EU platform is to become a competence/knowledge centre for food safety in Europe, supporting the transformation towards a safe and sustainable food system. Looking at the new sustainability framework from the food safety angle, the FoodSafety4EU project structured a multi-actor dialogue by applying the social lab methodology, to discuss present and future challenges to be addressed for ensuring a safe transition towards increased sustainability. Through this participatory process, selected experts were guided in the co-creation of a Food Safety Strategic Research and Innovation agenda (FS4EU SRIA). The FS4EU SRIA has a strong focus on emerging food safety hazards and risks, and faces two main questions: (i) what are the future research needs to be addressed to ensure a safe transition towards sustainable food systems under the edge of the new sustainability regulation; and (ii) what tools, methodologies, knowledge do we need to address food safety hazards and risks (re)-emerging in circular, bio-based, sustainable food systems of the future. Identification and prioritization of future research needs were first carried out by a multi-actor group in a social lab (over an 18-month process), delivering a transparent and shared priority list of topics for future research. Then, an open consultation to collect input for the new SRIA was conducted, which could reach 311 persons. Finally, feedback by high level experts from EC, JRC, EFSA and other running projects was collected in a cross-fertilization workshop. The topics identified for the new SRIA address 8 challenges, where mycotoxins (re)emerged as cross-cutting issue: (i) climate change and food security; (ii) food supply chain—traceability and transparency; (iii) integration and improving risk assessment methodologies; (iv) rapid technological advancements and emerging technologies; (v) sustainable production/processing; (vi) ethics and one health systemic approach; (vii) science-based decision-making; and (viii) food safety related issues sharing information and resources. Social lab pathway, as well as the results of the public consultation, outlining short/medium/long term actions will be shown. 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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WHO DOES WHAT ALONG THE CASSAVA VALUE CHAIN AND HOW DO THE PRACTICES ALONG THE CHAIN INFLUENCE MYCOTOXIN CONTAMINATION IN UGANDA?

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Cassava is the second most important staple food crop for Uganda and is prone to contamination with mycotoxins. This study aimed at identifying key practices that may result in mycotoxin contamination and to assess the knowledge about aflatoxins within the cassava value chain actors in Uganda. Data were collected from 210 value chain actors (farmers, wholesalers, and processors), 34 key informant
interviews and 4 focus group discussions. The findings revealed that mycotoxin contamination starts at harvest. A significant 51% of farmers peel cassava directly on bare ground resulting in direct contact with soil that potentially harbours mycotoxin-producing species, such as those belonging to the *Aspergillus* section *Flavi*. At the post-harvest stage, 51.62% of farmers dry cassava chips directly on bare ground. Majority (95.24%) of wholesalers’ pack cassava chips in local gunny bags and place them on ground surface instead of pallets. Processing is mainly done at local level, only one out of 14 machines assessed is certified with the Uganda National Bureau of Standards (UNBS). In terms of consumption, 50.77% of the interviewed cassava consumers admitted consuming cassava flour regardless of contamination, and 73% blend cassava flour with flour derived from mycotoxin-susceptible crops mainly maize, millet, and sorghum. Results from knowledge on mycotoxins along the value chain significantly revealed that mycotoxins are a new term to many (96.18%), and aflatoxins are the most known mycotoxins. While more cassava value chain actors (82.86%) were significantly aware of the methods for reducing aflatoxin build-up, only 40.95% were really putting such methods into practise. This study highlights the critical areas of mycotoxin contamination within the cassava value chain in Uganda and underscores the need to improve the knowledge among value chain actors especially farmers.

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**MYCOTOXINS RISK ASSESSMENT FOR CEREALS AND MAIZE IN EUROPE**

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Mycotoxins are natural contaminants caused by fungi attacks on crops. Because of possible harmful effects, these toxins are regulated in Europe and worldwide. At harvest, mycotoxins may be detected and sometimes at high level in crops. Syngenta has monitored a large database to assess recommendation of Good Agriculture Practices, such as variety tolerance, residue management, crop protection, ... Statistical models have been also developed to predict the mycotoxins risk before harvesting with a tool called Qualimetre. Combination of all in a holistic approach contributes to mycotoxins risk mitigation for food safety at farmers and grain collectors but also at food industry level in Europe.

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**PREDICTION MODELS FOR FUNGAL SPOILAGE AND MYCOTOXIN PREVALENCE IN STRAWBERRIES**

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The project FRIETS aims to develop novel processed soft fruits (strawberry, raspberry and blackberry) with superior quality and nutritional characteristics, as well as extended shelf-life. As a contribution to the project, the WP5 – Model-based process optimization and shelf-life assessments – carried out by the University of Malta aims to: (i) identify the most mycotoxin contaminants in strawberries; (ii) construct a comparative risk assessment system for evaluating and ranking fungal and mycotoxin hazards; and (iii) build model-based process optimization for the production of safe and stable products. The methods used were based on (i) benchmarking data (HPLC analysis) and (ii) meta-analysis study. To assess the presence/absence of toxins in local fruits, samples of strawberries from two Maltese farms (Mgarr and Swieqi) were analysed for aflatoxins, ochratoxin A and patulin. The methods were developed and optimised in-house using HPLC-FLD-PDA and showed robustness, accuracy with a reliable linear range ($r^2 \geq 0.99$) and were improved for short runs (<15 min). However, while the methods developed showed that small amounts of toxins could be quantified, the mycotoxins were not detected. Indicating that the samples were harvested and stored in good conditions not allowing fungal development and mycotoxin production. To build the initial prediction models, studies from literature (n=71) were used by transforming data on fungi presence and mycotoxin contamination into presence (1) or absence (0). A logistic regression model was used to describe the probability of mycotoxin production in strawberries. The adjustment and performance of the models were evaluated according to the degree of agreement between observations and predictions, and the receiver operating curve (ROC). The sensitivity (true positive rate) versus specificity (false positive rate) for the prediction of mycotoxin presence in strawberries shows that the model has some discriminatory power (ROC with AUC=0.70), but further optimization is needed to improve its performance. Further work will give us more information to develop the risk of contamination in the chain production. The risk and prediction models in each step of strawberry chain production (post-harvest, retail storage and domestic storage) will be developed.
Ochratoxin A (OTA) is a potent pentaketide nephrotoxin diffusely distributed in food and feed products; it is also carcinogenic, neurotoxic, teratogenic and immunotoxic. The mycotoxin is produced by species of genus *Aspergillus* and *Penicillium*. OTA is the primarily mycotoxin risk in wine and dried wine fruits, and the main source of OTA contamination in grapes is *A. carbonarius*, followed by *A. niger* and *A. welwitschiae*. Geographical regions, climatic conditions, crop and pest management, and grape genotypes influence the contamination risk. So, the availability of validate predictive models could be very useful in supporting the optimization of grape management for the mitigation of OTA content in grapes. So far, the only predictive model for OTA risk in grapes is that developed by Battilani et al. (2015). It uses hourly data on air temperature, relative humidity and rainfall as inputs and provides a risk assessment during the growing season. The model has not yet been validated with real field data. In this framework, the OTA model was implemented in a digital platform, developed within the project ‘Digital Grape’, aiming at supporting the management of main agronomic and phytosanitary practices for precision viticulture (https://digitalgrape.it). The model was elaborated and preliminary tested with experimental data, and successively used to predict OTA risk on 43 Apulian vineyards in two consecutive years (2021-2022). Grape samples were collected at harvest from each field and analysed for OTA content and *A. carbonarius* contamination. The results were compared with the Toxin Index generated by the OTA model at harvest time. Chemical analysis evidenced for both years an OTA content always below the legal limit of 2 μg/kg according to Commission Regulation (EC) No 1881/2006 and *A. carbonarius* contamination ranging from 0 to 1.1x10^7 cfu/g must, while the toxin index varied from 75 to 6,097. In general, the correlation between the Toxin Index and the OTA level was low, mainly for the vineyards located in Salento (South of Apulia), while a higher correlation was observed for vineyards in North of Apulia, especially in 2022. On the opposite, a good correlation between the OTA level and *A. carbonarius* contamination was observed in most of the vineyards in both years. These preliminary results highlighted that the OTA predictive model need to be improved through fine-tuning on experimental data for being useful in managing OTA risk in a smart agriculture system. **Acknowledgements.** This work was partially funded by Puglia Region - Project ‘Digital Grape’ – New Digital Technologies and Decision Support Systems for the Improvement of Quality and Sustainability in Viticulture – P.S.R. Puglia 2014/2020 – Misura 16 – Cooperazione – Sottomisura 16.2 ‘Sostegno a progetti pilota e allo sviluppo di nuovi prodotti, pratiche, processi e tecnologie’.

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**A JOINED-UP APPROACH TO THE IDENTIFICATION, ASSESSMENT AND MANAGEMENT OF EMERGING FOOD SAFETY HAZARDS AND ASSOCIATED RISKS (FoodSafeR)**

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In Europe, foodborne hazards, which include biological and chemical hazards, such as bacteria, parasites, toxins, and allergens, cause already approximately 23 million cases of illnesses and 5,000 deaths per year. Food safety is further under pressure by a range of drivers like climate change, emerging raw materials, an increase in susceptible population groups, and dietary changes. This shows the need for an adaption of food safety management systems, which were established over the past decades in European farmers and food businesses, as well as European food safety governance, to make them more robust in the face of changes that affect our global food systems. To achieve such an
This approach has the potential to enhance pet food safety measures and protect the health of animals. Biagio Zaffora, Biagio.Zaffora@rd.nestle.com

ASSOCIATED WITH MYCOTOXINS IN CEREALS
INTERCOMPARISON OF MACHINE LEARNING APPROACHES TO ESTIMATE RISKS

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MACHINE LEARNING FOR PREDICTING ZEARALENONE OCCURRENCE IN PET FOOD
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Cereals commonly used in commercially available pet foods are susceptible to mycotoxin contamination, which can have deleterious effects on pet health. Among several mycotoxins, zearalenone (ZEN) and T-2 have been detected in both pet food ingredients and final products, leading to acute toxicity and chronic health issues in pets. Hence, the early detection of mycotoxin contamination in pet food is crucial for ensuring the safety and well-being of animals. This study aims to establish a fast and cost-effective method utilizing an electronic nose (E-nose) and machine learning algorithms to predict whether ZEN levels in pet food exceed regulatory limits. A total of 118 pet food samples were collected from different brands between 2021 and 2022. These samples were analysed for ZEN contamination using the liquid chromatography-tandem mass spectrometry (LC-MS-MS) method. Additionally, the ‘AIR PEN 3’ E-nose, equipped with 10 metal oxide sensors, was employed to identify volatile substances within the pet food samples, categorized into 10 different groups. Machine learning algorithms, including k-Nearest neighbour (k-NN), support vector machines, decision tree, random forests, naive bayes, XGBoost, and multi-layer perceptron, were utilized to classify the samples based on the ZEN contamination level (250 μg/kg), as specified by Chinese pet food legislation. The multi-layer perceptron algorithm exhibited the highest discrimination accuracy of 74.56% in differentiating between pet food samples exceeding the ZEN threshold and those that were below it. The accuracy of other machine learning algorithms ranged from 55.62 to 71.83%, indicating their moderate performance in predicting ZEN contamination in pet food. The findings of this study suggest that the integration of an E-nose and machine learning algorithms can provide a rapid and cost-effective method for screening ZEN contamination in pet food at the market entry stage. The multi-layer perceptron algorithm demonstrated the highest accuracy in classifying samples according to the ZEN contamination status. This approach has the potential to enhance pet food safety measures and protect the health of animals.

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INTERCOMPARISON OF MACHINE LEARNING APPROACHES TO ESTIMATE RISKS ASSOCIATED WITH MYCOTOXINS IN CEREALS

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Cereals naturally harbour fungi that can produce mycotoxins, making them susceptible to contamination. Numerous regions across the world grow cereal crops facing a risk of fungal contamination, an issue that climate change is amplifying. The consumption of contaminated cereals can yield adverse effects on both human and animal health. Mycotoxins removal during food and feed processing is complex and often not feasible, therefore their timely detection or prediction is crucial for managing risks. In recent years, a growing body of scientific research has examined the applicability of machine learning to forecast mycotoxin risk levels, highlighting their ability to provide superior predictive performance compared to traditional statistical or mechanistic models. This study undertakes a comparative analysis of the implementation and performance of multiple machine learning classification algorithms. These algorithms are used to assess risks associated with the presence of deoxynivalenol and zearalenone, exceeding defined acceptance levels, in wheat for a selected regional area in Europe. The objective is...
to illustrate minimal data quality requirements and features engineering steps such as missing value replacement, up-sampling and features creation or transformation, as well as to discuss performance metrics. The exploration performed includes a critical review of accuracy-based metrics for classification tasks in which the prevalence of positive samples is low, which is often the case for classification tasks involving mycotoxin risk estimation. The machine learning pipeline outlined in this study, while tailored to specific cereal and mycotoxin combinations within a binary classification framework – low vs. high risk – can be readily extended to encompass other raw materials, additional mycotoxins, and multi-class classification scenarios – e.g., low vs. medium vs high risk.

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MYCOTOXIN RISK MANAGEMENT IN MAIZE GLUTEN MEAL
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Maize gluten meal (MGM) is a by-product of maize starch and ethanol, produced by the wet milling process. Its high protein content makes it a preferred ingredient in feed. Given the high prevalence of mycotoxins in maize globally, they pose a significant challenge to use of MGM for feed: wet milling could concentrate certain mycotoxins in gluten components, and mycotoxin consumption affects animal health and can contaminate animal-source foods. To help confront this issue, mycotoxin occurrence in maize, distribution during MGM production and mycotoxin risk management strategies for MGM were summarized. Available data emphasize the importance of mycotoxin control in MGM and the necessity of a systematic control approach, which includes: good agriculture practices (GAP) in the context of climate change, degradation of mycotoxin during MGM processing with SO2 and lactic acid bacteria (LAB) and the prospect of removing or detoxifying mycotoxins using emerging technologies. In the absence of mycotoxin contamination, MGM represents a safe and economically critical component of global animal feed. With a holistic risk assessment-based, seed-to-MGM-feed systematic approach to reducing and decontaminating mycotoxins in maize, costs and negative health impacts associated with MGM use in feed can be effectively reduced.

P96
PRODUCTION OF MEALWORM LARVAE BIOMASS WITH THE USE OF FEED MATERIALS WITH A HIGH CONCENTRATION OF DEOXYNIVALENOL
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Global warming and changing environmental conditions contribute to the increased prevalence of crop pathogens. As a result, agricultural produce is often contaminated with mycotoxins, highly dangerous compounds that lead to intoxication in humans and animals. One of the strategies for mycotoxin control is to use mycotoxin-adsorbing (sorbents) or mycotoxin-degrading (enzymes) agents as feed additives. The development of innovative feed additives and the assessment of their activity against toxins are among the main research areas in the food processing and animal feed industries. Toxin-producing fungi of the genus Fusarium are most prevalent in countries with temperate climates. In the group of Fusarium mycotoxins, particular attention should be paid to deoxynivalenol (DON), which is most frequently detected and most important in agriculture and livestock production in Central and Northern Europe. The aim of this study was to perform DON biodegradation and to determine whether DON-contaminated feedstuffs can be used in the production of mealworm larvae biomass for animal feed. In a feeding trial, mealworm larvae were fed diets contaminated with DON at two concentrations (D1=663 μg/kg, D2=919 μg/kg) and a control diet (C). The larvae were reared for two weeks under controlled environmental conditions. At the end of the experiment, the larvae and their faeces were analysed for the presence of DON by liquid chromatography–mass spectrometry (LC-MS/MS). It was found that mealworm larvae fed contaminated diets were characterized by normal growth rates, and their faeces contained high concentrations of DON. Mycotoxin absorption from the digestive tract of the analysed larvae was very low, as confirmed by the carry-over factor of DON from feed to larvae biomass (D1, 1.97%; D2, 3.50%). The results of the study indicate that DON-contaminated diets can be used in the production of mealworm larvae biomass, and that the obtained biomass can be safely fed to livestock.
EU LEGISLATION REQUIRES AN OPTIMIZED/VALIDATED METHOD FOR THE OFFICIAL CONTROL OF BENTONITES AS AFLATOXIN INACTIVATORS

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In Europe, bentonites are allowed as feed additives for aflatoxin mitigation (1m558) provided they have specific mineralogical characteristics and an aflatoxin-binding capacity ($BC_{Afb1}$) $>90\%$. $BC_{Afb1}$ is determined by an official adsorption assay using an aflatoxin solution (4 mg/l) in acetate buffer (pH 5.0) and a bentonite at 0.02% (w/v). To date, the robustness of this method has not been investigated. In this work, we addressed this challenge and performed a robustness study by analysing six bentonites that met the mineralogical requirements for claim code 1m558. Fixing experimental conditions of the EU official assay, main leading parameters of this method were analysed and varied one-by-one. Leading factors selected for robustness testing were: (i) preparation mode of bentonite suspension; (ii) residual amount of acetonitrile in the test trial; (iii) acetate buffer concentration; (iv) incubation time; and (v) centrifugation. In some cases, different levels of each parameter were evaluated. Thereof, bentonites were first tested according to the experimental condition described in the EU method. Then, they were tested again by making some changes, each one in independent, triplicate experiments. It was statistically evinced that although some variations in the protocol, i.e., the optimization of acetonitrile concentration in the working solutions, centrifugation conditions, and buffer concentration, produced an effect on the AFB1 adsorption by materials, only a combination of all variations determined a strong, significant effect on $BC_{Afb1}$ values. Due to its weakness, the method excluded four out of six bentonites from being marketed in the EU because their $BC_{Afb1}$ values were $>90\%$. A new protocol was developed by keeping the main experimental parameters of the official assay and was in-house validated according to international harmonized guidelines. This protocol yielded $BC_{Afb1}$ values $>90\%$ for all test bentonites and showed satisfactory precisions with a RSD of 3.4% and HorRat$<2$. This value falls within the acceptability criteria stated in the AOAC guidelines for standard method performance requirements. The validity of the optimized method was proven by the Langmuir isotherm approach, which allowed the calculation of the maximum adsorption capacity ($B_{max}$) and affinity ($K_d$) of test bentonites. These adsorption parameters were compared with $BC_{Afb1}$ values measured with both the official and optimized assays. The study helped us rank the best aflatoxin-adsorbing bentonites and confirm the suitability of the optimized protocol for $BC_{Afb1}$ measurement. An interlaboratory study for the validation of this method is recommended. Application of the protocol to bentonites other than montmorillonite was demonstrated.

Acknowledgements. This work was supported by the FoodSafety4EU Project (European Union’s Horizon 2020 Research and Innovation Programme) under Grant Agreement No. 101000613.

A DNA-BASED BIOSENSOR FOR THE FAST AND SENSITIVE DETECTION OF OCHRATOXIN A IN URINE WITH ISOTHERMAL ROLLING CIRCLE DNA AMPLIFICATION

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Ochratoxin A (OTA) is a toxic and teratogenic metabolite produced by fungal species of the genera Penicillium and Aspergillus. Analysing OTA in food and monitoring its presence in biological samples is recommended to assess individual exposure to the mycotoxin. The primary technique used for OTA detection in biological samples is LC-MS/MS. However, biosensors provide a viable alternative for mycotoxin detection due to their high portability potential and ease of use. Various types of biosensors have been developed for OTA detection, relying on the specific recognition of OTA by DNA aptamers. DNA's engineering versatility makes it a powerful and programmable element for constructing micro-scale systems that find numerous applications in biosensing. In our study, we present a DNA-based biosensor for detecting OTA in urine. The sensor consists of a DNA-based capture system and a detection system. We created paramagnetic microbeads carrying a capture aptamer for OTA, enabling its specific capture in liquid samples. A detection complex, which triggers an isothermal rolling circle amplification (RCA), was assembled using the same aptamer annealed to a circularized probe. This complex was used to detect the occurrence of toxin capture. We designed the RCA to generate autocatalytic units with peroxidase activity (DNAzyme). In the presence of OTA, the circular DNA initiates its isothermal amplification at 30°C, producing a single-stranded and tandemly repeated long
homologous copy of its sequence. Within the amplified DNA strand, a peroxidase self-catalytic structure induces a colour reaction that is visible to the naked eye. The resulting biosensor exhibited high sensitivity and selectivity for detecting OTA, with a limit of detection as low as 1.09×10^{-12} ng/ml. Furthermore, we tested the biosensor for OTA detection in naturally contaminated urine. Accuracy and repeatability data obtained from recovery experiments showed recoveries exceeding 95%, with relative standard deviations ranging from 3.6 to 15%. For the first time, an aptasensor has been successfully applied to detect OTA in biological fluids. It can be used for mycotoxin biomonitoring and assessment of individual exposure.

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VALIDATION OF MILKSAFE™ AFLAM1 RAPID TEST KIT FOR AFLATOXIN M1 RESIDUES IN MILK
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The MilkSafe™ AflaM1 (Chr. Hansen S/A, Hørsholm, Denmark) was validated at ILVO concerning the evaluation of detection capability, discordant results, selectivity, robustness, and suitability for various milk types and milks from various species. The test provides a quantitative value except for blank milk indicated as '<15 ng/l' or milk with a AFM1 concentration above 150 ng/l indicated as '>150 ng/l'. The theoretical cut-off (mean value 50 ng/l – 2×SD) which discriminates samples with aflatoxin M1 in a concentration that possibly is exceeding the maximum level (ML) is 36.8 ng/l (95% detection). Results show that at 25 ng/l AFM1, no false positive results were obtained. On a total of 100 farm and 100 tanker milk samples, a 2% rate of discordant (false positive) results was obtained. It is worth noting that retesting of the presumptive positive milk samples resulted in a negative result. The test is selective for aflatoxins and shows no cross-reactivity with other mycotoxins. Cross-reactivity was noted for aflatoxin B1, G1, G2 and M2 at 50 ng/l, for B2 cross-reactivity was observed only at high concentrations. Test and reader repeatability values were very good for a quantitative lateral flow device, with a coefficient of variation of 9.7 and 4.7%, respectively. Changes to the test protocol could result in small influences on the quantitative results, it is therefore recommended to follow manufacturers’ guidelines. Also, the impact of milk quality (somatic cell count, aerobic plate count), milk composition (fat and protein content), milk pH, milk type (UHT, sterilized milk, reconstituted milk powder and whey products) and milk from other species than cow (goat and ewe) was tested. Out of the results could be concluded that in raw cows’ milk with a high somatic cell count or high protein content, sweet whey powder and ewes’ milk the detection capability is diminished. More details will be given on the poster. Summarising, MilkSafe™ AflaM1 is a fast (10 min), simple and reliable highly specific quantitative test for screening for aflatoxin M1 in raw commingled cows’ milk at EU ML of 50 ng/l AFM1 (Commission Regulation (EU) 2023/915).

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ANALYSIS OF ERGOT ALKALOIDS IN A VARIETY OF SIMPLE AND COMPLEX MATRICES BY LIQUID CHROMATOGRAPHY-TANDEM QUADRUPOLE MASS SPECTROMETRY
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Ergot alkaloids are mycotoxins produced by fungi including Claviceps spp. and Claviceps purpurea. Ergot alkaloids are most commonly found in rye, wheat, barley, oats, and other cereal grains as well as forages. Ergot bodies can be identified visually as dark brown to black growths at the seed heads of grasses and grains. The presence of ergot dates back to 600 BC where an Assyrian tablet mentions ‘noxious pustules’ on grain seeds and in the Middle Ages when people were eating contaminated rye bread that caused thousands of people’s deaths. On January 1st of 2022, the EU published maximum levels of ergot sclerotia and ergot alkaloids at the new maximum of 0.5 g/kg of ergot sclerotia in unprocessed rye and 0.2 g/kg of ergot sclerotia in unprocessed cereals with the exception of maize, rye and rice, and 150 ppb ergot alkaloids in milling products of barley, wheat, spelt, and oats; maximum level of ergot alkaloids in wheat gluten of 400 μg/kg and 20 μg/kg for processed cereal based food for infants and young children. A method was developed to identify and quantify ergot alkaloids in various matrices including, rye, wheat, barley, oats, finished feeds, feed ingredients, and other matrices. The method developed utilizes liquid chromatography coupled with tandem quadrupole mass spectrometry. The alkaloids included in this analysis include ergometrine, ergosine, ergotamine, ergocornine, ergocryptine, ergocristine, and ergovaline as well as their corresponding -inine isomers.
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IMPROVEMENT OF AN INTEGRATED AND FAIR (FINDABLE, ACCESSIBLE, INTEROPERABLE, REUSABLE) WORKFLOW FOR LC–HRMS METABOLOMICS STUDY. CASE STUDY: METABOLOMICS ANALYSIS OF FUSARIUM VERTICILLIOIDES MUTANTS

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An integrated and open-source workflow for liquid chromatography-high resolution mass spectrometry (LC-HRMS) metabolomics studies based on open-source data processing tools was proposed in a previous work [Ciasca et al., 2020] and tested through a case study focusing on Fusarium verticillioides (host/pathogen) interaction. The key steps of the proposed workflow were as follows: (i) experimental design; (ii) sample preparation; (iii) LC-HRMS analysis; (iv) data processing; (v) custom database search; (vi) statistical analysis; (vii) compound identification; and (viii) biochemical interpretation. The present work describes a significant improvement of the previous workflow, to make it fully compliant with the FAIR principles. The FAIR principles – Findability, Accessibility, Interoperability, and Reusability – have been formulated, and then boosted to promote open science, maximize access to and re-use of research data generated [Wilkinson et al., 2016]. To this purpose, the following aspects were improved: a framework for the description of the metabolomic experiments such as information about the sample preparation protocol, sample collection and LC-HRMS condition based on the standard metabolomics reporting structure (SMRS) was designed; data processing was implemented with the latest software version (MZmine 3.0); automatization of feature annotation by fragmentation pattern comparison and annotation of MS2 spectra; integration of new modules of MetaboAnalyst (v 5.0) for the biochemical interpretation. Furthermore, one additional step was added to the workflow, enabling acquisition and analysis of MS2 spectra either in data independent analysis (DIA mode), and data dependent analysis (DDA mode). The applicability of the developed approach was explored through the investigation and comparison of the metabolic profile of two mutant strains of F. verticillioides, blocked at different steps in the fumonisin biosynthetic pathway, grown on malt extract agar. Metadata and raw data will be shared on MetaboLights according to the FAIR principles.

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SIDE BY SIDE COMPARISON OF HPLC AND AN AUTOMATED MYCOTOXIN DETECTION PLATFORM

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Fusarium species is a significant toxin-producing fungi. Of the more than five hundred mycotoxins, several trichotheccenes, fumonisins and zearalenone have global significance. They are toxic to humans and the animals causing significant health hazards, some have also carcinogenic effect. Deoxynivalenol is a representative of trichotheccenes, appearing on cereals including wheat, maize, barley. According to a recent survey in Hungary, deoxynivalenol is the most common mycotoxin in small grain cereals (e.g., wheat, barley, oat, rye). When infected grain is digested by animals, it reduces food intake causing weight loss. In general, they adversely affect offspring growth and they increase susceptibility to infections. In humans, deoxynivalenol can cause nausea, vomiting, diarrhoea, abdominal pain, headaches, and dizziness. Lengthy exposure leads to compromised immune and hematopoietic system and may cause death. Even low-level exposure can alter immune functions. There is a global demand for more accurate, robust, simpler, sensitive and faster assays. Multimycotoxins analysis is preferable as it is less labour-intensive. Currently, the most frequently used mycotoxins detection methods are ELISA, LFD, TLC, HPLC-MS, and GC-MS. To deal with climate change, a cost-effective alternative to HPLC is in demand. Methods are available for simultaneous quantitative analysis for multiple mycotoxins. They are immunoassay-based multiplexed assays performed with flow cytometry applying fluorescent microspheres. Finally, there is a platform solution which integrates sample preparation with multimycotoxin analysis. This study compared UPLC-MS/MS with an automated platform called MycoFoss™ analysing the mycotoxin DON. MycoFoss™ is the first fully automated mycotoxin analysing method that combines preparation and analysis. It combines sample preparation with multiplexed fluorescent microsphere technology using flow cytometry. For the UPLC-MS/MS analysis, the methodology of the accredited Bonafarm Feed Laboratory Nagyigmánd (Hungary) was used. Bland-Altman plotting, generally used to compare different measurements methods, was used to determine agreement. In this test, untreated and artificially contaminated samples
both containing nine samples coming from natural inoculation were tested by the MycoFoss detection platform on maize in February 2023 for DON. The data showed a close correlation between the UPLC-MS/MS technology and the MycoFoss™ method. As the tests takes 8 min and its exactness is close to UPLC-MS/MS, the praxis has a new and rapid instrument to test toxin contamination at harvest to separate the lots below and higher than the toxin limits of the country and provide grain with low toxin contamination granted.

P103 DEVELOPMENT OF AN UPLC-MS/MS METHOD FOR THE SCREENING AND QUANTITATIVE DETERMINATION OF MULTIPLE MYCOTOXINS IN EGG YOLK AND WHITE IN ORDER TO EVALUATE THE CARRY-OVER FROM NATURALLY CONTAMINATED FEED TO EGGS

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Mycotoxins are toxic compounds that are naturally produced by different types of fungi. If animals consume contaminated feeds, mycotoxins can occur as the parent components and/or metabolites in animal derived food products, such as eggs, which ultimately can reach the human food chain and pose a substantial health risk. The most common mycotoxins that pose a concern to human or animal health include aflatoxins, ochratoxin A, and Fusarium toxins, for which the European Union has set maximum levels in certain foodstuffs (Commission Regulation (EU) No 1881/2006). A high-throughput multi-method was optimized and validated for the screening and quantification of 37 selected mycotoxins in chicken egg yolk and white using ultra-high performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). The investigated mycotoxins belong to the regulated groups and to groups of emerging mycotoxins, such as Alternaria mycotoxins and enniatins. Sample preparation of egg yolk and white consisted of a liquid extraction using 0.1% formic acid in acetonitrile, followed by a further purification using Oasis® Ostro. Chromatography was performed on an Acquity Premier BEH C18 column (50x2.1 mm i.d., dp, 1.8 μm) using 0.1% acetic acid in water and methanol as mobile phase A and B, respectively. A gradient elution was performed. ¹³C-labelled internal standards were used for most groups of mycotoxins. The UPLC-MS/MS method was validated in accordance with the VICH GL49 guidance (European Medicines Agency) and the following parameters were evaluated: linearity, accuracy, within-day and between-day precision, limit of quantification (between 0.05 and 2.5 ng/g), limit of detection, selectivity and carry-over. The applicability of the method was tested by the analysis of egg samples that were taken in Jimma, Ethiopia as part of an animal trial that was performed to evaluate the carry-over of mycotoxins from naturally contaminated feed into animal-derived food products, such as eggs. Results will be presented.

P104 RAPID SCREENING OF DEOXYNIVALLENOL IN WHEAT BRAN BY LOW-COST POCKET FLUOROMETER

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Durum wheat bran for direct human consumption is one of the most commonly added components in high-fibre breakfast cereals, bread and baked goods. Deoxynivalenol (DON) is a Fusarium mycotoxin commonly occurring in wheat and derived products with several adverse and toxic effects in animals and humans. Although bran fractions produced by milling of wheat have numerous health benefits, cereal bran is the part of the cereal with the highest concentration of DON, thus representing a risk for consumers. In order to protect the health of consumers from the exposure of DON through the consumption of cereals and cereal-based products, the European Commission has set DON maximum permitted levels in these food products, and specifically a level of 750 μg/kg for wheat bran. Reliable screening methods, to assess the compliance of the food with the legislation in force and reduce the number of samples to be analysed by confirmatory methods, is highly demanded. In the present work, the use of a low-cost and pocket-size fluorometer, using a LED array for illumination and a miniaturized spectrometer for detection, is described for a “green” screening of DON-contaminated durum wheat bran samples. Three wavelengths were used for fluorescence excitation, i.e., 355 nm, 365 nm, and 375 nm. Principal Component-Quadratic Discriminant Analysis was employed as classification technique, using a cut-off value of 400 μg/kg DON for distinguishing samples in two contamination classes. Furthermore, an intermediate level data fusion was employed, for combining information coming from...
the single and multiple excitation wavelengths. The resulting models were rated in terms of accuracy (64-92%), sensitivity (78-100%), and specificity (48-100%). The model, combining the 365 nm and 375 nm wavelengths, showed the best scores with accuracy, sensitivity and specificity of 92%, 100% and 80%, respectively. For the first time, fluorescence spectroscopy, carried out by means of a pocket-size fluorometer, was successfully used for screening wheat bran naturally contaminated by DON and assess their compliance with EC regulation. **Acknowledgements.** The present work has received funding by the European Union’s HORIZON 2020 – EU.4.b. Twinning of research institutions under Grant Agreement No. 952337 (MycoTWIN).

P105 **MYCO-TECH: A PLATFORM FOR MYCOTOXIN DIAGNOSTICS AND TECHNOLOGIES**

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Mycotoxins are naturally occurring fungal metabolites, contaminating a variety of food and feed commodities. They form a heterogeneous group of contaminants exerting a broad range of toxic effects (carcinogenicity, hepatotoxicity, immunotoxicity,...) on humans and animals. Mycotoxins are produced during pre-harvest and/or post-harvest (storage, transportation, processing practices of food & feed). Contamination results in reduced crop yields and livestock productivity, food and feed waste, causing drastic economic losses and trade implications on national and international level. Recent studies have shown that the prevalence of mycotoxins in food and feed is up to 60-80% of which 50% of all crops worldwide exceed the guidance levels or maximum allowed levels. Despite strong efforts to control fungal growth, mycotoxin contamination remains a global problem which demands a concerted and sustainable approach. Additionally, climate change and global warming are worsening the mycotoxin threat. Myco-Tech is a valorised platform for mycotoxin diagnostics and technologies, linked to MYTOX (www.mytox.be), which is coordinated by the Centre of Excellence in Mycotoxicology and Public Health at Ghent University (Belgium). Myco-Tech offers customized mycotoxin testing and analyses in feed, food and biological samples. Furthermore, tailor-made methodologies for efficacy testing of detoxifying agents (such as binders and modifiers) are included. By adding these detoxifying agents to feed commodities, the toxic effects of mycotoxins may be prevented. As current research is more and more focusing on mycotoxin metabolization and identification of biomarkers, Myco-Tech will deliver in the near future standards of mycotoxin derivatives (i.e., biomarkers) and conjugates. On the current market, these standards are lacking and are absolutely of crucial importance to fill this huge gap in mycotoxin research. Moreover, products such as synthetic and natural (antibodies) recognition elements for implementation in mycotoxin diagnostics and technologies will be delivered.

P106 **VALIDATION OF THE AQUALOG® SYSTEM FOR RAPID AFLATOXIN QUANTIFICATION IN MAIZE SAMPLES**

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Aflatoxins are toxic fungal metabolites frequently contaminating human foodstuffs such as cereals, beans, fruits, nuts and spices. These toxins are strongly carcinogenic and can cause both acute and chronic toxicity in humans. Therefore, it is important to keep the exposure through food as low as possible and to have fast and reliable analytical methods which can monitor and quantify the presence of aflatoxins. For this study a unique optical spectrometer is used to monitor and quantify the amount of aflatoxins (AFB1, AFB2, AFG1, AFG2) in maize samples. It simultaneously acquires fluorescence excitation-emission matrix (EEM) (few seconds), absorbance and transmittance. The instrument uses the absorbance data to correct the fluorescence EEM for the inner filter effects (IFE). The applied technique is referred to as: absorbance, transmittance and excitation-emission matrix, an A-TEEM™ molecular fingerprint. Compared to the conventional chromatographic methods A-TEEM™ is faster, more sustainable and has a lower per-measurement cost, while sharing the same low limits of detection, reproducibility, and repeatability. The aim is to evaluate the applicability and valorisation of this spectrometer system for analysis of aflatoxins in maize. An uncomplicated method will be designed and optimized for the instrument for on-site analysis of crops and food products at the farmer, processing and food production sites, respectively. After extraction from maize, the supernatants are diluted and analysed with the Aqualog®. PARAFAC analysis is then applied to decompose the signal. Preliminary
results indicate a limit of detection of aflatoxin B1 in the ppt range. This approach shows great potential and is a good first step towards a rapid, sensitive and inexpensive detection method for mycotoxins.

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RESULTS OF A COLLABORATIVE PRE-TRIAL FOR THE EVALUATION OF A NEW ANALYTICAL METHOD FOR THE DETERMINATION OF AFLATOXIN M1 IN CHEESE PRODUCTS
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Aflatoxin M1 (AFM1) is the major hydroxylated metabolite of aflatoxin B1 and is excreted by urine and milk. AFM1 is a concern when found in milk and dairy products originating from animals who ingested AFB1 contaminated feed. Commission Regulation (EU) 2023/915 has set maximum levels for ‘raw milk, heat-treated milk and milk for the manufacture of milk-based products’. Milk and dairy products are a food category of great importance in diet, consumption figures of dairy and cheese products in g per day, are high not only in Italy but also in the majority of EU countries where percentages of consumers only among all subjects in the national consumption surveys are above 70%. In order to support the official laboratory network in the burden of multiple matrices analyses, the Italian National Reference Laboratory for mycotoxins, in cooperation with the National Reference Center for cow milk quality (IZSLER), has organised a collaborative pre-trial based on the analytical method developed by the IZSVe for the analysis of AFM1 in cheese. The method enables the determination of AFM1 by ELISA assay or by liquid chromatography with FLD or MS/MS detectors, after immunoaffinity clean-up. Twenty independent laboratories were enrolled and asked to analyse with the proposed analytical protocol three samples (sample A (LOQ), sample B (naturally contaminated) and sample C (spiked)) belonging to two different types of cheese (semi hard and hard) in blind duplicate. Overall, the pre-trial was positively evaluated both in terms of results obtained and in terms of participating laboratories, given that all 20 participants who joined the trial sent their results. Regarding performances of repeatability, reproducibility and accuracy, the values obtained for the samples B and C were excellent showing RSD of 4 and 7%, RSDR of 21 and 16% and recovery equal to 88%, thus demonstrating the good performances of the method applied. The ‘LOQ sample’ showed certain inconsistency for reproducibility parameter (56%) due to the high variability of AFM1 value probably for not sufficient homogeneity. The method applicability is in the range 0.05 up to 0.50 µg/kg of AFM1. In conclusion, the method demonstrated to be suitable for the determination of AFM1 in cheese at contamination levels that are consistent with the ML in milk and will, therefore, be used for the organization of a more exhaustive interlaboratory study which will include the analysis of several samples of different types of cheese.

P108
COMPREHENSIVE ASSESSMENT OF THE EFFECTIVE HOMOGENIZATION OF THE AGGREGATE SAMPLE PREPARED FOR TESTING MYCOTOXINS IN OFFICIAL CONTROL
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Mycotoxins are toxic compounds naturally produced by different types of fungi belonging mainly to the genera Aspergillus, Penicillium and Fusarium. In particular environmental conditions of temperature and humidity these fungi proliferate on vegetable substrates and produce mycotoxins. The highly heterogeneous distribution of mycotoxin in agricultural commodities poses a challenge for the collection of representative samples to be tested during official controls. European sampling regulations addressed to mycotoxins establish procedures to be strictly followed to guarantee representativity of the aggregate sample (AS). In this study, four different approaches to the aggregate sample preparation (hand-mixing of the raw grains; coarse milling; fine milling; water-slurry mixing) were evaluated and compared, and, by means the measure of the variability of the contaminant and the assessment of the sample comminution efficacy, the procedure which better reduces the variance to acceptable levels was established. From each of the four homogenised samples, 10 testing aliquots, 25 g each, were taken and analysed by HPLC-FLD in order to measure the concentration of aflatoxins (B1, B2, G1 and G2) and fumonisins (B1 and B2). The level of variability of aflatoxins and fumonisins content in the AS was measured by means of relative standard deviation of repeatability (RSDr) obtained by 10 independent
analyses. RSDr showed similar trend for each mycotoxin class in the four different procedures, with RSDr in grain > RSDr in coarse > RSDr in fine ≈ RSDr in slurry. Prescriptions for analytical variability in terms of method precision are reported in Commission Regulation (EC) No 401/2006, which states for aflatoxins a RSDr obtained calculating the 0.66xRSDr of 22% (i.e., RSDr ≤15%) and for fumonisins a value ≤20%. Taking these RSDr values as the acceptable criteria of the homogeneity testing, the performance obtained for aflatoxins in grain (242%) and in coarse (138%) are considered unsatisfactory, while values obtained for fine (2.1%) and for slurry (1.7%) are considered satisfactory. Comparably, for fumonisins while the RSDr values obtained for grain (38%) is considered unsatisfactory, the values for coarse (8%), for fine (7%) and for slurry (6%) are considered satisfactory, suggesting that fumonisins reach homogeneity with a lower comminution grade. These outputs enable comprehensive assessment of the effectiveness of sample preparation to achieve complete homogenization. Competent Authority will direct official laboratories to pursue sample preparation of only fine materials or water-slurry by adopting a suitable procedure for the preparation of AS of unprocessed grain materials for testing mycotoxins in official control.

P109
DEVELOPMENT OF METHOD OF ENZYMATIC HYDROLYSIS FOR INDIRECT QUANTIFICATION OF MYCOTOXIN-GLYCOSIDES IN CEREALS
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After infection of crops by microscopic filamentous fungi, mycotoxins are produced and further modified by plant enzymes. The most common mechanism of fusarium mycotoxins modification is conjugation with glucose, but also with oligosaccharides and polysaccharides. Although this reduces the toxicity of mycotoxins to plants, enzymes present in the gastrointestinal tract of animals or enzymes involved in certain food processing techniques are able to hydrolyse the glycosidic linkage between the carbohydrate and the mycotoxin. While the analysis of free mycotoxins is nowadays common practice, the quantification of oligo-/polyglycosides is rather difficult. This leads to an underestimation of the real risk estimate. There is thus an urgent need to develop an effective way for their quantification. Our study was aimed to develop an analytical method for indirect quantification of modified trichothecenes by using enzymatic hydrolysis of cereal matrix and subsequent analysis of the free mycotoxin forms. Our previous studies have shown that hydrolysis with the gradual addition of enzymes in a specific order is very effective. Therefore, in this study we aimed to evaluate the yields of enzymatic hydrolysis under different conditions of physicochemical pre-treatment and to characterize in detail the mono-/oligoglycoside residues of mycotoxins after hydrolysis in order to elucidate their primary native structure. In addition to the relatively widely discussed deoxynivalenol-glycosides, also the less studied glycosides of nivalenol, HT-2 toxin, T-2 toxin, neosolaniol and diacetoxyscirpenol were also targeted. The analyses were performed by ultra-performance liquid chromatography with high-resolution mass spectrometric detection.

P110
DEVELOPMENT OF A MULTI-TOXIN UPLC-MS/MS METHOD FOR 50 MYCOTOXINS AND TROPANE ALKALOIDS IN CEREAL COMMODITIES
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Mycotoxins are naturally occurring, notoriously toxic compounds to both humans and animals. They can occur in high frequency and concentrations in cereals, and in other food and feedstuffs. The demand for testing for masked, modified, and emerging mycotoxins has significantly increased over the last decade, as ongoing studies provide a steady stream of insights about newly discovered mycotoxin metabolites - as do plant breeding efforts adapting to a changing climate. Hence, there is a need to extend the scope of analysis to cover these compounds not already legislatively regulated. A multi-toxin UPLC-MS/MS method for 50 regulated and emerging mycotoxins, atropine, and scopoline in cereal-based products was developed. A mixture of wheat, barley, rice, and maize flours were extracted using a simple “dilute-and-shoot” protocol, without clean-up or internal standards. Calibration curves were plotted using solvent standards and matrix-matched calibration. Coefficients of determination (r²) were almost all >0.99. The calibration range covered three orders of magnitude for most analytes, and values for relative standard deviation (%RSDr) were ≤10% for all analytes. Matrix effect ranged from -100% to +83%. Data was imported into the waters_connect for Quantitation software and processed with the MS Quan app for an improved efficiency in data processing and review. Ion ratios and retention times
from the spiked test portions agreed well with the criteria specified in the guidance document on identification of mycotoxins in food and feed (SANTE 12089/2016) for all compounds. The sensitivity of the Xevo TQ-XS allows considerable dilution of the sample extract while still reaching extremely low limits of quantification – the lowest method limit of quantification (m-LOQ) was for aflatoxins (0.1 μg/kg).

P111
SUSTAINABLE ANALYSIS OF DEOXYNIVALENOL IN WHEAT VIA ATTENUATED TOTAL REFLECTION INFRARED SPECTROSCOPY
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Cereal production is significantly influenced by extreme temperature fluctuations and unpredictable rainfalls, which pose a challenge for in-field fungal growth control. Species such as *Fusarium graminearum* and *Fusarium culmorum* are highly prevalent in pre- and post-harvesting stages, producing a high-incidence mycotoxin in cereal commodities, known as deoxynivalenol (DON). DON is a concern for food producers and authorities due to its potential causing exposure-related diseases to consumers [Paterson et al., 2010]. Therefore, food safety control assurance relies on innovative and sophisticated analytical methods for reliable detection of DON in cereal products [Femenis et al., 2022]. This study introduces a novel approach utilizing simultaneous optimized solvent-based DON extraction from wheat and attenuated total reflectance Fourier transformed infrared (ATR-FTIR) spectroscopy. Advanced chemometric data analysis, such as Principal component analysis (PCA) was utilized for reducing data dimension and identifying outliers. Additionally, classification models via sparse partial least squares discriminant analysis (SPLS-DA) were implemented. The classification threshold was established at 1250 μg/kg, in accordance with the European Union regulation for DON. Hence, classification models on water extracts achieved a cross-validation accuracy of 91%, whereas for mixtures of ethanol:water (30:70) and methanol:water (30:70), the accuracy stood at 88.5% and 84.6%, respectively. The results suggest that the utilization of water extraction and ATR-FTIR analysis makes the entire process environmentally sustainable, a crucial consideration in an era of increasing challenges due to evident climatic changes. Acknowledgments. This work was supported by the EU Horizon 2020 project PHOTONFOOD [#101016444] which is part of the PHOTONICS Public Private Partnership.

P112
ON-SITE ANALYSIS OF MYCOTOXINS VIA INFRARED SPECTROSCOPY
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Deoxynivalenol (DON) is a secondary fungal metabolite produced primarily by the plant pathogens *Fusarium graminearum* and *Fusarium culmorum*. The excessive consumption of DON causes serious health problems in humans and animals (the tolerance daily intake for humans is 1 μg/kg of body weight per day). Acute exposure to DON causes diarrhoea, headaches, dizziness, fever, and vomiting. Because of these dangers, DON has become a global food safety issue. Therefore, it is important to have robust analytical techniques to detect DON contamination in cereals on-site to avoid health problems of humans and animals as well as economic losses throughout the food chain. In the current study, we introduce attenuated total reflection infrared (ATR-IR) spectroscopy as a reliable, rapid, and sustainable tool for mycotoxin screening in cereals, aiming DON detection in wheat. We tested a handheld, portable mid-fidelity (Mi-Fi) spectrometer in order to prove the scalability of IR spectroscopy towards on-site analysis. In the present study 26 samples with DON concentrations between blank and 10600 μg/kg were obtained by blending two wheat varieties, extracted with ethanol:water (30:70) mixture and water (100) subsequently analysed via Mi-Fi spectrometer. To evaluate the performance of the device, the advanced chemometric method sparse partial least squares discriminant analysis (SPLS-DA) was applied to build classification models. The European Union limit for DON in wheat of 1250 μg/kg was used as a threshold to establish the models and to differentiate high and low DON-contaminated wheat samples. The obtained results demonstrate a cross-validated classification accuracy of 84.6 % for ethanol:water (30:70) and 70.1% for water (100). The achieved results demonstrate great potential of infrared spectroscopy for the rapid, reliable on-site analysis of DON in
wheat. **Acknowledgments.** This work was supported by the EU Horizon 2020 project PHOTONFOOD [#101016444] which is part of the PHOTONICS Public Private Partnership.

P113 INTERCONNECTABLE 3D-PRINTED SAMPLE PROCESSING MODULES FOR PORTABLE MYCOTOXIN SCREENING OF INTACT WHEAT

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The increasing demand for food and feed products is stretching the capacity of the food value chain to the extent that in the forthcoming years it will reach its limit. An essential measure to ensure food safety involves the assessment of mycotoxin contamination in wheat. However, this evaluation typically involves laborious and costly chromatographic techniques, like liquid chromatography-tandem mass spectrometry (LC-MS/MS). These approaches require extensive sample preparation, which is not convenient at various stages of the food distribution process. Therefore, the rapid screening of mycotoxins, performed on-site and by non-experts, is an important asset for future food safety management. To address difficulties in sample handling, we designed interconnected 3D-printed modules capable of on-site integrated sample preparation. This includes activities such as grinding wheat kernels and solvent-based extraction. Subsequently, we analysed these modules for their effectiveness in mycotoxin screening, and we compared them against a laboratory mill using a commercially available lateral flow screening device (LFD) and LC-MS/MS analysis as reference method. We evaluated different sieve configurations integrated into the system based on their grinding efficiency. Ultimately, a 2 mm sieve size was chosen, enabling the grinding of 10 g of wheat within a 5-minute timeframe. By utilizing the developed 3D-printed prototype, we successfully detected deoxynivalenol (DON) in naturally contaminated samples below the relevant legal limit set by the European Commission (1.25 mg/kg). The entire process, from sample preparation to screening outcome, was accomplished in just 15 min. We found a linear correlation ($r^2=0.96$) between the results obtained from the LFD screening and LC-MS/MS reference analysis. This proof-of-concept shows the potential of 3D-printed equipment for sample handling, offering a valuable expansion of existing analytical protocols. The advances reported here facilitates the practical implementation of rapid on-site screening techniques for mycotoxin assessment in grains. The developed prototype is cost-effective and employs biodegradable 3D printing material and can be printed for 2.5 € material costs. This can be produced using readily available consumer-grade printers and subsequently shows potential for distributed manufacturing. In the future, the modular nature of our innovative toolkit could be exploited towards the adaptation to other solid food commodities, beyond the scope of DON determination in wheat. **Acknowledgements.** This research is part of the PHOTONFOOD project, which received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No. 101016444 and is part of the PHOTONICS Public Private Partnership.

P114 DETERMINATION OF MYCOTOXINS AND DERIVATIVES IN BIOLOGICAL FLUID SAMPLES USING LC-MS/MS AND HPLC METHODS

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Myco toxins are secondary metabolites produced by some fungi that are usually present in feeds and forages. Many species of animals that eat these products are exposed to these compounds. Toxic effects of the mycotoxin have been described in farm animals. The toxicological effect manifests itself by gastrointestinal disorders such as vomiting and diarrhoea and reproductive disorders [Pierron et al., 2016; Jakovac-Strajn et al., 2009]. Therefore, monitoring the presence of these mycotoxins and their metabolites in fluids, such as blood plasma, milk or urine, can be useful to prevent disease. Hence, many analysis methodologies for biological samples from different animals have been proposed [Sørensen and Elbæk, 2005; Razzazi-Fazeli et al., 2003; Stastny et al., 2019; Broekaert et al., 2014]. A mycotoxins LC-MS/MS method for the simultaneous detection of aflatoxin B1, fumonisin B1, deoxynivalenol, 3-acetyl-deoxynivalenol, fusarenone X, tenuazoc acid, nivalenol, zearalenone, and T-2 and HT-2 toxin was developed for blood plasma or urine. Strategies for the elimination of interfering substances, such as fats or proteins, were used for each type of sample. Different extraction and preconcentration methods were used for each of the matrices. Samples were pre-concentrated by...
evaporation under nitrogen pressure to increase sensitivity. In the case of milk, the principal contaminant is aflatoxin M1 [Iqbal et al., 2015]. The method used for its determination used a different preconcentration system. In this case, immunoaffinity columns were used to preconcentrate the sample and thus carry out the determination using HPLC-FLD. Good sensitivity and selectivity were observed for the studied mycotoxins. The analysis of different biological spiked matrices provided a good basis for the evaluation of the mycotoxin exposure at the animal farm.

P115
DETERMINATION OF AFLATOXINS (AFB1, AFB2, AFG1, AFG2, AFB2) IN PISTACHIOS COLLECTED IN SOUTHERN ITALY (SICILY)
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Aflatoxins (AFs) are a group of potent naturally occurring mycotoxins that pose a significant threat to food safety and human health due to their carcinogenic and mutagenic properties [Pickova et al., 2021]. Pistachios can be contaminated with AFs for harvesting techniques, drying methods, storage conditions and more [Set and Erkmen, 2010]. AFs presence in pistachios is regulated in Europe with the Commission Regulation (EU) 2023/915. The maximum level (ML) is 8 μg/kg of AFB1 and 10 is the sum of B1, B2, G1 and G2 for pistachios that are placed on the market for the final consumer use as an ingredient in food. A total of n=178 pistachios were analysed in Southern Italy (Sicily) in 2022. Analyses were made with high-pressure liquid chromatography with a fluorescence detector (HPLC-FLD). The limit of detection (LOD) and the quantification limit (LOQ) expressed as μg/kg were AFB1 (1.10, 1.20), AFB2 (0.37, 0.42), AFG1 (1.20, 1.31), AFG2 (0.36, 0.39), respectively. Of all samples analysed, n=15 (8.42%) were over the detection limit with a detectable amount of AFs. None of the samples were above the MLs allowed by the Commission Regulation. The highest concentration of AFB1 detected was 5.7±0.9 μg/kg, while 1.24±0.54 was the lowest quantifiable concentration. A single co-occurrence of AFs occurred, with a sample that presents 2.3±0.6 μg/kg of AFG1 and 1.47±0.40 μg/kg of AFG2. The incidence of positive samples is lower than in countries such as Türkiye, Iran, and Spain [Soares Mateus et al., 2021] indicating that Sicilian manufacturer industries (such as Bronties) are characterized by high-quality practices. However, the pooled sample is low, and more studies and more samples are needed to assess better the incidence of AFs in pistachios.

P116
DEVELOPMENT OF AN ULTRA-RAPID LC-MS/MS SCREENING METHOD FOR THE DETERMINATION OF REGULATED AND MOST IMPORTANT EMERGING MYCOTOXINS IN THE FEED AND FOOD INDUSTRY
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Due to global warming, climate change and resulting environmental stresses, mycotoxigenic fungi and their secondary metabolites (mycotoxins) have become more widespread globally. These have the opportunity to contaminate crops across the food and feed chain. There is a need to accurately quantify any mycotoxins present which have regulatory/guideline values as set by the European Food Safety Authority (EFSA) and European Commission (EC). Due to the prevalence of numerous ‘emerging’ mycotoxins in crops and crop-related products, it would be beneficial to detect and quantify these also. To assist producers, rapid testing kits (LFDs) are available, enabling samples to be screened on-site, allowing a quick decision on whether a consignment is compliant or not. These LFDs however are for regulated mycotoxins only, and a separate LFD is required for each individual (class) of mycotoxin. Other rapid diagnostic tools on the market can run multiplex analyses of regulated mycotoxins, but again, provide no insight into any emerging mycotoxins potentially present. Additionally, none of the aforementioned provide accurate identification of the individual mycotoxins present. Furthermore, research conducted by IGFS (QUB) on broiler chickens indicated that low doses of mycotoxins at levels below EU regulatory limits impacted animal performance, and subsequently an associated economic and environmental impact. Additionally, co-occurrence of emerging mycotoxins such as the enniatins (ENNs), diacetoxyscirpenol (DAS) and beauvericin (BEV) may also be a contributing factor. Therefore, the aim was to develop a rapid screening (LC-MS/MS) method for the accurate detection of regulated, masked and (important) emerging mycotoxins in one run. This would potentially lead to less samples requiring analysis using a fully quantitative, confirmatory LC-MS method following screening. Ultimately,
this would ease the economic impact in reducing the cost of analysis annually, while also being environmentally friendly due to a reduction in solvent waste and energy costs of operating these systems. To facilitate this, an LC-MS/MS multiclass method was developed using a guard-column only for the chromatography, with analysis time drastically reduced from 13 to 4 min. Overall, 28 mycotoxins were incorporated in the method and a mini validation performed to verify the methods feasibility. Complementing this, work conducted in collaboration with Agilent using their automated BRAVO platform could lead to a fast, robust, and reliable screening method that could be used across the feed and food industry.

P117
AUTOMATED MULTIPLEX MYCOTOXIN CONTROL FOR SAFE RAW MATERIAL INTAKE
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The significant change in climate, other environments, and human factors have resulted in a higher prevalence of mycotoxin contamination in grain. Thus, the necessity to deliver fast and efficient analytical methods to improve the assessment of mycotoxin risk management programs has risen. While it is becoming a prerequisite that such secondary metabolites are counteracted and thoroughly monitored along the food supply chain, the mycotoxin analysis continues to present a ubiquitous challenge. For many years, rapid immunoanalytical methods greatly contributed to ensuring food safety. During the mycotoxin test procedure: preparing the sample, pipette the extract on the strip, and reading the result after some minutes, have been considered well-founded, but rely on several factors that limited the application. Besides sampling, sample preparation involves time-consuming and complicated processes and has been demonstrated that it increases the variability in the mycotoxin concentration. Therefore, novel methods to analyse in a simpler way by reducing the variation for each step of the mycotoxin test procedure, have emerged. An automated solution (MycoFoss™) has been developed for analysing up to six mycotoxins simultaneously in wheat, corn and barley. The technology behind the solution is an immunoassay on microscopic beads detected by a flow cytometer. By using a set of different beads, it is possible to detect multiple mycotoxins simultaneously. The presentation will describe this technology in more detail. The MycoFoss™ solution has been thoroughly tested using natural wheat, corn and barley samples, to make sure it meets specifications. This data as well as data from external proficiency tests will also be presented.

P118
ACCELERATION OF AN HPLC-MS/MS MULTI-CLASS METHOD FOR THE ANALYSIS OF >1,200 BIOTOXINS, PESTICIDES, AND VETERINARY DRUGS
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In our laboratory, a quantitative multi-method that has an annual throughput of 7500 samples and 20,000 injections is used. This method is capable of measurement of >1000 analytes in just 40 minutes using a scheduled multiple reaction monitoring (sMRM) algorithm. This is achieved within two injections in positive and negative ionization modes, with a total run time of 20 min each while using a flow rate of 1 ml/min. However, this high flow rate requires a significant amount of eluents, which is not eco-friendly. To contribute to the ongoing shift towards environmentally sustainable analytical methods, we have opted to adopt fast polarity switching (FPSW) to accelerate the current method and significantly reduce eluent and energy consumption. The objective of this study was to condense the method into a single 20-minute injection without compromising its accuracy and precision. The software-calculated dwell time based on the number of concurrent MRMs, the retention time window width, and the cycle time is a limited factor in the scheduled MRM mode. The application of FPSW significantly reduced the dwell time from 25 to 10 ms for the analytes eluting from 8 to 16 min. Such a low dwell time may potentially influence the precision of the method. To evaluate the repeatability of the method, repeated injections of a neat-solvent standard (n=15) were conducted. The acquired data set was compared with the original method. The repeatability of injections of FPSW with 5.8% on average was not significantly worse than 4.7% in the case of the original method comprising two separate injections. In order to decide whether the implementation of this accelerated method is feasible without a significant decline in the data quality, the impact of FPSW on the absolute and relative matrix effects in food and feed matrices will be examined as the next step.
Caciocavallo is a cheese traditionally manufactured in southern Italy, that can be ripened in caves by artisanal dairies, by exploiting fungal population naturally occurring on cave walls, without adding any fungal starter culture. These autochthonous moulds spontaneously colonize cheeses by attributing both rheological and sensorial characteristics, highly appreciated by consumers. Mycobiota occurring in caves could be composed by several *Aspergillus* species, including *Aspergillus westerdijkiae*, one of the most notorious species producing ochratoxin A (OTA), contaminating ripened cheese products. It has been reported as the cause of cheese contamination by OTA, able to migrate inside cheese from the surface, where fungi grow during ripening. Early detection of ochratoxigenic moulds growth in cheese is highly recommended to prevent human exposure to OTA. The availability of rapid methods for monitoring these fungal species is crucial and constitutes a key stage for the production of safe ripened foods, mainly when carried out under not-controlled environmental conditions. The mass spectrometry-based electronic nose (MS-eNose) technique, based on the use of headspace solid-phase microextraction directly coupled to MS, is a most innovative approach that can be used to analyse volatile organic compounds (VOCs) of complex matrices. VOCs are often used for early detection of food spoilage, fungal growth and also for distinguishing between toxigenic and non-toxigenic strains. The aim of this study was to develop a rapid and non-invasive MS-eNose method for the early detection of *A. westerdijkiae* on traditional Italian caciocavallo during ripening process. MS-eNose analyses were carried out on caciocavallo cheeses inoculated with OTA non-producing strains and artificially contaminated with an *A. westerdijkiae* OTA producing strain. The inoculated caciocavallo cheeses were ripened at laboratory-scale and sampled at 1, 15, 30, 45, 60 and 90 days. Partial least squares discriminant analysis (PLS-DA) and principal components linear discriminant analysis (PC-LDA) were used to discriminate cheese samples in two classes based on contamination by toxigenic and non-toxigenic fungal strains. Accuracy values, in the different sampling days for both statistical approaches, were in the range 87-100% and 86-100%, in calibration and validation, respectively. The best results were obtained at 15-ripening days with 98 and 100% of accuracy in validation for PLS-DA and PC-LDA, respectively. Moreover, GC-MS analyses showed that eighteen VOCs of the two classes were significantly different (t-test, p<0.05) at 15-ripening days. These results show that MS-eNose represents a useful tool for a rapid screening in preventing *A. westerdijkiae* and related OTA contamination in caciocavallo cheese during the ripening process.

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**P120**

SURFACE-ENHANCED RAMAN SPECTROSCOPY FOR THE RAPID DETECTION OF DEOXYNYIVALENOL IN AGRICULTURAL PRODUCTS

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Mycotoxin contamination in food and feed crops remains one of the greatest global health concerns. Therefore, there is a need for rapid, low-cost, and sensitive techniques to detect mycotoxins in agricultural products. Surface-enhanced Raman spectroscopy (SERS) is a vibrational surface-sensitive technique which enhances conventional Raman scattering through the adsorption of molecules close to the surface of roughened noble metals, commonly made of gold (Au) or silver (Ag). Its unique fingerprinting ability allows for individual analytes to be detected and identified at low levels. In this study, four nanosubstrates were fabricated and applied to determine the limit of detection (LOD) for deoxynivalenol (DON). The SERS enhancement of fabricated Au nanoparticles (AuNPs), Ag nanoparticles (AgNPs), Au nanostars (AuNSs) and core-shell nanoparticles composed of a Ag core and Au shell (Au@Ag CSNPs) was determined in the presence of a fluorescent dye; Rhodamine 6G (R6G). The particles were applied to detect the fingerprint spectra of DON standards either by mixing in solution or by assembling the mixture onto a solid substrate (filter paper or aluminum tin foil). The LOD for DON
was determined as 3 μg/kg (ppb), which is much lower than the European recommended limits for DON in cereals intended for human consumption (750 μg/kg). Wheat samples naturally contaminated with DON were extracted and detected using the same approach. It was found that matrix interferences from the wheat sample reduced the SERS intensity of the DON peaks and therefore this is an area that requires future improvements. To observe whether chemometric modelling could improve matrix effects and multiplexing capabilities two interfering mycotoxins; aflatoxin B1 (AFB1) and ochratoxin A (OTA) were also analysed. By exploiting supervised orthogonal projections to latent structures discriminant analysis (OPLS-DA), the model could successfully discriminate between three mycotoxins: DON, OTA and AFB1. The internal validation of the OPLS-DA model produced a R2 and Q2 value of 0.974 and 0.979. Overall, the results from this preliminary study reveal that there is substantial merit in developing SERS-based applications combined with statistical modelling to help quantify levels of mycotoxins within agricultural products.

P121
DETECTION OF T-2 TOXIN IN WHEAT AND MAIZE WITH A PORTABLE MASS SPECTROMETER
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Direct methods for toxin measurement, such as mass spectrometry, are generally confined to traditional laboratories, while indirect methods, such as immunoassays, are often used for rapid estimation in the field. The potential of a portable scanning mass spectrometer was explored as a means to enable direct detection of T-2 and HT-2 toxins in settings outside of traditional laboratories. In order to detect T-2 and HT-2 toxins the inlet of the atmospheric pressure ionization source of a commercially available portable linear ion trap mass spectrometer was modified. Modification allowed extracts of wheat or maize that had been cleaned up using MycoSep 225 columns to be infused into the instrument, using air to nebulize the samples. T-2 toxin was detected in soft white wheat, hard red wheat, and yellow dent maize with limits of detection of 20 to 28 μg/kg. The cut-off value in hard red winter wheat was 110 μg/kg. The appearance of an interfering compound at the same m/z as HT-2 prevented the measurement of that toxin, which undermined the use of the method for meeting EU performance criteria. Nevertheless, the results demonstrate that it is possible to develop rapid, field portable, mass spectrometric methods for detecting mycotoxins.

P122
COMPARATIVE PERFORMANCE OF RAPID TEST KITS FOR THE DETECTION OF T-2 AND HT-2 TOXINS IN OATS PRODUCED ON THE ISLAND OF IRELAND
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The Island of Ireland is an important and increasingly large producer of the cereal crop, oats and while some of the output is used for animal feeds, a significant proportion is used in the food industry. As a cereal crop, oats are susceptible to natural contamination with fungal pathogens that produce mycotoxins, therefore contaminated produce may present a risk to human health. Previous studies have highlighted contamination of oats with the Fusarium mycotoxins T-2 toxin and HT-2 toxin in Ireland and the United Kingdom, underlining the need for regular testing of unprocessed cereals and cereal-based foods to ensure product compliance with regulatory controls. To achieve this, rapid tests have been increasingly promoted as a means to validate food safety management systems used in the agri-food industry. While these more user-friendly, inexpensive, and rapid techniques are favoured by growers, suppliers, and processors along the supply chain, they must be accurate, reproducible and provide the required sensitivity for regulatory compliance. The performance of four commercially available rapid diagnostic kits for the determination of the sum of T-2 and HT-2 (three lateral flow kits and one enzyme linked immunoassay) were assessed against state-of-the-art LC-MS/MS. Oats, sampled according to Commission Regulation (EC) No 401/2006 were provided by industry stakeholders and analysed according to the manufacturers’ instructions. The results demonstrated that, in general, all kits underestimated the concentration of the sum of T-2 and HT-2 in the samples. In terms of false negatives (according to the current EU guidance limits for the sum of T-2 and HT-2 toxins), both Test Kits 1 and 3 fell within the accepted EU criteria (≤5%). Results observed for test kits 2 and 4 did not meet the criterion for false negative rates, both exceeded 5%. False positive rates for all kits were ≤2.2%. An alternate means of evaluating the data was to assess the level of agreement between each test kit and LC-MS/MS. Cohen’s Kappa was calculated for each and the observed results showed that test kits 1 and 3
displayed almost perfect agreement with LC-MS/MS (K=0.93 and K=0.85, respectively). In contrast, both test kits 2 and 4 displayed only fair agreement with K values calculated as 0.21 and 0.38, respectively. Based on performance, speed, and cost per sample of the kits evaluated, test kit 1, the Neogen Reveal® Q+ MAX for T-2/HT-2 Kit, would be the recommendation to industry.

P123
USING SAMPLES COMPOSED OF DIFFERENT GRAIN TYPES TO ESTIMATE AFLATOXIN CONTAMINATION IN MAIZE LOADS
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Aflatoxin contamination in maize loads was estimated using three different sampling protocols, one containing all kinds of grains present in maize loads (whole sound, damaged, and broken), another composed of grains of high density, and a third one with grains of low density. The samples were obtained from randomly selected maize trucks loads (18 trucks) that supplied a Brazilian corn wet milling plant. From each load, 16 samples of 8 kg were withdrawn using the same procedure. All the 8 kg samples were processed using a grain divider, each resulting in three samples, one named total grains sample (TGS) weighing 5 kg, another intended for density segregation weighing 1 kg, and a third one designated for the company’s quality control weighing 2 kg. In this study, only the 5 and 1 kg samples were used. The TGS was coarsely ground and subsampled to 500 g using a subsampling mill, after that the subsample was wholly ground by hammer mill to obtain a material with particle size below 0.85 mm. The sample used for density segregation was divided into high- and low-density grains using 5 l of a saline solution (NaCl 30% w/v) contained in a 20-liter bucket. To perform the segregation, the whole sample was transferred to the bucket, the solution and grains were homogenized and after 10 min of rest the supernatant grains were removed and designated as the low-density sample (LDS). These grains were subsequently dried in an air circulation oven at 50°C, weighed, and milled by hammer mill. The decanted grains were designated as the high-density sample (HDS), which underwent processing identical to that of the low-density material to obtain the final ground material. After milling and homogenization, one analytical sample (25 g) was withdrawn for aflatoxin analysis, whose extraction and purification steps were carried out using immunoaffinity columns, followed by detection and quantification by high performance liquid chromatography with fluorescent detection, automatic sample injection, and post-column photoreaction. The number of positive results and the aflatoxin B1 contamination levels observed for each sample type were used to calibrate probabilistic models. Based on the calibrated probabilistic models and the maximum tolerable aflatoxin contamination limit for maize (20 μg/kg) established in Brazil, operating characteristic curves (OCs) were designated for each type of sample (TGS, LDS and HDS). The LDS presented the highest number of positive results and contamination levels; however, its OC showed the largest risk area for both buyers and sellers. Acknowledgements. Financial support: FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo), grant number 07/59366-3.

P124
MULTI-TOXIN ANALYSIS: SINGLE EXTRACTION IMMUNOAFFINITY COLUMN CLEAN-UP FOR THE ANALYSIS OF 11 MYCOTOXINS IN PSEUDO CEREALS
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Nowadays, consumers are more conscious about nutritional benefits of foods which has led to an increased interest in pseudo cereal grains, such as quinoa and buckwheat and “ancient grains” such as spelt. There are indications that mycotoxicogenic moulds require different conditions to grow on pseudo cereals compared to modern cereals. Prevention techniques have been more focused on modern cereals however, methods of analysis for pseudo cereals are also required to enable monitoring these novel foods. The aim of this study was to develop a method for the analysis of 11 legislated mycotoxins in four pseudo cereal samples. The samples were spiked at EU levels (total AFTs 10 ppb, OTA 5 ppb, total FUM 1000 ppb, DON 750 ppb, ZON 100 ppb, and total T-2/HT-2 100 ppb) and the mycotoxins were successfully extracted using 11+ Myco MS-PREP® with no interfering peaks being observed and recoveries for all mycotoxins ranging from 88-117 % for all samples.
THE ASSESSMENT OF 11 TOXINS IN MILK ALTERNATIVES USING IMMUNOAFFINITY COLUMN CLEAN-UP PRIOR TO LC-MS/MS DETECTION

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Dairy-free milk alternatives are becoming more and more popular and even though aflatoxin M1 is not a concern, other mycotoxins may still be present. EU regulations for mycotoxins are complex with varying limits applying to specific commodities. This, along with the fact that mycotoxins can co-exist in commodities, has created 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 and ensure compliance with EU method performance criteria. The present study evaluates 11 toxins in various dairy-free milk products using R-Biopharm Rhône’s multi-toxin immunoaffinity column, 11+ Myco MS-PREP®. Of the finished product, only 1-20% is made up of the key ingredient and therefore, assessment was done at 1/10th of the usual maximum legislative limits. Testing was also conducted at EU legislative limits with spiked recovery values and % RSD being assessed against EU criteria. The method proved to be suitable for a range of alternative milks with acceptable range recoveries.

AUTOMATED ANALYSIS OF OCHRATOXIN A IN HERBAL DRUGS AND HEALTH SUPPLEMENTS

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As consumers develop a greater interest in adopting healthy habits, pharmaceutical products are becoming increasingly popular with consumers. As a result, the need for analytical methods to test for mycotoxins, including OTA, in herbal drugs and health supplements is also growing. In response, R-Biopharm Rhône developed immunoaffinity clean-up methods for the analysis of ginger root, Boswellia serrata, ginseng, milk thistle-based supplements, turmeric-based gummies, ashwagandha based capsules and tablets and turmeric and black pepper capsules. The methods were developed and validated using IMMUNOPREP® ONLINE OCHRATOXIN immunoaffinity cartridges which are used in conjunction with a CHRONECT Symbiosis RIDA®CREST handling system prior to HPLC-FLD system. All results met with EU method performance criteria (Commission Regulation (EC) No 401/2006) in terms of recovery and %RSDs when spiked at 0.5x, 1x and 2x the EU legislative levels for OTA. The methods also gave precise results when matrices were extracted and analysed on three non-consecutive days, resulting in acceptable %RSDs, as per the Commission Regulation (EC) No 401/2006 method performance criteria. Methods were found to be selective to OTA with no interfering components observed on the chromatograms.

ANALYTICAL METHODS FOR MONITORING MYCOTOXINS IN HUMAN URINE AND EXPOSURE ASSESSMENT – A REVIEW OF THE LAST 15 YEARS

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Mycotoxins pose a serious risk to human and animal health worldwide. Generally, mycotoxins cause toxic responses including carcinogenicity, hepatotoxicity, nephrotoxicity, endocrine disorders, metabolic and biochemical deficiencies, allergic reactions, immune alterations, reproductive deficiencies, foetal alterations or death. Mycotoxin exposure assessment has been traditionally evaluated combining food consumption and occurrence data (indirect approach), this method presenting few limitations due to the heterogeneous distribution of mycotoxins in foods, the limited accuracy of food consumption data and the inter-individual differences in mycotoxin metabolization. These limitations could be overcome with the measurement of specific urinary biomarkers (direct approach named biomonitoring), since biomarker excretion correlates very well with the intake of some mycotoxins and leads to a more realistic scenario to assess the exposure to mycotoxins. The aim of this study was to provide an overview of the occurrence of mycotoxins in human urine as a biomonitoring tool. Relevant studies published from 2009 to 2023 were selected after a literature search in PubMed, Scopus and Science Direct. Human urine samples are extremely complex matrices in which mycotoxins can be found in traces or very small
quantities, sometimes being masked by other compounds. Thus, from an analytical point of view, during mycotoxin analysis in urine a clean-up step is necessary. Different techniques such as liquid–liquid extraction, dispersive liquid–liquid micro extraction, solid phase extraction, QuEChERS, and immunoaffinity columns have been reported for mycotoxin determination in human urine. The most used method for the determination of different mycotoxins or their biomarkers in human urine was LC-MS/MS, while the most studied mycotoxins were deoxynivalenol and aflatoxin B1. In conclusion, human urine analysis for mycotoxin detection, as part of the biomonitoring process, can be an effective tool for establishing the global health status. **Acknowledgments.** This work was supported by a grant of the Romanian Ministry of Education and Research, CNCS - UEFISCDI, project number PN-III-P1-1.1-PD-2019-1171, within PNCDI III.

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**ANALYSIS OF MYCOTOXIN OCCURRENCE AND FEED STORAGE PRACTICES IN SMALL-HOLDER BROILER FARMS IN KENYA**

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Poultry keeping is a major contributor to livelihood and food security in Kenya, with at least 79% of households rearing chicken. Broilers consist of a large proportion of the poultry population, with a projected increase from 38 million birds in 2019 to 166 million birds by 2050. A major challenge to poultry feed safety in sub-Saharan Africa is the contamination with mycotoxins, whose presence is often increased due to the favourable environmental conditions, e.g., humidity and temperature. Additionally, pre-and post-harvest mitigation strategies for the feed and feed ingredients may be insufficient in preventing the presence of mycotoxins. Despite these challenges, surveillance data on the occurrence of these toxins in poultry feed is lacking. This study aims to determine the occurrence and levels of mycotoxin contamination in broiler feed in Kenya, and its possible association to storage of feed practices on farms. To investigate this, broiler feed samples were collected from 124 small-holder broiler farms (those keeping between 200 to 2000 birds) in three peri-urban counties in Kenya. Farm data was obtained through semi-structured questionnaires administered to the farmers. Feed samples were analysed using a validated multi-mycotoxin liquid chromatography-tandem mass spectrometry method (LC-MS/MS). Preliminary data from 40/124 farms shows that all these samples had at least one mycotoxin present. The most prevalent toxins in these samples are *Fusarium* mycotoxins including deoxynivalenol (90%; range 96.5-981 μg/kg), fumonisins (90%; range 83.1-741 μg/kg), and zearalenone (50%; range 35.8-460 μg/kg). Aflatoxin B1 was the highest *Aspergillus* mycotoxin found (50%; range 3.2-30.6 μg/kg), followed by aflatoxin B2 (32.5%; range 4.6-6.1 μg/kg), aflatoxin G2 (15%, range 4.2-5.8 μg/kg), and one sample with aflatoxin G1 (7.00 μg/kg). Ochratoxin A was also found in two samples (39.9 and 53.9 μg/kg). Ninety-two percent of these samples had co-occurrence of more than one mycotoxin, indicating the need for increased monitoring of these toxins in the country. Only 63% of farmers stored their feed elevated above the ground, while more than half of them (53%) stored the feed within the poultry coop. Similarly, only 46.5% of the farmers had ensured that the feed was stored in an area proofed against water, birds, and vermin. These findings indicate that there is need to create awareness among farmers on mycotoxin mitigation strategies during storage and use of animal feeds.

P129
**VALIDATION OF A FLOW-THROUGH RAPID TEST FOR THE QUICK AND EASY DETECTION OF OCHRATOXIN A IN WINE**

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Ochratoxin A (OTA) is a nephrotoxic and hepatocarcinogenic toxin produced by fungi. OTA has been shown to occur in various cereals and plant products such as coffee and grapes. OTA also occurs in wine produced from contaminated grapes. In the European Union the maximum limit for OTA in wine is 2 μg/l in accordance with Commission Regulation (EU) 2022/1370. Wine products should be tested with appropriate methods to assure the contaminated wine is not offered to customers. In order to reach the detection level of 1 μg/l of OTA (half of the regulatory limit) in wine a new immune-based flow-through rapid test was developed and validated. Furthermore, an additional application was optimised for the cut-off value of 2 μg/l of OTA. The performance of the method was tested with a set of 21 blank wine samples and a set of 21 wine samples spiked at 1 μg/l with OTA. The samples were analysed using
three different batches of ochratoxin A wine test kit. Additionally, sets of samples were also spiked at lower and higher levels, that is 0.5 µg/l and 1.5 µg/l. Visual interpretation of each result (in total 252 results) was performed by three different analysts and the results were classified as screen negative (2 lines visible) or positive (only 1 line visible). The specificity was determined to be 100% meaning all samples not containing any OTA (negative) gave correctly negative result. The overall sensitivity was 98% meaning 2% of samples gave false negative result. This false negative rate was lower than the acceptable false negative rate for a screening method (≤5%). The test was also shown to be highly robust and small deviations from the recommended testing protocol did not affect the test result. The application for the cut-off value of 2 µg/l was validated in a similar way. In this case the specificity was determined to be 100% meaning all samples not containing any OTA (negative) gave correctly negative result. The overall sensitivity was 100% meaning there was no false negative result. The test is applicable for analysis of red, white or rosé wines in just 15 min and can be used on site at wineries for in-process and final products testing. It can be performed by an inexperienced user and does not require any additional reagents or equipment. The end-user can choose to screen at the level of 1 µg/l or 2 µg/l, depending on the requirements.

P130
VALIDATION OF TWO FLOW-THROUGH RAPID TESTS FOR THE QUICK AND EASY DETECTION OF OCHRATOXIN A IN GREEN AND ROASTED COFFEE WITH A CUT-OFF VALUE OF 3 mg/kg
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Ochratoxin A (OTA) is a nephrotoxic and hepatocarcinogenic toxin produced by fungi. OTA can occur in contaminated green coffee beans (incoming product) and due to thermostability also in the roasted coffee beans (end product). In the European Union the maximum limit for OTA in roasted coffee is 3 µg/kg in accordance with Commission Regulation 2022/1370. In order to test green coffee beans or roasted coffee beans as end product for the presence of OTA two new immune-based flow-through rapid tests were developed with a screening target concentration (STC) of 3 µg/kg in line with legislation for roasted coffee. The performance of these methods was tested with a set of 21 blank green or roasted coffee samples and a set of 21 green or roasted coffee samples spiked at 3 µg/kg with OTA. Additionally, sets of samples were also spiked at 3 lower and 1 higher level, that is 0.75, 1.5, 2.5 and 3.5 µg/kg. The samples were analysed using 3 different batches of each specific ochratoxin A test kit and visual interpretation of each result (in total 198 results) was performed by three different analysts. Both assays showed good specificity of 100% meaning the samples which do not contain any OTA (negative) gave a correct screen negative result (two visible lines). Besides this, the assays showed excellent overall sensitivity of 100% and a 0% false negative rate meaning the assays give a screen positive result (one line visible) when the sample contains ≥3 µg/kg of OTA. The assays also showed high robustness as small deviations to the recommended testing protocol did not cause any adverse effect to the test result. The test kits are fast and reliable tools for the analysis of incoming green coffee beans as well as the end product roasted coffee on-site. The test can be performed by users with limited experience and do not require use of any equipment.

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ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY TO ADDRESS THE INDUSTRIAL NEEDS FOR THE DETECTION OF ERGOT ALKALOIDS IN SEVERAL CEREAL PRODUCTION CHAINS
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Within the last decades, in the EU, there has been an increasing interest in toxic plant alkaloids as food contaminants, especially after the growing consumption of plant-based foods compared to food of animal origin. The once neglected emerging ergot alkaloids have now been under the focus of the European Food Safety Authority (EFSA), highlighting the lack of data and the need to develop risk assessment strategies. In this regard, according to the specific needs of the food industry, the emphasis has been placed on detecting their occurrence in several types of cereals (rye, wheat, oat, barley, and spelt) through the development of accurate and sensitive analytical methods. An ultra-high performance liquid chromatography coupled to tandem mass spectrometry method is presented for the determination of the regulated ergot alkaloids (ergometrine, ergosine, ergotamine, ergocornine, ergokryptine, ergocristine) and their epimers (ergometrinine, ergosinine, ergotaminine, ergocorninine, ergokryptinine
T-2 and HT-2 toxins are primarily produced by *Fusarium* fungi during the field growth, but also during the harvesting or storage. They are known to exert toxicity to humans and animals, and after reviewing of new *in vivo* toxicity study in rats in 2017 EFSA has established the tolerable daily intake (TDI) of T-2 and HT-2 at 0.02 μg/kg body weight (bw) [EFSA, 2016]. To estimate the concentration of these toxins (metabolites) in urine, procedures developed by Narváez et al. (2021), Gratz et al. (2019) and information from Welsh et al. (2012) were used as a starting point. A specific clean-up using IAC columns was found to be necessary to achieve sufficiently low LOQs. During the method development, T-2 tetraol and neosolaniol were not retained by the IAC columns applied, and it was noted that T-2 triol and its corresponding internal standard were not stable during the sample preparation process. Finally, sample analysis of T-2, HT-2 toxins and T-2 triol in urine involved a deconjugation step using β-glucuronidase from *Escherichia Coli* in a buffer at pH 6.8 (overnight, 37°C), extraction/cleanup on an immunoaffinity column (IAC) dedicated to T-2 and HT-2 toxins and analysis by LC-MS/MS. Quantification was carried out by external calibration of standards in solvent after normalization of the response to the isotopically labelled internal standards (IS) 13C24 T-2 toxin, 13C22 HT-2 toxin and 13C20 T-2 triol. The LOQ of the method is 0.05 ng/ml urine for T-2 and HT-2 toxins and 0.3 ng/ml for T-2 triol. The developed procedure was applied for analysis of 26 urine samples. Only HT-2 toxin was detected, with the concentrations from below LOQ to max. 0.312 ng/ml of urine. T-2 toxin and T-2 triol were not detected in any of the analysed samples. Moreover, when the two highest contaminated samples (0.312 and 0.230 ng/ml of HT-2 triol) were analysed without the use of β-glucuronidase enzyme, and using the Narváez et al. (2021) sample extraction technique, neither T-2 nor HT-2 toxins were detected. This would suggest that all the HT-2 toxin fraction found using the developed procedure was conjugated with glucuronide. In the literature, the discrepancies between the results obtained for T-2 and HT-2 toxins in human urine by different authors are substantial. From negative [Warth et al., 2015; Heyndrickx et al., 2015; or positive [De Ruyck et al., 2020; Narváez et al., 2021; Ndaw et al., 2021; Niknejad et al., 2021; (oesophageal cancer patients)] results for both toxins, to studies detecting only T-2 [Rodríguez-Carrasco et al., 2014; Fan et al., 2019] or HT-2 toxin [Niknejad et al., 2021 (control group); Rodríguez-Carrasco et al., 2014; here presented study]. The area of this research definitely requires further investigations to explain the discrepancies. The above can be possibly justified by other than the differences in methods extraction or detection techniques, too high LODs/LOQs, which is not always the case when reviewing the papers. It could be, e.g., related with the (time of) exposure and thus the availability of T-2 toxin to produce certain metabolites or regional differences in urinary biomarker excretion patterns.

**P133**

**DETERMINATION OF BIOMARKERS OF EXPOSURE OF T-2/HT-2 TOXINS IN HUMAN URINE**

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T-2 and HT-2 toxins are primarily produced by *Fusarium* fungi during the field growth, but also during the harvesting or storage. They are known to exert toxicity to humans and animals, and after reviewing of new *in vivo* toxicity study in rats in 2017 EFSA has established the tolerable daily intake (TDI) of T-2 and HT-2 at 0.02 μg/kg body weight (bw) [EFSA, 2016]. To estimate the concentration of these toxins (metabolites) in urine, procedures developed by Narváez et al. (2021), Gratz et al. (2019) and information from Welsh et al. (2012) were used as a starting point. A specific clean-up using IAC columns was found to be necessary to achieve sufficiently low LOQs. During the method development, T-2 tetraol and neosolaniol were not retained by the IAC columns applied, and it was noted that T-2 triol and its corresponding internal standard were not stable during the sample preparation process. Finally, sample analysis of T-2, HT-2 toxins and T-2 triol in urine involved a deconjugation step using β-glucuronidase from *Escherichia Coli* in a buffer at pH 6.8 (overnight, 37°C), extraction/cleanup on an immunoaffinity column (IAC) dedicated to T-2 and HT-2 toxins and analysis by LC-MS/MS. Quantification was carried out by external calibration of standards in solvent after normalization of the response to the isotopically labelled internal standards (IS) 13C24 T-2 toxin, 13C22 HT-2 toxin and 13C20 T-2 triol. The LOQ of the method is 0.05 ng/ml urine for T-2 and HT-2 toxins and 0.3 ng/ml for T-2 triol. The developed procedure was applied for analysis of 26 urine samples. Only HT-2 toxin was detected, with the concentrations from below LOQ to max. 0.312 ng/ml of urine. T-2 toxin and T-2 triol were not detected in any of the analysed samples. Moreover, when the two highest contaminated samples (0.312 and 0.230 ng/ml of HT-2 triol) were analysed without the use of β-glucuronidase enzyme, and using the Narváez et al. (2021) sample extraction technique, neither T-2 nor HT-2 toxins were detected. This would suggest that all the HT-2 toxin fraction found using the developed procedure was conjugated with glucuronide. In the literature, the discrepancies between the results obtained for T-2 and HT-2 toxins in human urine by different authors are substantial. From negative [Warth et al., 2014; Heyndrickx et al., 2015; or positive [De Ruyck et al., 2020; Narváez et al., 2021; Ndaw et al., 2021; Niknejad et al., 2021; (oesophageal cancer patients)] results for both toxins, to studies detecting only T-2 [Rodríguez-Carrasco et al., 2014; Fan et al., 2019] or HT-2 toxin [Niknejad et al., 2021 (control group); Rodríguez-Carrasco et al., 2014; here presented study]. The area of this research definitely requires further investigations to explain the discrepancies. The above can be possibly justified by other than the differences in methods extraction or detection techniques, too high LODs/LOQs, which is not always the case when reviewing the papers. It could be, e.g., related with the (time of) exposure and thus the availability of T-2 toxin to produce certain metabolites or regional differences in urinary biomarker excretion patterns.

**P132**

**PENTAHELICENE DERIVATIVE COMPOUNDS: FLUORESCENT ORGANIC DYES THAT GIVE SIMPLICITY TO POINT-OF-NEED MYCOTOXIN DETECTION**

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Among all components in point-of-need diagnostic test kits, signal reporting material plays a significant role in the performance of detection. Herein, aiming to develop in-house signal reporting materials for lateral flow technique, a group of pentahelicene derivative compounds was synthesized through convenient procedures and successfully validated as novel fluorescent reporter molecules for mycotoxin detection application. The core chemical structure of the compounds is composed of five six-membered rings connected at ortho position to produce a helical shape molecule resulting in a long τ-electron conjugating system. Injunction with additionally having a variety of proper chemical moieties, the compounds exhibit excellent fluorescent emission in the range of 430-680 nm. The said compounds meet the analytical testing needs of the industry for ergot alkaloids in order to ensure compliance with the recent European legislation, with a particular focus on vulnerable population groups. Several commercial samples were analysed to further verify the applicability of this analytical approach, providing insights into the level of contamination on the market and also comparing our findings with those of a qualified external laboratory.
also contain reactive chemical functional groups, i.e., carboxyl and formyl, which are ready to link with biomolecules. Moreover, they are soluble in a variety of solvent systems that are used in crosslinking processes with biomolecules. The organic compounds show highly stable under storage and working environments. The lateral flow strip test using a selected fluorescent pentahelicene dye was demonstrated for detection of five mycotoxins including AFB1, DON, FUMB1, T-2, and ZON. The results clearly implied that the organic dyes based on pentahelicene derivative compounds can be conveniently used as optical reporting molecules for point-of-need mycotoxins quantitative diagnostic test.

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MYCOSMART READER: MICROARRAY READER FOR MULTIPLE MYCOTOXIN DETECTION
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Recently, a microarray lateral flow immunoassay strip test (mLFIA) utilizing novel fluorescent dyes was successfully developed to simultaneously detect many regulated mycotoxins. While the mLFIA allows cost-effective, rapid, portable, and multiplex mycotoxins detection, it requires a portable, quantitative, and affordable detector to fully harvest its point-of-care benefits. However, most of the devices available commercially are expensive and not portable. Overcoming these challenges, MycoSMART Reader, a device for detecting and analyzing signals from the mLFIA, has been developed with portability, ease-of-use, and affordability in mind. MycoSMART Reader assists provide easy-to-understand analysis results in real-time, allowing non-technical users to operate. With optimized software and hardware, MycoSMART Reader allows multiplex and quantitative analysis of different mycotoxins, with good signal repeatability and robustness. A user-friendly graphical user interface (GUI) was also designed for the end users to control MycoSMART Reader directly via a built-in touchscreen. Most importantly, MycoSMART Reader supports online data management. Analysis results are uploaded to the online database based on their geographic information, where data analytics can be applied later. Moreover, MycoSMART Reader only consists of off-the-shelf components lowering its cost, The developed MycoSMART Reader would bridge the gap in translating the lab-based mycotoxin detection to commercialization, making it more accessible and useful to a wider group of users resulting in safer food for humankind.

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THE QUANTITATION OF MYCOTOXINS IN FOOD AND FEED USING A SIMPLE EXTRACTION AND LC-MS/MS WITH FAST POLARITY SWITCHING AND SCHEDULED MRM
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Mycotoxins are produced by several strains of fungi both in the field, during storage, mixing and delivery of grain, human and animal food. Mycotoxins fall into several major classes and those which can affect the health of humans or animals include the aflatoxins, ochratoxins, Fusarium toxins, including fumonisins, zearalenone (ZON), trichothecenes, and ergot alkaloids. Regulations for mycotoxin contamination for some of the major classes have been set in different countries. Mycotoxin analysis needs to be comprehensive and able to deliver accurate and consistent results across a wide range of matrices. In this poster we introduce an improved approach to testing Mycotoxins, their metabolites and emerging masked mycotoxin compounds using LC-MS/MS with fast polarity switching for different matrices. Results and detection limit for regulated mycotoxin compounds are determined on different SCIEX platforms. Workflow that incorporates mycotoxins, their metabolites and emerging masked mycotoxins in one comprehensive method using Scheduled MRM™ Algorithm will be demonstrated.
Mycotoxins are a major problem in the food supply chain. Different methods are available to qualitatively/quantitatively evaluate them in several food matrices. Rapid methods, such as lateral flow immunoassay (LFD), and enzyme-linked immunosorbent assay (ELISA), are more and more used particularly in the food industry, with the limitation that in general these methods are usually not able to detect more than a single mycotoxin at once. Besides sampling, sample preparation involves time-consuming and complicated processes, and it has been demonstrated that it increases the variability in the mycotoxin concentration. Therefore, novel methods to analyse in a simpler way by reducing the variation for each step of the mycotoxin test procedure, have emerged. To simultaneously quantify six different mycotoxins in cereals, an automated bead-based flow cytometric immunoassay instrument (https://www.fossanalytics.com/it-it/products/mycofoss), MycoFoss™, recently made commercially available on the market, has been successfully tested on the field for measuring aflatoxin (AFB1, AFB2, AFG1, AFG2), fumonisins (FB1, FB2, FB3), deoxynivalenol (DON), ochratoxin A (OTA), zearalenone (ZEA), and T-2 toxin in important matrices of the food supply chain, i.e., durum wheat and maize. From the extraction to the quantification, the instrument is fully automated, and it is able to give results within 8 minutes with very little sample preparation. Precision, accuracy and uncertainty were evaluated for the different mycotoxin in the two different matrices, in order to estimate the overall performance of the instrument. Good results were comprehensively obtained in view of a rapid screening approach; the instrument could be beneficial for the food industry, where a lot of samples are usually tested, providing an easy-to-use method almost able to also minimize the human error.

In recent years, *Fusarium* mycotoxins, such as deoxynivalenol (DON), T-2 toxin and HT-2 toxin, have been the most commonly found in oats. Oat and oat-based products are expected to present the highest levels of type A trichothecenes, being T-2 and HT-2 toxins the ones with the greater incidence. Moreover, their consumption is expanding, so it is all the more relevant to control their mycotoxin content at the reception of the food and feed companies. Near-infrared hyperspectral imaging (NIR-HSI) is considered a promising technique for determining mycotoxins in cereals because it is quicker, cheaper and more sustainable than the conventional techniques. However, it has never been used to assess DON in naturally contaminated oats nor to predict T-2 and HT-2 toxins content in any cereal. In the present study, NIR-HSI has been applied to 119 oat samples to quantify DON and T-2+HT-2 toxins and classify them according to the EU legal limit for DON (1,750 µg/kg) and EU recommended legal limit for T-2+HT-2 (1000 µg/kg) for unprocessed oats. Hyperspectral images were obtained for unground samples. The reference methods for determining mycotoxins were HPLC-DAD for DON and ELISA for the sum of T-2 and HT-2 toxins content. The data were pre-treated and evaluated by multivariate tools, applying PLS regression for quantification and four classification models. The most efficient DON prediction model was obtained with the 1st derivative plus SNV pre-treatment (r²=0.85 and RMSECV=312.64 µg/kg), and the best-fitted T-2+HT-2 toxins prediction model was achieved with the SNV plus 1st derivative pre-treatment (r²=0.64 and RMSECV=157.80 µg/kg). The correlation between the predicted and observed values was 0.92 and 0.80 for DON and T-2+HT-2 toxins, respectively. The most characteristic wavelengths were 1178, 1368 and 1393 nm for DON prediction and 1038, 1110 and 1393 nm for T-2+HT-2 prediction. Other studies affirmed that these wavelengths are associated with changes in the hull structure and β-glucan content in oats [Serranti et al., 2013; Meenu et al., 2022]. Besides, other authors found that oat with high content of DON tended to have greater hull or a lower groat percentage than oat with low content of DON [Yan et al., 2017]. The maximum classification accuracies based on the EU legal limits were 83.2 % for DON (random forest) and 94.1 % for T-2+HT-2 (neural network). These findings suggest that trichothecenes (DON, T-2 and HT-2 toxins) detection by NIR-HSI is possible in oat grains, even though further investigations are needed.

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THE QUANTITATIVE MYCOTOXINS LATERAL FLOW TESTS IN FINISHED ANIMAL FEED AND PET FOOD
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Lateral flow strip tests have been successfully used to detect mycotoxins in grains, nuts and other animal feed ingredients for years. However, these tests were unable to be used to test complete feed formulations due to challenges with complex sample types such as pelleted and mixed feeds. Afla-V™ ONE and DON-V™ ONE tests eliminate this barrier to on-site testing using the world’s FIRST Universal Calibration for fully quantitative aflatoxin and deoxynivalenol (DON) results in 15 categories of finished feeds and pet foods, including poultry, cattle, swine, horse, rabbit, cat, dog, goat and more. Afla-V™ ONE and DON-V™ ONE tests offer 5-minute results for total aflatoxins and DON. The approach eliminates complex pipetting, rinsing, shaking, and incubation steps needed for traditional ELISA testing, while improving test safety by eliminating the need for toxin-dependent calibration. The ability to obtain actionable results for total aflatoxin and DON in finished feeds and pet foods enables quality managers to simplify on-site testing while managing feed and pet food quality and safety with greater confidence. The Afla-V ONE test kit was validated using spiked samples (2.5-300 ppb) of naturally contaminated feed and pet food samples. Sixteen feed samples spiked with aflatoxin standards at 5 and 20 ppb, the recoveries are between 76.1 and 111.8%. The percent CV of reproducibility from three operators by using two strip lots in two different matrices are all less than 12.9%. The DON-V ONE test kit was validated using spiked samples (0.3-40 ppm) of naturally contaminated feed, pet food and feed ingredient samples. Fifteen grain and feed samples were tested by DON-V ONE test with three different lots and the results are accurate (matching well with target values obtained through internal and external testing), the recoveries are between 84 and 111%. The CV% of reproducibility three operators for four different types of feed are between 7.5 to 10.6%.

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EASY AND FAST – A SMART SAMPLE CLEAN-UP FOR MULTI-MYCOTOXIN ANALYSIS OF VARIOUS MATRICES USING LC-MS/MS
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Matrix removal and analyte concentration are the main challenges for multi-mycotoxin analysis. Many easy sample clean-ups, e.g., QuEChERS-like approaches, struggle with matrix interferences or reduction of analytes due to a selective binding to the dispersive reagents employed, which were added to trap matrix interferences. As a consequence, a compensation by internal standards is needed. An easy non-dispersive SPE clean-up facilitates the sample clean-up and works for different types of matrices, such as cereals, dried fruits or nuts as a general procedure. The high sugar content of dried fruits and the fat and oil from nuts, which are present in many matrices, interferes with the sample clean-up. The CrossTOX® column allow a differentiated clean-up with removal of fat and oil or sugar from crude extracts without rendering the content of mycotoxins. This could be demonstrated for quality control materials from various origins. A sample clean-up just by pressing the crude extract through the cartridge via a plastic syringe with a standard Luer outlet is simple and easy and thus dedicated for high sample throughput. Due to several frits and the sorbents, the SPE cartridge substitutes the use of a syringe filter and accelerates the sample clean-up dramatically. All regulated mycotoxins from aflatoxin to zearalenone, including all highly regulated and trichothecene mycotoxins could be analysed by the clean-up procedure using the CrossTOX® column. High recoveries and correct toxin quantifications were achieved easily, while the detectable toxin amounts indicate a suitability for high sample throughput even at demanding levels. Recovery rates for various mycotoxins (aflatoxins B/G, ochratoxin A, zearalenone, fumonisins B1/B2, nivalenol, deoxynivalenol and derivatives thereof, T-2/H-T2 toxin) comply with any food and feed analysis. The detected levels of individual mycotoxins in QC material are comparable to traditional clean-up methods. The proposed multi-mycotoxin analysis covers the need of food, and feed testing routine laboratories, and is fit for changing challenges due to different prevalence of mycotoxins that originate from the global climate change or seasonally changing weather conditions. The fast and easy sample processing facilitates the clean-up and analysis of various sample matrices within the shortest time possible, reducing downtime of analytical devices and matrix interferences. Furthermore, can this SPE column successfully be used for other analytes as well, e.g., parameters in
food fraud (polyphenols and Whiskey-lactones), and chloramphenicol/malachite green in shrimps and meat samples, giving the CrossTOX® column new features to analyse food and feed samples beyond mycotoxins.

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LOW LEVEL DETECTION OF AFLATOXINS AND OCHRATOXIN IN CHALLENGING MATRICES – MEDICAL HERBS, SPICES, COCOA, AND COFFEE
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The challenge of mycotoxin analysis for aflatoxins, ochratoxin A or zearalenone is, besides the low tolerance of toxins in foodstuff, the occurrence of matrix effects in the analysis in particular at low regulatory levels. These factors are influencing the analytical results either by interferences or by masking analytical sensitivity. An increase of sample weight-in negatively influences the extraction efficiency and in general high sample loads proved to be disadvantageous for analysis. The clean-up using antibody-based technologies allows a selective concentration of analytes, a differentiated sample processing to reduce matrix interferences and finally concentrates analytes to increase analytical sensitivity. This avoids time-consuming evaporation processes and analyte losses due to adsorption effects on vials. The complete transfer of eluate into the analytical device allows a higher analytical sensitivity and facilitates downscaling in the entire sample processing. This reduces sample/solvent waste and accelerates the sample clean-up process. The special thermal denaturation technology used by the FREESTYLE ThermELUTE® increases selectivity and sensitivity of analysis in combination with full automated sample processing. Furthermore, downscaling of the sample volume by using highly selective SMART cartridges significantly accelerates the sample processing. This work presents the advantages of a thermal elution process in combination with a chromatographic focusing and, beyond nut and cereal matrices, focuses on difficult matrices, such as spices, medical herbs, cannabis, cocoa, and coffee. Matrix interferences of these samples are highly affecting the analytical performance. With the presented methodology, no compensation with analytical references as well as quality control samples is necessary. The complete, quantitative transfer of the cleaned sample into the LC system using the FREESTYLE ThermELUTE gains further analytical sensitivity in combination with the antibody based efficient and selective clean-up (SMART cartridges) and thus allows the usage of HPLC-FLD instead of highly sophisticated instruments. The low matrix load, but complete transfer into the chromatographic system overcomes the low sensitivity of traditional clean-up cartridges and improves sensitivity without losing selectivity. Additionally, there is no need for prolonged run times in order to overcome matrix impurities in the chromatography. Sample clean-up could be achieved by preparing the diluted sample in an automated manner 24/7 with online chromatography and data analysis as fast as 20 minutes for clean-up and subsequent chromatography. The technology is designed for all regulated mycotoxins, which need best sensitivity due to their regulatory levels (aflatoxins B/G/M1, ochratoxin A, zearalenone) and improves the sample throughput in food and feed testing laboratories.

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LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY DETERMINATION OF PATULIN USING ATMOSPHERIC PRESSURE CHEMICAL IONIZATION
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Patulin is a mycotoxin produced by Penicillium, Aspergillus, and Byssochlamys moulds that grow on fruit, grains, and cheese. The best-known example is patulin in juice or cider made from apples. The FDA has set an action level for patulin in apple juice and apple juice products at 50 ppb. Presently, FDA field laboratories use AOAC 995.10 for the quantification of patulin in apple juice by liquid chromatography with optical detection, and confirmation by mass spectrometry. The development of an LC-MS/MS based method provides a modernized approach to enable the quantification and confirmation of patulin in a single method, further expanding testing capabilities for monitoring the occurrence of patulin in apple juice and apple-derived food products. In this study, a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method with atmospheric pressure chemical ionization (APCI) was developed to determine patulin in apple juice and apple-based food. The method was single-laboratory validated following the FDA Guidelines for the Validation of Chemical Methods for the FDA Foods Program (3rd Ed.). Apple juice, apple cider, apple puree, apple-based baby food, applesauce, fruit rolls, and fruit jam were fortified with 13C-patulin and extracted using dichloromethane (DCM), followed by centrifugation to facilitate phase separation. The resulting DCM extracts were
analysed by LC-APCI-MS/MS. The identity of patulin was further confirmed using an information-dependent acquisition-enhanced product ion (IDA-EPI) MS/MS scan. Preliminary data demonstrates that the performance of the LC-APCI-MS/MS method is appropriate, without a requirement for time-consuming sample preparation or matrix-matched calibration standards. The linear calibration range was 1.0 to 1000 ppb ($r^2>0.99$), and the limit of quantitation (LOQ) was estimated to be 5 ppb. The use of $^{13}$C-patulin allowed quantitation using solvent calibration standards with satisfactory accuracy and precision. Average recoveries and relative standard deviations (RSD) in the seven spike matrices were 104% (15% RSD) at 10 ppb, 110% (5% RSD) at 50 ppb, 92% (9% RSD) at 200 ppb, and 102% (6% RSD) at 1000 ppb (n=28). The evaluation of measurement uncertainty using certified reference materials will also be assessed. Finally, the operation efficiency of the method will be investigated using an automated sample preparation system to evaluate feasibility of use for routine surveillance or survey activities.

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COST AND BENEFIT ANALYSIS: LC-MS VS. TLC/LC-UV/FLD FOR MYCOTOXIN DETERMINATION IN FOODS

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As the FDA mycotoxin program works toward implementing liquid chromatography-mass spectrometry (LC-MS) for regulatory analysis, we performed a cost analysis to assess the cost and benefit of using conventional thin layer chromatography (TLC) and liquid chromatography-ultraviolet/fluorescence (LC-UV/FLD) based single mycotoxin methods to the FDA compendial LC-MS based multi-mycotoxin method. The analysis was performed over a predefined time horizon of 10 years and includes variables such as the number of target mycotoxins (6), hourly labour cost ($60.12/hr), opportunity costs (at 3 and 7% discount rates), and sample throughput capacities (100, 1000, 10,000 samples/year), using total present value of costs and cost per analysis/sample. We estimate that the implementation of advanced LC-MS technology will produce significant benefits in the form of cost savings as opposed to TLC and LC-UV/FLD. With the throughput of 1000 sample/year, the LC-MS method could save approximately $1.47 million (7% discount rate), $1.80 million (3% discount rate), and $2.12 million (no discount rate) compared to TLC, and $1.56 million (7% discount rate), $1.89 million (3% discount rate), and $2.21 million (no discount rate) compared to LC-UV/FLD. With the throughput of 10,000 samples/year, LC-MS could save $1.90 million (7% discount rate), $2.23 million (3% discount rate), and $2.56 million (without discount rate) compared to TLC, and $1.91 million (7% discount rate), $2.24 million (3% discount rate), and $2.56 million (without discount rate) compared to LC-UV/FLD. However, for a laboratory with a low sample throughput, LC-MS would be a more costly method. For 100 samples/year, LC-MS costs about $0.2 million more than TLC and $0.14 million more than LC-UV/FLD over the course of 10 years. The cumulative costs associated with the three yearly sample capacities (100, 1,000 and 10,000 sample per year) clearly illustrate the benefit of LC-MS for the analysis of large number of samples (e.g., 1,000 or 10,000 per year). The use of Monte Carlo simulation further demonstrates the cost effectiveness in terms of cost per sample by factoring in the variability of key parameters within our model.

ADDENDUM

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AFLATOXINS AND OCHRATOXIN A OCCURRENCE IN DARK CHOCOLATE BARS MARKETED IN SOUTHERN ITALY

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Dark chocolate, renowned for its indulgent flavour and potential health benefits, is a globally cherished treat. Cocoa beans, derived from the cacao tree (Theobroma cacao L.) represent the raw material used in the manufacture of chocolate and confectioneries. Although it contains a high amount of bioactive compounds, such as flavanols and procyanidins, some research has shown that chocolate might be susceptible to fungus contamination, which could result in the formation of potentially dangerous mycotoxins. The increasing trend in its consumption highlights the significance of quality control...
procedures to minimize potential contamination risks in the final product. Based on the above, the aim of the current investigation was to optimize a simple method for the identification and quantification of aflatoxins (n=4) and ochratoxin A and their occurrence afterward in dark chocolate bars marketed in the Southern Italy region, Campania through a UHPLC Q-Orbitrap HRMS analysis. The method was optimized in accordance with the regulation in force (Commission Implementing Regulation (EU) 2021/808) and applied for the analysis of 18 dark chocolate samples differing in cocoa percentage. Validation parameters were optimized in terms of selectivity and specificity (mass accuracy<5 ppm), linearity was $r^2>0.990$, average recoveries were from 71 to 93%, and the intra-/inter-day precision was below 18 and 16%, respectively. Results showed contamination in 3 out of the total analysed samples. In particular, aflatoxin B2, produced by *Aspergillus* species, was detected in 16.67% of the samples under the limit of quantification (LOQ, 0.390 ng/g). Among mycotoxins, aflatoxins arouse particular concern due to their classification as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC). Hence, constant monitoring of final products intended for human consumption is needed to ensure food safety for consumers. **Acknowledgments.** This work was supported by the Project ‘ON Foods – Research and Innovation Network on Food and Nutrition Sustainability, Safety and Security – Working ON Foods’ funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3 – Call for proposals No. 341 of 15 March 2022 of the Italian Ministry of University and Research funded by the European Union – NextGenerationEU.

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**OPTIMIZATION OF A SIMPLE METHOD FOR DEOXYNIVALENOL ANALYSIS IN ITALIAN GRAIN SAMPLES**

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Mycotoxins are insidious toxins produced by certain fungi that represent a threat that not only jeopardizes human health but also inflicts considerable economic losses upon the industry. Since ever, cereals have been the main source of calories for a large part of the world’s population. Wheat is widely cultivated worldwide due to its adaptability to diverse climatic conditions. During the stages of food production, from pre-harvest to distribution, including post-harvest, processing, and storage phases, wheat grains can encounter fungal contamination. Deoxynivalenol (DON) contamination of wheat is a great concern to animal and human health. Prevention of *Fusarium* toxins has been intensively studied all over the world because genera *Fusarium* are typical field fungi infecting plants on the field. To examine the issue, a comprehensive method for the investigation of deoxynivalenol (DON) in Italian cereal grains samples (n=219) by ultra-high-performance liquid chromatography coupled with quadrupole Orbitrap high-resolution mass spectrometry (UHPLC Q-Orbitrap HRMS) was developed. The proposed method combines a simple sample preparation process with advanced technology, achieving rapid processing of results while maintaining exceptional sensitivity being the main features. The method was optimized in accordance with the regulation in force (Commission Implementing Regulation (EU) 2021/808). Comparisons between calibration curves built in a blank matrix and in neat solvent were performed. Calibration and matrix-matched curves were prepared over a concentration range from LOQ to 400 µg/kg. The correlation coefficient ($r^2$) was >0.988. Based on the results of matrix effects (101%), the external calibration curves were used for quantification purposes. The good specificity of the HRMS made it possible to have no signal interferences in the blank matrix. In addition, the LOQ obtained was more times lower than the limit reported for DON in Commission Regulation (EC) No 1881/2006. DON contamination was found in 1.4% of total analysed samples in concentration levels between 104.08 and 292.62 µg/kg. Although the obtained results do not exceed the maximum limit reported for unprocessed durum wheat and oats (1,750 µg/kg), analysis of contaminants in food grain is essential for minimizing health human risk. **Acknowledgments.** This work was supported by the Project ‘ON Foods – Research and Innovation Network on Food and Nutrition Sustainability, Safety and Security – Working ON Foods’ funded under the National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.3 – Call for proposals No. 341 of 15 March 2022 of the Italian Ministry of University and Research funded by the European Union – NextGenerationEU.
CONTAMINATION BY AFLATOXINS IN DIFFERENT FOOD MATRICES PRODUCED AND CONSUMED IN MOZAMBIQUE

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Mycotoxins are toxic metabolites produced by various moulds that frequently contaminate food worldwide, being significant contributors to food losses in developing countries. In Mozambique, there is no comprehensive knowledge of the risk of mycotoxins in the country, nor structured actions to reduce the impacts of mycotoxins and promote health and food security in disadvantaged populations. This research aimed to analyse the level of contamination by aflatoxins in different food matrices produced and consumed in southern Mozambique. Ten samples were collected from each matrix (maize, rice, and peanut) in each of the 3 districts (Chongoene, Manjacaze and Chókwe) of Gaza province, and 10 peanut samples in each of the 3 districts (Massinga, Inhambane and Inharrime) of Inhambane province, in a total of 120 samples. Samples were collected between January and June 2023 from local markets and producers. Samples were analysed for total aflatoxins using the lateral flow strip, AgraStrip® Pro WATEX® (Romer Labs) method. Results showed that, from all matrices, the highest levels of aflatoxins were found in maize, with averages ranging from 369.2 (in Manjacaze) to 1,972.6 μg/kg (in Chokwe). Average aflatoxin levels in rice ranged between 1.2 (Chongoene) and 63.08 μg/kg (Manjacaze). Peanuts from the province of Inhambane were more contaminated than those from Gaza, with averages ranging from 5.6 (Manjacaze, Gaza) to 95 μg/kg (Inhambane). Considering that the maximum admissible levels for total aflatoxins recommended by the Codex Alimentarius Commission for cereals and pulses is 15 μg/kg, the level of aflatoxin contamination in food produced and consumed in southern Mozambique is high and constitutes a public health risk for the population. Therefore, risk mitigation strategies are urgently needed. Acknowledgements. The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) and to the Aga Khan Development Network for the financial support to the project Ref. FCT AGA-KHAN / 541590696 / 2019 ‘MYCOTOX-PALOP – Multi-actor partnership for the risk assessment of mycotoxins along the food chain in African Portuguese-speaking countries (PALOP)’, and to FCT for financial support through national funds FCT/MCTES (PIDDAC) to CIMO (UIDB/00690/2020 and UIDP/00690/2020), SusTEC (LA/P/0007/2020), CiTAB (UID/AGR/04033/2020), CEB (UIDB/04469/2020), LABBELS (LA/P/0029/2020), and Inov4Agro (LA/P/0126/2020). Cláudio Matusse thanks FCT for the PhD grant PRT/BD/15483/2022.
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